DISTRIBUTION OF ENSET BACTERIAL WILT IN SOUTHWEST ETHIOPIA, CHARACTERIZATION OF THE PATHGEN (*Xanthomonas campestris* Pv. *musacearum*) AND VARIATIONS IN HOST PATHOGEN INTERACTION

M.Sc. THESIS

BEFEKADU HAILE DESTA

OCTOBER, 2012
JIMMA, ETHIOPIA

DISTRIBUTION OF ENSET BACTERIAL WILT (*Xanthomonas campestris* Pv *musacearum*) AND VARIATIONS IN HOST-PATHOGEN INTERACTION IN SOUTHWEST ETHIOPIA

By

BEFEKADU HAILE DESTA

A Thesis Submitted to Department of Horticulture and Plant Sciences, Jimma University College of Agriculture and Veterinary Medicine, in Partial Fulfillment of the Requirements for Master of Science in Plant Pathology

OCTOBER, 2012

JIMMA, ETHIOPIA

APPROVAL SHEET OF THESIS
SCHOOL OF GRADUATE STUDIES JIMMA UNIVERSITY

As Thesis research advisor, I herby certify that I have read and evaluated this Thesis prepared, under my guidance, by Befekadu Haile Desta, entitled **"**Distribution of Enset Bacterial Wilt (*Xanthomonas campestris* Pv *musacearum*) and Variations in Host-PathogenInteraction in Southwest Ethiopia". I recommend it be submitted as fulfilling the Thesis requirement.

Girma Adugna, (PhD)	_____	_____
Name of Major-Advisor	Signature	Date
Fikire Handoro, (PhD)	_____	_____
Name of Co-Advisor	Signature	Date

As members of the Examining Board of the Final M.Sc Thesis Open Defense Examination, we certify that we have read, evaluated the thesis prepared by Befekadu Haile Desta and examined the candidate. We recommend that it be accepted as fulfilling the Thesis requirement for the degree of Master of Science in Plant Pathology.

_____	_____	_____
Name of Chairperson,	Signature	Date
_____	_____	_____
Name of Internal Examiner	Signature	Date
_____	_____	_____
Name of External Examiner	Signature	Date

DEDICATION

This M.Sc. thesis work is dedicated to my Wife W/ro Asmarech Desseno.

STATEMENT OF AUTHOR

I declare that this piece of work is my own and all sources of materials used for this thesis work have been duly acknowledged. The thesis has been submitted in partial fulfillment of the requirements for the degree of Master of Science at Jimma University and is reserved at the University Library to be made available to users. I solemnly declare that this thesis work is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

With accurate acknowledgment of the source, users are free to use this thesis without special permission. Permission for extended quotation or duplication of the manuscript in whole or in part may be granted by the Horticulture and Plant Science Department Head, or Dean of School of Graduate Studies of Jimma University. In all other instances, however, permission should be obtained from the author.

Name: Befekadu Haile Desta
Place: Jimma, Ethiopia
Signature_____
Date of Submission_____

BIOGRAPHICAL SKETCH

Befekadu Haile Desta was born in June, 1986 at Gecha town, Sheka Zone of Southern Nations, Nationalities and Peoples' Region. He attended his elementary and junior secondary schools at Gecha and High School at Masha secondery high school. Following the completion of his secondary education, he joined Addis Ababa University, Science Faculty and graduated with B.Sc. Degree in Biology in August, 2006. He was employed by Sheka Zone Water, Mineral and Energy Department where he has been working as water and mineral resource study and management main job process coordinator, until he joined the graduate studies program of Jimma University College of Agriculture and Veterinary Medicine to pursue his study leading to a Master of Science degree in Plant Pathology.

ACKNOWLEDGEMENTS

All praises and thanks goes to almighty of GOD, who is the entire source of all knowledge and kinds of wisdom endowed to mankind. His bounteous blessing that flourished my thoughts and fulfilled my ambitions and my modest efforts in the write up and made this material contribution towards the deep ocean of scientific knowledge already existing.

This thesis owes its existence to the help, support, and inspiration of many people. In the first place, I am profoundly indebted to my major advisor Dr. Girma Adugna and co-adviser Dr. Fikire Handoro for their unreserved advice, guidance and valuable suggestions in each and every step of my research work and thesis write up. Without the encouragement, insight and professional expertise of my advisors, the completion of this work would not have been possible.

I gratefully acknowledge the immense contribution made by Jimma University College of Agriculture and Veterinary Medicine postgraduate coordination office for its facilitation and fruitful guidance during class and research work.

My special gratitude is extended to my dearest brothers Takele Abeto, Isral Haile, Yared Haile and Efrem Gelito for their unreserved effort to cooperate with me from the beginning to the end of the field experiments.

I would like also to express my sincere gratitude to my mother Shageche Wotto, my brothers Asfaw Haile and Mulugeta Haile; and my sisters Atalelech Haile, Serawit Haile and Atinesh Haile for their dedication in bringing me up and for their strong support throughout my life and my academic career. I would like to express my deepest gratefulness to my wife Asmarech Desseno for constant support, understanding, and love I received from her. She has been the source of my strength and encouragement. I consider this academic achievement as a success for her as well.

ACRONOMY

^{0}C	degree centigrade
AUDPC	area under the disease progress curve
c.v	coffiecient of variation
cfu	colony forming unit
DI	disease incidence
DP	Disease prevalence
EBW	enset bacterial wilt
g	Gram
IP	incubation period
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
m.a.s.l	metres above sea level
ml	milliliter
PSI	Percentage severity index
Pv.	pathovar
RCBD	randomized completely block design
Rep-PCR	repetitive polymres chain reaction
RFLP	restriction fragment length polymorphism
SD	standerd deviation
SNNPR	Southern Nations, Nationalities and Peoples' Region
Sq. Km	square kilometer
Uv	ultra violate
YPSA	yeast, peptone, sucrose, agar

TABLE OF CONTENTS

Contents **Pages**

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF TABLES

Tables **Page**

LIST OF FIGURES

Figures **Pages**

LIST OF PLATES

Plates **Pages**

LIST OF TABLES IN THE APPENDICIES

Appendix Tables **Pages**

ABSTRACT

Enset bacterial wilt (EBW) caused by Xanthomonas campestris Pv. *musacearum is one of the highly destructive diseases that affect enset plants in the enset growng areas. However its extent of damage and distribution, the characteristics and variability of the pathogen, and the host-pathogen interactions are little studied in southwest Ethiopia. Thus, objective of this study were to assess the distribution of enset bacterial wilt, to isolate and characterize strains of the causal pathogens, and to study host-pathogen interactions of enset clones and Xanthomonas campestris* Pv. *musacearum strains collected in these areas. Survey and field studies were conducted in three enset growing areas of Sheka, Keffa and Bench-magi zones of southwest Ethiopia and the laboratory and greenhouse experiments were conducted in Jimma University College of Agriculture and Veterinary Medicine. Completely randomized design (CRD) with three replications were used for the laboratory study while randomized complete block design (RCBD) with 3 isolates x 4 enset clones factorial treatment combinations, replicated three times, were used for host-pathogen interaction studies in the field experment. The disease incidence ranged from 25.56% to 59.63% in enset growing areas of Sheka, Keffa and Bench-Magi zones in southwest Ethiopia. EBW symptoms were more severe in two Weredas, Masha and She-bench, each having percentage severity index (PSI) of 55.56% than in other Weredas (<50%). The most important factors responsible for spreading the disease include infected planting materials, contaminated farming and processing tools, and human and animal vectors. In cultural and physiological characterization of Xanthomonas campestris* Pv. *musacearum populations, almost all of the 19 isolates slightly differ in colony color and growths types with similar pathogenicity. In the field experiment, there were highly significant differences ($p<0.001$) in disease incidence (DI), incubation period (IP), percentage severity index (PSI) and area under disease progress curve (AUDPC) values; and significant differences ($p<0.05$) in date to complete wilting (DW) in the host-pathogen interactions study. Based on the result, the enset clones Nobo and Gudiro revealed complete and high resistance reaction, respectively, to all isolates with low mean percentage severity index of 0.0% and 6.7%, respectively; whereas enset clones Yeko and Chikaro were susceptible to all the isolates and showed high mean percentage severity index of 100% and 85.2%, respectively. Among the isolates, Sheka (YeLYe) isolate was highly pathogenic to Yeko and Chikaro enset clones as compared to the moderately pathogenic Keffa (GiHSh) and Bench- magi (ShMGe) isolates. In comparing clones by isolate interactions, all isolates caused equal percentage severity index on enset clone Yeko (100%), and isolate YeLYe induced higher percentage severity index on enset clone Chikaro (100%) than the isolate ShMGe (71.2%) and GiHSh (68.9%). In conclusion, enset bacterial wilt is prevalent and important disease of enset in southwest Ethiopia although there exists variation in host reaction to the pathogen. The bacterial population collected in the enset growing areas showed basically similar cultural, physiological and biochemical nature of the species Xanthomonas campestris* Pv. *musacearum although clear difference in some features like colony color, growth nature, salt tolerances and pathogenicity. Variations in isolate-clone interaction are suggestive of the need to evaluate the response of several number of Xanthomonas campestris* Pv. *musacearum isolates and characters of enset clones. Therefore, for further work by including more number of Xanthomonas campestris* Pv. *musacearum isolates and enset clones as well as their genetic backgrounds associated with pathogenicity of the Xanthomonas campestris* Pv. *musacearum strains and resistance of enset clones are important.*

Key words: *enset clone, enset bacterial wilt*, *Xanthomonas campestris* Pv. *musacearum*

1. INTRODUCTION

1.1. Background information

Enset (*Ensete ventricosum* (Welw.) Cheesman) is a monocarpic, herbaceous plant belonging to the *Musacea family* as it strongly resembles banana morphologically (Taye, 1980) and the genus *Ensete*. Wild enset (*E. ventricosum*) is common and widespread in Ethiopia and along the rift valley in eastern Africa, all the way south to Mozambique (Simmonds, 1958). However, it is only in Ethiopia that enset has been domesticated and is cultivated for food, animal feed and fiber (Bezuneh *et al*., 1967). The cultivation of enset is concentrated in the southern and southwestern part of Ethiopia (Bezuneh and Feleke, 1966).

It is estimated that a quarter or more than 15 million of Ethiopia's population depends on enset as staple and co-staple food source, for fiber, animal forage, construction materials and medicines (Brandt *et al.,* 1997) and the area of enset production in Ethiopia is estimated to be over 279,000 hactares (CSA, 2009).

Based on the level of priority given to enset cultivation in different zones and regions, three enset based farming systems have been identified. Enset is the main food source in Gurage, Kembata, Sidama, Gedeo, Hadya, Jemjem, Arero, Keffa and Sheka zones. It is the second important crop as co-staple food in Wolaita, Gofa and Yem special *weredas*. It is planted as the third most important food crop in Wollega, Illubabor and in some parts of Southern region. In the second and third farming systems, cereals and other root crops take the primary and secondary importance (Admasu *et al*., 1997).

For various reasons, its production system is still traditional and tiresome. Among others different management practices starting from propagation to harvesting and processing demand high labor. Furthermore, diseases, insect pests and wild animals are also among the important production constraints of enset production. Various diseases and insect pests of enset have been reported including leaf damaging fungal diseases, corm rot, sheath rot and dead heartleaf rot of enset with unknown causal agents and root knot, root lesion and black leaf streak nematode diseases (Quimio and Mesfin, 1996). There are also viral diseases of enset known as mosaic and chlorotic leaf streak diseases. Insects damaging enset leaves such

as Jassid fly, spider mites, mealy bugs and wild animals such as porcupine, mole rat and wild pigs have been reported (Brandt *et al.,* 1997). However, based on the distribution and the damage incurred on enset production, enset bacterial wilt disease caused by *Xanthomonas campestris* Pv *musacearum* is known to be the most threatening and important problem to enset production in Ethiopia. The disease is widely distributed in many enset growing regions of the country and affects the crops in all stages (Dereje, 1985). Natural epidemics of the disease were also observed in banana fields at different enset growing area (Archaido and Mesfin, 1993).

Disease eradication efforts for enset bacterial wilt could not be effective because of gaps in the basic research including understanding nature of the disease, host-pathogen interactions; mode of transmission etc. has not been studied in-depth. The nature and use of the crop is also one of the reasons that affect the effectiveness of sanitary measures and resistant clone screening activities. This is because enset is a perennial crop with an average length of 10 meter and pseudostem circumference of about one meter and it is very difficult to easily practice sanitary measure. Uprooting and burying of infected plants may not be a simple task for the farmers. In addition, selecting tolerant clones and distributing among enset growing farmers will not be easy because of the fact that farmers use different enset clones for different purposes. For example, enset clones such as *Arkiya* and *Suite* (in Wolaita) are preferred for their corm, *Astara* (in Gurage) for its medicinal value and *Hala* (in Wolaita) for its good kocho yield; *Yeshirekinke* (in Gurage) and *Genticha* (in Sidama) are preferred for their kocho and especially for their disease tolerance (Kidist, 2003).

Some studies indicated variations in the occurrence and incidence of enset bacterial wilt in major enset growing areas. This is perhaps the implication of the existence of variability within *X. campestris* Pv *musacearum* population. Therefore detection of this variation could contribute for the development of technologies to control enset bacterial wilt mainly with identifying bacterial wilt resistant clones (Gizachew, 2000). In a study conducted at Awassa, for example, some enset clones that are tolerant to some isolates of the causal agent become susceptible to other strains of the bacteria. While some enset clones showed consistently better reaction to enset wilt pathogen under different experiments and at varying times (Fikre and Gizachew, 2007).

Thus, the incidence and prevalence of enset bacterial wilt, diversity within the bacterial pathogen populations and the reaction of local enset clones against the pathogen strains are not studied in southwest Ethiopia.

1.2. Objectives

1.2.1. General objectives

To assess enset bacterial wilt, to isolate and characterize strains of the causal pathogens, and to study host-pathogen interactions of enset clones and *Xanthomonas campestris* Pv *musacearum* strains in southwest Ethiopia.

1.2.2. Specific objectives

1. To assess the distribution, prevalence, incidence and severity of enset bacterial wilt disease in southwest Ethiopia.
2. To isolate and characterize strains of the pathogen collected in southwest Ethiopia.
3. To study host-pathogen interactions of enset clones and *Xanthomonas campestris* Pv. *musacearum* strains by artificial inoculation in the field under natural conditions.

2. Literature Review

2.1. Taxonomy and History of Enset

Enset (*Ensete ventricosum* (Welw) Cheesman) is a Monocot that belongs to order *Schistaminae* and family *Musaceae*. Enset was considered as member of the genus *Musa* as it strongly resembles banana morphologically (Taye, 1980). It was Cheesman (1947) who separated enset from banana on the basis of differences in pseudostem morphology and chromosome numbers. Different hypothesis are proposed on the origin of enset agriculture. Agronomists and biogeographers have long considered the Ethiopian high lands to be the primary center of origin for enset agriculture. In relation to this, anthropologists, archaeologists, historians and their scholars have also developed hypothesis that argue for the domestication of enset in Ethiopia as early as 10,000 years ago (Brandt *et al*., 1997).

Currently enset distribution is restricted to south, southwest and central part of Ethiopia and it is not known as food crop in the northern part of the country. However, historical evidences suggested that enset may have once played a much more important role in the agricultural practices of central and northern Ethiopia before the mid-19th century (Brandt *et al.,* 1997). The possible reasons for total disappearance of enset culture in the North could be disease, drought and instability in the sociopolitical events between the 1700 and the 1800 (Brandt *et al*., 1997).

2.2. Enset Morphology and Ecology

Enset looks like a large, thick, single- stemmed banana plant usually larger than banana and 6-12 meters tall. The leaves are 5-7 meters tall and up to1 meter in diameter and are more erect than a banana plant (Brandt *et al.,* 1997). The stem has three parts. The pseudostem, which is made of tightly clasping leaf sheaths, is 2-3 meters in height and with an average of 1meter diameter containing an edible pulp and quality fiber. The underground corm is an enlarged lower portion of the stem with an average of 0.7meter length and diameter, the fibrous root system of enset grows out from this part. The true stem is between the pseudostem and corm near the ground. The plant usually grows up during maturity and initiates a single flower head, which forms multiple flower fruits and seeds. The small banana-like fruits produce

several irregularly shaped black seeds. Most wild enset plants are produced from seeds unlike the domesticated ones, which are propagated from suckers (Brandt *et al.,* 1997).

Enset cultivation is restricted at altitudes ranging from 1600 to 3100 m.a.s.l (Taye and Asrat, 1966). But recent investigations indicated that it grows in areas as low as 1200m.a.s.l (Endale, 1990). However, the best elevation for enset cultivation is between 2000 and 2750 m.a.s.l with an annual rainfall of 1100 to 1500 mm where the majority falls between March and September (Brandt *et al.,* 1997). The average temperature of enset growing areas varies between 16 to 20°c and the relative humidity 60 to 80 % (Yohannes and Mengel, 1994). Soil types in the enset growing areas of Ethiopia are moderately acidic to slightly basic with pH reactions ranges of 5.6 to 7.3. These soils contain 0.10 to 0.15% total nitrogen and 2 to 3% organic matters (Taye and Asrat, 1966).

2.3. Economic Importance and Uses of Enset

Enset is a multipurpose crop of which every part is thoroughly utilized (Shigeta, 1996). The corm and the pseudostem are the most important sources of food. It is a good source of starch. The types of food from these parts are known as '*Kocho*', '*Bulla*' and '*Amicho'* (Spring *et al.,* 1996). *Kocho* is the bulk of the fermented starch obtained from the decorticated (scraped) leaf sheathes and grated corm. *Bulla* is obtained by squeezing out the liquid containing starch from scraped leaf sheathes and grated corm and allowing the resultant starch to concentrate into white powder. Amicho is boiled corm of young enset plants known for best quality of corm. It is prepared and consumed in a similar manner to preparation of other root and tuber crops (Brandt *et al.,* 1997). Fiber is the by-product of enset that is left after decorticating the leaf sheathes. Its strength is found to be equivalent to the important fiber crop *Musa texstalis* (*abaca*) (Taye, 1984). Fiber is used for making bags, ropes, twines, cordage, mats, etc where the variety, the age of the plant, and the way in which the fiber is extracted and stored determine its length and quality (Yohannes and Mengel, 1994).

Enset is one of the major crops that can significantly help to ensure food security in a country like Ethiopia (Brandt *et al.,* 1997). The average yield of refined enset product kocho ranged from 7 to 12 tons/ha/ year. The amount of food attainable from 50-60 enset plants per year could provide enough food for an average family of 5-6 persons (Zeweldu and Ludders,

1998). Enset products are available throughout the year and can be stored in pits for long periods of time without spoiling. Enset is rich in carbohydrate and mineral substances like calcium and iron (Shigeta, 1996). The energy yield of enset is by far higher than that of several cereals. A mature enset plant could yield 20 x10^6 cal / ha/ year which is 20 times higher than that of barley (Terefe, 1991). Enset energy yield was also reported to be higher than potato, sweet potato and banana (Pijls *et al.,* 1995). This shows that cultivation of enset can significantly improve food security at household and at national level.

Some clones and parts of enset plants are reported to have medicinal value for both human and animals. These clones are claimed to heal bone fractures, for treatment of diarrhea and delivery problems i.e. assisting to discharge the placenta (Spring *et al.,* 1996). Even some farmers believe that eating *bulla* from clones like *Boliae* (in Wolaita) after taking traditional medicines against tapeworm protects the liver from the side effect of the medicine. *Bulla* supplemented with milk and milk products is also known as important for quick recovery of women after child delivery (Kefale and Sandford, 1991).

The fresh and dried leaves of enset have various uses. They are used as food wrappers, serving plates and pit liners during *kocho* storage. Dried petioles and midribs are used as fire wood, to make mats and tying materials (Brandt *et al.,* 1997). In the dry season, the fresh leaves are used as cattle feeds. Fekadu and Ledin (1997) reported that the degradability of *Ensete ventricosum* lamina given for rumen animals was found to be better than that of straw and banana leaf and similar with that of stoker and *Chloris gayana* hay. Other parts of enset such as leaf midribs, pseudostem sheath, pseudostem core and corm were all found to have high degradability than green *Chloris gayana*, setaria grass, elephant grass and Guatemala grass.

Furthermore enset contributes to higher reduction of losses of plant nutrients particularly nitrogen loss through leaching as compared to annual crops (Brandt *et al.,* 1997). Research conducted on fields where enset is continuously cultivated has revealed that there is a higher soil nutrient status in the enset fields than in other crops. This indicates that enset cultivation is sustainable system with regard to maintaining soil fertility (Asnaketch, 1997). Eyasu (1998)

has also confirmed that soil fertility is maintained and even increased around the garden areas in enset and taro fields.

2.4. Major Production Constraints of Enset

The agronomic practices from field preparation and propagation to harvesting and processing are laborious and time consuming. It is mainly women who carry out enset harvesting and processing using local tools like machete. However, diseases and insect pests are the major constraints in all enset production systems. Some major diseases and insect pests of enset are discussed below.

2.4.1. Insect pests and wild animals

According to Terefe and Endale (1989) banana aphid, leafhopper, spider mites and mealy bug were frequently observed on both healthy and wilting enset plants and Jassid flies in virus-infected plants. Usually these insects were suspected in transmitting bacterial wilt. However, recent survey on enset root mealy bug damage has revealed that it is incurring great loss in enset production especially in Gedio and Sidama zones. These soft bodied insects feed on the corm and roots and the infested enset plants show stunted growth (Brandt *et.al*., 1997). The wild animals usually feed on the corm and pseudostem of enset and among them mole rat is reported to cause considerable damage.

2.4.2. Enset diseases

There are many diseases that attack different parts of enset caused by fungi, bacteria and nematodes. Their importance also varies depending on the damage they incur. According to Quimio and Mesfin (1996), fungal foliar diseases are numerous and widespread. Some are destructive on suckers, seedlings, young transplants and rapidly growing plants up to two years old. However, infected plants normally tolerate these diseases. *Phyllostica s*p., *Piricularia s*p., and *Drechslera s*p. are suspected to cause these leaf spot diseases on suckers and young plants and *Cladosporium s*p. and *Deightoniella s*p. infect older plants. *Mycosphaerella musicola*, which causes sigatoka in banana, is also known to cause destructive leaf spot on enset.

According to Mesfin *at al.,* 2009, twelve leaf and two root-rots, one bacterial wilt and nine nematode diseases were recorded on enset. Bacterial wilt caused by *Xanthomonas campestris* PV. *musacearum (*Dagnachew and Bradbury, 1968) is the major enset production constraint. It is followed by bacterial corm rot caused by unidentified bacterium reported in 1991 as important bacterial disease affecting the enset production (Brandt *et.al*., 1997). Bacterial corm rot attacks both young and mature plants and in advanced stage of the disease, the plant easily topples down when pushed and a rotten corm is observed (Quimio and Mesfin, 1996). Another reported bacterial disease is sheath rot of enset, which is manifested by patches of watery rot in the outer leaf sheaths (Quimio, 1991).

The common nematodes that attack enset are the root lesion nematode, *Pratylenchus goodeyi* and the root knot nematode, *Meloidogyne s*p. where the former is often found in association with bacterial wilt. Therefore, it is suspected in transmission of enset bacterial wilt disease (Peregrine and Bridge, 1992). The leaf nematode disease of enset caused by *Aphelechoides s*p. was discovered in 1991 (Quimio, 1992). It attacks leaves of suckers and young seedlings and characterized by linear black leaf streaks usually occurring on leaf margins and near the base of the newly expanded leaves (Quimio and Mesfin, 1996). The mosaic and chlorotic streak viral diseases were first observed in 1991 and resemble those of mosaic and infectious chlorosis of banana caused by strains of cucumber mosaic virus. The mosaic is more destructive than chlorotic streak as it causes severe stunting of affected plants (Quimio and Mesfin, 1996).

2.5. *Biology and Epidemiology of* *Xanthomonas campestris* PV. *musacearum*

2.5.1. Taxonomy and characteristics of *Xanthomonas campestris* PV. *musacearum*

The International Committee on Systematic Bacteriology (ICSB) formed in 1980, for the first time compild approved list of bacterial nomenclatures which include only names of bacteria (Dye *et al.,* 1980). They also suggested several species of the genus *Xanthomonas* to be reduced to the pathovar level under the type species *Xanthomonas campestris* and the pathovars were distinguishable with only host range (Dye *et al.,*1980) rather than by the usual biochemical tests used in Bergy's manual (Schaad and Stall, 1988). Accordingly, the genus *Xanthomonas* is classified under the kingdom: Bacteria, phylum: Proteobacteria, class:

Gamma proteobacteria, order: Xanthomonadales, family: Xanthomonadaceae, genus: *Xanthomonas,* species: *campestris and* pathovar: *musacearum*; and the yellow-pigmented plant pathogen of this family have been unified in this genus (Bradbury, 1984).

Cells of *Xanthomonas campestris* Pv.*musacearum* are straight rods usually within the range 0.4 - 0.7 wide X 0.7 – 1.8 um long. They are gram negative, aerobic and motile by a single polar flagellum. The optimum temperature for growth is usually 25 - 30 ^{o}C. Colonies are yellow, smooth and butyrous or viscid (Kidist, 2003). Phenotypic and genetic variations were observed in different *X. campestris* pathovars. For example, biochemical and pathogenic variation was observed in strains of *Xanthomonas campestris* Pv. *musacearum* collected from southern regions of Ethiopia (Kidist, 2003). Gizachew (2000) indicated that yellowish colony color with mucoid growth was exhibited by all isolates of X.cm. Gram negative nature of the bacterium and its motility was quite evident for all *Xanthomonas campestris* Pv. *musacearum* isolates. Studies on the utilization of carbohydrates by *Xanthomonas campestris* Pv. *musacearum* isolates indicated that all *Xanthomonas campestris* Pv. *musacearum* isolates did not use lactose, maltose and manitol. Pathogenicity tests with *Xanthomonas campestris* Pv. *musacearum* isolates obtained from different locations on different enset clones showed no significant variations and no clone by isolate interaction; but mean incidences ranged from 95.7-100% (Mesfin *at al.,* 2009).

Phenotypically distinct strains of *X. campestris* Pv. *mangiferaeindicae* as yellow pigmented and apigmented were isolated in Brazil (Pruvost *et al.,* 1998). White pathovars of *X. campestris* were also observed (Sugimori and Oliveira, 1994). Genetic variations in different isolates of several *X. campestris* pathovars were also observed and measured using different molecular techniques such as RFLP and rep-PCR (Restrepo *et al*., 2000). Antibacterial substances from *Xanthomonas* species have been described. For example, antibacterial substances were produced from 32 isolates of *X. oryzae* Pv. *oryzae* when treated with chloroform vapor; heat and UV light (Hwang and Lim, 1998). The production of a bacteriocin called glycinecin from *X. campestris* Pv. *glycines* that was antagonistic to related bacteria *X. campestris* Pv. *vesicatoria* was also reported (Jung *et al*., 1998).

2.5.2. Disease symptoms

The typical symptoms of bacterial wilt of enset are wilting of the heart-leaf followed by the wilting of the neighboring leaves. When petioles and leaf sheaths are dissected, pockets of yellow or cream colored bacterial mass are clearly observed in the air pockets, and bacterial slime oozes out from cut vascular tissues. Once an enset plant shows wilting symptoms, a total yield loss is common as the whole pseudo-stem rots inside (Mesfin *at al.,* 2009). Eventually, infected plants wither and the plant rots. Symptom development is rapid under favorable conditions and typically evident within 3 to 4 weeks under field conditions and 2 to 3 weeks after inoculation under screen house condition (Dereje, 1985).

2.5.3. Host range of the Pathogen

In addition to enset, the disease affects banana under natural condition and epidemics of the disease was reported in the former Keffa province (Dagnachew and Bradbury, 1974). According to Archaido and Mesfin (1993) disease symptoms were observed on enset, *Musa* spp. and *Canna archoides* within four to six weeks after inoculation indicating that these three plant species are hosts of *X. musacearum*. Wilt symptom (yellowing and wilting of leaves, wilting and shriveling of bud, yellow ooze from rachis and cut pseudostem) was also observed in banana through artificial inoculation of the disease (Dereje, 1985) and recently a screening trial on 45 banana cultivars for resistance to enset bacterial wilt disease revealed that all cultivars were found susceptible (Anonymous, 2000).

2.5.4. Disease cycle, distribution and damage

Bacteria cannot enter plants via intact cuticles, and entry is either through wounds or natural openings such as hydathodes and stomata (Manners, 1993). For example, *X. campestris* Pv. *campestris* enters to *Arabidopsis thaliana* leaves through hydathodes (Hugouvieux, *et al*., 1998). With regard to *Xanthomonas campestris* Pv. *musacearum,* mechanical damage as an entrance for initiation of enset bacterial wilt disease was demonstrated by cutting enset leaves with contaminated knives (Dereje, 1985). Once the bacteria enter into the plant, they multiply in the intercellular spaces and move through the tissues. Cell death of the plant may follow due to toxins or pectolytic enzymes produced by the bacteria. In general the rate of spread of the disease depends on the rate of multiplication of the pathogen, its motility, its ability to

produce pectolytic enzymes and the structure of the host. All the parameters are also affected by the environmental conditions especially on temperature and on the extent to which the host produces stimulants or inhibitors for bacterial growth and activity (Manners, 1993). Usually, vascular bacterial pathogens multiply rapidly in the xylem vessels adjoining the point of entry, and are then passively dispersed in the transportation stream (Manners, 1993).

Xanthomonas campestris Pv. *musacearum* was first reported in Ethiopia on enset in 1968 (Yirgou and Bradbury, 1968). It was later described on banana in Ethiopia on cultivar Casse hybrid (Yirgou and Bradbury, 1974), with incidence between 70% and 80%. Until 2001, it was restricted to Ethiopia, where it attacked Musa (banana and plantain) and enset plant. The situation has since changed drasticaly. In 2001, the disease was recorded for the first time in Uganda (Tushemereirwe et al., 2003) and by the end of 2003; it was reported in the eastern part of Democratic Repubilic of Congo (Ndungo et al., 2006). It has been also reported in Rwanda (Biruma et al., 2007), Tanzania (Mgenzi et al., 2006) and Kenya (Aritua et al., 2008).

Surveys conducted in the early 1980's and 1990's in major enset growing zones revealed the occurrence of enset bacterial wilt in all zones with different degree of incidence (Anonymous, 2000). It is very destructive as it kills enset plants at all growth stages including 4 to 7 years old plants ready to harvest. Once it appears in a field, it is easily transmits from infected enset plant to healthy plants through different mechanisms and in some areas where the severity of the disease and loss is high, farmers are obliged to abandon the whole field and replace it with another crop. Recent surveys conducted on enset wilt incidence in 24 different localities in 1997/98 and 1998/99 crop seasons indicated that the percentage incidence was high in Gera and Suntu in 1997/98, while in Waka, Gera and Solemo the incidence was high in 1998/99 (Mesfin *at al.,* 2009).

2.6. Enset Bacterial Wilt Management

2.6.1. Cultural control measures

Bacterial wilt constitutes a potential threat to the cultivation of enset in Ethiopia. Because of its severe damage to enset, farmers undertake a variety of traditional practices (smoking bones, tires, burning porcupine body including local spiritual believes such as '*Dua'* prayer

ceremony and slaughtering black goat etc.) for the control of the disease. Some farmers also uproot and throw away-infected plants on the road or near the enset farm which further spread the disease. Farmers in Peasant associations where some training on sanitary measure was advocated also do not practice the measure correctly (Million *et al*., 1999), although sanitation has been recommended for Enset bacterial wilt management by several researchers (Brandt *et al*., 1997). This measure includes the use of disease free suckers as planting material, uprooting and burying of diseased plants far from the field, cleaning and flaming of equipment that has come in contact with diseased plants and rotation of crops if the damage is severe (Brandt *et al*., 1997). Such measures should be taken in a manner of campaign and as regular practice in all enset-growing areas.

2.6.2. Host plant resistance

Resistance to pathogens is a genetically inherited character similar to other attributes such as height, yield and leaf size and it is used as a means to control losses caused by plant pathogens in most crops. Enset farmers know that certain enset clones such as *Yeshirekinke* in Gurage, *Ado* and *Genticha* in Sidama, Siskela and *Gimbo* in Hadya and *Mezia* in Wolaita have relatively high tolerance against bacterial wilt. Gizachew *et al.,* (2008) reported that, enset clones *Abate, Arkya, Heila, Mezya* and *Sorpie* showed low infection levels, hence indicating a high degree of tolerance to the disease.

2.6.3. Chemical control measures

So far no bactericide has been recommended against enset bacterial wilt although various *in-vitro* trials were done on antibiotics and plant extracts against *Xanthomonas campestris* pathovars that cause diseases in different crops. For example, streptomycin, oxytetracycline, chloroamphenicol and rifampicin were tested for the control of black rot of cauliflower caused by *X. campestris* Pv. *campestris* and streptomycin was the most effective, giving 100% control followed by oxytetracycline (Lenka and Ram, 1997). *In-vitro* test was also done on *X. campestris* Pv. *mangiferaeindicae* and all the chemicals and antibiotics used, aureofungin, bavistin, erythromycin, streptocycline, streptomycin and tetracycline inhibited the bacterium (Talwar *et al*., 1996). Efficiency of copper oxychloride and a mixture of oxytetracycline and streptomycin sulfate were also evaluated in controlling angular leaf spot of cotton caused by

X. campestris Pv. *malvacearum* in fields and 643.3 g/ha oxytetracycline and streptomycin sulfate was recommended (Araujo and Siqueri, 1999). Nisin 50µg/ml was also reduced the growth of *X. campestris* Pv. *campestris* by greater than 90% (Wells et al., 1998).

2.6.4. Biological control measures

Several studies have also indicated the potential of plant extracts in the control of diseases caused by *X. campestris* in several important crop plants. Akhtar *et al.*, (1995) tested about 208 diffusates from various plants such as forest trees, shrubs, herbs, fruit seeds etc. against *X. campestris* Pv. *citri*. and diffusates from various parts of *Phyllanthus emblica, Acacia nilotica, Sapindus mukorossis* and *Terminalia chebula* exhibited an inhibition zone 4.83-6mm at 50 g/liter appeared to be the most effective. Extracts from *Acacia arabica*, *Achras zapota*, and from other 6 higher plants were also found inhibitory to various pathovars of *X. campestris* (Satish *et al*., 1999). In some enset growing areas in Ethiopia, particularly in Gurage and Hadya, farmers plant a herb locally called *'Fanfo', 'Yeriyo'* or *'Olomo'* in Silte, *Oromiya* and Wolaita, respectively near infected enset plant roots and in the middle of the enset field. Farmers believe that, the plant prevents transmission of the disease from infected to healthy enset plants. The crude extracts from the bract and the leaf of *'Yeriyo'* (*Pychnostachis abyssinica*) showed better inhibithion to *Xanthomonas campestris* Pv. *musacearum* (Kidist, 2003).

2.6.5. Integrated disease management (IDM)

Management of diseases is a challenge due to continuous association of host and the pathogen inoculum over a long period of time (Ploetz, 2007). The recommended measures for *Xanthomonas campestris* Pv. *musacearum* management involves a mixture of approaches combining regular sanitation, eradication, use of tolerant enset clones, and protection. Control of *Xanthomonas campestris* Pv. *musacearum* and similar bacterial diseases of enset depends on prevention of disease spread, reduction of disease impact in affected farms (management), and rehabilitation of previously affected areas.

3. MATERIALS AND METHODS

3.1. Descriptions of the study area

The Sheka zone is one of the southwest zones of Southern Nations, Nationalities and Peoples' Region (SNNPR) with a total area of 2134.13 sq km. and lies between 7^0.12'-7^0.89' latitude and 35^0.24' to 37^0.90' longitudes, with elevations ranging 1000 – 3000 meters above sea level. The zone has three *weredas* (= districts), namely Masha, Andiracha and Yeki with a total population of 198,406 (CSA 2007). Regarding the agroecology of the zone, out of the total land size 55.6% is *kolla* (lowland), 41.4% *Weinadega* (mid altitude) and 3% *Dega* (highland). The annual mean temperature ranges between 15.1-27.5^{o}C and the annual total rainfall ranges 1200-1800mm.

From the neighboring zones of Sheka, two different zones namely Keffa (1,350 - 1,750 m.a.s.l.) and Bench Maji (1,350 – 1,750 m.a.s.l.) were included in the study. In Keffa zone, two representative districts *i.e*. Gesha and Bita, and in the Bench maji zone one representative district *i.e.* Shebench were selected. Therefore, six "*Weredas"* (Amharic terminology which is equivalent to district) namily Masha, Andiracha, Yeki, Gesha, Bita and Shebench were considered for the study of bacterial wilt based on production and consumption of enset as staple food.

The field experiment was conducted at Tugiri, Andiracha *Wereda*, Sheka Zone of SNNPR on a land owned by a farmer during 2011 main cropping season. Aderacha *wereda* is located 36km away from Masha town (Administrative center of the zone) and 712km south west of Ethiopia from Addis Ababa. The experimental field is at elevation of 1960 masl and 8 Km and 28 Km from the center of the *Wereda* (Gecha) and Masha, respectively. The site receives an average annual rainfall between 1800 and 2200 mm with multimodal distribution and experiences annual mean temperature ranging between 15.1 and 27^0C (Bedru, 2007).

3.2. Disease survey and assessment

The survey of enset bacterial wilt was undertaken in two stages, depending on the objectives to collect baseline information related to the crop production and factors that could accelerate disease spread or severity. The first stage was the administration of the designed questionnaire through oral interview with the farmers, extension agents and experts. Fifty four (54) farmers were randomly selected from the whole survey area on the basis of their having enset plantation and near the homestead for adequate sampling. The questioners addressed, enset clones grown, cultural practices, presence and status of bacterial wilt, farmer awareness (first disease observation, how the disease spread, management practices including indigenous knowledge and beliefs), and any other issues relevant to the disease spread and management.

The second stage involved survey and assessment of the disease through visiting sample enset farms to make disease diagnosis and detect bacterial wilt of enset based on typical symptoms and signs of the disease. Assessment were made in 18 randomly selected major enset growing peasant associations (PAs) in the six *weredas* through a random inspection of three enset farms with at least 30 enset trees (divided in to three blocks each with 10 trees per plot). The enset clone types and age were identified and plants were assessed for the bacterial wilt disease. So that, disease prevalence were recorded by counting the number of farms having the disease, and disease incidence was recorded by counting the number of plants showing diseases symptoms per plot.

Where the formula is,

$$DI = \frac{\text{Number of infected enset trees}}{\text{Total number of healthy and infected enset trees}} \times 100$$

$$DP = \frac{\text{Number of farms with enset bacterial wilt}}{\text{Total number of assessed farms}} \times 100$$

Whereas, disease severity was also assessed on three randomly selected diseased plants per farm using the following scale (Winstead and Kelman 1952):

0: no visible disease symptom,

1: 1 leaf wilted,

2: 2-3 leaves wilted,

3: 4 leaves wilted,

4: all leaves wilted and

5: plant dead.

Each plant was assessed on this severity scale and percentage severity index for each clone calculated as:

$$PSI = \frac{\sum(\text{Scores} \times \text{Number of enset plants with this score})}{(\text{Total numbe of enset plants} \times \text{Greater score in the scales})} \times 100$$

3.3. Sample specimen collection

Bacterial samples were collected from infected enset plant leaf petioles, which show early stage of the disease symptom to avoid some saprophytic microorganisms that grow in tissues killed by the primary pathogen (Quimio, 1992). A total of 19 representative samples, i.e. bacteria cells oozing out of the vascular tissues of infected enset plant, grouped from three altitude ranges (highland, midland and lowland areas) were randomly taken using sterile toothpick and then suspended in sterilized distilled water in half filled screw-capped vials according to Quimio (1994). Each sample was labeled with location (zone, *wereda, kebele* and enset farm) altitude and date of collection (Appendix Table 1).

3.4. Isolation, identification and preservation of the pathogen

From each of the 18 bacterial samples that were collected, a loopful of the suspension was streaked on Yeast peptone sucrose agar (Yeast extract, 5 g; Peptone, 10 g; Sucrose, 20g; Agar, 12-15g in 1 liter distilled water with pH 7.4 and autoclaved at 121 ^{o}C for 15 min). The plates were incubated at 28 ^{o}C for 48 - 72 hours according to Schaad and Stall (1988). Bacterial colonies from each plate were further sub cultured and transferred to YPSA slants incubated at 28 ^{o}C for 48 - 72 hours. The identification of isolated target bacteria was based on the cultural (colony color were given based on RGB color chart according to internet document Anonymous (2006)) and morphological descriptions on Bergey (1930); Dagnachew and Bradbury (1974) and Dye *et al.*, (1980), in comparison to previous work. And preserved at

4 ^{o}C for further work in Plant Pathology laboratory at Jimma University College of Agriculture and Veterinary Medicine.

3.5. Characterization of *Xanthomonas campestris* Pv. *musacearum*

3.5.1. Gram staining reaction

The Gram-reaction of each isolate of 19 bacterial samples was determined following the staining procedure (Schaad 1988). First, thinly spread bacterial smear was prepared on a clean slide, dried in air and fixed by heating. The dried smear was flooded with crystal violet solution for one minute and washed in tap water for few seconds. It was again flooded with iodine solution for one minute and washed and blot-dried. It was then decolorized with 95% ethyl alcohol by applying drop by drop until no more color flows from the smear and washed and blot dried. Finally slides were counter stained for about 10 seconds with safranin, washed and examined under microscope using oil immersion objective of compound microscope. Accordingly, those isolate that appeared pink considered being Gram negative bacteria and they were subjected for further tests.

3.5.2. KOH solubility test

The test was carried out by placing a drop of 3% KOH (w\v) on a microscope slide and part of a single colony from an YPSA was removed using sterile loop and mixed with a drop of KOH solution on the slide until an even suspension was obtained. When mucoid thread was lifted with the loop from the slide, it was designed as a gram-negative bacterium but when a completely dissolved suspension was produced, it was designed as a gram positive bacterium (Fahy and Hayward, 1983).

3.5.3. Growth on Asparagine medium

All Gram-negative isolates were allowed to grow on Asparagine medium (Asparagine, 0.5g; $KH_2PO_{4,}$ 0.1g; $MgSO_4.7H_2O$, 0.2g; $KNO_{3,}$ 0.5g; $CaCl_2$, 0.1g; NaCl, 0.1g and agar, 12-15g (for plates) in 1 liter distilled water with P^H =7 and autoclaved at 121 ^{o}C for 15 minutes) at 28 ^{o}C for 48-72 hours without any other carbon and nitrogen sources (Dye *et al.*, 1980). This is used

as a diagnostic test for *Xanthomonas* because they are not able to grow on Asparagine medium while others like yellow *Enterobacteriaceae* and many *Pseudomonads* can grow on it. The growth of the bacteria on Asparagine agar plates and broth was recorded and those isolates that unable to grow on the medium were taken for further tests. In all cases, uninoculated medium was included as negative controls.

3.5.4. Growth on Nutrient agar with 5% Glucose

Each isolate was streaked on nutrient agar with 5% glucose (Nutrient agar, 23g; 5% Glucose in 1 liter distilled water with P^H =7 and autoclaved at 121^{o}C for 15 minutes) and incubated at 28^{o}C for 48-72 hours. Mucoid and yellow colony growth on this medium is one of the characteristics that differentiate *X. campestris* from other *Xanthomonas* species (Bradbury, 1984). Therefore the growth and colony color of each isolate was recorded.

3.5.5. Catalase test

Few drops of 3% hydrogen peroxide was added on the surface of 48 hours old culture of each isolate on YPSA medium and bubble formation was recorded as positive for catalase activity (Dickey and Kelman, 1988).

3.5.6. Nitrate reduction

The ability of the isolates to reduce nitrate to nitrite was evaluated in a test medium that contains NO_3,1g; peptone, 5g;yeast extract, 3g and agar, 3g in 1 liter distilled water, sterilized at 120^{0}C for 15 minutes in tubes. Each isolate was inoculated by stabbing and sealing with 3ml-sterilized molten agar to avoid false positives and incubated at 28^{0}c. The growth of each bacterial isolate and bubble formation beneath the upper agar layer was observed and recorded as positive result for nitrate reduction after 3,5 and 7 days of inoculation (Dickey and Kelman, 1988).

3.5.7. Salt tolerance

Isolates were inoculated in to nutrient broth with 0%, 1%, 2%, 3%, 4% and 5% NaCl concentration to evaluate their salt tolerance (Hayward, 1964). The nutrient broth without salt

(0%) was positive control and the presence or absence of bacterial growth was recorded on the inoculated broth with different salt concentration.

3.6. Hypersensitivity test

Forty-eight hours old cultures of eight bacteria isolates were suspended in sterilized distilled water and adjusted to 0.3 O.D at 460 nm (equivalent to 10^7-10^8 cfu/ml bacterial cell concentration) using spectrophotometer. An aliquot of 2 ml of bacterial culture suspension was injected using sterilized hypodermic syringe into the intercellular spaces of expanded leaves of a one-month tobacco plant (*Nicotiana tabacum* var. white burley). Injection of sterilized distilled water was used as negative control. All the tobacco plants were kept in green house at 25-30^{o}C and 15-18 ^{o}C day and night temperature until symptom developed and a complete collapse of tissues occurred with yellow chlorosis to brown necrosis around the injection point was taken as positive for the test (Quimio, 1992).

3.7. Pathogenicity Test

3.7.1. Growing susceptible clones

A locally known susceptible Enset clone known as '*Yeko*' was used for pathogenicity test. One year old suckers vegetatively propagated from a single enset plant were transplanted in 25 lit pots with sun-dried soil, sand and composted manure mixture with the ratio of 3:1:1 (Quimio, 1992). The suckers were grown for six months in greenhouse at 25 - 30 ^{o}C day and 15 - 18^{o}C night temperature at Jimma University College of Agricaltur and Veternary Medicine.

3.7.2. Inoculum preparation and inoculation

Among the already preserved representative strains of Enset bacterial wilt pathogen (*Xanthomonas campestris* PV. *musacearum*) eight isolates that showed hypersensitivity reactions were selected for further pathogenicity test. They were grown on YPSA medium at 28 ^{o}C for 48 hours and then bacterial cells were independently harvested by scratching and suspending in sterilized water in a sterile beaker and adjusted to 1x10^8 cfu/ml concentration using spectrophotometer (Fikre and Gizachew, 2007). An aliquot of three ml of the bacterial

cell suspension were inoculated using a sterile hypodermic syringe to the second innermost leaf petiole of young (6 month old) Enset plant. Three plants were used as replication and sterilized distilled water inoculated plants were included as negative control. The typical wilting symptom developments were observed and recorded at weekly interval for three months; and finally the bacterial strains were also reisolated and compared with the respective parent colony characteristics.

3.8. Host-pathogen (enset clones and *Xanthomonas campestris* Pv. *musacearum* isolates) interaction study

Based on survey and questionnair results, four enset clones with varying levels of reaction to enset bacterial wilt and representative of the major enset growing zones of the study areas were selected for this study. The clones are locally called by farmers as "*Nobo*", "*Gudiro*" "*Chikaro*" and "*Yeko*". Besides the selected enset clone was described based on their morphological characters, and propagated and then transplanted in the field around Gecha town (1960 m.a.s.l). A total of 48 enset suckers per clone were planted in each plot (including control and the three Enset plants/plot) by spacing 1 m x 1.5 m between plants and rows (Fikre and Gizachew, 2007). Thus a total of 144 Enset trees were transplanted on a plot size of 12 x 16.5m for whole experiment. Based on the results of hypersenstivity and pathogenicity tests and each representing high, mid and low altitude ranges, three *Xcm* isolates were selected for the host-pathogen interaction study. The experiment was arranged in 3 x 4 factorial in three blocks, where factor one was baterial straines (3 types) and factor two, enset clones (4 types)

As briefly described above (section 3.7.2) bacterial suspensions from pure culture of the three *Xanthomonas campestris* Pv. *musacearum* isolates, were prepared for artificial inoculation. From each Enset clone, three plants were inoculated with 10 ml of *Xcm* suspension of each selected strain at the bases of leaf petioles using sterile hypodermic syringe according to Fikre and Gizachew (2007)). The control plants were inoculated with the same amount of sterile distilled water. The data on disease symptom development, incubation period, disease incidence, disease severity and date to complete wilting (death) were recorded at 15, 30, 45, 60, 75, 90,105 and 120 days after artificial inoculation until completion of the trial. Each Enset plant was run on the severity scale for perecentage severity index as described above

(section 3.2).

An area under disease progress curve (AUDPC) value was computed by mid-point rules between assessment dates using Van der plank's (1963) equation.

$$\left[AUDPC = \sum_{i=1}^{n-1} \left(\frac{Xi + Xi + 1}{2} \right) (ti + 1 - ti) \right]$$

Where 'n'= the number of observations, $(t_i+1 - t_i)$ = days after emergency for the i^{th} disease assessment is the interval between eight consecutive assessments; and Xi = the percentage severity index.

4. EXPERIMENTAL DESIGNS

The design used for the field survey are purposive sampling for Zone, *wereda* and PAs selection; and the random sampling technique for the farmer, enset farm and the enset plant in the farm, while completely randomized design (CRD) with three replications in laboratory studies and the pathogencity test and host-pathogen interaction studies were conducted in randomized complete block design (RCBD) with three *Xanthomonas campestris* Pv. *musacearum* isolates x four enset clone factorial treatment combinations replicated three times. Hence, in the cases the experiment had two way factorial combinations with fixed effect model. If the two factors are represented as:

Enset clone = Factor A with **i** levels

Xanthomonas campestris Pv. *musacearum* strains = Factor B with j levels, the wilt (disease) development response variable can be modeled as,

Y_{ijk} = MODEL+ ERROR

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

$$\begin{cases} i=1, 2\ldots a \\ j=1, 2\ldots b \\ k=1, 2\ldots n \end{cases}$$

Where:-

Mean Model Components:

μ = the overall mean of the scores

Main Effect Model Components:

α_i =the effect of being in level **i** of Factor A

β_j =the effect of being in level **j** of Factor B

Two-way interaction model Components:

$(\alpha\beta)_{ij}$ =the effect of being in level i of Factor A and level j of Factor B

Error Components:

ε_{ijk} = the unexplained part of the score(random error).

5. STATISTICAL ANALYSIS

The disease incidence and severity data were transformed by Arc sine (angular) transformation, because the data were collected in percentages. The importance of data transformation is to stabilize the response variance to the normal distribution and to fit the model to the data.

Field survey data for enset bacterial wilt disease was analyzed using descriptive statistics. While evaluations of enset clones and host pathogen- interaction studies were analyzed by analysis of variance (ANOVA) using the SAS software version 9.2 (SAS Institute Inc. 2008), and means were compared using Tukey's test according to Gomez and Gomez (1984). Significant differences between and among means were examined at appropriate levels of 5 % probability.

6. RESULTS

6.1. Enset bacterial wilt symptoms as observed on enset and banana plants in the field visit

Repeated field observation and interview indicated that Enset bacterial wilt symptoms first appeared on the central heart leaf or one of the inner leaves of enset whose tip becomes yellowish, limp and drop. These symptoms spread gradually to the remaining leaves. A cut made through the petioles of a newly infected enset plant reveals browning of the vascular strands and yellowish or grayish masses of bacterial ooze in the strand. Cross sections at the base of the pseudostem and corm show discoloration of the vascular strand with large bacterial pocket and grayish or yellowish exudates with brownish to black spot. Such bacterial oozes differ in color, from light-yellow to deep-yellow, from one Enset plant to other. In a more advanced stage of disease development, most of the leaves wilt, breaks at the petiole and wither. Eventually, the whole plant dies and rots to the ground. Similar symptoms were also observed on the infected banana plants during the field visits (Plate 1A - G).

Plate: 1. Healthy enset plants (A); Enset plot with typical bacterial wilt symptoms (B & C); bacterial ooze in strands of infected enset leaf petiole and pseudostem (D & E); a banana plant with bacterial wilt symptoms (F); bacterial ooze in cross sections of banana pseudostem (G).

6.2. Distribution of enset bacterial wilt in southwest Ethiopia

The survey results indicated that enset bacterial wilt was widely distributed and a serious problem in the enset growing regions of southwest Ethiopia (Fig.1). In this regard, the disease was 96% prevalent in the survey region with an average incidence and severity of 39.86% and 48.89%, respectively. Incidence of enset bacterial wilt varied across the *weredas* with Masha had the highest percentage of the plants affected by the disease (59.63 %), followed by Andiracha (55.93%) and Gesha (52.22%) (Fig. 2). She-bench *wereda* had the lowest percentage of infected plant (25.56%). The disease were more severe in Masha and Shebench, each having percentage severity index (PSI) of 55.56% than in other *Weredas* (<50%). Gesha *Wereda* had the lowest severity recorded (Fig. 3).

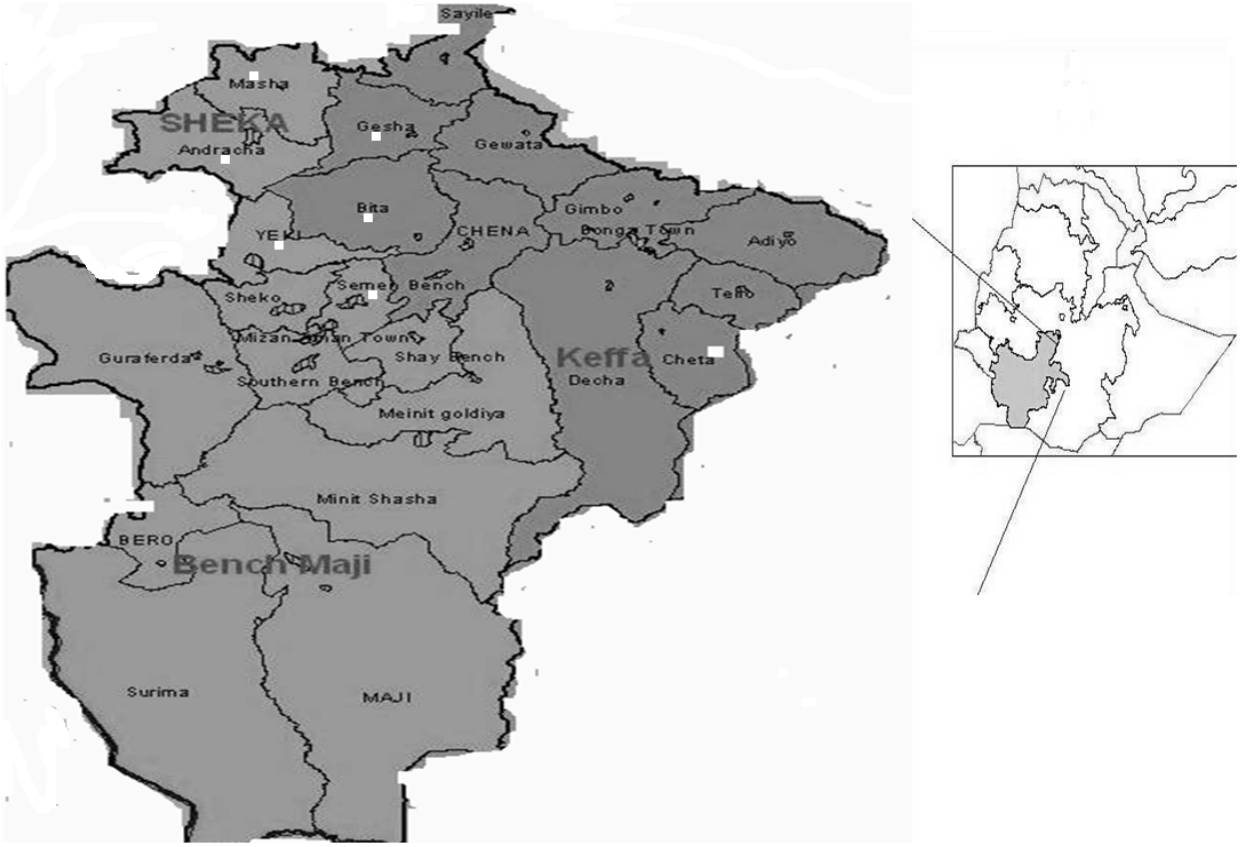

Figure 1: Prevalence and distribution of enset bacterial wilt in Sheka, Kaffa, and Benchi-Maji zones of southwestern Ethiopia (2011**)**

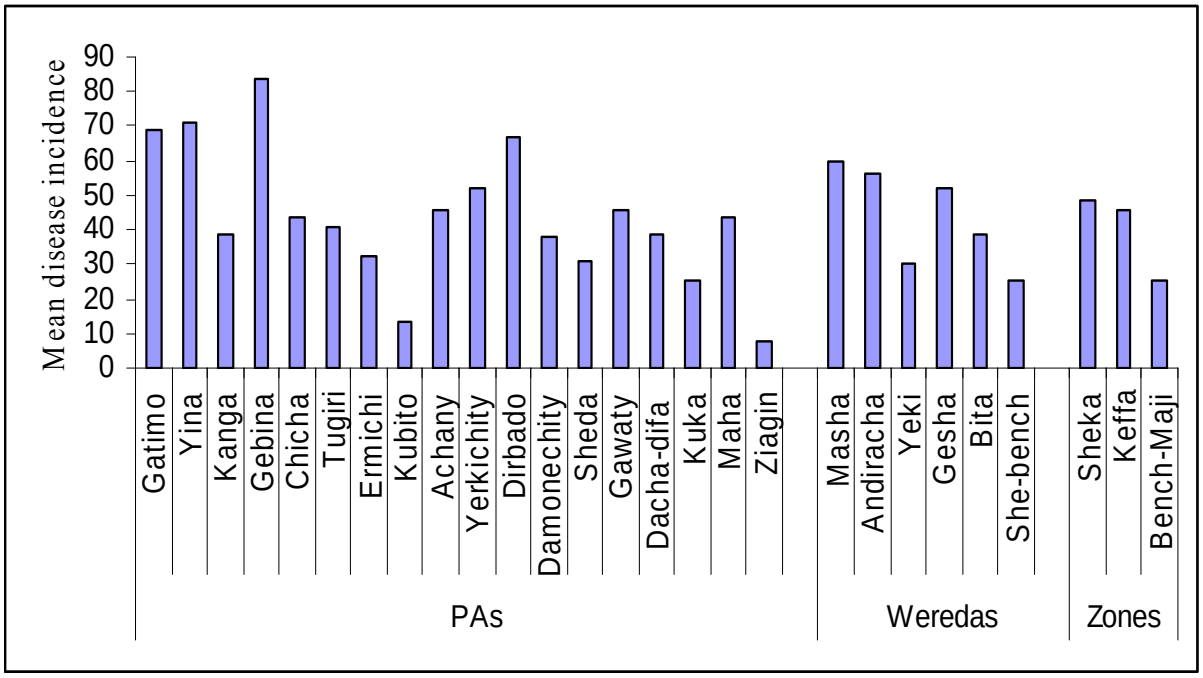

Figure 2: Enset bacterial wilt incidence in Sheka, Kaffa, and Benchi-Maji zones of southwestern Ethiopia (2011)

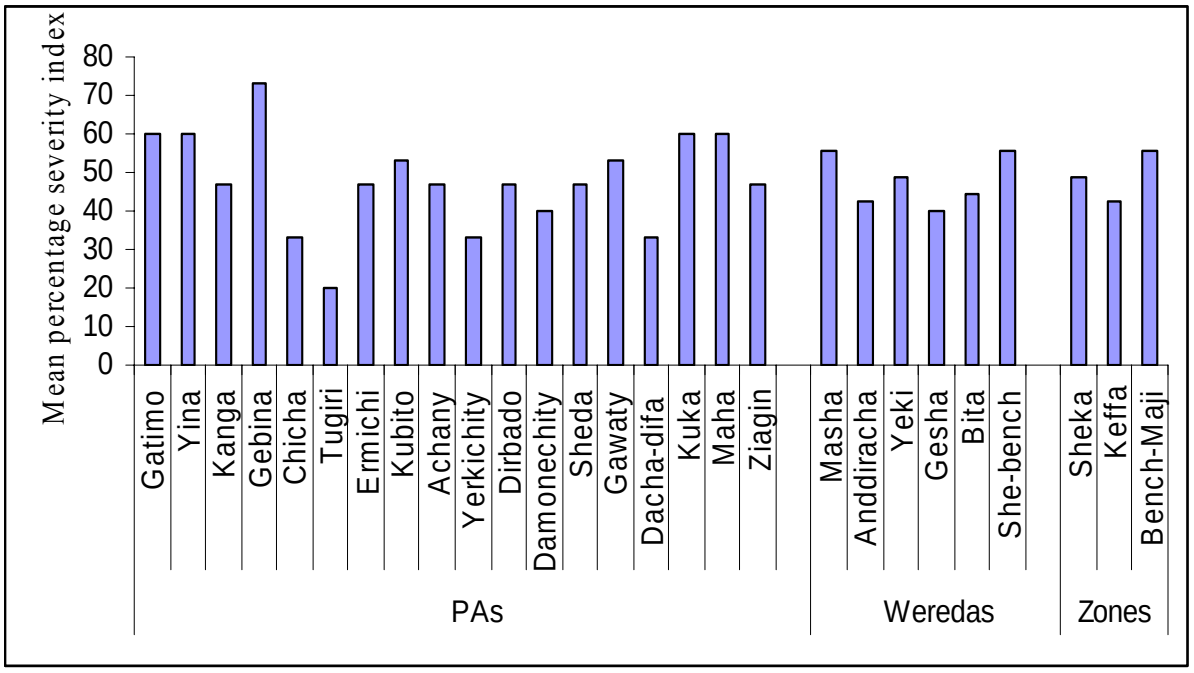

Figure 3: Enset bacterial wilt percentage severity index in Sheka, Kaffa, and Benchi-Maji zones of southwestern Ethiopia (2011)

The results of questionnaire showed that about 74.07% of the sample farmers reported that enset bacterial wilt in the study area with increasing trends, only 14.81% of them reported that the diseases is currently decreasing, and the remaining sample farmers reported as no change of disease in the farm. Even though enset bacterial wilt (EBW) reported to affect most enset colones, some of them were also reported to have better tolerance reaction to the diseases (Table:1). About 76% of the sample farmers reported that clone *Nobo* as tolerant/resistant one followed by clone *Gudiro* against EBW (Table 1).

Table 1: Proportions of respondents on tolerant/resistant enset clones to enset bacterial wilt (2011)

No	Enset clone	Number of respondent	Percentage (%)
1	*Charallo*	4	7.41
2	*Nobo*	41	75.93
3	*Gaji-boso*	1	1.85
4	*Gudiro*	8	14.81
	Mean		25±34.37

The results obtained from this study indicated that some of the clones were reported to show highly susceptible reaction to EBW. About 24% and 19% of the sample farmers reported that clone *Yeko* and *Chikaro* is more susceptible to EBW as compared to other clones, respectively (Table 2).

Table 2: Proportions of respondents on susceptible enset clones to enset bacterial wilt (2011)

No	Enset clones	Number of respondent	Percentage (%)
1	*Chikaro*	10	18.52
2	*Yeko*	13	24.07
3	*Boso*	9	16.67
4	*Barasho*	7	12.96
5	*Ataro*	5	9.26
6	*Tafaro*	4	7.41
7	*Kekaro*	4	7.41
8	*Gushiro*	2	3.7
	Mean		12.5± 6.84

In the study area, the occurrence of enset bacterial wilt was reported to occur in wet and dry seasons. In this regard, about 68.52% of the sample farmers reported that enset bacterial wilt disease occurs in both dry and wet seasons while 18.52% and 12.96% of the respondents indicated that enset bacterial wilt occurs in wet and dry seasons, respectively. It was also indicated that EBW attack all stage of enset plants, and occurred in all altitude ranges. However, about 16.67% and 14.81% of the sample farmers reported that EBW were frequently occurred in low lands and high lands, respectively.

The results obtained from this study indicated that the most important factors responsible for spreading disease of bacterial wilt were use of contaminated tools, grazing cattle in the infected field, using diseased planting material and air transmission (Table: 3).

Table 3: Farmers cultural practice that increases the spread of enset bacterial wilt in south west Ethioia

No	Cultural practice	Number of respondent	Percentages (%)
1	Grazing cattle in the infected field	14	25.93
2	Use of contaminated tools	25	46.30
3	Air transmission	6	11.11
4	Using diseased planting material	8	14.81
5	No information	1	1.85
	Mean		20 ± 17.05

In the study area, 41%, 35% and 7% of the respondents' reported that they rouge out (uproot), use tolerant enset clone and burry infected enset plants, respectively for EBW control practices (Table: 4). Some farmers also reported that, rotating with none related crops, separating infected enset plant from others and burying the diseased plants are some of the control practices for the disease, and the entire remaining sample farmers reported, as they do not practice any of control measures against enset bacterial wilt.

Table 4: Farmers cultural control practice for enset bacterial wilt in southwest Ethiopia

No	Control practice	Number of respondent	Percentage (%)
1	Separating infected enset plant from others	2	3.7
2	Rouging out	22	40.74
3	Use of tolerant enset clone	19	35.19
4	No action	3	5.56
5	Burning	4	7.41
6	Burying	1	1.85
7	Rotating with none related crops	3	5.56
	Mean		14.29±16.35

6.3. Pathogen isolation and identification

A total of 19 *Xanthomonas campestris* Pv *musacearum* isolates (17 isolate from enset and 2 isolate from banana plant) were identified from the entire sample of enset bacterial wilt infected enset and banana plants randomly collected from the six enset growing *Weredas*, namely, Masha, Andiracha, Yeki, Gesha, Bita and She-bench in southwest Ethiopia (Appendix Table 1).

6.4. Morphological, Biochemical and Physiological Characteristics of *Xanthomonas campestris* Pv. *musacearum* isolates

6.4.1. Colony growth characteristics

In total 19 bacterial isolates were grown on YPSA and most of them showed less to highly mucoid growth with light to deep yellow and creamy colony color. The colonies were also dome-shaped, mucoid; circular and shiny that conform with *Xanthomonas campestris* Pv. *musacearum* (Plate 2 and Table: 5).

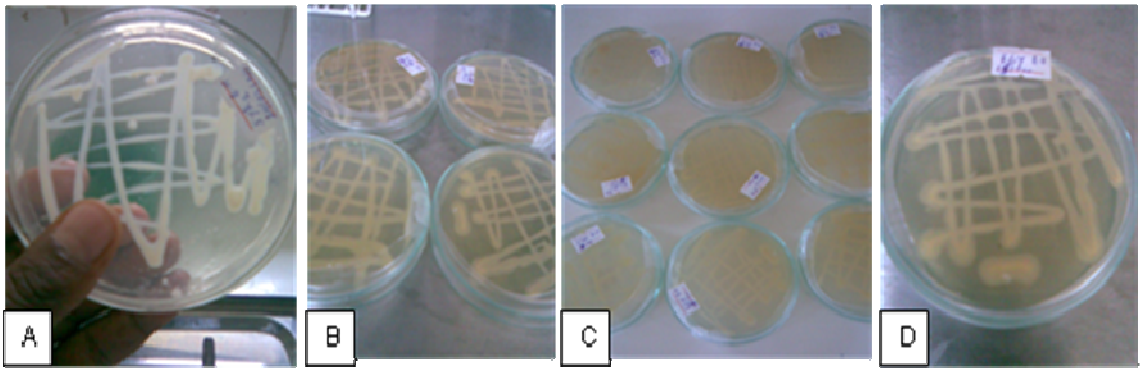

Plate 2: Cultural characteristics of *Xanthomonas campestris* Pv *musacearum* isolates grown on YPSA after 48 hrs at 28^{o}C with light colony (Plate 2A & B) to deep yellow Plate 2C & D).

The growth of the bacterial isolates on nutrient agar with 5% glucose medium showed slight variation in color and growth character of colonies. As indicated in Table 5, most of the isolates such as MaHBa, MaHBo, AnHCh, AnHKe, YeLCh, YeLYe, GiHSh, GiHGu, BiMAr, BiMBo, BiMBan, ShHYe and ShHGe showed mucoid type of growth with yellow colony color that were collected from enset clones Barasho, Boso, *Chikaro*, Kekaro, *Chikaro*, *Yeko*, Shalako, Gushiro, Arako, Boso, Banana, Yedi and Gean, respectively; whereas isolates MaHTa, AnHOg, GiHAt and BiMBa showed less mucoid growth and yellow to creamy colony that were isolated from clone Tafaro, Ogiso, Ataro and Banana, respectively. And all the bacterial isolates did not grow on asparagines medium.

6.4.2. Biochemical reactions

All the isolates were tested for their reaction to Gram staining, they were found to be gram negative. In addition, KOH solubility test (as a supplementary test to Gram staining), the isolates did not dissolve in 3% KOH solution, they rather showed a thin strand of slime when the mixed bacterial culture in the solution was lifted with the inoculating loop and they were gram-negative (Plate 3A & Table 6). All the isolates did not reduce nitrate to nitrite (Plate 3B & Table 6). The strains of *Xanthomonas campestris* Pv. *musacearum* isolates formed gas bubbles when a 48 hr old colony mixed in a few drop of 3% H_2O_2; hence, the strains were catalase positive (Table: 6).

Table 5: *Xanthomonas campestris* Pv *musacearum i*solates and their growth on yeast peptone sucrose agar and nutrient agar with 5% glucose

No.	Isolate Code	yeast peptone sucrose agar media	Nutrient agar with 5% glucose media
1	MaHBa	Yellow colony and mucoid	Light yellow to deep yellow colony and mucoid growth
2	MaHTa	Light yellow medium colony, mucoid	Creamy (honey) colony and highly mucoid growth
3	MaHBo	Light yellow medium colony, mucoid	Yellow colony and mucoid growth
4	AnHOg	Yellow colony and mucoid	Creamy (honey) color and highly mucoid growth
5	AnHCh	Yellow colony and mucoid	Yellow colony color and mucoid growth
6	AnHKe	Yellow colony and mucoid	Yellow colony and mucoid growth
7	YeMWa	Creamy large colony, highly mucoid	Light yellow to deep yellow colony and mucoid growth
8	YeLCh	Light yellow small colony, less mucoid	Yellow colony and mucoid growth
9	YeLYe	Light yellow small mucoid	Yellow colony and mucoid growth
10	GiHSh	Yellow colony and mucoid	Yellow colony and mucoid growth
11	GiHAt	Yellow colony color and mucoid	Light yellow colony with less mucoid growth
12	GiHGu	Yellow colony and mucoid	Yellow colony and mucoid growth
13	BiMAr	Light yellow medium colony, mucoid	Yellow colony and mucoid growth
14	BiMBa	Creamy large colony, highly mucoid	Light yellow colony with less mucoid growth
15	BiMBo	Yellow colony and mucoid	Light yellow to deep yellow colony and mucoid growth
16	BiMBan	Yellow colony and mucoid	Yellow colony and mucoid growth
17	ShHBan	Yellow colony and mucoid	Yellow colony and mucoid growth
18	ShHYe	Light yellow medium colony, mucoid	Yellow colony and mucoid growth
19	ShHGe	Yellow colony and mucoid	Yellow colony and mucoid growth

MaHBa MaHTa and MaHBo(isolates originated from Sheka zone Masha worda Gatimo, Yina and Kanga peasant associations, respectively); AnHOg, AnHCh and AnHKe (from Sheka zone Andiracha Wereda Gebina, Chicha and Tugiri peasant associations, respectively); YeMWa, YeLCh and YeLYe (from Sheka zone Yeki Wereda Ermichi, Kubito and Achany peasant associations, respectively); GiHSh, GiHAt and GiHGu (from Keffe zone Giesha Wereda Yerkichity, Dirbado and Damonechity peasant associations, respectively); BiMAr, BiMBa, BiMBo and BiMBan (from Keffa zone Bita Wereda Sheda, Gawaty , Dachadifa and Dachadifa peasant associations, respectively); ShHBan, ShHYe and, ShMGe (from Benchmagi zone Shebench Wereda Kuka, Maha and Ziagin peasant associations, respectively).

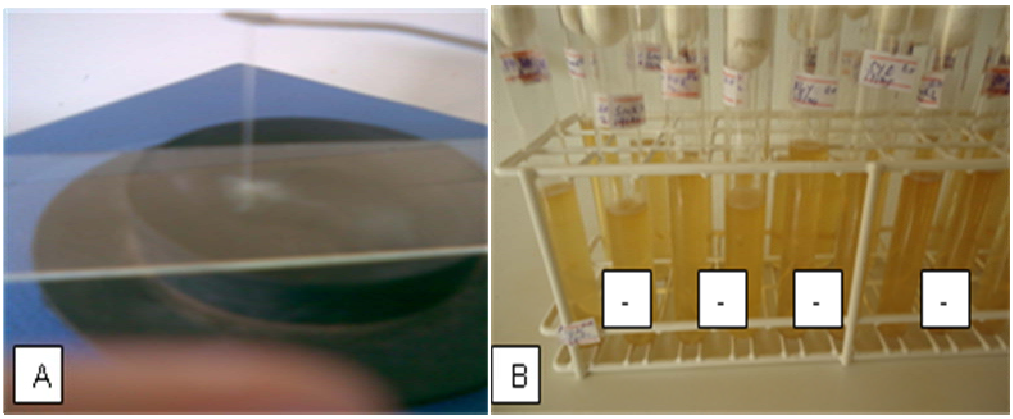

Plate 3: KOH test of enset bacterial wilt isolates showing the formation of thin strand of slime when the mixed bacterial culture in the solution on the microscopic slide and lifted with the inoculating loops (A); and no growth on nitrate reduction reaction (B).

6.4.3. Physiological tests

Variation among *Xanthomonas campestris* Pv. *musacearum* isolates was observed in physiological tests. For NaCl tolerance test, all isolates tolerated 1% and 2% NaCl except the isolates AnHOg, AnMKe and BiMBa which did not grow on 2% NaCl. Isolates MaHBo, YeLCh, GiHAt, GiHGu, BiMAr, BiMBo, BiMBan, ShMBan and ShMGe were found tolerant to 3%; MaHBo, YeLCh, GiHAt, BiMBo, ShMBan and ShMGe isolates to 4%; and YeLCh and BiMBo isolates were found tolerant to 5% NaCl. Most of the isolates growth were retarded by 4% NaCl and suppressed by 5% NaCl (Plate 4 & Table: 7).

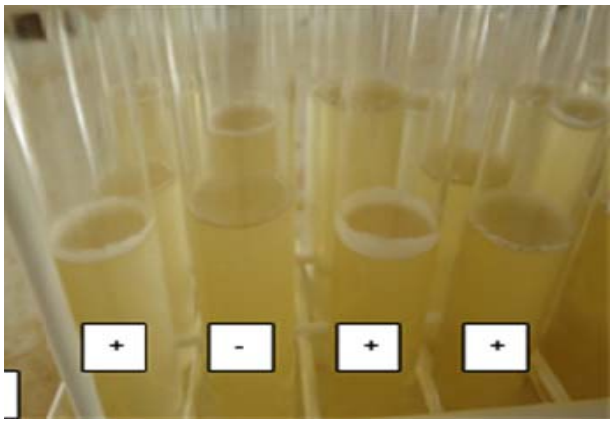

Plate 4: *Xanthomonas campestris* Pv. *musacearum* isolates showing physiological characteristics in relation to salt tolerance ranging from1 to 5% NaCl concentrations

Table 6: Reaction of *Xanthomonas campestris* Pv. *musacearum* isolates collected from southwest Ethiopia to different Biochemical testes in 2011

No.	Isolate code	Gram staining reaction[1]	KOH-test[2]	Growth on nutrient agar with 5% Glucose[3]	Catalase test[4]	Nitrate reduction reaction[5]
1	MaHBa	-	+	+	+	-
2	MaHTa	-	+	+	+	-
3	MaHBo	-	+	+	+	-
4	AnHOg	-	+	+	+	-
5	AnHCh	-	+	+	+	-
6	AnHKe	-	+	+	+	-
7	YeMWa	-	+	+	+	-
8	YeLCh	-	+	+	+	-
9	YeLYe	-	+	+	+	-
10	GiHSh	-	+	+	+	-
11	GiHAt	-	+	+	+	-
12	GiHGu	-	+	+	+	-
13	BiMAr	-	+	+	+	-
14	BiMBa	-	+	+	+	-
15	BiMBo	-	+	+	+	-
16	BiMBan	-	+	+	+	-
17	ShHBan	-	+	+	+	-
18	ShHYe	-	+	+	+	-
19	ShMGe	-	+	+	+	-

[1]Gram negative; [2]KOH positive; [3]there is a growth; [4]Catalase positive; [5]Could not reduce nitrate. MaHBa MaHTa and MaHBo(isolates originated from Sheka zone Masha worda Gatimo, Yina and Kanga peasant associations, respectively); AnHOg, AnHCh and AnHKe (from Sheka zone Andiracha Wereda Gebina, Chicha and Tugiri peasant associations, respectively); YeMWa, YeLCh and YeLYe (from Sheka zone Yeki Wereda Ermichi, Kubito and Achany peasant associations, respectively); GiHSh, GiHAt and GiHGu (from Keffe zone Giesha Wereda Yerkichity, Dirbado and Damonechity peasant associations, respectively); BiMAr, BiMBa, BiMBo and BiMBan (from Keffa zone Bita Wereda Sheda, Gawaty , Dachadifa and Dachadifa peasant associations, respectively); ShHBan, ShHYe and, ShMGe (from Benchmagi zone Shebench Wereda Kuka, Maha and Ziagin peasant associations, respectively).

Table 7: Salt (NaCl) tolerance tests of *Xanthomonas campestris* Pv *musacearum* isolates collected from southwest Ethiopia in 2011

No.	Isolate code	NaCl concentrations (%)					
		0	1	2	3	4	5
1	MaHBa	+	+	+	-	-	-
2	MaHTa	+	+	+	-	-	-
3	MaHBo	+	+	+	+	+	-
4	AnHOg	+	+	-	-	-	-
5	AnMCh	+	+	+	-	-	-
6	AnMKe	+	+	-	-	-	-
7	YeMWa	+	+	+	-	-	-
8	YeLCh	+	+	+	+	+	+
9	YeLYe	+	+	+	-	-	-
10	GiHSh	+	+	+	-	-	-
11	GiHAt	+	+	+	+	+	-
12	GiHGu	+	+	+	+	-	-
13	BiMAr	+	+	+	+	-	-
14	BiMBa	+	+	-	-	-	-
15	BiMBo	+	+	+	+	+	+
16	BiMBan	+	+	+	+	-	-
17	ShMBan	+	+	+	+	+	-
18	ShMYe	+	+	+	-	-	-
19	ShMGe	+	+	+	+	+	-

MaHBa MaHTa and MaHBo(isolates originated from Sheka zone Masha worda Gatimo, Yina and Kanga peasant associations, respectively); AnHOg, AnHCh and AnHKe (from Sheka zone Andiracha Wereda Gebina, Chicha and Tugiri peasant associations, respectively); YeMWa, YeLCh and YeLYe (from Sheka zone Yeki Wereda Ermichi, Kubito and Achany peasant associations, respectively); GiHSh, GiHAt and GiHGu (from Keffe zone Giesha Wereda Yerkichity, Dirbado and Damonechity peasant associations, respectively); BiMAr, BiMBa, BiMBo and BiMBan (from Keffa zone Bita Wereda Sheda, Gawaty , Dachadifa and Dachadifa peasant associations, respectively); ShHBan, ShHYe and, ShMGe (from Benchmagi zone Shebench Wereda Kuka, Maha and Ziagin peasant associations, respectively).

6.5. Hypersensitivity and pathogenicity tests of *Xanthomonas campestris* Pv. *musacearum* isolates collected from Sheka, Keffa and Bench-Magi zones, southwest Ethiopia

6.5.1. Hypersensitivity reaction on tobacco plant

All the inoculated bacterial isolates on the tobacco leaves (*Nicotiana tabacum* var. white burley) induced varying types of hypersensitive reactions ranging from chlorosis to brown necrosis around the injection point within 48 to 72 hours (Plate 5A). Among the isolates ShMGe, GiHSh and YeLYe showed aggressive reaction of deep brown necrosis earlier than the other isolates. Those leaf parts inoculated with sterilized distilled water remained green.

6.5.2. Pathogenicity test of the isolates on susceptible young enset and banana plants

The bacterial isolates MaHTa, AnHKe, YeLYe, GiHSh, BiMAr, ShMGe, ShHBan and BiMBan which showed better result in hypersensitivity reaction also resulted in positive pathogenic reaction on susceptible enset clone within two to three month after inoculation. The inoculated leaves of the enset plants showed light yellow to dark brown necrosis around the inoculated areas of the leaves and those leaves then became yellowish brown and finally dried from apex end till the petiole collapsed. These typical symptoms started on the inoculated leaf and spread gradually to the remaining leaves of the plant leading to complete death (Plate 5C & D). There was typical oozing of bacterial cells in dissected petioles of symptomatic leaves and in cross-section cut of pseudostem piths of dying enset plants (5F & G). These symptoms were consistent with bacterial wilt of enset that observed in the field during disease assessment. Re-isolation and identification confirmed their similarities with the parent isolates. Those leaf parts inoculated with sterilized distilled water remained healthy until end of the study period.

Moreover, in the case of banana plants; first symptoms were externally observed on leaves of inoculated plantlets. The earliest symptoms observed was collapses of the leaf blades along the midrib with two halves touching each other. Then, there was appearance of dull green color and the leaf apex begun folding down wards. The leaf then turned deeper yellow and begun drying from apex end till the petiole collapsed. The symptoms were observed first on the inoculated leaf that spread gradually to the reaming leaves (Plate 5E & H).

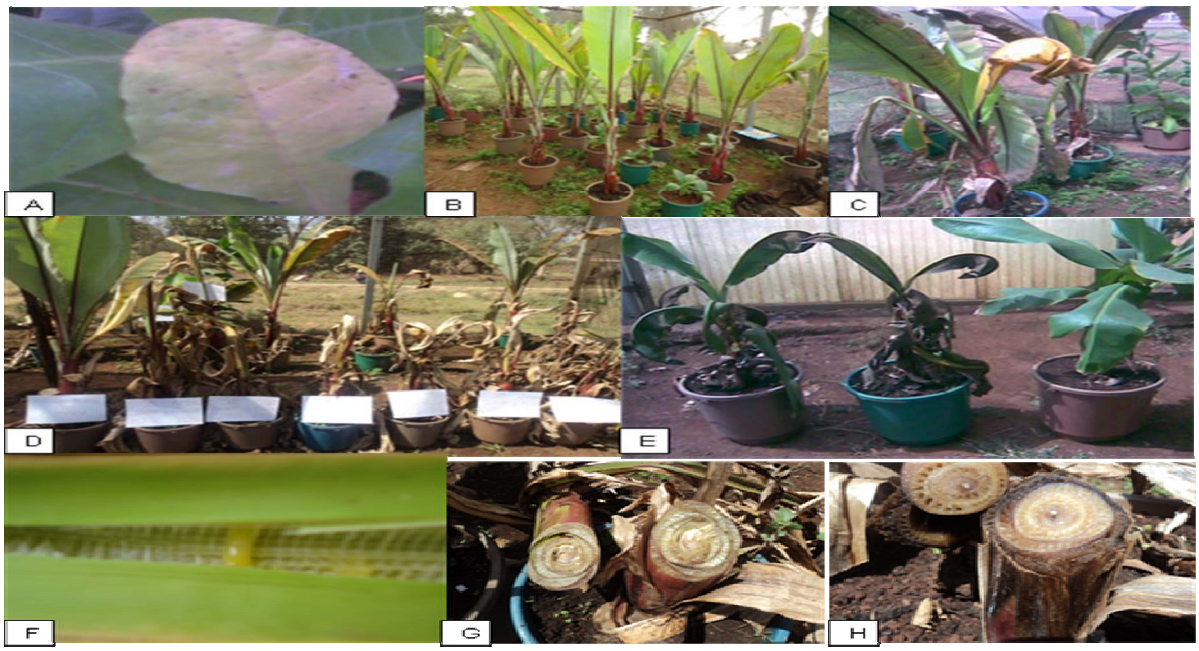

Plate 5: Hypersensitivity reaction on tobacco plant (A); uninoculated susceptible enset clone *Yeko* (B); disease starting from inoculated leaf petiole (C); completely dead enset clone *Yeko* by different *Xanthomonas campestris* Pv. *musacearum* isolates as compared to the control plant (D); compelitily dead banana plant by different *Xanthomonas campestris* Pv. *musacearum* isolates as compared to the control plant (E) ; *Xanthomonas campestris* Pv. *musacearum* oozing out through dissections of enset plant leaf petiole (F); yellow colored bacteria oozing out while cutting the pseudostem of the infected enset plant (G) and banana plant (H)

The isolates YeLYe, GiHSh, ShMGe, ShHBan and BiMBan started causing by showing leaf wilting symptoms on plants within 30-45 days after inoculation; but isolates MaHTa, AnHKe and BiMAr started symptom development by taking longer time of 50 – 60 days after inoculation. The isolates YeLYe, GiHSh and ShMGe caused a complete wilting of plants in a shorter time of 55-70 days after inoculation; while isolates MaHTa, BiMBan, BiMAr, AnHKe and ShHBan caused complete wilting in a longer time of 75-90 days after inoculation. The isolates generally showed variability in disease incubation period and date to complete wilting as assessed four months after inoculation. Isolates YeLYe, GiHSh, ShMGe and ShHBan which have a shorter disease incubation period and shorter date to complete wilting were considered highly pathogenic (Table 8).

Table 8: Comparison of *Xanthomonas campestris* Pv. *musacearum isolates* for appearance of first disease symptoms, complete wilting of plants and wilt incidence after inoculation on enset clone and banana cultivar in green house for pathogencity test at JUCAVM

Isolates and enset clone/ banana plant combination		Mean number of days to first symptom appearance (Incubation period)	Mean number of days to complete wilting	Wilt incidence (%)
Isolates	Enset clone/ banana plant			
MaHTa	*Yeko*[1]	50^{AB}	75^{a-d}	100
AnHKe	*Yeko*	55^{A}	85^{ab}	100
YeLYe	*Yeko*	35^{AB}	55^{de}	100
GiHSh	*Yeko*	45^{AB}	65^{b-e}	100
BiMAr	*Yeko*	50^{AB}	80^{abc}	100
ShMGe	*Yeko*	45^{AB}	70^{a-d}	100
ShHBan	*Yeko*	60^{A}	90^{a}	100
BiMBan	*Yeko*	45^{AB}	75^{a-d}	100
ShHBan	Butiza[2]	30^{B}	60^{c-e}	100
BiMBan	Butiza	30^{B}	45^{e}	100

[1]Local susceptabile enset clone; [2]Banana cultivar
MaHTa, AnHKe, YeLYe, GiHSh, BiMAr, ShMGe are Xanthomonas campestris Pv.*musacearum isolates isolated from enset plant; and ShHBan and BiMBan isolates isolated from banana plant.*
Means in the column followed with the same letter(s) are not significantly ($P<0.05$) different from each other according to Tukey's test. LSD values for the mean number of days to appearance of symptom and mean number of days to complete wilting 20.95 and 22.40, respectively.

6.6. Host- Pathogen Interaction Study

6.6.1. Description of local enset clones used in host-pathogen interaction study

There were four enset clones widely grown by farmers and commonly known with their vernacular (local) names in different *weredas* of the study areas of southwest Ethiopia. As there might be clones similarity across surveyed locations, the clones used in this study were morphologically described based on Tesfaye (2008) as follows.

'*Nobo':* trees of this enset clone has erect, stiff, broad and long leaves, with green or light green petiole, lamina, and midribs, and long and thick pseudostem with tightly clasping leaf

sheaths. This clone is believed by farmers to be resistant to enset bacterial wilt throughout the surveyed areas.

'Gudiro': has a stiff, broad, long and horizontal leaf with light-red petiole and midribs. Lamina folds backward, pseudostem curves at the base, thick, medium in length, and leaf sheath lifts from it. The plant can recover from an infection of enset bacterial wilt even if sometimes it shows the disease symptoms.

'Yeko': has an erect, soft and narrow leaf, with red petiole and midrib. Short and a slenderical pseudostem with tightly clasping leaf sheath. It was known to be highly susceptibility to enset bacterial wilt.

'Chikar': has an erect, soft, narrow and short leaves, deep red petiole and midrib. Short and a slenderical pseudostem with tightly clasping leaf sheath. This clone is also believed to be susceptible to enset bacterial wilt.

6.6.2. Description of symptoms on artificially inoculated enset plants

Enset clones *Yeko* and *Chikaro* started symptoms development after artificial inoculation with *Xanthomonas campestris* Pv. *musacearum* isolates with mean days of 52.22 and 59.17, respectively. The symptoms developed were similar to those observed in naturally infected enset plants during field visit. That were, initial symptoms first appear on the inoculated leaf petioles of enset whose leaf turned from green to deeper yellow. These symptoms spread gradually to the remaining leaves. The infected leaf tips become limp and droop. A cut made through the petioles of a newly infected enset plant revealed browning of the vascular strands and yellowish or grayish masses of bacterial oozes out from the strand. Such bacteria oozes were different in color, from light-yellow to deep-yellow, from one enset plant to other. This may be due to variations in *Xanthomonas campestris* Pv. *musacearum* strains (populations). Gradually all the leaves wilt, bend over, and wither. These symptoms were similarily observed on only inoculated leaves of clone *Gudiro* with *Xanthomonas campestris* Pv. *musacearum* isolates; but it is not observed on the tolerant clone *Nobo.* The control plants only exhibited a scar at the point of inoculation with water (Plate 6A - E).

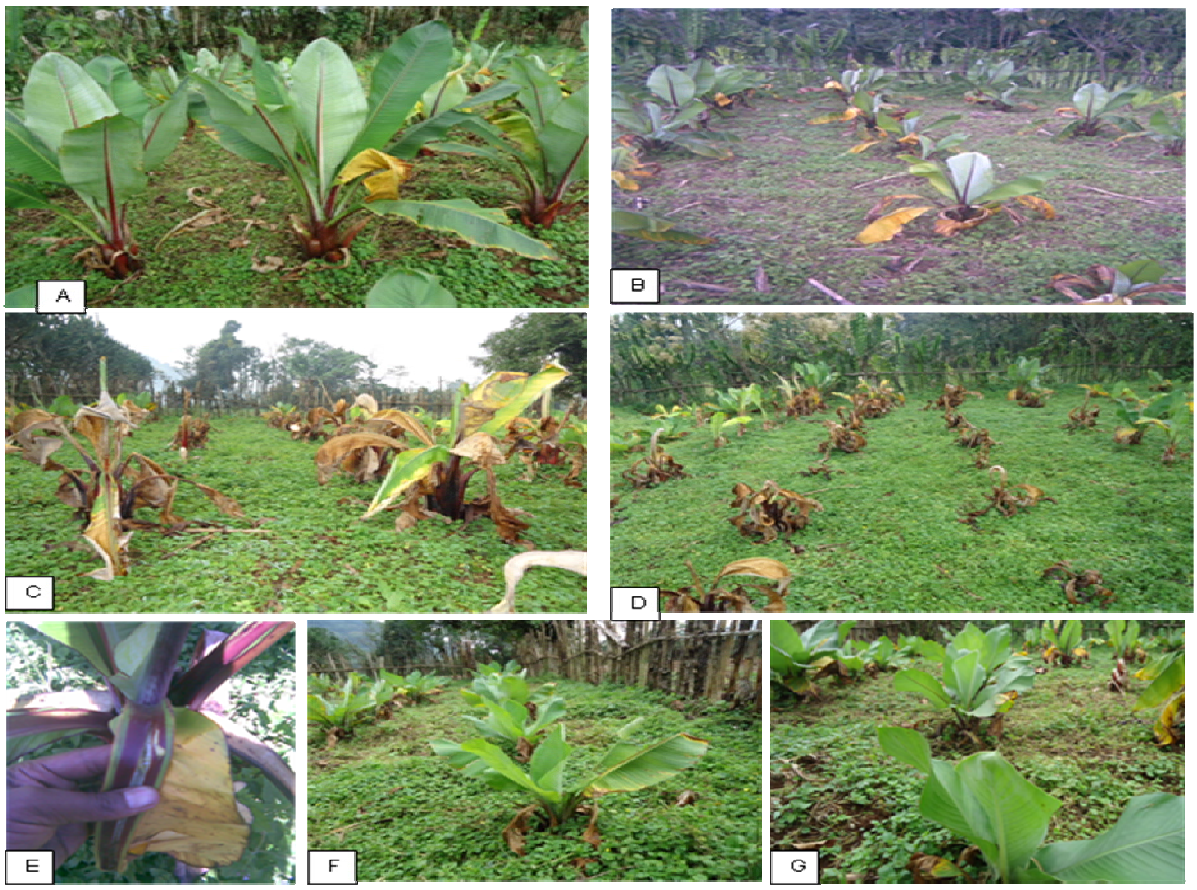

Plate 6: Enset bacterial wilt disease symptom appearing on first inoculated leaf petiole (A); disease progressed to the remaining leave petioles and yellowing of leaves (B); completely wilted enset clone *Chikaro* (C); completely wilted enset clone *Yeko* (D); yellowish bacteria oozing out while opening the leaf petiole (E); Enset clone *Nobo* and *Gudiro* tolerant to enset bacterial wilt disease (F & G), respectively.

6.6.3. Interactions of enset clones by *Xanthomonas campestris* Pv. *musacearum* strains

Three *Xanthomonas campestris* Pv. *musacearum* isolates namely ShMGe, GiHSh and YeLYe representing the three major enset growing *weredas* of Bench-Magi, Keffa and Sheka zones, respectively, were pathogenic to the inoculated enset clones *Yeko* and *Chikaro* in the field experiment.

There were highly significant ($P < 0.001$) differences in disease incidences and incubation periods (Appendix Table 2, 3 and 4). Enset clones *Yeko* and *Chikaro* showed significantly

($p<0.05$) higher mean disease incidence of 100% and 88% (Table 9) with incubation period of 52.22 and 59.17 days, respectively (Table 10). On the contrary, there was no disease incidence observed on Enset clones *Nobo* and *Gudiro*, but clone *Gudiro* showed symptoms on inoculated leaf petioles only after 60 days incubation (Table 10). Enset clones *Nobo* and *Gudiro* exhibited complete and higher resistance reaction, respectively, to the three tested bacterial wilt isolates that may implay horizontal resistance reaction, while clone *Yeko* and *Chikaro* showed highly susceptible reaction to all isolate (Table 9 and Plate 6).

Xanthomonas campestris Pv. *musacearum* isolate ShMGe, GiHSh and YeLYe caused significantly ($p<0.05$) different mean disease incidence with 35.50%, 40.30%, and 50% and with mean incubation periods of 44.79, 43.75 and 40 days, respectively (Table 9, Table 10 and Plate 6 C&D). Among the isolates, isolate YeLYe was more pathogenic to *Yeko* and *Chikaro* enset clones as compared to the moderately pathogenic isolate ShMGe and GiHSh (Table 9 and Plate 6C &D). In enset clone by *Xanthomonas campestris* Pv. *musacearum* isolate interaction, an enset clone *Chikaro* was moderately susceptible to ShMGe and GiHSh isolate (66.67% and 83.40%, respectively) but infected by the *Xanthomonas campestris* Pv. *musacearum* isolate YeLYe (100%) (Table 9 and Plate 6C).

The entire tested plants of enset clones *Yeko* and *Chekaro* except for *Nobo* and *Gudiro* eventually wilted completely. The period between inoculation and complete wilting (plant death) varied significantly ($P<0.05$) between enset clones (Table 11 and Appendix Table 5). No plants of enset clone *Nobo* and *Gudiro* wilted completely, even if some plants of the clone *Gudiro* which showed the disease symptom on its inoculated leaf petioles was revived (recovered) from the disease, and eventually these plants resembled healthy control plants. In contrast, all the plants of enset clone *Yeko* wilted completely at lower mean 88.33 days after inoculation than the clone *Chikaro*, its complete wilting was observed in 69.7% of plants 98.89 days after inoculation.

Xanthomonas campestris Pv. *musacearum* isolate ShMGe, GiHSh and YeLYe caused significantly ($p<0.05$) different mean number of date to complete wilting (death) 47.5, 49.58 and 43.34 days respectively (Table 11). Among the isolate, isolate YeLYe had shortest time to cause complete death to *Yeko* and *Chikaro* enset clones as compared to isolate GiHSh, but

isolate ShMGe was statistically similar with both isolates (Table 11).

Table 9: Mean disease incidence (%) on four enset clones inoculated with three *Xanthomonas campestris* Pv. *musacearum* isolates under field conditions at Gecha, Sheka (2011)

Enset clones	Bacterial Isolates[1]			Mean
	ShMGe	GiHSh	YeLYe	
Gudiro	1.6^{c}(0.0)[2]	1.62^{c}(0.0)	1.6^{c}(0.00)	1.6^{2C}(0.0)
Nobo	1.6^{c}(0.0)	1.62^{c}(0.0)	1.6^{c}(0.00)	1.6^{C}(0.0)
Yeko	88.4^{a}(100)	88.4^{a}(100)	88.4^{a}(100)	88.4^{A}(100)
Chikaro	54.76c(66.7)	66.0^{b}(83.4)	88.4^{a}(100)	69.7^{B}(88.0)
Mean	36.6^{Y}(35.50)	39.4^{XY}(40.3)	45^{X}(50.0)	

Values in parenthesis are the original/untransformed data
[1]ShMGe, GiHSh and YeLYe were Xanthomonas campestris Pv. *musacearum isolates obtained from Bench-magi, Keffa and Sheka zones of southwest Ethiopia.*
[2](0.00) indicate no disease incidence was observed until end of the trial.
Disease Incidence % = [No. of Plants wilted / total number of plants inoculated] x 100
Means followed with the same letter(s) are not significantly (P<0.05) different from each other according to Tukey's test. LSD values for the enset clones, bacterial isolates and the interactions comparisons were 7.33, 5.75 and 3.24, respectively.

Table 10: Incubation periods (mean number of days) of three *Xanthomonas campestris* Pv. *musacearum* isolates inoculated with four enset clones under field conditions at Gecha, Sheka (2011)

Enset clones	Bacterial Isolates[1]			Mean
	ShMGe	GiHSh	YeLYe	
Gudiro	62.5^{ab}	55.83^{bc}	61.67^{ab}	60^{A}
Nobo	0.0^{e2}	0.00^{e}	0.00^{e}	0.00^{C}
Yeko	53.33^{cd}	51.67^{cd}	51.67^{cd}	52.22^{B}
Chikaro	63.33^{a}	67.5^{a}	46.67^{d}	59.17^{A}
Mean	44.79^{Y}	43.75^{XY}	40.00^{X}	

[1]ShMGe, GiHSh and YeLYe were Xanthomonas campestris Pv. *musacearum isolates obtained from Bench-magi, Keffa and Sheka zones of southwest Ethiopia.*
[2]0.00 indicate no incubation periods (no external symptom) was observed until end of the trial.

Means followed with the same letter(s) are not significantly (P<0.05) different from each other according to Tukey's test. LSD values for the enset clones, bacterial isolates and the interactions comparisons were 5.19, 4.06 and 2.29, respectively.

Table 11: Date to complete wilting (mean number of days) of three *Xanthomonas campestris* Pv. *musacearum* isolates inoculated with four enset clones under field conditions at Gecha, Sheka (2011)

Enset clones	Bacterial Isolates[1]			Mean
	ShMGe	GiHSh	YeLYe	
Gudiro	[2]0.00[c]	0.00 [c]	0.00 [c]	0.00[C]
Nobo	0.00 [c]	0.00 [c]	0.00 [c]	0.00[C]
Yeko	90[b]	88.33 [b]	86.67 [b]	88.33[B]
Chikaro	100[ab]	110[a]	86.67 [b]	98.89[A]
Mean	47.5[X]	49.58[X Y]	43.34[Y]	

[1]ShMGe, GiHSh and YeLYe were Xanthomonas campestris Pv. *musacearum isolates obtained from Bench-magi, Keffa and Sheka zones of southwest Ethiopia.*
[2]0.00 indicate no complete wilting of plant was observed until end of the trial.
Means followed with the same letter(s) are not significantly (P<0.05) different from each other according to Tukey's test.
LSD values for the enset clones, bacterial isolates and the interactions comparisons were 7.64, 5.98 and 3.37, respectively

Enset bacterial wilt symptoms appeared 52.22 days following the first inoculation. The progress of the symptoms evolved slowly or none on certain enset clone but at a much greater rate on other clones (Plate 6 and Figure 4). There were very highly significant ($P < 0.001$) differences in overall percentage severity index and the AUDPC values under field conditions (Appendix Table 2, 6 and 7). Enset clones *Yeko* and *Chikaro* showed significantly ($p<0.05$) higher mean percentage severity index of 100% and 85.20% (Table 12) with AUDPC values of 2702.67 and 1656.33, respectively (Table 13); on the contrary, significantly ($p<0.05$) lower overall mean percentage severity index and AUDPC values of 15 and 570.07 were recorded on enset clone *Gudiro*, but no percentage severity index and AUDPC values were recorded on enset clone *Nobo*.

Xanthomonas campestris Pv. *musacearum* isolates ShMGe and YeLYe caused significantly (0.05) different mean percentage severity index with 40.63 and 48.35 and with mean AUDPC values of 1197.94, and 1392.04, respectively, but isolate GiHSh was statistically similar with ShMGe (Table 12 & 13). Among the isolate, YeLYe was more pathogenic to *Yeko* and *Chikaro* enset clones as compared to the moderately pathogenic isolate ShMGe and GiHSh (Table 12).

Table 12: Mean percentage severity index (PSI) of three *Xanthomonas campestris* Pv. *musacearum* isolates inoculated with four enset clones under field conditions at Gecha, Sheka (2011)

Enset clones	Bacterial Isolates			Mean
	ShMGe	GiHSh	YeLYe	
Gudiro	15^{c}(6.70)	15^{c}(6.70)	15^{c}(6.70)	15^{C}(6.70)
Nobo	1.62^{d}(0.00)[2]	1.62^{d}(0.00)	1.62^{d}(0.00)	1.62^{D}(0.00)
Yeko	88.38^{a}(100)	88.38^{a}(100)	88.38^{a}(100)	88.38^{A}(100)
Chikaro	57.51^{b}(71.20)	56.14^{b}(68.90)	88.38^{a}(100)	67.34^{B}(85.20)
Mean	40.63^{Y}(43.40)	40.29^{Y}(41.80)	48.35^{X}(55.80)	

Values in parenthesis are the original/untransformed data
[1]ShMGe, GiHSh and YeLYe were Xanthomonas campestris Pv. *musacearum isolates obtained from Bench-magi, Keffa and Sheka zones of southwest Ethiopia.*
[2]0.00 indicate no perecentage severity index value was recorded until end of the trial.
Severity on the 1-5 disease scoring scale, where 1 stands for 1 inoculated leaf wilted, 2 for 2-3 leaves wilted, 3 for 4 leaves wilted, 4 for all leaves wilted, and 5 for plant dead.
Means followed with the same letter(s) are not significantly (P<0.05) different from each other according toTukey's test.
LSD values for the enset clones, bacterial isolates and the interactions comparisons were 3.93, 0.98 and 0.55, respectively.

Table 13: Mean area under disease progress curve (AUDPC) of three *Xanthomonas campestris* Pv. *musacearum* isolates inoculated with four enset clones under field conditions at Gecha, Sheka (2011)

Enset clones	Bacterial Isolates[1]			Mean
	ShMGe	GiHSh	YeLYe	
Gudiro[3]	536.6[d]	603.5[d]	570.1[d]	570.07[C]
Nobo	85.05[e2]	85.05[e]	85.05[e]	85.05[D]
Yeko	2750[a]	2613[a]	2745[a]	2702.67[A]
Chikaro	1420[c]	1381[c]	2168[b]	1656.33[B]
Mean	1197.94[Y]	1170.55[Y]	1391.94[X]	

[1]ShMGe, GiHSh and YeLYe were Xanthomonas campestris Pv. *musacearum isolates obtained from Bench-magi, Keffa and Sheka zones of southwest Ethiopia.*
[2]85.05, is equvalent to the number 0 which is its original/untransformed data, indicate no area under disease progress curve value was recorded until end of the trial.
[3]it's recorded area under disease progress curve values was only from inoculated leaf petiole (at the end of the trial period the enset clone is revived).
Means followed with the same letter(s) are not significantly (P<0.05) different from each other according toTukey's test.
LSD values for the enset clones, bacterial isolates and the interactions comparisons were 162.46, 127.28 and 71.65, respectively.

Different in the progression of the disease also was apparent in differences of enset clones, *Xanthomonas campestris* Pv. *musacearum* isolates and their interactions. For example, dead or infected plants were not observed in any enset clone *Nobo* while, it was observed in *Yeko* throughout the assessment period; even 100 % percentage severity index was recorded at the end of assessment period (Fig.4). The progress of the symptoms evolved slowly on enset clone *Yeko* by *Xanthomonas campestris* Pv. *musacearum* isolate GiHSh (after 30 days of inoculation) than *Xanthomonas campestris* Pv. *musacearum* isolates YeLYe and ShMGe (both started 15 days after inoculation). In all the causes the disease progressions were highly

increasing between 30 and 90 days after inoculation and the entire enset clone *Yeko* dead after 90 days of inoculation (Fig.4).

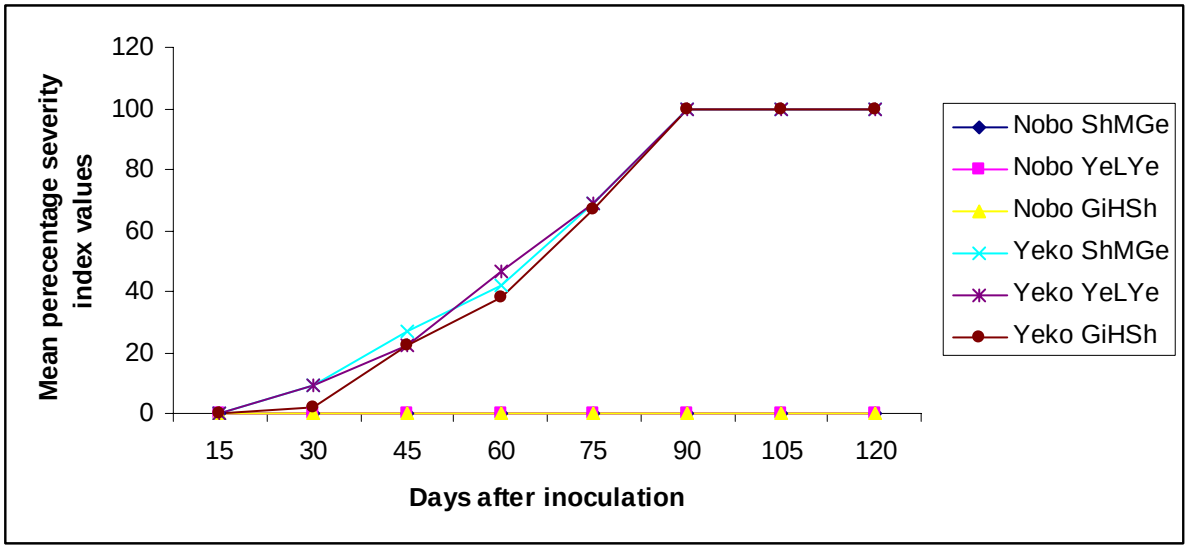

Figure 4: Comparison of disease progress curves of enset clone *Nobo* (the most tolerant) and *Yeko* (the most susceptible) with three *Xanthomonas campestris* Pv*. musacearum* isolate combinations.

6.6.4. Correlation analysis of enset bacterial wilt parameters

Correlation analysis showed that disease incidence had highly significantly and strongly positive relationship with date to complete wilting (r =92**), percentage severity index (r =98**) and AUDPC (r=95**). On the other hand, date to complete wilting was found to be strongly positively and highly significantly correlated with percentage severity index (r=90**) and AUDPC (r=84**). Moreover, percentage severity index and AUDPC were highly significantly and strongly positive relationship(r=0.98**) to each other. On the contrary, the incubation period was highly significantly and weakly positively correlated with all enset wilt parameters (Table 14).

Table 14: Pearson Correlation coefficient of enset Bacterial Wilt parameters

	DI	IP	DW	PSI	AUDPC
DI	1	0.44**	0.92**	0.98**	0.95**
IP		1	0.52**	0.53**	0.52**
DW			1	0.90**	0.84**
PSI				1	0.98**
AUDPC					1

*** indicate significant at 1% probability level*
DI=disease incidence, IP=incubation period, DW=date to complete wilting, PSI=perecentage severity index and AUDPC=area under disease progress curve

7. DISCUSSION

Enset bacterial wilt (EBW) occurs in the enset growing areas of southwest Ethiopia with increasing order of importance varying in the extent of damage among and within enset farms and distribution. These variations were due to different interacting factors, such as susceptibility of enset clones, intensity of cultural practices which increase the spread of the inoculums and environmental condition. In the present studies the incidence and severity of diseases were assessed in three major enset growing zones of southwest Ethiopia. During the survey incidence was higher (59.63 %) in enset growing areas of Masha *wereda* followed by Andiracha and Gesha. She-bench *wereda* had the lowest (25.56%) percentage of plant infected. The mean disease incidence during the assessment was 55.93% at Andiracha, 52.22% at Gesha, 38.52 at Bita and 30% at Yeki *weredas* (Figure 2).

According to the observation in sample fields, the differences in disease incidence across the locations were due to differences in farmer' cultural practices. For example, in Masha (Sheka) and Gesha (Keffa) *weredas* farmers accustomed to work in the farm with contaminated tools without taking care for enset plant not to contaminate with pathogen, even some times they uproot the diseased enset plant and throw at the nearby healthy enset fields due to lack of awareness, to possibility of contaminating the enset plant with the pathogen. In Andiracha (Sheka) flocks of cattle have grazed and moved along the enset field carrying the inoculum on their bodies (tooth's) from area to area. In addition, in all surved areas people intensively use infected enset plant leaf petioles as wrapping material for '*kocho'* for sale in market that could be the means for distribution and spread of diseases; and the infected enset plant were found standing in the field even when they had long died with the bacterium which serve as sources inoculums for infection. And also the incidence of EBW varied among geographic areas, between altitude ranges that depends on physical requirements of pathogen or diseases such as temperature and relative humidity. Dagnachew and Bradbury (1974) similarly, noted that the epidemic of bacterial wilt in banana in Keffa and Sheka zones, southwestern Ethiopia and indicated that the disease was becoming a serious problem for enset and banana production. Moses (2007) also stated, field observations indicate that the disease reduces yields of banana plants to varying levels, depending on prevailing climatic conditions.

Enset bacterial wilt was more sever in Masha and She-bench than in other *Weredas*. The mean severity index during the survey period was 55.56% at Masha and She-bench, 48.89% at Yeki, 42.22% at Andiracha, 44.44 at Bita, and 40% at Gesha *Weredas* (Figure 3). This variation was due to the presence of more susceptible enset clones in the *weredas* with the highest disease severity index recorded; and in *weredas* with low disease severity index, where plantations are mixed with tolerant and less tolerant enset clones. During field observation, enset fields in Masha and She-bench *weredas* were found to be with more number of enset clones *Yeko* and *Chikaro* (susceptible to EBW); while Gesha *wereda* had enset fields with more number of enset clones *Nobo* and *Gudiro* (tolerant to EBW). Moreover, high disease was due to availability of more bacterial inoculum and conducive environmen for development of disease at Masha and She-bench *weredas* and less in others. Gizachew *et al.* (2008) reported that enset farmers commonly grow combinations of clones in the same fields, which show varying reactions against EBW disease. Tushemereirwe *et al.* (2003) also indicated that, the variability of bacterial wilt disease severity with clone.

From the survey result, the respondents' reported that, use of contaminated tools (46%), grazing cattle in the infected field (26%), using diseased planting material (15%) and air transmission (11%) were considered mainly as the most important factors responsible for spreading of enset bacterial wilt. Transmission from plant to plant within a field is mechanically accomplished by contaminated farming and processing tools. According to Dereje (1985), the bacteria were found on the surface of contaminated tools for up to 4 days under humid conditions and up to 3 days under dry conditions. Farmers in southwest Ethiopia were planting suckers either from the same field or from neighbors' fields. The latent nature of *Xanthomonas campestris* Pv. *musacearum* especially in the early stages may lead to farmers planting the already infected suckers and these may serve to spread diseases across farms and regions. Hayward (2006) suckers are an important means of spread for systemic bacterial diseases. Animals such as cattle that move through the infected field, could also contribute to the spread of the enset bacterial wilt. During field observation in most surved areas, that is not reported by the farmers, the mole rats were seen within the farms that tunneling from one enset plant to others. Similarily Brandt *et al.,* (1997) claimed that mole rats can also transmit the enset bacterial wilt as they tunnel from one plant to the other. Eshetu

(1981) also noted that all diseases in which the pathogen is carried internally or externally by one or a few specific vectors, dissemination of the pathogen depends to a large extent or entirely on that vector.

In the study area, 41%, 35% and 7% of the respondents' reported that they rouge out (uproot), use tolerant enset clone and burry infected enset plants, respectively for disease control practices. Like wise, Brandt *et al*., (1997) reported that the only recommended control measures for the enset bacterial wilt is cultural practices which include the use of healthy, disease-free suckers for planting material, destruction and controlled movement of diseased plants, cleaning of equipment that has come in contact with diseased plant material and rotation of crops. Similarly Karamura *et al*., (2005) stated that, early detection and destruction of the diseased plants is a key step in preventing disease spread. In addition to that, during survey, in most locations the farmers have commented that "the disease (EBW) unable to kill the enset clone *Nobo* and *Gudiro* easily and it is very strong and hard to withstand some adverse conditions". Hence, enset clone *Nobo* and *Gudiro* were considered as resistant/tolerant clones to pathogen and these materials can also be used as a bacterial wilt management component.

Xanthomonas campestris Pv. *musacearum* isolates were identified from the entire sample of infected enset plants randomly collected from enset growing areas of southwest Ethiopia. Accordingly, 19 isolate (17 isolate from enset and 2 isolates from banana plant) were characterized from enset growing areas of southwest Ethiopia. The cultural and morphologicual identification of the pathogen was supported by previous work of Dagnachew and Bradbury (1974), while pathological characteristics during pathoginicity test on the susceptible enset clone was in line with characterization works of bacterial isolates that were collected from six *weredas* in Gurage and North-Omo zones in southern Ethiopia by Gizachew (2000).

Detection of variation is the one of the objectives of this work. Hence, morphological characteristics of *Xanthomonas campestris* Pv. *musacearum* isolate were observed on YPSA and Nutrient broth with 5% glucose. The color and growth conditions were variable among most isolates (Table 5 and Plate 2). Among isolates, most of them showed light yellow to

deep yellow colony color and there were isolates that grew faster and isolates with slow growth that is one character of *Xanthomonas campestris* in general. Similarily Kidist (2003) also showed the same results during characterization of *X.campestris* Pv. *musacearum* isolates that were collected from enset growing areas of southern and Oromia regions in Ethiopia. In contrast to these, Gizachew (2000) did not found any differences between *X.campestris* pv. *musacearum* isolates that were collected from six *weredas* in Gurage and North-Omo zones in southern Ethiopia during biochemical characterization.

According to the result in the current study, all the bacterial isolates were gram negative, catalase positive and could not reduce nitrate to nitrite. These finding was in agreement with Gizachew (2000) and Kidist (2003) studies on characterization of *X. campestris* Pv. *musacearum*; and are also in line with general characteristic of *X. campestris* described in Bergey (1930) and Bradbury (1984).

All isolates were found tolerant to NaCl 1-2% where about 42 % were found tolerant to 3-5% NaCl but in the previous work by Eshetu (1981), 3% NaCl retarded and 4% NaCl suppressed the growth of all *X. campestris* Pv. *musacearum* isolates. However, these results were in agreement with salt tolerance ability of isolates during physiological test in Kidist (2003).

Positive reaction to the non-host, tobacco (*Nicotiana tabacum*) is a confirmation for the isolates to be pathogen. It is commonly used as preliminary test before testing for their pathogenicity in the host of origin. This is also true for *X. campestris* Pv. *musacearum* (Quimio, 1992; Gizachew, 2000; Kidist, 2003). The isolates AnHKe, YeLYe, GiHSh, BiMAr, ShMGe, ShHBan and BiMBan infiltrated to tobacco leaves had shown variation in time for symptom development (48-72 hours). For example, isolates YeLYe, GiHSh and ShMGe were reacted fast and showed very deep brown necrosis around the area of injection; and the remaining isolates were reacted slowly and showen light brown necrosis on the injected leaf. Probably, one possible reason for failure of isolates in hypersensitivity reaction could be the effect of the growth media (YPSA). Although YPSA was recommended for all *Xanthomonas campestris* pathovars, there could be mutation or loss of virulence of the bacterial isolates while growing on this medium. The same problem was faced while pathogenicity testing of *X.campestris* Pv.*musacearum* isolates, during characterization of *X. campestris* Pv.

musacearum isolates from enset growing regions of southern Ethiopia (Kidist, 2003) and screening enset clones for resistance to enset bacterial wilt in Awasa Agricultural Research Center, Pathology section (Anonymous, 1997). While conducting this experiment, the field samples collected were grown on YPSA and inoculated to enset clones and all showed negative reaction but when the samples were inoculated directly without growing them in artificial medium (YPSA), all clones showed wilt symptom and died within 1 to 2 months time except few enset clones which are tolerant. This implies that there is a need to develop or evaluate the available semi selective or selective media of other pathovars for *X. campestris* PV. *musacearum* that could provide a growing condition similar to the natural environment.

The promising isolates during hypersensitive reaction were subjected to pathogenicity test on susceptible enset clone. The reaction to pathogenicity tests was found to be positive to all tested isolates. They did show complete wilt symptoms to all plants (Plate 5 and Table 8). In line wth this, Gizachew (2000) stated that, *Xanthomonas campestris* PV. *musacearum* isolates that were collected from six *weredas* in Gurage and North Omo zones were shown similar hypersensitive and pathogenicity reactions; on the other hand Kidist (2003) reported, the failure of *Xanthomonas campestris* PV. *musacearum* strains in symptom development during pathogenicity test.

The enset bacterial wilt symptoms developed in this finding were turning of inoculated leaf petioles from green to deeper yellow; then spread gradually to the remaining leaves; and the leaf tips become limp and droop. Finally, through cut point, the brown vascular strands and yellowish masses of bacteria oozes were recorded. This was supported by the works of Eshetu (1981) and Dereje (1985); who stated as initial symptoms appear on the central heart leaf or on one of the inner leaves of enset whose tip becomes yellowish, limp and droop. A cut made through the petioles of a newly infected enset plant reveals browning of the vascular strands and yellowish or grayish masses of bacteria ooze out from the strand. Cross sections at the base of pseudostem and corm show discoloration of the vascular strand with large bacterial pocket and grayish or yellowish exudates with brownish to black spot, respectively. Moreover, Archaido (1992) further observed, in a more advanced stage of disease development, most of the leaves wilt, breaks at the petiole and wither. Eventually, the whole plant dies and rots to the ground

Dagnachew and Bradbury (1974) reported first disease symptoms on banana plant as development of dull green color in the lamina followed by scalding; and Wandimagene *et al.* (1987) reporting first symptoms as browning of vessels and surrounding tissues beginning with the point of inoculation. In addition to these, the symptom development in this finding were similar with Ssekiwoko *et al.* (2006), that was earliest symptom is collapsing of the leaf at the midrib.

The results of host-pathogen interaction showed that there were highly significant differences among enset clones, *X. campestris* Pv. *musacearum* isolates, and the interaction between the enset clone and bacteria isolates in disease incidence, incubations periods, date to complete wilting, percentage severity index and AUDPC. This study indicated the existence of horizontal resistance in the host; and pathogencity in the bacterial strains (i.e. main effects). In this case the enset clones *Nobo* and *Gudiro* revealed horizontally high resistance with mean disease incidence of 0.00% (Table 9). The YeLYe isolate was more pathogenic with mean percentage severity index of 55.80% than the other isolates ShMGe and GiHSh (Table 12). All the *X. campestris* Pv. *musacearum* isolates were more pathogenic to enset clone *Yeko*, but isolate ShMGe and GiHSh moderately pathogenic on enset clone *Chikaro*, and all isolates became non- aggressive to enset clones *Nobo* and *Gudiro*. Similarly Fikre and Gizachew (2007) found low (insignificant) bacterial wilt disease infection on artificially inoculated *Meziya* enset clone. The *Meziya* clones, inoculated with Sidama (S-I), Dawro (D-I) and Kembata (K-I) isolates showed bacterial wilt infection of 8.3, 5.7 & 2.6 percent with slight yellowing symptoms if compared with 100% infection on *Arkia* enset clone (Fikre and Gizachew, 2007). At the end of disease assessments (after 4-6 months depending on locations), *Meziya* clones which showed bacterial wilt symptoms were observed to recover/revive from infection and became healthy. All of *Arkia* clones artificially inoculated with all *X. campestris* Pv. *musacearum* isolates were showed typical bacterial wilt symptoms and the mean percentages of infection ranged from 58.33 to 100% indicate that *Arkia* clone is highly susceptible to all *X. campestris* Pv. *musacearum* isolates tested.

During assessment period enset bacterial wilt symptoms were observed in inoculated leaf of *Gudiro* and these symptoms did not spread, and there after appeared healthy and no *X. campestris* Pv. *musacearum* was recovered from these plants. This could be possible that the

bacteria stay confined to the leaf petiole and leaf sheath of this inoculated leaf. May be the bacteria cannot enter in the corm and hence cannot infect adjacent leaves as the vascular connection between leaves passes through the corm. Possibly this is due to the hypersensitive reaction of enset clone. According to Gizachew *et al*. (2008) this apparent recovery may be explained by the un-systemic nature of the disease development after an artificial inoculation in the leaf petiole of a newly formed leaf. The hypersensitive reaction is characterized by the rapid death of individual plant cells which come into contact with pathogenic bacteria, and is generally associated with disease resistance of the whole plant to the pathogen (Kiraly, 1980). This would result in the disappearance of the disease when the inoculated leaf eventually wilts and dies. Fikre and Gizachew (2007) also observed that artificially inoculated enset clones *Genticha* and *Meziya* recovered from *X. campestris* Pv. *musacearum* infection.

On the contrary enset clone *Yeko* and *Chikaro* were susceptible to all isolates as they showed high percentage severity index (Table 12). Sheka isolate was most pathogenic than the both Keffa and Bench-Magi isolates. In comparing the combined enset clone and *X. campestris* Pv. *musacearum* isolate interactions a percentage severity index showed that Sheka (YeLYe) isolate induced a higher percentage severity index on enset clone *Chikaro* than Keffa (GiHSh) and Bench- magi (ShMGe) isolates with YeLYe (100%), ShMGe (43.40%) and GiHSh (41.80%). Gizachew (2000) reported similar result on variation on isolates of enset wilt pathogens that were collected from six *weredas* of Gurage and North Omo zones in southern Ethiopia.

In this study disease symptoms only developed on enset clones *Yeko, Chikaro* and on inoculated leaves of enset clone *Gudiro* within an incubation period of 52.22, 59.17 and 60 mean days; no disease symptoms developed on enset clone *Nobo* during the assessment periods. This implies the differences in host resistance to the pathogen in accordance with incubation periods.

Comparatively, the enset clone *Yeko* showed enset bacterial wilt symptom with short period of time in both green house and field conditions (incubation period of 45 - 52.22 days). Generally, the disease incubation period in inoculated plants less than 6 months is short (Wandimagegne *et al*., 1987). So that, conformation of pathogenicity of *X. campestris* Pv.

musacearum isolates can be performed and proved within 45 - 52.22 days on enset clone *Yeko*.

Tripathi *et al. (*2008) found that, no plants of banana cultivar *Musa balbisiana* wilted completely; and in contrast, all the plants of *Pisang Awak* wilted completely 24–28 days after inoculation. In agreement with this, we found that no plants of enset clone *Nobo* and *Gudiro* wilted completely, even if the clone *Gudiro* which showed initial disease symptom on its inoculated leaf petioles and it revived (recovered) from the disease, and eventually these plants resembled healthy control plants. On the other hand, all the plants of enset clone *Yeko* wilted completely at lower mean 88.33 days after inoculation than the clone *Chikaro*, its complete wilting was observed in 69.7% of plants 98.89 days after inoculation.

Enset clone *Nobo* was found to be the most resistant to bacterial wilt, while the enset clone *Yeko* was the most susceptible with the remaining enset clones comprising a stratified intermediate with clone *Gudiro* close to the resistant *Nobo* and the clone *Chikaro* close to the susceptible *Yeko.* The significantly interaction between the enset clone and bacteria isolates implicated some horizontal resistance in the host population; and horizontal pathogenicity in pathogen strains. In this case the enset clone *Nobo* reveled horizontally complete resistant with mean AUDPC value of 0.00 and enset clone *Yeko* reveled horizontally susceptible with mean AUDPC value of 2702.67 (Table 13). The YeLYe isolate induces more mean of AUDPC values (1391.94) than the statistically similar isolates ShMGe (1197.94) and GiHSh (1170.55). Enset clone *Yeko* was highly infected by all the *X. campestris* Pv. *musacearum* isolates, but isolate ShMGe and GiHSh moderately infects enset clone *Chikaro*, and all isolates could not infect enset clone *Nobo*. This was probably due to high genetic diversity within enset clones and narrow genetic diversity within *Xanthomonas campestris* Pv *musacearum* isolates. Similarly, Aritua *et al*., (2008) found that, the genetic diversity amongst *Xanthomonas campestris* Pv *musacearum* strains has been shown to be very narrow; and thus investigation for host resistance is some what simplified in that host/strain interactions are unlikely and any variation in disease expression recorded is most likely to be attributed to the host genotype.

From Pearson correlation analysis, the result may indicate that the peresence of direct relationships between the enset wilt parameters. If the percentage severity index increases, the bacterium multiplies in the tissues of the plant within short period of time and severely affects it; and results in complete wilting of the plant.

The investigation on enset bacterial wilt disease and its casual pathogen in enset growing areas of southwest Ethiopia indicated that the disease is increasingly becoming equal important as the case in other previously studied part of enset growing regions. The bacterial isolates are found to be very pathogenic to the available enset clones except to enset clone *Nobo* and *Gudiro* even more pathogenic than those *X. campestris* Pv. *musacearum* isolates reported by Kidist (2003), that was collected from 12 *weredas* of southern and oromia regions, and unable to infect the susceptible enset clone *Arica* during pathogeniciy test that was collected from other parts of southern Ethiopia. The difference among the sample farms along with variations obtained in enset clone and *X. campestris* Pv. *musacearum* isolate interaction studies revealed that there are some promising resistance enset clones (*Nobo* and *Gudiro*) to be used for variety development.

8. SUMMERY AND CONCLUSIONS

Enset is the main source of food for over 15 million people. It is also used for fiber, animal forage, construction materials and medicines. However, enset plant is susceptible to most diseases mainly fungal, bacterial and viral causes. Among these, enset bacterial wilt, caused by *Xanthomonas campestris* Pv *musacearum* is the most important disease affecting yield of enset. The disease has been inflecting great losses to enset production by totally killing the plant in Ethiopia since it was first reported and described in 1968 but has been largely contained with host resistance and in some instance with cultural control practices. *Xanthomonas campestris* Pv *musacearum* is also known to attack banana plants in this country. Other than Ethiopia, it has been also reported by causing a great loss in banana plantations in Africa countries such as Uganda, Rwanda, the Democratic Republic of Congo, Tanzania, and Kenya.

Enset bacterial wilt was widespread in enset growing areas of Sheka, Keffa and Bench-magi zones in southern Ethiopia. However, the distribution was not known, *Xanthomonas campestris* Pv *musacearum* isolates were not collected and characterized, and the host-pathogen interaction was not well studied yet from these zones; although the damage was significantly influencing enset clones. Thus, the objectives of this study were to assess enset bacterial wilt, to isolate and characterize strains of the causal pathogens, and to study host-pathogen interactions of enset clones and *Xanthomonas campestris* Pv *musacearum* strains collected from Sheka, Keffa and Bench-magi zones in southwest Ethiopia.

The survey was conducted in the enset growing arease of Sheka, Keffa and Bench-magi zones and host-pathogen interactions study at Gecha, Sheka zone, in southwest Ethiopia; while the laboratory and the green house studies were condacted at Jimma University College of Agricalture and Veternary Medicine. In this study period (2011), the disease incidence and severity was assessed and enset bacterial wilt samples were collected and brought to the laboratory for further study. In addition, two banana bacterial wilt samples were included.

Enset bacterial wilt was prevalent in assessed enset growing areas with significantly increasing disease incidence that ranged from 25.56% to 59.63%. This disease is more sever in Bench-magi (55.56%) than Sheka (48.89%) and Keffa (42.22%) zones. The survey result

showed that enset bacterial wilt occure in different altitude ranges (1470 to 2422 masl) and seasons by affecting the enset plant in all growth stages with an increasing trend in the areas.

The most important factors responsible for spreading disease of bacterial wilt include contaminated farming and processing tools, and human and animal vectors. The only research recommended control measures for diseases are cultural measures to prevent the movement of the causal agent. For bacterial wilt, these measures include the use of healthy, disease free suckers for planting material; cleaning of equipment (by burning on fire or washing with alcohol) that has come in contact with diseased plant material; and rotation with none related crops. However, developing and use of resistant/tolerant enset clones is one of the best approaches in the management of enset bacterial wilt, cheaper to farmers and safer to environments. According to the study results, enset clone *Nobo* and *Gudiro* have exhibited better reaction to bacterial wilt, under both natural epidemic and artificial inoculation conditions in south western Ethiopia. Hence, enset clone Nobo and Gudiro should be considered as a resistant/tolerant clone to pathogen and this material can be also used as a bacterial wilt management component. So that, use of resistant/tolerant clones along with cultural practices and sanitary control measures were some of the advisable enset bacterial wilt management components.

In general, bacterial diseases of plants once established are difficult to control owing to the lack of an effective chemical or other curative treatment. Early detection and destruction of the diseased plants is a key step in preventing disease spread. In the case of enset bacterial wilt, the situation is complicated since most enset clones examined are apparently susceptible and no single control measure has been found to be effective. Consequently, management must focus on methods that reduce the initial inoculums and subsequent spread of the pathogen between host plants.

The casual agent of the disease was confirmed to be *Xanthomonas campestris* Pv *musacearum.* In cultural, morphological and physiological characterizations of *Xanthomonas campestris* Pv *musacearum* population, most isolates from the southwest enset growing areas had different appearances in colony color, growth types and tolerance abilities in different NaCl concentrations, but similar in most biochemical characterizations. In these regard,

those southwest isolates ranged from light to deep yellow in colony color, isolates that grew faster and isolates with slow growth, and all isolates were found tolerant to 1-2% NaCl and about 42 % were found tolerant to 3-5% NaCl. But they are similar in gram reaction, KOH solubility reaction, nitrate reduction and catalase enzyme production.

During hypersensitivity test, the *Xanthomonas campestris* Pv *musacearum* isolates infiltrated to tobacco leaves had shown variation in time for symptom development (48-72 hours) although all were positive to this test. And in pathogenicity test on a susceptible enset clone *Yeko* was found to be positive (100% diseace incidence) to all the tested isolates and showed variability in disease incubation period and date to complete wilting.

There were highly significant ($P<0.01$) difference in disease incidence, incubation periods, date to complete wilting, percentage severity index and area under disease progress curve values; the host – pathogen interaction study where by plants of four enset clones were inoculated with three *Xanthomonas campestris* Pv *musacearum* isolates representing the three Zones of southwest Ethiopia. The significant variations among enset clones, isolates and their interactions indicated horizontal host resistance and horizontal pathogenicity in the pathogen isolates collected from enset growing zones of southwest Ethiopia.

Based on the result, the enset clones *Nobo* and *Gudiro* revealed resistance reaction to all isolates with low mean perecentage severity index of 0.00% and 6.70%, respectively, where as enset clones *Yeko* and *Chikaro* were susceptible to all isolate and showed high mean percentage severity index (PSI) of 100% and 85.20%, respectively. Isolates YeLYe was more pathogenic than the isolates ShMGe and GiHSh. In enset clones by bacterial isolate interaction, all isolates showed an equal PSI value on enset clone Yeko (100%); but isolate YeLYe was in variably showed PSI value on enset clone *Chikaro* (100%) than the isolates ShMGe (71.20%) and GiHSh (68.90%) which were statistically similar.

In conclusion, enset bacterial wilt is an important disease of enset plants although there exist variation in host reaction to pathogen. The bacterial population collected in the enset growing areas showed basically similar cultural, morphological, physiological and biochemical nature of the species *Xanthomonas campestris* Pv. *musacearum* although clear difference in some

feature like colony color, growth nature, salt tolerances and pathogenecies. Generally, enset clone *Nobo* and *Gudiro* showed very low disease severity, as compared to enset clone *Yeko* and *Chikaro* against enset bacterial wilt. This indicates that in the course of resistant enset clone development it is possible to get some resistant genetic material from enset growing regions of southwest Ethiopia.

In the current study, enset clones *Nobo* and *Gudiro* found to be the only resistant clones, considering only three *Xanthomonas campestris* Pv *musacearum* pathogenic isolates. However, the response of these clones to large number of *Xanthomonas campestris* Pv. *musacearum* isolates have to be confirmed under natural disease epidemics in different hot spot areas in enset growing regions of the country and artificial inoculation in the field and green house conditions. The enset clone *Yeko that* showed the highest disease incidence with lowest incubation period for enset bacterial wilt could be used as a susceptible check during clonal evaluation study.

In spite of the fact that we used a limited number of *Xanthomonas campestris* Pv *musacearum* isolates and enset clones, the result obtained clarifies the variations in pathogenicity in some of three *Xanthomonas campestris* Pv. *musacearum* isolates to some of the four enset clones used in this study. Variation in isolate-clone interaction are suggestive of the need to evaluate the response of several number of *Xanthomonas campestris* Pv. *musacearum* isolates before one can draw meaningful inferences about the characters of enset clones and genotypes of *Xanthomonas campestris* Pv. *musacearum* isolates. Therefore, there seems a need for further work by including more number of *Xanthomonas campestris* Pv. *musacearum* isolates and enset clones as well as their genetic backgrounds associated with pathogenicity of the *Xanthomonas campestris* Pv. *musacearum* strains and resistance of enset clones. So that clear understanding of the variability situation in *Xanthomonas campestris* Pv. *musacearum* population might be determined in the major enset growing areas of southwest regions as well as the country.

Simultaneously with conducting basic studies on the causal agent, it is important to encourage and support the enset farmer in application of sanitary measures in organized way to minimize the loss incurred by the disease. Besides, since enset is one of the major crops in Ethiopia

attention for research and development of technologies that improve the production system of enset could contribute for better food security in Ethiopia.

9. REFERENCES

Admasu T., Asnaketch W/T., Assefa A., Bakalu M., Bizuayehu H., Brandt A.S., Michael D; Endale T., Gizachew W/M., Clifton K.H., Kelsa K., Kidist B., McCabe T.J., Mulugeta D., Ousman S., Seble S., Senait B., Shiferaw T., Shigeta M., Spring A., Tamirat L., Tariku M., Tesfaye H., Tesfaye T. and Yewelsew A. 1997. Phase II Final Report of the GTZ funded enset Needs Assessment Project.

Akhtar M.A., Rahber-Bhatti M.H. and Aslam, M. 1997. Antibacterial activity of plant Diffusate against *Xanthomonas campestris* Pv. *Citri*. *International Journal of Pest Management.* 43(2): 149-153.

Anonymous, 1997. Awassa Agricultural Research Center Progress Report of Plant Protection ResearchDivision for 1997. Awassa, Ethiopia

Anonymous, 2006. Internet document-http.//HTM helps centeral-com. Hexid-RGB color chart -filels. Accessed on 25/12/2009.

Araujo A.E. and Siqueri F.V. 1999. Evaluation of the efficiency of the use of copper oxychloride and amixture of oxytetracycline + streptomycin sulfate to control angular leaf spot in cotton. *ComunicadoTecnico Embrapa Algodao*. 105:3.

Archaido J.Q. and Mesfin T.1993. Disease of enset. In Proceedings of the first international workshopon enset spp. (Tsedeke Abate, Clifton Hiebsch and Steve Brandt eds.).Dec.13-21.IAR, Addis Ababa, Ethiopia.

Archaido J.Q. 1992. Annual report of the plant pathologist: July17,1991 to July 16,1992. Enset team support project Sidamo and Gamo-Goffa Peasant Agricultural Development program-PADEP III. Awassa Research center (IAR). Awassa, Ethiopia.

Aritua V., Parkinson N., Thwaites R., Heeney J.V., Jones D.R., Tushemereirwe W., Crozier J., Reeder R., Stead, D.E. and Smith J.J. 2008. Characterization of the *Xanthomonas* sp. causing wilt of enset and banana and its proposed reclassification as a strain of *X. vasicola.* Plant Pathology 57:170–177.

Ashagari D.1985. Studies on bacterial wilt of enset (*Ensete ventricosum*) and prospects for its control. Ethiopian journal of Agricaltural Science 7: 1-14.

Asnaketch, W.1997. The ecology and production of *Ensete ventricosum* in Ethiopia: Doctoral Thesis, Swedish University of Agricultural Sciences, Uppsala.

Atkinson M. M., Huang J. S., & Knopp J. A. (1985). The hypersensitive reaction of tobacco to Pseudomonas syringae Pv. Pisi. Plant Physiology, 79: 843–847.

Bedru S. 2007. Land Cover Changes in Andercha and Masha *Weredas* of Sheka Zone SNNP Regionalstate inforest of sheka; Pp: 22-57.

Bergey, D.H. 1930. Bergey's Manual of Systematic Bacteriology, 2nd ed., Vol. II . (Krieg, N. R. and Holt, J.G. eds). Williams and Wilkins, Balimore. Pp.63-112.

Bezuneh, T. And Feleke, A. 1966. The production and utilization of the genus *Ensete* in Ethiopia.*Economic Botany* 20 (1): 65-70.

Bezuneh, T., Feleke, A. and Bayie, R. 1967. The cultivation of the genus *Ensete* in Ethiopia. *Soil and Crop Science Society of Florida* 27:133-141.

Bradbury, J.F. 1984. *Xanthomonas* Dowson. 1939. In: *Bergey's manual of systematic Bacteriology*, Vol.1. (Krieg, N. R. and Holt, J.G. eds). Williams and Wilkins, Balimore. Pp.199-210.

Brandt, S.A., Spring, A., Hiebsch, C., McCabe, S.T., Endale, T., Mulugeta, D., Gizachew, W/M., Gebre, Y., Shigeta, M. and Shiferaw, T. 1997. *The 'Tree against Hunger'. enset-based Agricultural Systems in Ethiopia.* American Association for the Advancement of science. 6pp.

Biruma, M., Pillay, M., Tripathi, L., Blomme, G., Abele, S., Mwangi, M., Bandyopadhyay, R., Muchunguzi, P.,Kassim, S., Nyine, M., Turyagyenda, F.L. and Eden-Green, S. 2007. Banana*Xanthomonas* wilt: a review of the disease, management strategies and future research directions. Afr. J. Biotech. 6:953–962.

Cheesman, E.E. 1947. Classification of the Banana. I. The genus *Ensete. Horan. Kew Bulletin.* **2**: 97-106.

Central Statistics Authority (CSA). 2009. Annual report of 2008/2009. Addis Ababa, Ethiopia.

Dagnachew, Y., and Bradbury, J.F. 1968. Bacterial wilt of enset (enset ventricosum) incited by *Xanthomonas campestris* sp. Phytopathology 59:111-112

Dagnachew, Y., and Bradbury, J.F. 1974. A note on wilt of banana caused by enset wilt organism.Xanthomonas campestris. East African Agricultural and Forestry Journal. 40: 111-114.

Dereje, A. 1985. Studies on the bacterial wilt of enset *(Ensete ventricosum*) and prospects for its control. *Ethiopian Journal of Agricultural Science*. 7(1): 1-14.

Dickey, R. S. and Kelman, A. 1988. 'Caratovora' or soft rot group. In: Laboratory guide for

identification of plant pathogenic bacteria 2nd ed. (ed. N.W. Schaad.). APS Press St. paul, Minnesota. Pp.81-84.

Dye, D.W., Bradbury, J.F., Goto, M., Hayward, A.C., Lelliot, R.A. and Schroth, M. N. 1980. International Standards for naming pathovars of phytopathogenic bacteria: a list of pathovars names and pathotype strains. Review of Plant Pathology. 59: 153-168.

Endale, T. 1990. Enset in Ethiopian Agriculture. Institute of Agricultural Research News. Areka Research Center.

Eshetu W. 1981. The role of Poecilocarda nigrinervis, pentanolia nigronervosa, and Plantococcus ficus in the transmission of enset wilt pathogen Xanthomonas *musacearum* sp. n. in Wolaita, Ethiopia. M.Sc. Thesis, Addis Ababa, Ethiopia

Eyasu, E.1998. Is soil fertility declining? Perspectives on environmental change in Southern Ethiopia. Managing Africa's soils Drylands Programme, IIED. No.2:38 p.

Fahy P. and Hayward A. 1983. Media and methods, p. 337-378. In: P.C. Fahy and G.J. Persley (eds.). Plant bacterial disease: Adiagnostic guide. Academic, New York.

Ferdu A., Temesgen A., Tameru A., Sisay L., Eyob T., Mesele G. and Guy B. (2009). An IPM Guide For enset Root Mealybug (*Cataenococcus Ensete*) In enset Production.

Fekadu, D., and Ledin, I. 1997. Weight and chemical composition of the plant parts of enset (*Ensete ventricosum*) and the intake and degradability of enset by cattle. *Livestock Production Science*. 49(3): 249-257.

Fikre H. & Gizachew W. (2007). Evaluation of enset clone meziya against enset bacterial wilt. African Crop Science Conference Proceedings Vol. 8. Pp. 887-890.

Gizachew W., K. Bobosha1, G. Blomme, T. Addis, T. Mengiesha (2008): Evaluation of enset Clones Against enset Bacterial Wilt. African Crop Science Journal, Vol. 16, No. 1, pp. 89 – 95.

Gizachew W/M. 2000. Variation in isolates of enset pathogen (*Xanthomonas campestris* Pv. *musacearum*) and reaction on enset
clones (*Ensete ventricosum* (Welw) Cheesman to this disease. MSc.Thesis. Alemaya University, Ethiopia.

Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedures for AgriculturalResearch*. John Wiley & Sons, Singapore. Pp. 139–153.

Hayward, A.C. 1964. Characteristics of *Pseudomonads solanacearum. Journal of applied bacteriology*. 27:265-277.

Hayward, C. 2006. Fruit rots of banana caused by *Ralstonia solanacearum* race 2:

questions of nomenclature, transmission and control. *InfoMusa* 15 **(1-2),** 7-10.

Hugouvieux, V., Barber, C.E. and Daniels, M.J. 1998. Entry of *Xanthomonas campestris* Pv. *Campestris* into hydathodes of Arabidopsis thaliana leaves: a system for studying early infection events in bacterial pathogenesis. *Molecular Plant Microbe Interactions*. 11(6): 537-543.

Hwang, I. and. Lim, S.M. 1998. Pathogenic variability in isolates of *Xanthomonas campestris* Pv. *Glycines*. *Korean Journal of Plant Pathology*.14 (1**)**:19-22.

Jung, W., Heu, S.G. and Cho, Y.S. 1998. Influence of growth conditions for the production of bacteriocin, glycinecin, produced by *Xanthomonas campestris* Pv. *Glycines* 8 ra. *Korean Journal of Plant Pathology*. 14(5): 376-381.

Karamura E, Osira M, and Blomme G. 2005. Containing banana *Xanthomonas* wilt. InfoMusa 14(1):45-46.

Kefale, A. and Sandford, S. 1991. Enset in North Omo region. Farmer's research technical Pamphlet, No.1. Addis Ababa, Ethiopia.

Kidist B. 2003. Characterization of *Xanthomonas campestris* Pv. *musacearum* Isolates: Causal Agent of enset bacterial wilt
disease. MSc.Thesis. Addis Ababa University, Ethiopia.

Kiraly, Z. (1980). Defenses triggered by the invader: Hypersensitivity.In J. G. Horsfall, & F. B. Cowling (Eds.) Plant disease: An advanced treatise Vol 5, (pp. 201–224).

Kocks, C.G., Ruissen, M.A., Zadoks, J.C. and Duijkers, M.G. 1998. Survival and extinction of *Xanthomonas campestris* Pv. *Campestris* in soil. *European Journal of Plant pathology*. *104(9**)**:*911-923.

Lenka, S., and Ram, S. 1997. A note on the efficacy in vivo of various antibiotics and fungicide chemicals against *Xanthomonas campestris* Pv. *Campestris* causing black rot of cauliflower. *Orissa Journal of Horticulture*. 25(1):90-92

Manners, J.G. 1993. Principles of Plant Pathology. 2nd ed. Cambridge University Press.
Mesfin T., Wondirad M., and Bekele K.(2009). Review of Research on Disease of Root and Tuber cropes in Ethiopia.Vol. II.

Mgenzi, S.R.B., Eden-Green, S.J. and Peacock, J.J. 2006. Overview of banana *Xanthomonas* wilt in Tanzania.Proc. 4th Intl. Bacterial wilt symposium. York, England 17–20, July. p.107.

Million, T., Kidist, B. and Mulugeta, D. 1999. Working with farmers to control enset bacterial wilt disease using sanitary measure. Summary of data on farmers' interview conducted before application of sanitary measures in Gurage Zone.

Moses B., Michael P., Leena T., Guy B., Steffen A., Maina M., Ranajit B., Perez M., Sadik K., Moses N., Laban T., and Simon E.,(2007): Banana *Xanthomonas* wilt: a review of the disease, management strategies and future research directions- African Journal of Biotechnology Vol. 6 (8), pp. 953-962.

Ndungo, V., Eden-Green, S.J., Blomme, G., Crozier, J. and Smith, J.J. 2006. Presence of banana *Xanthomonas* wilt (*Xanthomonas campestris* PV. *musacearum*) in the Democratic Republic of Congo (DRC).Plant athology 55:294–294.

Pergrine, W.T.H., and Bridge, J. 1992. The lesion nematode *Pratylenchus goodeyi*, an important pest of Ensete in Ethiopia. Tropical Pest Management. 38(3) : 325-326.

Pijls, L.T.J., Timmer, A.M., Wolde-Gebriel, Z. and West, C.E. 1995.Cultivation, preparation and consumption of Ensete (*Ensete ventricosum*) in Ethiopia. Journal of the Science of food and Agriculture.67(1):1-11

Ploetz, R. C. 2007. Diseases of tropical perennial crops: Challenging problems in diverse environments.Plant Dis. 91:644-663.

Pruvost, O., Couteau, A., Perrier, X. and Luisetti, J. 1998. Phenotypic diversity of *Xanthomonas* sp. *Mangiferaeindicae*. *Journal of Applied Microbiology***.**84(1):115-124.

Quimio, J.A. and Mesfin,T. 1996. Diseases of enset. In enset-based Sustainable agriculture in Ethiopia (Tsedeke Abate, Clifton Hiebsch and Steve Brandt eds.). Proceedings of the First international workshop on enset. IAR, Addis Ababa, Ethiopia. Pp.188-203

Quimio, J.A. 1991. First quarter report of the plant pathologist: July 1 to September 30,1991. enset Team Support Project Sidamo GamoGofa. Peasants Agricultural Development Program-PADEPIII. Awasa Research Center (IAR). Awasa, Ethiopia.

Quimio, J.A. 1992. Annual report of the plant pathologist: July 17, 1991 to July 16,1992. EnsetTeam Support Project Sidamo GamoGofa. Peasants Agricultural Development Program-PADEPIII. Awasa Research Center (IAR). Awasa, Ethiopia.

Quimio, J.A. 1994. Final technical report of the World Bank (IDA) -funded enset pathology progect. September 15, 1993 to July 15, 1994.135pp.

Restrepo, S., Velez, C.M. and Verdier, V. 2000. Measuring the genetic diversity of *Xanthomonas axonopodis* PV. *manihotis* within different fields in Colombia. Phytopathology. 90(7): 683-690.

SAS Institute Inc. 2008. SAS/STAT ® 9.2 User's Guide. Cary, NC: SAS Institute Inc.

Satish, S., Raveesha, K.A. and Janardhana, G.R. 1999. Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. Letters in Applied Microbiology.28 (2): 145-147.

Schaad N.W. and Stall R.E. 1988. *Xanthomonas.* In: Laboratory guide for identification of plant pathogenic bacteria 2nd ed. (ed. N.W. Schaad.). APS Press St. paul, Minnesota.Pp.81-84.

Schaad, N.W. 1988. Initial Identification of Common Genera. In: Laboratory guide for identification of plant pathogenic bacteria 2nd ed. (ed. N.W. Schaad.). APS Press St. paul, Minnesota. Pp.81-84.

Shigeta, M. 1996. Folk in-situ conservation of Ensete (*Ensete ventricosum* (Welw) Cheesman): towards the interpretation of indigenous agricultural science of the Ari, Southwestern Ethiopia. *African study monographs* (Japan). 10(3): 93-107.

Simmonds, N. W. 1958. Enset cultivation in thesouthern highlands of Ethiopia. TropicalAgriculture (Trinidad) 35: 302-307.

Spring A., Hiebsch C., Endale T. and Gizachew, W/M. 1996. Enset needs assessment project phase I Report. Awasa, Ethiopia.

Ssekiwoko F, Taligoola HK, Tushemereirwe WK. 2006. *Xanthomonas campestris* Pv *musacearum* host range in Uganda. Afr. Crop Sci. J.14: 111-120.

Sugimori, M.H. and De-Oliveira, A.R. 1994. Serological characterization of white pathovars of *Xanthomonas campestris*. *Summa Phytopathologica*. 20(3-4): 168-170.

Talwar, M., Kumar, P. and Saxena, S.C. 1996. Evaluation of chemicals including antibiotics against *Xanthomonas campestris* Pv. *Mangiferaeindicae*, the bacterial canker pathogen of mango. *Advances in Plant Sciences*. 9(2): 235-237.

Taye, B. 1984. Evaluation of some Ensete ventricosum clones for food yield with emphasis on the effect of length of fermentation on carbohydrate and calcium content. Tropical Agriculture. 61(2): 111-*116.*

Taye B. 1980. Morphology of Ensete embryo in vitro culture. Ethiopian Journal of Agricultural Science 2: 23-28.

Taye, B. and Asrat, F. 1966. The production and utilization of the genus Ensete. Economic Botany. 20 (1):65-70.

Terefe, B and Endale, T.1989. A review of the available research recommendations and future strategies on enset. Proceedings of the 19th National Crop Improvement conference. April 1987. Addis Ababa. Institute of Agricultural Research.

Terefe, B. 1991. The position of enset (*Ensete ventricosum*) Research in Ethiopia. In. *INIBAP* (International Network for the improvement of Banana and Plantain) Regional Network for Eastern Africa, proceedings of the Regional Advisory Committee Meeting. Kampala, Uganda

23-25 Sept 1991.pp. 29-32.

Tesfaye B. 2008. On Sidama folk identification, namining and classification of cultivated enset (*Ensete ventricosum*).

Tripathi L, Odipio J, Tripathi JN, Tusiime G (2008). A rapid technique for screening banana cultivars for resistance to *Xanthomonas* wilt. Eur.J. Plant Pathol. 121: 9-19.

Tushemereirwe, W., Kangire, A., Smith, J.J., Ssekiwoko, F., Nakyanzi, M., Kataama, D., Musiitwa, C.and Karyaija, R. 2003. An outbreak of bacterial wilt on banana in Uganda. InfoMusa 12:6–8.

Van der plank, J.E., 1963. Diseases epidemics and controls. Academic press. London.

Wandimagegne E., Korobko A. and Dilbe C, 1987. The modes of transmit ion of bacterial wilt of enset. Scientific phythopathological laboratory (SPL) of the state Agro-industrial Committee of the USSR.Progress report for the period of 1986/1987. Pp.375-383.

Wells, J.M., Liao, C. and Hotchkiss, A.T. 1998. In-vitro inhibition of soft-rotting bacteria by EDTA and nisin and in vivo response on inoculated fresh cut carrots. Plant Disease. 82 (5): 491-495.

Winstead N. and Kelman A. 1952. Inoculation techniques for evaluating resistance to Pseudomonas solanacearum. Phytopathology 42:628-634

Yirgou D. and Bradbury J. 1974. A not bacterial wilt of banana caused by the enset wilt organism, *Xanthomonas musacearum.* East Africa Agric. Forest. J. 40: 111-114.

Yohannes, U. and Mengel, K. 1994. Response of enset (*Ensete ventricosum* (Welw.) Cheesman) to mineral fertilizers in southwest Ethiopia. *Fertilizer* Research 37:107-113.

Zeweldu, T and Ludders, P. 1998. Prelimenary tissue culture investigation in Ensete (Ensete spp.). Angewandte-Botanik. 72(1-2): 25-27.57.

10. APPENDICES

Appendix Table 1: Enset bacterial wilt disease samples and locations of collection

No.	ISOLATES	CODE*	Zone/*Wereda*/PA	Altitude(m)	CLONE	Date of collection	Isolation
1	Masha-Barasho	MaHBa	Sheka-Masha-Gatimo	2319	Barasho	06/10/11	14/11/11
2	Masha-Tafaro	MaHTa	Sheka-Masha-Yina	2422	Tafaro	06/10/11	14/11/11
3	Masha-Boso	MaHBo	Sheka-Masha-Kanga	2342	Boso	06/10/11	14/11/11
4	Andiracha-Ogiso	AnHOg	Sheka-Andiracha-Gebina	2353	Ogiso	05/10/11	14/11/11
5	Andiracha-*Chikaro*	AnHCh	Sheka-Andiracha-Chicha	2097	*Chikaro*	07/10/11	14/11/11
6	Andiracha-Kekaro	AnHKe	Sheka-Andiracha-Tugiri	2092	Kekaro	07/10/11	14/11/11
7	Yeki-Wango	YeMWa	Sheka-Yeki-Ermichi	1602	Wango	28/10/11	14/11/11
8	Yeki-*Chikaro*	YeLCh	Sheka-Yeki-Kubito	1482	*Chikaro*	28/10/11	14/11/11
9	Yeki-*Yeko*	YeLYe	Sheka-Yeki-Achany	1470	*Yeko*	28/10/11	14/11/11
10	Giesha-Shalako	GiHSh	Keffe-Giesha-Yerkichity	2450	Shalako	04/11/11	14/11/11
11	Giesha-Ataro	GiHAt	Keffa-Giesha-Dirbado	2372	Ataro	05/11/11	14/11/11
12	Giesha-Gushiro	GiHGu	Keffe-Giesha-Damonechity	2317	Gushiro	05/11/11	14/11/11
13	Bita-Arako	BiMAr	Keffa-Bita-Sheda	1888	Arako	13/11/11	14/11/11
14	Bita-Balaso	BiMBa	Keffa-Bita-Gawaty	1891	Balaso	13/11/11	14/11/11
15	Bita-Boso	BiMBo	Keffa-Bita-Dachadifa	1981	Boso	13/11/11	14/11/11
16	Bita- Banana	BiMBan	Keffa-Bita-Dachadifa	1981	Banana	13/11/11	14/11/11
17	Shebench-Banana	ShHBan	Benchmagi-Shebench-Kuka	2081	Banana	13/11/11	14/11/11
18	Shebench-Yedi	ShHYe	Benchmagi-Shebench-Maha	2083	Yedi	13/11/11	14/11/11
19	Shebench-Gean	ShMGe	Benchmagi-Shebench-Ziagin	1993	Gean	13/11/11	14/11/11

**The first two letters of each code are abbreviated wereda names,the letters L, M and H indicate low, medium and high altitudes (L≤1500; M > 1500 & ≤ 2000; and H > 2000) and the last two letters are abbreviated clone names.*

Appendix Table 2: Mean squares for enset bacterial wilt parameters

Source of variation	*DF*	*Mean Squares*				
		DI	*IP*	*DW*	*PSI*	*AUDPC*
Block	*2*	*31.39*	*10.59*	*25.69*	*1.42*	*6757.19*
Clones	*3*	*18505.37****	*7452.95****	*26456.25****	*15444.65***	*12281988.75****
Isolates	*2*	*219.78***	*76.22**	*121.53**	*249.28****	*174800.01****
*Clones*Isolates*	*6*	*219.78****	*110.24****	*99.31**	*249.28****	*145504.80****
Error		*31.39*	*15.70*	*34.03*	*0.90*	*15402.08*
SE±		*5.60*	*3.96*	*5.83*	*0.95*	*124.11*
C.V		*13.89*	*9.25*	*12.46*	*2.21*	*9.90*

, **, * indicate significant at 5%, 1% and 0.01% probability level, respectively; DF=degree of freedom; SE=standered error and C.V=coefficient of variation.*

Appendix Table 3: Analysis of variance (ANOVA) table for Disease Incident (DI)

Source of variation	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Clones	3	55516.12	18505.37	589.39	<.0001
Isolates	2	439.56	219.78	7.00	0.0044
Clones*Isolates	6	1318.69	219.78	7.00	0.0003
Error	22	690.74	31.39		
Total	35	58027.91			
R-Square	C.V	Root MSE	DI Mean		
0.98	13.89	5.60	40.33		

DF=degree of freedom; SS=sum of squares; MS=mean squares; R-Square=root square; C.V=coefficient of variation and DI=disease incidence.

Appendix Table 4: Analysis of variance (ANOVA) table for Incubation Period (IP)

Source of variation	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Clones	3	22358.85	7452.95	474.59	<.0001
Isolates	2	152.43	76.22	4.85	0.0179
Clones*Isolates	6	661.46	110.24	7.02	0.0003
Error	22	345.49	15.70		
Total	35	23539.41			
R-Square	C.V	Root MSE	IP Mean		
0.98	9.25	3.96	42.85		

DF=degree of freedom; SS=sum of squares; MS=mean squares; R-Square=root square; C.V=coefficient of variation and IP=incubation period.

Appendix Table 5: Analysis of variance (ANOVA) table for Date to complete Wilting of plant (DW)

Source of variation	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Clones	3	79368.75	26456.25	777.49	<.0001
Isolates	2	243.06	121.53	3.57	0.0454
Clones*Isolates	6	595.83	99.31	2.92	0.0301
Error	22	748.61	34.03		
Total	35	81007.64			
R-Square	C.V	Root MSE	DW Mean		
0.99	12.46	5.83	46.81		

DF=degree of freedom; SS=sum of squares; MS=mean squares; R-Square=root square; C.V=coefficient of variation and DW=date to wilting.

Appendix Table 6: Analysis of variance (ANOVA) table for Perecentage severity index (PSI).

Source of variation	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Clones	3	46333.95	15444.65	17074.70	<.0001
Isolates	2	498.57	249.28	275.60	<.0001
Clones*Isolates	6	1495.71	249.28	275.60	<.0001
Error	22	19.89	0.90		
Total	35	48350.99			
R-Square	C.V	Root MSE	PSI Mean		
0.99	2.21	0.95	43.09		

DF=degree of freedom; SS=sum of squares; MS=mean squares; R-Square=root square; C.V=coefficient of variation and PSI=percentage severity index.

Appendix Table 7: Analysis of variance (ANOVA) table for AUDPC values

Source of variation	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Clones	3	36845966.26	12281988.75	797.42	<.0001
Isolates	2	349600.03	174800.01	11.35	0.0004
Clones*Isolates	6	873028.78	145504.80	9.45	<.0001
Error	22	338845.76	15402.08		
Total	35	38420955.22			
R-Square	C.V	Root MSE	AUDPC Mean		
0.99	9.90	124.11	1253.47		

DF=degree of freedom; SS=sum of squares; MS=mean squares; R-Square=root square; C.V=coefficient of variation and AUDPC=area under disease progress curve.