BANANA *XANTHOMONAS* WILT: INCIDENCE, TRANSMISSION, PATHOGEN CHARACTERIZATION AND MANAGEMENT OPTIONS IN KAGERA, MWANZA AND MARA REGIONS

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ABSTRACT

Field surveys were conducted from December, 2011 to January, 2012 in Kagera, Mwanza and Mara regions to assess the current status of banana *Xanthomonas* wilt (BXW) disease. Twenty eighty banana samples with BXW disease symptoms were randomly collected from a total of 147 surveyed fields. Farmers' knowledge of BXW disease was assessed using a checklist, discussions and direct field observations. Isolation of bacteria was done on Yeast Peptone Glucose Agar and identification was done based on morphological, biochemical, polymerase chain reaction (PCR) and pathogenicity tests. Results indicated that the incidence of BXW disease was highest (56.7 %) at Ihangiro and the lowest (10 %) at Ruhija and Mulela, in Muleba District; Nakamwa and Busagami, in Ukerewe District. Such results implied that, BXW disease is a constraint to banana production in the surveyed districts. The results also indicated that BXW pathogen transmission was through infected farming tools (65.4 %) in Muleba District and infected planting materials in Tarime (50.5 %) and Ukerewe (45.8 %) Districts, implying that these were the major means by which BXW disease was spread in the study area. About 58.33 % and 41.67 % of farmers at Ibare and Kishanda villages, respectively, in Muleba District farmers associated pied crow (*Corvus albus* L.) with the transmission of BXW causing pathogen. Results based on morphological, biochemical and PCR test indicated that, four out of 16 bacteria isolated from infected banana samples were most likely *Xanthomonas campestris* pv. *musacearum* (*Xcm*). This study recommends farmers training through seminars on BXW disease identification and proper management. Further studies on the relationship between the pied crow and *Xcm* transmission in Kagera, Mara and Mwanza regions, are also recommended.

DECLARATION

I, **Ibrahim Hashim,** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Ibrahim Hashim Date

The above declaration was confirmed by

_____ _____

Professor Robert B. Mabagala Date

(Supervisor)

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DEDICATIONS

To my sister Rahima Hashim Mvungi who passed away while I was preparing this dissertation and to my mother Nuru Bahati Hussein Mvungi.

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LIST OF ABBREVIATIONS AND SYMBOLS

a.s.l	Above Sea Level
ARI	Agricultural Research Institute
AfSHC	African Seed Health Centre
BXW	Banana *Xanthomonas* Wilt
cfu	Colony Forming Unit
COSTECH	Commission for Science and Technology
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
FAOSTAT	Food and Agriculture Organization Statistics
GPS	Global Positioning System
h	Hour
Ha	Hectare
KOH	Potassium Hydroxide
MSc	Master of Science
mM	Milmolar
MOAC	Ministry of Agriculture and Cooperatives
O.D.	Optical Density
PCR	Polymerase Chain Reaction
spp.	Species
SPSS	Statistical Package for Social Sciences
pv.	Pathovar
SUA	Sokoine University of Agriculture
Xcm	*Xanthomonas campestris* pv. *musacearum*
TBE	Tris Borate EDTA
UV	Ultraviolet
YPGA	Yeast Peptone Glucose Agar

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Banana and plantain (*Musa* spp.) are the world's fifth most important food crops after maize, rice, wheat, and cassava (Tripathi and Tripath, 2009; Tripathi, 2011). The annual production of banana in the world is estimated to be 130 million tons; nearly one-third is grown in sub-Saharan Africa, where the crop provides more than 25% of the food energy requirements for over 100 million people (FAOSTAT, 2008; Tripathi, 2011).

Most of the bananas in Africa are produced and consumed in East Africa (Tripathi, 2011). Tanzania is the second biggest banana producer in East Africa after Uganda (FAOSTAT, 2008). The country cultivates 480 000 ha of banana, producing about 3.5 million metric tons per year (FAOSTAT, 2008). There are five main agro-ecological zones producing banana in Tanzania, namely; Lake zone (Kagera, Mwanza and Mara Regions), Northern zone (Arusha and Kilimanjaro Regions), Southern-Highlands (Mbeya, Iringa and Ruvuma Regions), Eastern zone (Morogoro, Tanga and Cost Regions) and Zanzibar Islands (MOAC, 2000).

Currently, banana production in these regions is decreasing due to declining soil fertility, increased pressure of insect pests, diseases and poor agronomic practices (Nkuba *et al.*, 2003). Of these factors, banana diseases such as banana *Xanthomonas* wilt (BXW) caused by *Xanthomonas campestris* pv. *musacearum,* is considered to be the biggest constraint in banana production particularly in the Lake zone regions where banana is the main staple food (Nkuba, 2007). The BXW disease was discovered first on Ensete (*Ensete ventricosum*), a plant closely related to banana in Ethiopia (Yirgou and Bradbury, 1968

and 1974). It was then reported in Uganda and the Democratic Republic of Congo in 2001, and one year later BXW disease was reported in Rwanda (Karamura *et al*., 2005). The disease was reported in 2005 in Muleba district, Kagera region, Tanzania (Mgenzi *et al*., 2006). The disease has also been reported in Kenya and Burundi (Karamura, 2006) and continues to spread to other banana growing areas in East Africa.

1.2 Justification

Banana *Xanthomonas* wilt disease is a major constraint in banana production in the Lake zone regions of Tanzania where banana plays an important role as a staple food and cash crop (Mgenzi *et al.,* 2006). Infected plants die and fruits rendered inedible (Tushemereirwe *et al.*, 2002). Since its first discovery in Muleba district, the disease has spread rapidly to the neighbouring districts of Missenyi, Bukoba, Ngara and Biharamulo (Kagera), Tarime (Mara) and Ukerewe (Mwanza) (Mgenzi *et al*., 2006; Mbega, E. personal communication, 2011). A survey conducted in 2011 by Agricultural Research Institute Maruku showed that about 112 ha of bananas have been uprooted due to BXW disease, causing an annual loss of $ 840 000 in Kagera region alone (Mkulila, S. personal communication, 2012). In the neighbouring countries of Kenya, Rwanda and Uganda, disease incidence of 61.9, 75.6 and 80 %, respectively, have been reported, causing yield losses of up to 100 % (Karamura *et al.,* 2005, Muhinyuza *et al.*, 2007). The BXW disease is spread by contaminated tools, sunbirds, insects such as bees and infected planting materials (Tinzaara *et al.,* 2006). However, little or no information is available on the contribution of these sources of inocula on the spread of the pathogen in Tanzania particularly in the areas where BXW disease has been reported. Furthermore, little or no information is available on the diversity of BXW-causing organism and on the current management options of the disease. Therefore, there is an urgent need to understand the

status of BXW disease in the Lake zone regions so that the results can be used in future BXW disease management programmes.

1.3 Objectives

1.3.1 Overall objective

To assess the current status of banana *Xanthomonas* wilt disease in Kagera, Mwanza and Mara Regions, in order to provide information for future disease management.

1.3.2 Specific objectives

i. To determine the incidence and mode of transmission of BXW disease in Kagera, Mwanza and Mara Regions
ii. To assess the efforts of stakeholders in the management of BXW disease
iii. To isolate and characterize the BXW disease causing pathogen

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of Banana in Tanzania

Bananas are staple food for estimated 20-30 % of the total Tanzania's population (Walker *et al.,* 1984). In the heavily banana-based farming systems; such as Kagera and Kilimanjaro Regions, about 70-95 % of households grow banana for food and/or cash, and average field size of banana grown ranges from 0.5 to 2.0 ha per household (Byabachwezi and Mbwana, 1996). In other areas, only few banana plants are maintained by households mainly for desert and roasting (Kalyebara *et al.,* 2007). Apart from being a potential food and cash crop, the banana plant provides medicines, feed for animals, used for decorations, making utensils, mats, thatching and protect the soil against lateral erosion and leaching by both massive root system and aerial leaf cover (Nkuba and Mgenzi, 2003; ARI Maruku, 2010).

2.2 Factors Affecting Banana Production in Tanzania

Average yield of banana has declined from 18 tonnes per hectare in the 1960s to less than 6 tonnes per hectare in the 2000s (Nkuba *et al.,* 2003; ARI Maruku, 2010). The major cause of this decline in yield includes; declining soil fertility due to low use of external inputs, low adoption of modern agronomic practices versus the use of outdated traditional banana husbandry, low return to labour due to current market prices, yield levels of endemic cultivars and increasing pressure and wide spread of biotic problems such as; weevils, nematodes, black Sigatoka disease, Panama disease or *Fusarium* wilt and banana *Xanthomonas* wilt disease (Bosch *et al.,* 1996; Ndile *et al.,* 1999; ARI Maruku, 2010).

2.3 Discovery and Importance of Banana *Xanthomonas* Wilt Causing Organism

The causal agent of banana wilt disease was first described in 1960s in Ethiopia as *Xanthomonas musacearum* (Dagnachew and Bradbury, 1968) and later on in the 1970s, it was renamed as *Xanthomonas campestris* pv. *musacearum* (*Xcm*) (Young *et al.,* 1978). The bacterium affects all commonly grown banana cultivars (Tushemereirwe *et al.,* 2002). It attacks the vascular system of banana, causing wilting and death of the plants (Karamura *et al.,* 2005; Mwebaze *et al.,* 2006a; Biruma *et al.,* 2007).

2.4 Description of *Xanthomonas campestris* pv. *musacearum*

Xanthomonas campestris pv. *musacearum* is motile, possess a single polar flagellum, Gram-negative, oxidase negative, obligate aerobic, rod shaped and produces typical yellow, convex, mucoid colonies on nutrient agar, forms xanthomonadin pigment and reduces nitrate (Yirgou and Bradbury, 1968 and 1974).

2.5 Transmission of Banana *Xanthomonas* Wilt Disease

The disease is transmitted from plant to plant within a field by flying insects or birds visiting the flowers to feed on nectar and mechanically by contaminated tools used in farming operations (Ndungo *et al.,* 2005; Tumushabe *et al.,* 2006 and Tinzaara *et al.,* 2006). Long distance transmission is often man-induced through the movement of planting materials that are carrying latent infections (Tinzaara *et al.*, 2006).

2.6 Symptoms of Banana *Xanthomonas* Wilt Disease

The BXW disease is characterized by a progressive yellowing and wilting of leaves, uneven/premature ripening of fruits with sections showing unique yellowish blotches in the pulp and dark brown placental scars. Symptoms on floral parts include wilting of bracts, shrivelling, rotting of the male buds, and yellow brown flower stalks. When

pseudostems and rachis are cut, pockets of pale yellow bacterial ooze appear within 5 to 15 min (Tushemereirwe *et al.,* 2003; Tripathi and Tripathi, 2009).

2.7 Host Range

In addition to *Musa* spp. and *Ensete ventricosum,* an ornamental wild weed *Canna indica* serves as an alternative hosts of banana bacterial wilt causal organism (Ssekiwoko *et al.,* 2006).

2.8 Disease Epidemiology

Eden-Green (2004) reported that, *Xanthomonas campestris* pv. *musacearum* infects banana plants through the lower parts of the plant (roots) possibly from soil-borne inocula which is able to persist in the soil for not more than 3 months (Mwebaze *et al.,* 2006a). The entry of pathogens into plants is facilitated by mechanical injuries caused by soil borne organisms such as nematodes and insects. Transmission from plant to plant within a field is thought to be principally accomplished by flying insects from oozing male bud to the healthy inflorescences (Tinzaara and Tripathi, 2006) and mechanically by contaminated tools used in pruning operations and eroded contaminated soils to the healthy plants (Tushemereirwe *et al.,* 2002).

2.9 Management of Banana *Xanthomonas* Wilt Disease

Banana *Xanthomonas* wilt disease management depends largely on the combination of approaches such as; exclusion, eradication, host resistance and protection (Tushemereirwe *et al.,* 2002; Tripathi *et al.,* 2009). Field observation in Uganda suggested that, cultural practices including timely removal of the male bud to interrupt the insect transmission cycle, the use of healthy, disease-free suckers for planting material, destruction and controlled movement of diseased plants, cleaning of contaminated tools using disinfectant

or heat treatment and rotation of crops are the most effective BXW disease control measures (Brandt *et al.,* 1997; Tushemereirwe *et al.,* 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Field Surveys and Collection of Infected Banana Samples

Field surveys were conducted in Kagera, Mwanza and Mara regions using Mult-stage Random sampling procedures (Kagenzi *et al.,* 2006) with modifications. Modifications included conducting surveys only in districts and villages with BXW disease, whereas within the villages banana fields with or without BXW disease were selected for data collection. Three Districts with high disease pressure namely Muleba (Kagera region), Tarime (Mara Region) and Ukerewe (Mwanza Region) were covered. A total of 17 villages and 147 banana fields were surveyed and inspected for the presence of BXW disease. BXW disease symptoms were identified using a diagnostic guide by Karamura *et al.* (2008). A total of 28 banana samples with BXW disease symptoms were randomly collected from 17 surveyed villages (Table 1). In addition, the locations where samples were collected were marked by recording the altitude, latitude and longitude using the Global Positioning System (GPS).

3.1.1 Determination of banana *Xanthomonas* wilt disease incidence

In each randomly selected banana fields (previously described under section 3.1), 30 banana plants were randomly selected by making two diagonal transect walk as described by Mbaka *et al.* (2009) with modification, where fields with only few banana mats, infected banana plants were identified and all banana plants or mats near homestead were counted. From randomly selected 30 banana plants, infected plants were identified and counted. The BXW disease incidence was determined by dividing the number of diseased plants over the total number of observed plants in the field times one hundred to get percentage.

Table 1: Banana *Xanthomonas* wilt disease samples collected from different areas of Muleba, Tarime and Ukerewe Districts

S/N	Banana cultivar	Part Collected	Village	District	Longitude (°)	Latitude (°)	Altitude (m. a.s.l)
1	Nshakara	Stem	Ibare	Muleba	31.6173	1.7500	1544
2	Ndizi kali	leaf	Nshambya	Muleba	31.6078	1.7278	1490
3	Njoge	Fruit	Rubao	Muleba	31.6350	1.7083	1491
4	Nshakara	Stem	Ruhija	Muleba	31.6186	1.7021	1478
5	Nshasha	leaf petiole	Rubya	Muleba	31.6151	1.7498	1536
6	Talio	Fruit	Ihangiro	Muleba	31.5917	1.8017	1538
7	Ntobe	Leaf	Ihunga	Muleba	31.5595	1.7272	1537
8	Nshakara	bunch stalk	Kishanda	Muleba	31.5578	1.7196	1533
9	Nyoya	Stem	Mulela	Muleba	31.5543	1.7574	1519
10	Ndizi kali	Fruit	Rwagati	Muleba	31.6086	1.7639	1508
11	Buganda	bunch stalk	Mogabiri	Tarime	34.4263	1.3860	1661
12	Buganda	leaf petiole	Mogabiri	Tarime	34.4265	1.3769	1666
13	Buganda	leaf	Mogabiri	Tarime	34.4339	1.3775	1684
14	Buganda	leaf petiole	Mogabiri	Tarime	34.4250	1.3733	1669
15	Buganda	Stem	Mogabiri	Tarime	34.4127	1.3695	1619
16	Buganda	Stem	Mogabiri	Tarime	34.4153	1.3732	1625
17	Buganda Ng'o	leaf petiole	Mogabiri	Tarime	34.4144	1.3683	1623
18	Buganda	Stem	Mogabiri	Tarime	34.4139	1.3682	1621
19	Buganda	Stem	Mogabiri	Tarime	34.3810	1.3668	1617
20	Buganda	Stem	Mogabiri	Tarime	34.4270	1.3765	1658
21	Nchwa	leaf petiole	Murutirima	Ukerewe	32.8950	1.9104	1143
22	Mungala	Fruit	Murutirima	Ukerewe	32.8951	1.9104	1160
23	Guantama	Leaf	Bukonyo	Ukerewe	32.9251	1.9621	1190
24	Mungala	Stem	Namilembe	Ukerewe	32.9170	1.9635	1196
25	Guantama	Fruit	Busangu	Ukerewe	32.8964	1.9591	1163
26	Nchwa	leaf petiole	Busagami	Ukerewe	32.9446	1.9758	1189
27	Nchwa	Stem	Nakamwa	Ukerewe	32.9371	1.9716	1178
28	Nchwa	leaf petiole	Namilembe	Ukerewe	32.9371	1.9718	1181

m.a.s.l = metres above sea level (°) =degrees

3.1.2 Means of transmission of banana *Xanthomonas* wilt disease

The means of transmission of BXW disease were determined by visual observation using a scale of 1-7 (Muhinyuza *et al*., 2007): where 1 = No symptom, 2 = Wilting symptoms and yellowing of young plants, 3 = Wilting leaves of flowering plants, 4= Dry male buds and

no wilting symptoms on plant, 5 = Wilting leaves on any plant of the mat and dry male bud, 6 = Heavy wilting, drying of male bud and premature ripening of fruits and 7 = Infected leaves were yellow/ necrotic and no bunch development/ discoloration. Scores 2, 3 and 7 were associated with transmission by infected tools or infected planting materials and scores 4, 5 and 6 were associated with insect transmission (Muhinyuza *et al*., 2007).

3.1.3 Assessment of management efforts for banana *Xanthomonas* wilt disease

A structured checklist (Appendix 1) was used to assess farmer's knowledge on BXW disease e.g. understanding of *Xanthomonas* wilt symptoms, first date of appearance of the disease in the field or village, means of spread, knowledge of disease control options, current uses of the control measures and sources of clean planting materials. In addition, the efforts of other stakeholders in the management of BXW disease were also assessed. Other methods used in the assessment include direct field observation and discussion with farmers.

3.1.4 Sample handling, isolation and characterization of *Xanthomonas campestris* pv. *musacearum*

3.1.4.1 Handling of collected samples

A small section of about 2 cm x 4 cm from *Xanthomonas* wilt symptomatic leaves, flower stalk, leaf petiole and stems were exercised using disinfected knife and packed with wool placed on silica gel in vials (Mbega, E. personal communication, 2011). A total of 28 samples were collected, packed on vials and labelled as shown on (Plate 1) and transported to the African Seed Health Centre (AfSHC) Laboratories, Sokoine University of Agriculture, Morogoro for further processing.

Plate 1: Banana samples packed in vials containing silica gel and cotton wool.

3.1.4.2 Isolation of bacteria from infected samples

Using the semi-selective media Yeast Peptone Glucose Agar (YPGA) (Yeast 5 g; Peptone 5 g; Glucose 4 g and Agar 12 g per litre) (Mwebaze *et al*., 2006b) to isolate bacteria from infected plant samples, small portions of 4 mm^2 were removed and placed into four drops of sterile distilled water and teased by sterile forceps. After 15 seconds a loopful of the extract was streaked, onto YPGA (Mwebaze *et al*., 2006b). Inoculated plates were incubated at 28°C for up to 72 h. Single yellow colonies were purified on Nutrient Agar ('Lab-Lemco' powder 1.0 g; Yeast extract 2.0 g; Peptone 5.0 g and Sodium chloride 15.0 g per litre) (at 28 °C for 48-72 h). The cultures were preserved at 4 ^{o}C until when used (Artua *et al*., 2007).

3.1.4.3 Identification of bacterial isolates

Pure bacterial isolates were identified using physiological and biochemical characteristics, including colony morphology on YPGA, Gram reaction, Kovac's oxidase reaction, nitrate reduction, pathogenecity test and polymerase chain reaction (PCR) (Aritua *et al*., 2008; Adriko *et al*., 2012).

(a) Gram reaction (3% Potassium hydroxide solubility test)

A sterile tooth pick tip was used to fetch a 28-h-old bacterial isolates from a pure colony and the isolates were mixed with one drop of 3% KOH aqueous solution on a clean glass slide. After mixing a tooth pick was raised a few centimetres from a glass slide to see a strand of viscid mucous material (Mortensen, 2005). If the strands of viscid material were observed the bacterial isolates in question were regarded as Gram-negative. Lack of strands of viscid material was recorded as Gram-positive.

(b) Kovac's oxidase reaction

A sterile tooth pick tip was used to fetch a 28-h-old bacterial culture on YPGA and the isolate was rubbed on a filter paper containing two drops of 1% aqueous N, N, N, N- tetra methyl -p-Phenylenediamine dihydrochloride solution. Development of purple colour on the isolates was observed within 10 seconds, 60 seconds and more than 60 seconds (Dickey and Kelman, 1988). Change in colour of the reagent to purple within 10 seconds of application of the culture was recorded as positive for oxidase reaction while a change in colour of the reagent to purple within more than 60 seconds was recorded as negative for oxidase reaction.

(c) DNA extraction

The DNeasy tissue and blood protocol was used to extract DNA from bacterial culture while the DNeasy plant kit (Qiagen, 2006) with modifications was used to extract DNA from infected banana plant samples. A modification included crushing of the tissue by adding 0.4 g of acid washed sand to 100 mg wet weight of banana samples and crushed using sterile mortor and pestle. The disrupted samples were put into an Eppendof tube and re-suspended, vortexed and centrifuged following the protocol of the manufacturers' recommendations (Qiagen, 2006).

(d) Detection of the bacteria using polymerase chain reaction

The DNA from bacterial isolates and infected plant samples were subjected to polymerase chain reactions (PCR) tests using the methodology by Adriko *et al.* (2012). Using *Xan* F7/R7 primers, the amplifications were carried out with a final volume of 25 µl (1.5 µl of 1.5 mM $MgCl_2$, 5 µl of buffer 5x, 4 µl of 1.25 mM dNTPs, 0.25 µl of 100 pM of each primer, 0.25 µl of 1.25 units of *Taq* polymerase, 1 µl of DNA extracted from banana sample/bacterial isolates and 12.75 µl of sterile distilled water). The reaction was run for 30 cycles, each consisting of 20 seconds at 95ºC, 15 seconds at 64 ºC and 15 seconds at 72 ºC. Samples of 8 µl from each amplified PCR product were run on a 1.0% agarose gel stained with 12 µl of 0.5 µg/ml ethidium bromide placed in electrophoresis buffer (TBE) at 50V for 45 min and visualized on a UV trans illuminator.

(e) Pathogenicity test

Pathogenicity tests to confirm the identity of the pathogen (*Xcm*) were done using a susceptible banana cultivar, Pisang Awak recommended for pathogenicity tests (Tripathi and Tripathi, 2009). Forty eight-hour-old bacterial isolates grown on Nutrient Agar were suspended in sterile distilled water and adjusted to 0.3 O.D at 460 nm (10^7-10^8 cfu/ml bacterial cell concentration) using a spectrophotometer (Phillip Sunnes: Model 21907). One ml of the bacterial suspension was injected onto the young leaf petiole of three months old suckers planted in pots with sun-dried soil, sand and manure mixture in the ratio of 3:1:1 (Quimio, 1992). Five isolates which were most likely member of the genus *Xanthomonas* were tested.

The pots were placed in the screen house. Two plants were inoculated per isolate. Control plants were inoculated with sterile distilled water. Inoculated plants were observed for

symptom development daily for five weeks and the bacteria were re-isolated as described under 3.1.4.2 – 3.1.4.3 sections and tested for pathogenicity based on Koch's postulates.

3.1.5 Data analysis

Banana *Xanthomonas* wilt disease incidence was calculated using Microsoft Excel 2007. To address objective 1 and 2, descriptive statistics (frequencies, means and cross tabulation) were used to describe the means of transmission and management options of BXW disease in Kagera, Mara and Mwanza regions. Data were analysed by using SPSS software for windows version 16.0.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Banana *Xanthomonas* Wilt Disease Incidence

The results show that the highest BXW disease incidence (56.7 % and 53.3 %) was found in Ihangiro and Ibare villages, respectively, and the lowest (10 %) BXW disease incidence was found in Ruhija and Mulela villages in Muleba District (Table 2). In Tarime District BXW disease incidence was 16.6 % and in Ukerewe District, the highest BXW disease incidence was found in the Busangu village (40 %) followed by Namilembe (30 %), Gallu (28.9 %) and Bukonyo (16.6 %) villages (Table 2). The lowest BXW disease incidence (10 %) in the Ukerewe District was observed in Nakamwa and Busagami villages (Table 2). Such results implied that, the BXW disease is a constraint to banana production in the surveyed Districts. Earlier reports by Mgenzi *et al*. (2006) and Mbega, E. personal communicationf, (2011) showed that, BXW is a major disease of banana in these Districts. Persistency of BXW disease on banana in these Districts may be attributed by improper BXW disease control methods (Mchunguzi, D.H. personal communication, 2012).

Addis *et al*. (2010) reported that, cultural practices that require intensive use of tools such as regular leaf removal and removal of excess suckers can contribute to increased BXW disease spread. During these cultural practices, bacteria can easily be transmitted by infected farm tools to health plants (Tushemereirwe, 2005; Addis *et al*., 2010).

Table 2: Banana *Xanthomonas* wilt disease incidence in banana fields in Muleba, Tarime and Ukerewe Districts in January, 2012

S/N	District	Village	Longitude (^{0})	Latitude (^{0})	Altitude (m.a.s.l)	Size (acres)	Sample Plants (mats)	No. of infected plants (mats)	BXW disease incidence (%)*
1	Muleba	Ibare	31.6151	1.7487	1520	2.0	30	16	**53.3**
2	Muleba	Nshambya	31.6104	1.7426	1490	1.5	30	10	**33.3**
3	Muleba	Rubao	31.647	1.7186	1489	2.0	30	4	**13.0**
4	Muleba	Ruhija	31.6194	1.7022	1469	1.2	30	3	**10.0**
5	Muleba	Rubya	31.6159	1.7502	1514	1.4	30	13	**43.3**
6	Muleba	Ihangiro	31.5902	1.8069	1529	1.5	30	17	**56.7**
7	Muleba	Kishanda	31.5886	1.7173	1527	2.0	30	12	**40.0**
8	Muleba	Ihunga	31.5631	1.7272	1529	1.6	30	11	**36.6**
9	Muleba	Mulela	31.5478	1.7678	1535	2.0	30	3	**10.0**
10	Muleba	Rwagati	31.5543	1.7574	1506	1.4	30	14	**46.6**
11	Tarime	Mogabiri	31.4178	1.3722	1658	1.6	30	8	**26.6**
12	Ukerewe	Bukonyo	31.9251	1.9621	1174	0.3	12	2	**16.6**
13	Ukerewe	Busangu	31.8894	1.9628	1174	0.7	30	12	**40.0**
14	Ukerewe	Nakamwa	31.9371	1.9383	1193	0.3	20	2	**10.0**
15	Ukerewe	Busagami	31.9471	1.9737	1186	0.3	10	1	**10.0**
16	Ukerewe	Namilembe	31.9371	1.9718	1188	0.3	10	3	**30.0**
17	Ukerewe	Gallu	31.8839	1.9303	1160	0.7	18	5	**28.9**

m.a.s.l = metres above sea level, BXW = Banana *Xanthomonas* Wilt, *BXW disease incidence = number of infected plants over total observed plants in the field times one hundred

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4.2 Means of Transmission of Banana *Xanthomonas* Wilt Disease

4.2.1 Transmission by infected tools

Using 1-7 scale (Muhinyuza *et al*., 2007) results show that 23.5 %, 65.4 % and 29.2 % of banana fields surveyed in Muleba, Tarime and Ukerewe districts, respectively, had typical BXW disease symptoms with scores of 2 and 3 indicating transmission by infected tools (Table 3). Such results implied that, infected farming tools have contributed to BXW disease transmission in the surveyed districts. These results comply with earlier reports by Addis *et al*. (2010) who indicated that infected farm tools can contribute to high disease incidence of up to 90 %.

4.2.2 Transmission by insects

Results indicated that 8.2 % of banana fields visited in Muleba District had BXW disease symptoms with a mean BXW disease score of 6, indicating transmission by insect (Table 3). Such low insect transmission may be due to a traditional practice of removing male buds which usually attract insects. Flying insects such as bees feeding on male inflorescences can move with BXW disease causing organism from infected male bud to the health inflorescences (Tinzaara *et al*., 2006). Farmers in Muleba District believe that the removal of male buds increases vigour on banana bunches (Mchunguzi, D.H. personal communication, 2012). Where male buds have not been removed, spread of BXW disease by insect increases (Tushemereirwe, 2005).

4.2.3 Transmission by planting materials

Using the same scale as in section 4.1.2.1 above, results showed that 50.5 %, 45.8 % and 15.4 % of the banana fields surveyed in Muleba, Ukerewe and Tarime districts, respectively, showed wilting symptoms with a mean BXW disease score of 2 (Table 3).

During the survey field observations showed that recently planted banana plants had BXW disease symptoms (Plate 2). This implied that, *Xcm* was probably transmitted by infected planting materials. Field observations indicate that, farmers use suckers harvested from their old fields or from neighbours for planting. If such planting materials are infected by *Xcm* the pathogen can spread across fields and regions (Mbaka *et al.*, 2009).

Table 3: Symptoms of banana *Xanthomonas* wilt disease observed in Muleba, Tarime and Ukerewe Districts in January, 2012

Types of symptoms observed	Fields visited			
	Muleba	Tarime	Ukerewe	$\bar{X}$
No symptoms	16.5	19.2	25.0	20.2
Wilting symptoms and yellowing of young plants	23.5	65.4	29.2	39.4
Wilting leaves of flowering plants	1.0	0.0	0.0	0.3
Wilting symptoms and yellowing on young plants and wilting leaves of flowering plants	50.5	15.4	45.8	37.2
Heavy wilting, dry male bud and premature ripening of fruits	8.2	0.0	0.0	2.7

$\bar{X}$ = Mean

The results also showed that, 84.5 % and 50 % of farmers visited in Muleba and Tarime district, respectively, obtained banana suckers from their own fields (Table 4). In the Ukerewe District, 50 % of the farmers visited obtained banana suckers either from their own fields or from their neighbours' fields (Table 4). Such results indicated that, farmers in Muleba, Tarime and Ukerewe Districts use plant materials from local sources. Such a situation can continue to transmit *Xcm* in infected banana suckers. Infected planting materials have been reported to be among the most important means of spreading systemic bacterial diseases including BXW (Hayward, 2006).

Plate 2: A recent established banana plants showing *Xanthomonas* wilt disease symptoms indicating that the disease was transmitted by suckers.

Table 4: Sources of banana planting materials in Muleba, Tarime and Ukerewe Districts in January, 2012

Source of planting materials (suckers)	Percentage of farmers reported			
	Muleba	Tarime	Ukerewe	$\bar{X}$
Own	84.5	50.0	25.0	53.2
Neighbours/friends only	9.3	15.4	25.0	16.6
Own and Neighbours/friends	6.2	34.6	50.0	30.3

$\bar{X}$ = Mean

4.2.4 Transmission by pied crow

Survey results revealed that 58.33 % and 41.67 % of farmers field infected with BXW disease at Ibare and Kishanda villages, respectively, in Muleba District were associated with pied crow (*Corvus albus* L.) (Plate 3a) with the increase in BXW disease incidence in their fields (Table 5). During field surveys, the pied crow was found resting on banana leaves (Plate 3a). Injuries on banana leaves caused by frequent visit by the pied crow on healthy and infected banana leaves might have provided an entry point for *Xcm* (Plate 3b

and 3c) carried by the bird's feet and the beak. In Ibare and Kishanda, the birds were often found near rubbish, slaughter houses, markets and farm lands feeding on rubbish, debris, and household leftovers. Small birds feeding on nectar on banana flowers are known to transmit *Xcm* (Tushemereirwe, 2005; Tinzaara *et al*., 2006; Addis *et al*., 2010). No solid evidence on the role of pied crow in transmitting *Xcm* but this was speculations only. There is a need to investigate the role of birds in transmission of *Xcm* in banana.

Table 5: Villages and banana fields visited by pied crow in Muleba District

Village	**No. of fields affected by pied crow / total**	**Fiels affected by pied crow (%)**	**BXW disease incidence (%)**
Ibare	7/12	58.33	42.20
Kishanda	5/12	41.67	39.70

BXW = Banana *Xanthomonas* Wilt

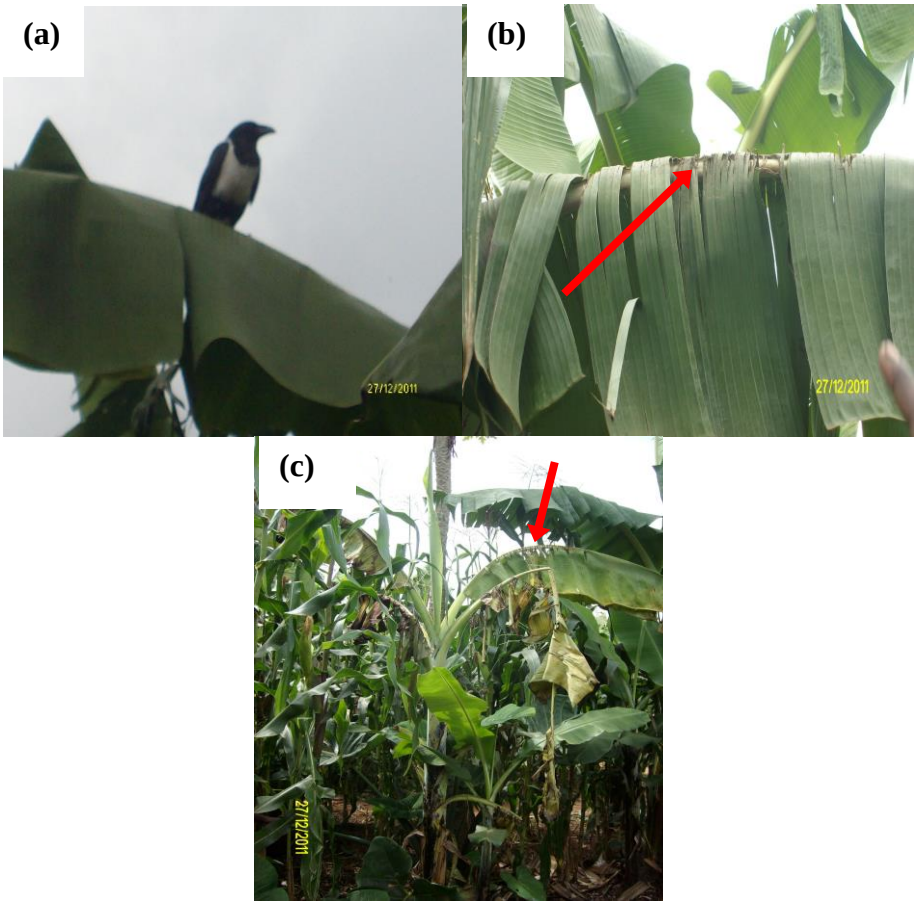

Plate 3: (a): Pied crow resting on banana leaf, (b): red arrow shows the damage caused by the same bird on banana leaf and (c): banana plant wilting due to infection by banana wilt causing xanthomonads.

4.3 Assessment of Management Efforts for Banana *Xanthomonas* Wilt Disease

Interviews with farmers in Muleba, Tarime and Ukerewe districts showed that, all (100 %) farmers visited obtained information about BXW disease outbreak from their neighbours or friends within the same District or from other areas already affected by BXW disease (Table 6). Such results imply that social networks such as farmer to farmer interactions contributed to awareness of BXW disease.

The results also showed that 54.6 % and 42.3 % of farmers in Muleba and Tarime Districts, respectively, obtained information about BXW disease management options from extension officers (Table 6). This can be attributed to prompt training of extension officers obtained from researchers during the outbreak of BXW disease (Nkuba, J. personal communication, 2012).

In Ukerewe District, 41.7 % and 29.2 % of farmers visited obtained information about BXW disease management options from neighbours/friends and researchers working at ARI Maruku in Kagera Region respectively (Table 6). Other sources of information for farmers about BXW control options are presented in Table 6. Such results demonstrate that, there is some interaction between farmers, extension staff, researchers and other governmental organizations in the dissemination of information about BXW disease control. Therefore, linkage between farmers, researchers and extension staff is important in the formulation and dissemination of BXW disease control technologies (Bagamba *et al*., 2006; Jogo *et al.*, 2011).

4.3.1 Current banana *Xanthomonas* wilt disease management options

Results indicated that 62.9 % and 26.9 % of the farmers visited in Muleba and Tarime districts, respectively, reported removal of male buds (Plate 4A), cutting down infected plants and disinfecting working tools as management options of BXW disease (Table 7). However, direct field observation during the surveys revealed that some farmers were cutting down infected plants, chopping them into small pieces and heaping them on one side of the field as management options of BXW disease (Plate 4B) and others tended to remove only the visible diseased plant parts such as leaves or plants within the banana mat (Plate 4C). Most of the farmers in Tarime (73.1 %) and Ukerewe (87.5 %) reported cutting

down of infected plants once the disease is observed in their fields (Plate 4D and Table 7). These results indicate that most of the farmers visited lack awareness on proper ways of eradicating BXW disease.

Table 6: Sources of information on banana *Xanthomonas* wilt disease outbreak and management options in Muleba, Tarime and Ukerewe Districts

Sources of information	The percentage of farmers reported			
	Muleba	**Tarime**	**Ukerewe**	$\bar{X}$
BXW disease outbreak				
Friends/neighbours	100	100	100	100
BXW disease control				
Own	0.0	0.0	12.5	4.2
Neighbours/friends	22.7	26.9	41.7	30.4
Own and neighbours/friends	1.0	0.0	0.0	0.3
Researchers	3.1	0.0	29.2	10.8
Extension officer	54.6	42.3	16.6	37.8
Village meeting	18.6	30.8	0.0	16.5

BXW = Banana *Xanthomonas* Wilt $\bar{X}$ = Mean

Different approaches for BXW disease management have been developed (Biruma *et al*., 2007; Mwangi *et al*., 2007a; Tushemereirwe *et al*., 2003). However, in this study it was found that approaches involving destruction of infected banana plants were poorly adopted. If the disease is not properly controlled, the *Xcm* can survive in corms and suckers. As a result infected suckers may carry the pathogen and become new sources of inocula for BXW disease in the field.

In the Ukerewe District, results from discussion with farmers indicated that, none of the farmers applied either removal of male buds and infected plants or disinfection of farm tools after use in the field (Table 7). Such results were probably due to lack of awareness of the importance of infected banana male buds in the transmission of BXW disease and the dangers of leaving them in the field.

In Tarime District, 26.9 % of farmers visited removed banana male buds for feeding cattle and not for BXW disease management purposes (Table 7). Male bud removal for control of BXW has been poorly adopted among the farming communities in the surveyed districts (Kagenzi *et al.*, 2006).

Table 7: Current banana *Xanthomonas* wilt disease management options in Muleba, Tarime and Ukerewe Districts

BXW disease management options	The percentage of farmers reported			
	Muleba	Tarime	Ukerewe	$\bar{x}$
Removal of male bud, cut down infected plant and disinfect tools	62.9	26.9	0.0	29.9
Removal of infected plant	29.8	73.1	87.5	63.5
Removal of male bud and avoid sharing working tools	5.3	0.0	4.2	3.2
Cut down infected plants and remove male bud	2.0	0.0	0.0	0.7
None of the above	0.0	0.0	8.3	2.8

BXW = Banana *Xanthomonas* Wilt $\bar{x}$ = Mean

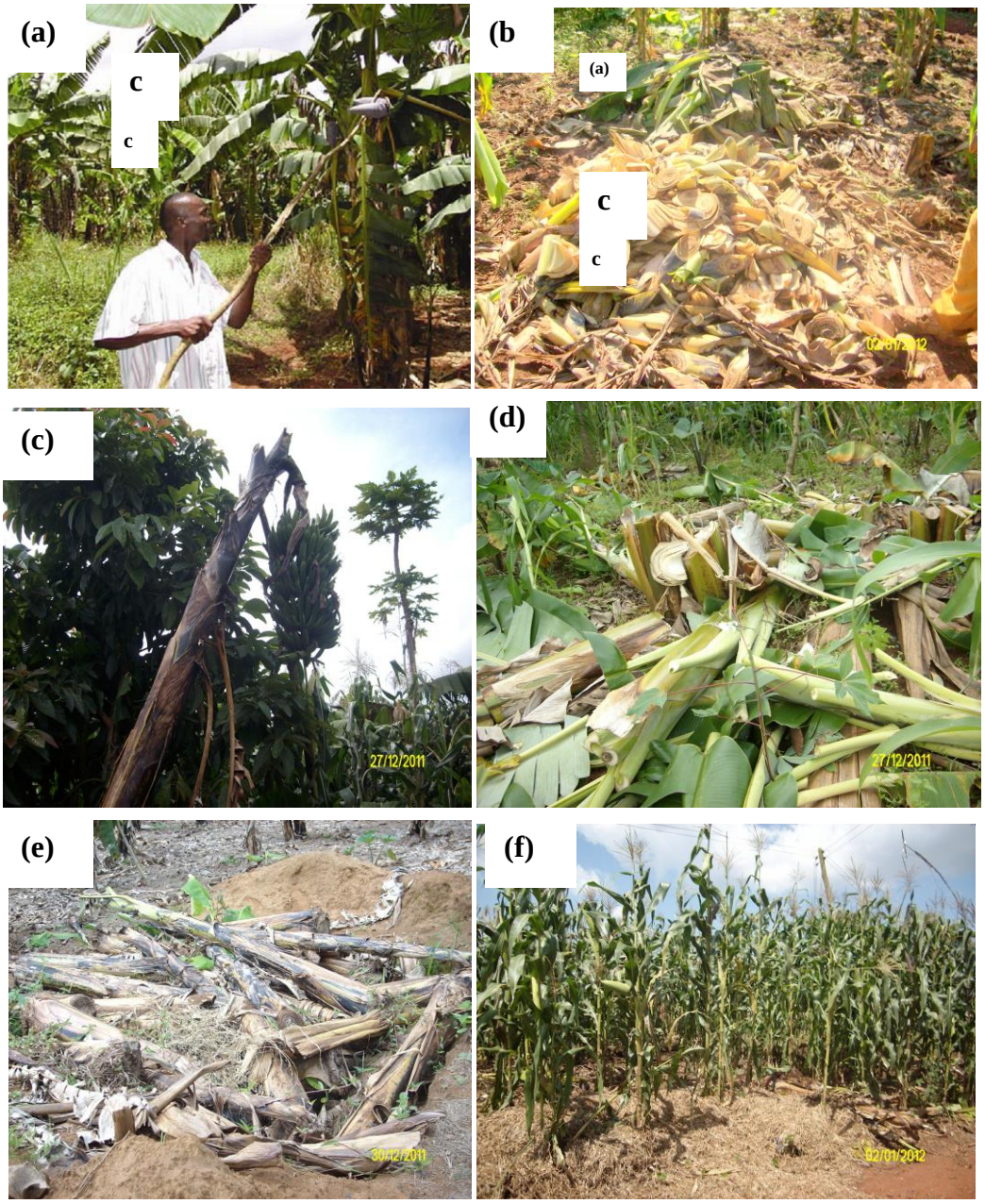

Plate 4: Different banana *Xanthomonas* wilt disease management options performed by farmers in the surveyed districts; (a): removal of male bud, (b): cutting down infected plants, chopping them into small pieces and heaping them on one side of the field (c): removing the visible diseased leaves, (d): cutting down all infected plants, (e): digging a hole and fill it with infected plants and (f): banana fields replaced with other crops such as maize during fallowing period.

Once BXW disease has been observed in the field its control depends largely on the immediate removal of sources of inocula such as infected plants and elimination of opportunities for further spread such as removal of male buds, disinfect garden tools and use of disease free suckers (Eden-Green, 2004).

4.3.2 Indigenous knowledge on the control of banana *Xanthomonas* wilt disease

Direct field observation and interview with farmers revealed that 8.2 % of the farmers in Muleba District use ash and human urine, while 6.2 % of farmers cut infected banana and cover the remaining base of banana stem (cut) with the soil to control BXW disease (Table 8 and Plate 5a). The use of soil and ash to cover the wounds of cut banana stems discourages the insects that may come to feed on the ooze and hence minimize disease transmission by insects (Tinzaara *et al*., 2006).

Table 8: Indigenous knowledge on the control of banana *Xanthomonas* wilt disease in Muleba, Tarime and Ukerewe Districts

	Percentage of farmers reported*			
Indigenous knowledge	**Muleba**	**Tarime**	**Ukerewe**	$\bar{X}$
Use of soil to cover wound of cut banana stem	6.2	0.0	0.0	2.1
Use ash to cover wound of cut banana stem	4.1	11.5	0.0	5.2
Use urine on cut banana stem	2.1	0.0	0.0	0.7
None of the above	79.4	88.5	100	89.3

*****Percentage of farmers reported = number of farmers using indigenous knowledge over total number of farmers surveyed times one hundred

$\bar{X}$ = Mean

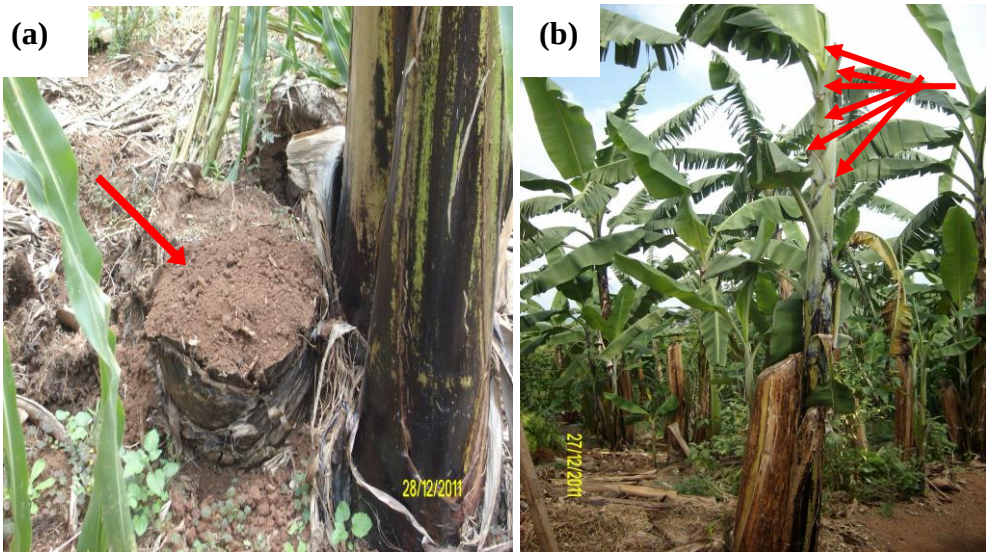

Plate 5: (a): The remaining part of the banana stem (after cutting down the aerial portion) covered with the soil and (b): removal of banana leaves showing banana *Xanthomonas* wilt disease symptoms as an indigenous disease management method (red arrows show scars on removed leaves).

4.4 Isolation and characterization of *Xanthomonas campestris* pv. *musacearum*

A total of sixteen bacterial isolates were isolated from 28 infected banana samples. Among 16 bacterial isolates grown on YPGA, five isolates were light to deep yellow convex colonies and 11 had yellow dry colonies (Table 9 and Plate 6a). This results indicated that there is a need to investigate the recovery of *Xcm* on YPGA as a selective medium.

4.4.1 Biochemical characteristics of the isolate

Results of biochemical test of the bacteria isolated from banana samples showed that 11 out of the 16 isolates were Gram-negative and five isolates were Gram-positive (Table 9 and Plate 6b). Seven out of the 16 isolates recovered were negative for nitrate reduction and nine isolates were positive for nitrate reduction. Oxidase test was negative for 13 of the 16 isolates and variable for three isolates (Table 9). Based on these results,

presumptive identification indicate that, five out of sixteen bacterial isolates were most likely members from the genus *Xanthomonas* (Yirgou and Bradbury, 1968). These five isolates were selected for further testing. Gram-positive, oxidase positive and yellow dry isolates were discarded.

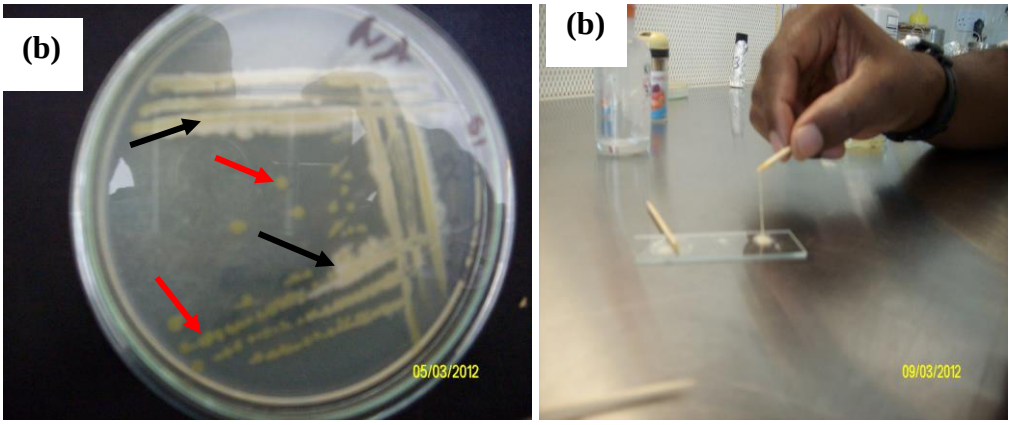

Plate 6: (a): Three days old colonies of bacteria from banana samples on the Yeast Peptone Glucose Agar showing a yellow color (red arrows) and cream pigmented bacterial contaminants (black arrow) and (b): the mucous thread produced by Gram negative bacteria on 3% Potassium hydroxide solubility test.

Table 9: Biochemical test (Gram reaction, nitrate reduction and oxidase test) and growth characteristic of the bacterial isolates on Yeast Peptone Glucose Agar medium

Isolate	Location	Part collected	Growth on YPGA medium	3% KOH test	Nitrate Reduction	Oxidase test
S5	Muleba	Stem	Yellow dry colonies	-	+	-
S0	Muleba	Leaf	Light, deep yellow convex colonies	-	-	-
S8	Muleba	Fruit	Yellow dry colonies	+	+	-
S1	Muleba	Leaf	Light, deep yellow convex colonies	-	-	-
S6	Muleba	bunch stalk	Yellow dry colonies	-	+	+/-
S10	Muleba	Fruit	Yellow dry colonies	-	-	-
S7	Tarime	bunch stalk	Yellow dry colonies	+	+	-
S16	Tarime	leaf petiole	Yellow dry colonies	_	+	+/-
S2	Tarime	Leaf	Light, deep yellow convex colonies	-	-	-
S11	Tarime	leaf petiole	Yellow dry colonies	+	+	-
S9	Tarime	Stem	Yellow dry colonies	_	+	-
S3	Tarime	Leaf	Light, deep yellow convex colonies	-	-	-
S15	Tarime	Stem	Yellow dry colonies	_	-	+/-
S12	Ukerewe	leaf petiole	Yellow dry colonies	+	+	-
S14	Ukerewe	Fruit	Yellow dry colonies	+	+	-
S4	Ukerewe	Leaf	Light, deep yellow convex colonies	-	-	-

(+/-) = Variable, (-) = Negative and (+) = Positive

4.4.2 Detection of bacteria using a polymerase chain reaction test

Polymerase chain reaction using *Xan* F7/R7 primers with DNA from banana samples collected from Ukerewe, Tarime and Muleba districts showed that, isolates S0, S1, S3 and S4 were amplified (Plate 7a and 7b) indicating that they were members of the genus *Xanthomonas*. DNA from bacteria isolate S2 was slightly amplified (Plate 7a).

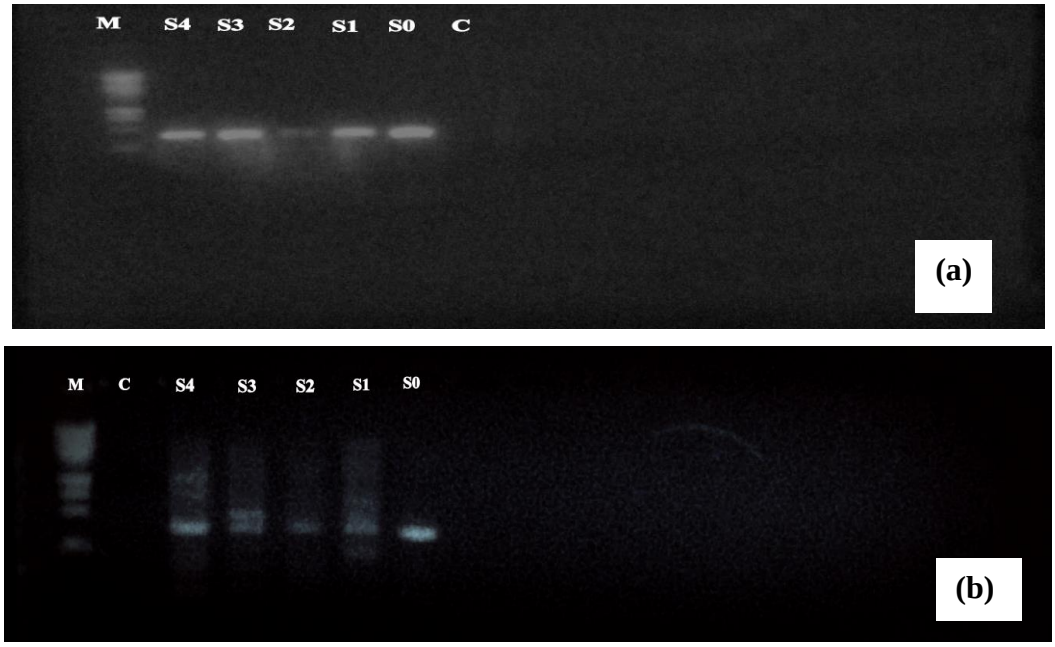

Plate 7: Polymerase chain reaction test results of; (a): DNA extracted from bacterial isolates and (b): banana samples collected from Muleba, Tarime and Ukerewe districts using *Xan* F7/R7 primers: genus specific primers for Xanthomonads, M= Molecular weight marker, S0 and S1= isolates from Muleba, S2 and S3= isolates from Tarime and S4= isolate from Ukerewe districts.

4.4.3 Pathogenicity tests

Pathogenicity tests done at three months old potted banana plants indicated that the leaves inoculated with isolate S3 developed water soaking brown necrosis at the leaf apex within 9 days followed by yellowing of the veins (Plate 8a and b). Plants inoculated with isolates S0, S1 and S4 showed yellowing around the inoculated area of the leaves within five weeks. Such symptoms were similar to those observed under field conditions. Among the five isolates; isolate S3 induced severe BXW disease symptoms with water soaking, brown necrosis and yellowing earlier than the other isolates. Necrotic symptoms were not observed on the control suckers inoculated with sterile distilled water (Plate 8c). The results from the re-isolated bacteria from banana plants inoculated with isolates S0, S1, S3

and S4 showed that the bacteria produced colonies with light to deep yellow colour and they were Gram-negative, Oxidase negative and the DNA from these isolates were amplified by PCR test. Such results confirmed Koch's postulates and implied that, these isolates were indeed pathogenic on banana (Plate 8b).

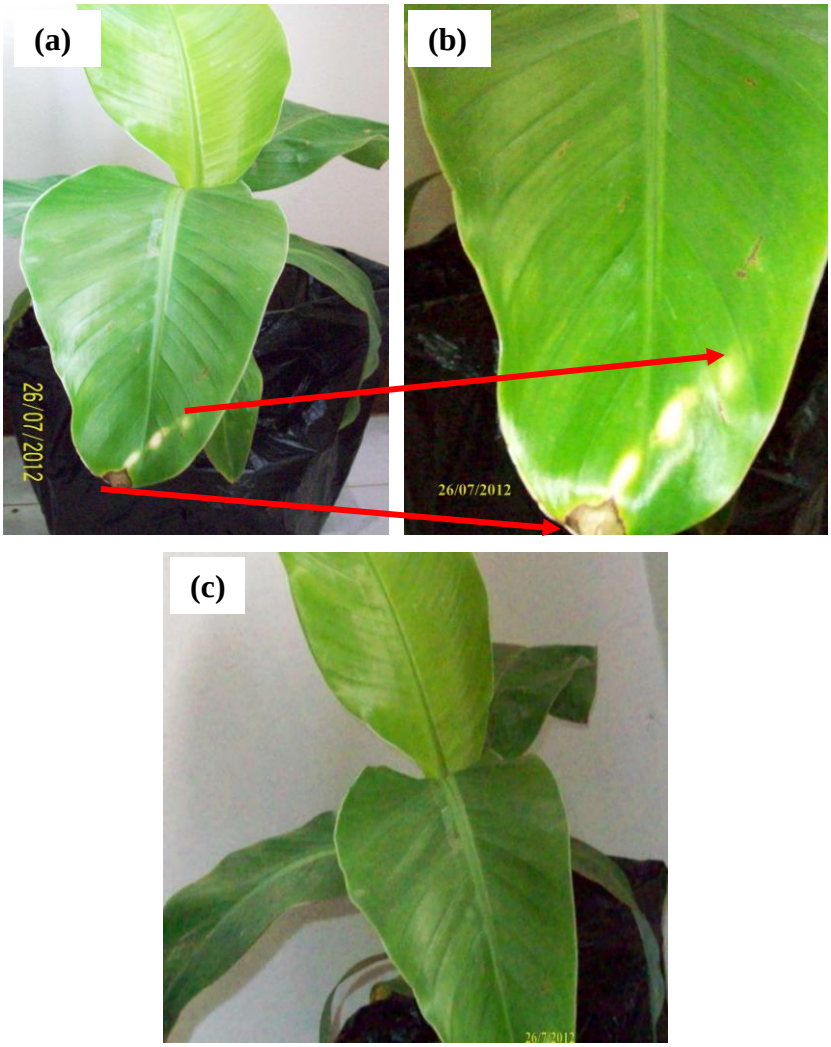

Plate 8: (a): Banana plant inoculated with isolate S3; (b): initial stage of wilting symptoms starting from the apex of the leaf that was inoculated; (c): plant inoculated with sterile distilled water.

CHAPTER FIVE

5.1 CONCLUSIONS AND RECOMMENDATIONS

5.2 Conclusions

In this study BXW disease incidence varied across and within the surveyed areas. The highest BXW disease incidence (56.7 %) was observed at Ihangiro in Muleba District and the lowest (10 %) was observed at Ruhija and Mulela in Muleba District and Nakamwa and Busagami in Ukerewe District. This implied that BXW disease is a major constraint to banana production in these Districts.

This study has also shown that the major means of BXW disease transmission were infected farming tools (65.4 %) in Muleba, infected planting materials (50.5 %) in Tarime and (45.8 %) in Ukerewe Districts indicating that these were the major means by which *Xcm* spread in these Districts. In Kishanda and Ibare villages, Muleba District farmers associated the pied crow (*Covus albus*) with BXW disease transmission. Such results indicate that the pied crow may be a potential vector for long distance transmission of BXW pathogen.

This study has also shown that social networks such as farmer to farmer interaction were important means of disseminating information about BXW disease and such network groups were aware of the BXW disease in banana production. The study also showed that management/control methods for BXW disease such as removal of male buds, cutting down infected plants and disinfecting tools were poorly applied by farmers in the study area.

Four of the 16 bacterial isolates obtained in this study were easily identified and confirmed as *X. campestris* pv. *musacearum* by PCR tests implying that the method can be used for fast, sensitive and reliable diagnosis of the bacteria from banana suckers and for monitoring movement of *Xcm* along the borders.

5.3 Recommendations

This study has shown that,

i. The major means by which *Xcm* spread in Muleba, Tarime and Ukerewe districts were infected farming tools and infected planting materials. Therefore, training of farmers through seminars on BXW disease management especially the use of disease free planting materials and proper disinfection of working tools is highly required.

ii. In Kishanda and Ibare villages in Muleba District farmers associated the pied crow bird with BXW disease transmission. Currently there is no evidence which show that pied crow can transmit *Xcm.* Therefore, further investigations on the effect of pied crow in transmission of *Xcm* in banana is highly required to confirm the current allegations.

iii. Four of the 16 bacterial isolates obtained in this study were confirmed as *X. campestris* pv. *musacearum.* Further studies on the recovery of *Xcm* on YPGA as a selective medium are recommended.

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APPENDICES

Appendix 1: A structured check list used for interviewing farmers during the field surveys

FIELD SURVEY CHECK LIST

A: Geographical location

Region …… District ……. Ward ……Village ……

B: Topography of the Area

Altitude……..… Latitude ……..…… Longitude ……

C: Farmers information

Name of the farmer…… Age …….. Sex (1= Male 2= Female)

Level of education …… (1= none, 2= primary, 3= secondary, 4=teritiary)

Number of years spent in this area ……years. Years spent in farming banana ……years

Size of your farm in acres…… Do you grow banana for (1= Food only, 2= Cash only 3= Both)

D: BXW diseasestatus on field and community

Have you ever heard of BXW disease (1= yes …, 2= no …..)

From where did you first heard of this disease (1= friends/neighbours, 2=radio/Tv, 3= news paper, 4= church/ mosque/school, 5= market place, 7= others)

Has the disease ever been observed in your:-

(1= yes, 2= no)	If yes; when did the disease first observed
Field	How many mats
Village	How many fields
Ward	How many fields

Name of banana cultivars grown		**Name of banana cultivars infected**	
Cooking	Brewing/ Ripening	Cooking	Brewing/ Ripening

Source of planting material (1=own, 2= neighbours/friends 3=both 1& 2, 4= researchers 5= others)

E: Control options by the farmer (farmers efforts)

Is there any indigenous knowledge used/ known to control BXW?

What do you do to control BXW disease in your field?

a…….

b……

c……..

d……

e……..

f…….

g……

Who informed you about these practices; (1=own, 2= neighbours/friends 3=both 1& 2, 4= researchers 5= extension officer, 6= others (specify))

F: Efforts of government and other stakeholders in management of BXW disease

What are the efforts of the government in controlling BXW disease?

Local government (Ward/Village level):-

Central government:-

What are the efforts of other groups (religious groups, NGOs, CBOs) in controlling BXW disease?