Effects of inorganic fertilizers and *Brassica* species leaf extract and green manure in controlling enset bacterial wilt in Gurage and Silte zones, Ethiopia

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A Thesis Submitted to the Department of Plant Biology and Biodiversity Management in Partial Fulfillment of the Requirements of Doctor of Philosophy in Biology.

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By

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Abstract

Enset (*Ensete ventricosum*) is one of the most important multipurpose crops grown in Ethiopia that is a staple food for approximately 20 million people in the country. The production and sustainability of enset agriculture is, however, threatened mainly by bacterial wilt of enset, which is caused by *Xanthomonas campestris* pv. *musacearum* (Xcm). This study was conducted in Gurage and Silte zones of Ethiopia with the objective of exploring the effects of inorganic fertilizers and *Brassica* species leaf extract and green manure in controlling enset bacterial wilt. The study began with an assessment of the prevalence and incidence of Xcm in relation to altitude i.e. low, mid and high altitudes. The assessments were done by random sampling of 60 farms. Qualitative analysis of leaf extracts was carried out using standard methods. The role of different levels of NPK fertilizers to control Xcm in tolerant (Yeshrakinkye) and susceptible (Ameratye) enset clones were evaluated under field condition. Again, the effects of selected *Brassica* species as a green manure and *Brassica carinata* seed extract residue on growth, physiology of enset clones and their effect on the incidence and severity of Xcm were evaluated under field condition. Field experiments were laid in Randomized Complete Block Design with three replications. Standard procedures were used to collect data for all studies. The data

were analyzed using descriptive and inferential statistics in SPSS and Microsoft Excel software. The result showed that mid altitude had the highest disease prevalence (DP) in 2014 (55%) and 2015 (43.8%), the highest disese incidence (DI) in 2014 (12.2%) and in 2015 (8.2%) compared to low and high altitude sites. This indicated that there were altitudinal based variations on enset bacterial wilt DP and DI in the study areas.

The extracts of *Brassica oleracea var capitata* and *Brassica oleracea var acepala* created the widest bacterial growth inhibition zone at (400 and 200 mg/mL) compared to other concentrations. This suggests that *Brassica oleracea var capitata* and *Brassica oleracea var acepala* controls the growth and development of Xcm. Qualitative phytochemical screening results revealed that the chemical constituents of extracts vary between *Brassica* species. The highest total phenolic content was recorded from *Brassica carinata* seed extracts residue*, Brassica oleracea var capitata* (Cabbage) and *Brassica oleracea var acepala* (Tekur Gomen).

The result of field experiment showed that inorganic fertilizers treatments with $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ significantly ($p<0.05$) decreased DI and disease severity (DS) and increased all growth parameters of both enset clones including plant height, pseudostem girth, green leaf number, leaf length, leaf width, leaf area and leaf area index at different measuring periods compared to positive controls. Application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ decreased the DI of tolerant enset clones by 6.8%, 7.7% and 13.8%, respectively compared to the positive control. In the same way, the DI of susceptible enset clones was decreased by 22.2%, 27.8%, and 33.1 %, respectively. Similarly, DS of tolerant enset clone was decreased with application of $N_{1/2}P_{1/2}K_{1/2,}$ NPK and $N_{3/2}P_{3/2}K_{3/2}$ by 12.4%,

17.3% and 35.2%, respectively as compared to positive control whereas that of susceptible enset clones was decreased by 10.1%, 15.7%, and 17.9 %, respectively. Similarly, the lowest AUDPC value (623) was recorded on tolerant clones treated with $N_{3/2}P_{3/2}K_{3/2}$ fertilizers while the highest AUDPC value (1455) was recorded in a susceptible clone compared with positive control.

Application of *Brassica* species as green manures such as *Brassica oleracea var capitata* (G_1) and *Brassica oleracea var acepala* (G_2) and *Brassica carinata* seed extract residue (G_3) did not bring significant difference at ($P > 0.05$) all growth parameters compared to positive controls. Similarly, compared to the positive control application of these Brassica plants as green manure and *Brassica carinata* seed extract residue didn't bring significant difference ($P < 0.05$) on all physiological parameters except for assimilation rate and on functional parameters of photosynthetic apparatus (chlorophyll content). However, compared to the positive control, G_1, G_2 and G_3 decreased the DI of tolerant enset clones by 19.4%, 23.3% and 23.1%, respectively. In the same way, application of G_1, G_2 and G_3 treatments decreased the DI of susceptible enset clones by 6.7 %, 12.3 %, and 4.6%, respectively. DS of tolerant enset clone with G_1, G_2 and G_3 decreased by 5.3 %, 7.8% and 11.4%, respectively as compared to positive control. Similarly, application of G_1, G_2 and G_3 treatments decreased the DS of susceptible enset clones by 11.4 %, 8.6 %, and 10 %, respectively. The lowest AUDPC value (976.5) was recorded on tolerant enset clone treated with G_3 while the highest AUDPC value (1828.9) was recorded in susceptible clone.

In general, the results of the present study showed use of recommended levels of NPK and $N_{3/2}P_{3/2}K_{3/2}$ amount improved the growth performance of enset and reduce the effect of

bacterial wilt on enset clones. Moreover, use of *Brassica oleracea var capitata* and *Brassica oleracea var acepala* as green manure and *Brassica carinata* seed extract residue were effective to control enset bacterial wilt. From these results, it can be recommended that combined NPK fertilizer and brassica plants green manuring is crucial to control bacterial wilt of enset.

Keywords: *Brassica* species; Disease incidence; Disease severity; *Ensete ventricosum;* Inorganic fertilizers; Phytochemicals; Seed extract residue; *Xanthomonas campestris pv. musacearum.*

Dedication

This dissertation is dedicated to:

My Brother, the late Dr. Biniam Desta

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Abbreviations and Acronyms

A	Net assimilation rate
AUDPC	Area Under Disease the Progress Curve
CFU	Colony forming unit
C_i	intercellular CO_2 concentration
CRD	Completely Randomized Design
CSA	Central Statistical Authority
DAI	Days after inoculation
DI	Disease incidence
DP	Disease prevalence
DS	Disease severity
DW	Dry weight
EBW	Enset Bacterial Wilt
EMSA	Ethiopian Meteorological Service Agency
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FW	Fresh weight
GLN	Green leaf number
gs	Stomatal conductance
HR	Hypersensitive response
LA	Leaf area
LAI	leaf area index
LL	Leaf length
LSD	Least Significant Difference

LW	Leaf width
M	Meter
m.a.s.l	meters above sea level
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
OD	Optical density
PAMP	Pathogen associated molecular pattern
PH	Plant height
PSG	Pseudostem girth
pv.	pathovar
RCBD	randomized completely block design
RWC	Relative water content
SNNPRS	Southern Nations, Nationalities and Peoples' Regional State
T	Transpiration rate
TPC	Total phenolics content
TW	Turgid weight
WUE	Water use efficiency
X.c.m	*Xanthomonas campestris* pv. *musacearum*
YPSA	Yeast peptone sucrose agar

CHAPTER 1

1. Introduction

1.1. Background

In Ethiopia, agriculture is the major economic activity for 85% of the population (CSA, 2016). It is a country of great altitudinal diversity ranging from extreme lowlands as low as 116 meters below sea level at Danakil to highlands more than 4620 meters above sea levels at Ras Dashin (IBC, 2014). This altitudinal diversity is responsible for the existence of diversified farming systems. There are four major farming systems in Ethiopia namely: pastoralism, seed-farming complex, shifting cultivation, and the enset-planting complex (Westphal, 1975). Of these, the enset-planting complex farming system is a system dominated by enset *(Ensete ventricosum* (Welw.) Cheesman) plant and mainly practiced in the highlands of southern and southwestern parts of the country (Admasu Tsegaye and Struik, 2002).

Enset *(Ensete ventricosum* (Welw.) Cheesman) resembles a banana plant, which is often referred as "False Banana". Taxonomically banana is classified into a separate genus *Musa*. Both *Ensete* and *Musa* have a large underground corm, a bundle of leaf sheaths (pseudostem), and large, paddle-shaped leaves (Abrham Besrat *et al.*, 1979).

Enset *(Ensete ventricosum* (Welw.) Cheesman) is typically a multi-purpose plant cultivated mainly as food and fiber in Ethiopia (Brandt, 1996; Tadessa Daba and Shigeta, 2016). It is

a staple food nourishing approximately 20 million people in the country (Temesgen Addis, 2005; Temesgen Magule *et al*., 2014). Enset production is largely for human food, fiber, animal forage, construction materials, medicine and for cultural practices (Yemane Tsehaye and Fassil Kebebew, 2006). The major foods obtained from enset are *kocho*, *bulla* and *amicho*. The energy content of enset is by far the highest compared to those of several cereals and Irish potato, sweet potato and banana (Pijls *et al*., 1995).

Enset is considered as a food security crop as it can withstand long periods of drought, heavy rains, and flooding, which normally devastate other crops (Getahun Degu and Tenaw Workayeu, 1990). However, a number of factors including land degradation and disease infestation (Quimio and Mesfin Tessera, 1996) threaten the sustainability of enset agriculture. Diseases are collectively the most severe biological problem for enset production. Some of diseases are bacterial, fungal diseases of corm rot, sheath rot and dead heartleaf rot and nematode diseases of root knot, root lesion and black leaf streak. There are also viral diseases of enset known as mosaic and chlorotic leaf streak diseases. In addition, insects such as jassid, spider, mites, mealy bugs and some vertebrate pests' damage enset plant and reduce its yield (Quimio and Mesfin Tessera, 1996). 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 system (Dagnachew Yirgou and Bradbury, 1968). The pathogen is very destructive as it kills the plant at all growth stages and regularly causes total losses (Kidist Bobosha, 2003; Bizuayehu Tesfaye, 2008).

Bacterial wilt attacks enset plants at any stage, including at full maturity. When bacterial wilt kills an enset plant late in its life cycle, it is particularly serious economic loss to farmers. Farmers have already invested several years of land, labor, and resources into the plant's production. Such situations have caused farmers to abandon their enset farming and replace it with annual crops in some enset growing areas of the country (Brandt *et al.*, 1997). Bacterial wilt is now recognized as a national problem and spread into most enset and banana growing agro-ecology zones of the country (Zerihun Yemataw *et al.,* 2017). It is mainly spread through infected farm tools, infected planting materials and insects (Dereje Ashagari, 1985; Mwangi *et al*., 2007). Therefore, the spread of the disease can be prevented by implementing cultural disease management practices. The 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, limitation of access of animals, laborers and equipment from and to the infected fields, and rotation of crops (Brandt *et al*., 1997; Mwangi *et al*., 2007; Gizachew Wolde- Michael *et al.,* 2008; Tripathi *et al*., 2009; Temesgen Addis *et al*., 2010; Blomme *et al*., 2017b). However, these methods are not effective as farmers are reluctant to employ and adopt labor-intensive disease controlling measures (Tripathi *et al*., 2009; Chemeda Dilbo *et al*., 2015). Therefore, studies for alternative disease controlling strategies that are effective, easily adopted by farmers and eco-friendly are very crucial.

In the past two decades, there has been an increasing interest in the investigation of various extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Bonjar and Farrokhi, 2004). Brassica vegetables have long been

known for their antimicrobial activity against various microorganisms, including Gram-positive and Gram-negative bacteria and fungi (Jaiswal *et al*., 2011). Recently, bio-fumigation as an approach to control multiple soil-borne pathogens using *Brassica* spp. as green manure or as seed meal amendment or as rotation has been receiving increased attention (Kirkegaard and Sarwar, 1998; Xiao *et al*., 1998; Smolinska, 2000; Matthiessen and Kirkegaard, 2003). In addition, crude extracts of *Brassica* spp. are effective in inhibiting the growth of pathogens. For instance, methanol extract of *Brassica oleracae* exhibited distinct zones of inhibition towards bacterial strains such as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Escherichia coli and Proteus* against the methanol control (Zamir *et al*., 2013). Lewis and Papavizas (1970) also reported that volatile products from decomposing cabbage tissues inhibit hyphal growth of *Aphanomyces euteiches* under *in vitro* studies. Similarly, Dandurand *et al*. (2000) showed that the volatile hydrolysis products of rapeseed meal (*Brassica napus*) strongly inhibited the soil-borne pathogens *Sclerotinia sclerotiorum* and *Aphanomyces euteiches*. It was also found that *S. sclerotiorum* sclerotic germination and prevented hyphal growth of *Aphanomyces euteiches was prevented by* the volatile products up to 77% (Dandurand *et al*., 2000). Moreover, incorporating cabbage tissue can significantly reduce pea root-rot under greenhouse experiment (Lewis and Papavizas, 1971). Greenhouse experiment on soil amended with seed meal of *B. napus* suppressed apple replant disease caused by *Rhizoctonia solani* and the nematode *Pratylenchus penetrans* (Mazzola *et al*.,2001). In a field study, a *Sinapis alba* (white mustard) cover crop significantly reduced Aphanomyces pea root rot in subsequent peas after incorporation of the white mustard tissues into *Aphanomyces euteiches*-contaminated soil. In Ethiopia, some works have been done to

manage white rot of garlic (*Sclerotium cepivorum* Berk.) using *Brassica carinata* (Ethiopian mustard). The findings of Tamire Zewde *at al.* (2007) showed that amendments with *Brassica carinata* seed meal; green manure and dried plant residue reduced the incidence of garlic white rot and increased bulb weight. The amendments of infested soil *Brassica carinata* cultivars seed meal also reduced the incidence of chickpea Fusarium wilt and increased yield of chickpea (Merkuz Abera *et al*., 2011). Moreover, nutrients are important for growth and development of plants and they are important factors in disease control (Agrios, 2005). In addition, plant nutrients can affect the development of a disease by affecting plant physiology or by affecting pathogens or both of them (Dordas, 2008). Berga Lemaga *et al*. (2005) reported soil amended with organic materials, inorganic materials (NPK fertilizers) or different combination of these amendments considerably affected bacterial wilt incidence of Irish potato. Phosphorus fertilization of wheat can have a significant effect and almost eliminate economic losses from pythium root rot (Huber, 1980). Similarly, Phosphorus application can reduce root rot of corn and reduce the incidence of soil smut in corn (Huber and Graham, 1999). A number of other studies have shown that Phosphorus application can reduce bacterial leaf blight in rice, downy mildew, blue mold, leaf curl virus disease in tobacco, pod and stem blight in soybean, yellow dwarf virus disease in barley, brown stripe disease in sugarcane and blast disease in rice (Reuveni *et al*., 1998; Huber and Graham, 1999; Kirkegaard *et al*., 1999; Reuveni *et al*., 2000). In addition, potassium fertilizer reduces the incidence of various diseases such as bacterial leaf blight, sheath blight, stem rot, sesamum leaf spot in rice, black rust in wheat, sugary disease in sorghum, cercospora leaf spot in cassava, tikka leaf spot in peanut, red rust in tea, cercospora leaf spot in mungbean and seedling rot caused by *Rhizoctonia solani* (Huber

and Graham, 1999; Sharma and Duveiller, 2004; Sharma *et al*., 2005). Moreover, under laboratory conditions, the application of potassium, nitrogen and calcium was identified as part of an integrated control package to reduce *Xanthomonas campestris* pv. *musacearum* disease incidence and lengthen incubation periods (Atim *et al*., 2013). However, in contrast, Ochola *et al*. (2014) reported that increasing fertilizer amounts did not significantly reduce disease incidence, wilt severity and plant mortality for artificially inoculated banana plantlets in pot experiments.

As far as it can be ascertained from a survey of the literature, there is limited studies on the effect of altitude on the incidence and severity of *Xanthomonas campestris* in Ethiopia. In addition, the *in vitro* antimicrobial activities and *in vivo* biofumigation effects of Brassica plants extracts against *Xanthomonas campestris* pv. *musacearum* have not been reported in Ethiopia. Furthermore, information on the effect of inorganic fertilizer application on *Xanthomonas campestris* control is scarce. Therefore, the main aim of the study was to assess the prevalence and incidence of *Xanthomonas campestris* across different altitude and evaluate the effects of inorganic fertilizers and green manure on the enset bacterial wilt control.

1.2. Research questions, hypotheses and objectives

1.2.1. Research questions

i. Does altitudinal variation have an effect on the prevalence and incidence of *Xanthomonas campestris* pv. *musacearum*?

ii. Does *in vitro* test of Brassica plant extracts and *Brassica carinata* seed extract residue show antibacterial activity against *Xanthomonas campestris* pv. *musacearum*?

iii. Is there a significant difference in disease incidence and severity between Yeshrakinkye and Ameratye enset clones?

iv. Do inorganic fertilizers affect the growth of enset and reduce bacterial wilt incidence and severity?

v. Do *Brassica* species as a green manure and *Brassica carinata* seed extract residue have an effect on the growth and physiology of enset and reduce bacterial wilt incidence and severity of enset?

1.2.2. Research hypotheses

i. Altitudinal variation does not influence the prevalence and incidence of *Xanthomonas campestris* pv. *musacearum* in different enset growing areas.

ii. Brassica plant extracts and *Brassica carinata* seed extract residue do not show antibacterial activity against *Xanthomonas campestris pv. musacearum.*

iii. A significant difference in disease incidence and severity does not exist between Yeshrakinkye and Ameratye enset clones?

iv. Application of inorganic fertilizers does not improve the growth of enset plant and does not reduce the incidence and severity of *Xanthomonas campestris* pv. *musacearum*.

v. Use of *Brassica* species as a green manure and *Brassica carinata* seed extract residue do not reduce the incidence and severity of *Xanthomonas campestris* pv. *musacearum* and do not increase the growth and physiology of enset plant.

1.2.3. Objectives of the study

1.2.3.1. General objective

The overall objective of this study was to understand the effect of inorganic fertilizer and *Brassica* species as a green manure and seed extract residue in controlling enset bacterial wilt caused by *Xanthomonas campestris* pv. *musacearum*.

1.2.3.2. Specific objectives

i. To assesses the prevalence and incidence of *Xanthomonas campestris* pv. *musacearum* at different altitudes.

ii. To evaluate the antibacterial activity and chemical composition of some Brassica plant leaf extracts and *Brassica carinata* seed extract residue against *Xanthomonas campestris* pv. *musacearum.*

iii. To evaluate difference in disease incidence and severity between Yeshrakinkye and Ameratye enset clones?

iv. To assess the effects of inorganic fertilizers on the growth of enset and the incidence and severity of *Xanthomonas campestris* pv. *musacearum.*

v. To understand the effects of *Brassica* species as a green manure and *Brassica carinata* seed extracts residue on growth and physiology of enset plant as well as the incidence and severity of *Xanthomonas campestris* pv. *musacearum.*

CHAPTER 2

2. Literature review

2.1. Taxonomy and history of enset

Enset (*Ensete ventricosum* (Welw.) Chessman) is a perennial, herbaceous, monocarpic and monocotyledonous crop that belongs to the order *Zingiberales,* the family *Musaceae*, and the genus *Ensete (*Smeds, 1955; Westphal, 1975). It is diploid (2N=18) plant and resembles the banana plant and for this reason is sometimes called "false banana" (Cheesman, 1947). Banana is in the same family as enset, but in the genus Musa (lye and Edwards, 1997; Kress *et al*., 2001; Simpson, 2005).

Horaninow (1862) was the first author to describe the genus *Ensete* creating a single species, *Ensete edule*. Later in 1947, Cheesman revised the genus *Ensete and* reported 25 species. Baker and Simmonds (1953) identified the synonyms whereas Simmonds (1960) with further work reported only six species including *E. glaucum* and *E. superbum (*in Asia), *E. gilletii* and *E. homblei (*in mainland Africa), *E. perrieri* (in Madagascar) and *E. ventricosum (*in Africa and Latin America).

Despite the extensive distribution of wild *enset* in the tropics, it is only in Ethiopia that the plant has been domesticated (Genet Birmeta, 2004; Nakato *et al*., 2018). Currently about one-fifth of the Ethiopian population (20 million) depend on this crop mainly in the

southern region and adjoining areas in Oromia and Gambella Regions (Million Tadesse *et al*.,2003; Zerihun Yemataw *et al.,* 2017). Majority of the people in southern parts of Ethiopia depend on *enset* for food, fiber, fodder, construction materials and medicines (Mohammed Beyhan *et al*., 2013; Asres Ayele and Sahu, 2014). The major enset growing areas in the Southern Ethiopia include Sidama, Gedeo, Gurage, Silte, Hadiya, Kembatta, Wolayita, Gamo, Gofa and Kefficho administrative zones (Bizuayehu Tesfaye, 2008). The total area covered with enset crop in Ethiopia has increased from 65,000 ha in the 1960s (Stanley, 1966) to 500,000 ha in 2015 (CSA, 2016).

Currently enset distribution is restricted to south, southwest and central part of Ethiopia and it is not known as a food crop in the northern part of Ethiopia (Admasu Tsegaye, 2002). However, historical evidences suggested that enset might 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; Bruce, 1790). Smeds (1955) reported that enset cultivation originated in the highlands of Ethiopia. The possible reasons for total disappearance of enset culture in the North and central part of the country could be disease, drought and instability in the sociopolitical events between mid-1700 and mid 1800 (Brandt *et al.,* 1997).

2.2. Morphology and ecology of enset

Both enset and banana are herbaceous perennial monocarpic crops; they produce flowers only once at the end of their life cycle (Endale Tabogie, 1997; Zerihun Yemataw *et al*.,

2017). They have an underground corm, a bundle of leaf sheaths that form the pseudostem, and large leaves. Enset, however, is usually larger than banana, reaching upto 11 meters and with a pseudostem up to one meter in diameter (Admasu Tsegaye, 2002). According to Brandt *et al*. (1997), the underground corm of enset is an enlarged lower portion of the stem with an average of 0.7-meter length and diameter. The fibrous rooting system of enset grows out from this part. The true stem is between the pseudostem and corm near the ground. Usually it grows up during maturity and initiates a single flower head, which forms multiple flower fruits and seeds (Fig 2.1).

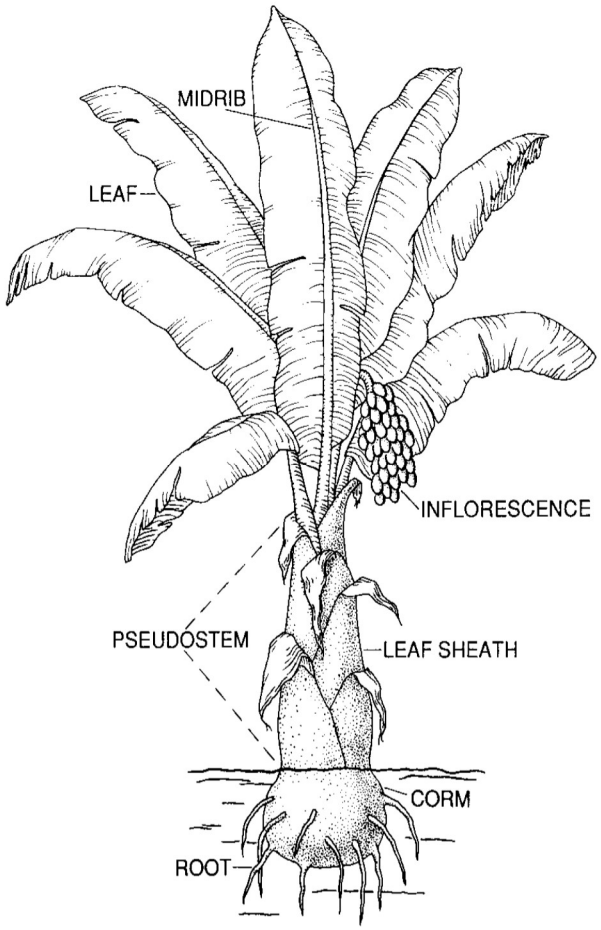

Figure 2. 1. Parts of mature enset plant. Source: Brandt *et al*. (1997)

The leaves are more erect than those of a banana plant, have the shape of a lance head and may be five meters long and nearly one meter wide. Its pseudostem dilates at the base to a circumference of 1.5 to 3.0 m (Zerihun Yemataw *et al*., 2017). Depending on the variety and ecological condition of its cultivation, the pseudostem length ranges from 2 to 5 m. The pseudostem and leaf midribs color vary considerably; some are purple to dark red but most are light green with variegated brown patches (Taye Bezuneh, 1984; Endale Tabogie, 1997; Admasu Tsegaye and Sturk, 2001; Endale Tabogie *et al*., 2003). When a banana plant dies, it is spontaneously replaced by new suckers sprouting from pre-existing buds in the corm. However, in enset, sucker production is induced only when the meristem is wounded. The main sources of food are the corm, pseudostem, and leaf petioles (Hildebrand, 2001).

Ensete ventricosum grows wild from Eritrea, Ethiopia and Sudan in the north, to Angola, South Africa and Mozambique in the South. Enset predominantly grows between an altitudinal range of 1200–3100 m.a.s.l. (Cheesman, 1947; Simmonds, 1962). However, it grows best at elevations between 2,000 and 2,750 meters (Brandt *et al.,* 1997; Admasu Tsegaye, 2002). For a certain range below 1,500 meters, the constraint to enset plant growth probably is more related to available water than to high temperatures. In most areas of Ethiopia below 1,500 meters, the total rainfall and the length of the rainy season decrease, and the potential water use by plants increases because of the greater evaporative demand. Most enset plantings below 1,500 meters have supplemental irrigation or are small that household wastewater may be applied (Admasu Tsegaye and Struik, 2001). Most enset-growing areas receive annual rainfall of about 1,100 to 1,500 millimeters, the

majority of which falls between March and September (Westphal, 1975). The average temperature of enset growing areas is between 10 and 21 degrees centigrade, and the relative humidity is 63 to 80 percent (Asnaketch Woldetensae, 1997). Enset grows well in most of the soil types if they are sufficiently fertile and well drained. Cattle manure is used as the main organic fertilizer. The ideal soils of enset growing areas are moderately acidic to slightly alkaline with pH of 5.5 to 7.3. Most soils in enset growing areas contain 0.10 to 0.15% total Nitrogen and 2 to 3% organic matters (Taye Bezuneh and Asrat Feleke, 1966). Enset requires considerable amount of mineral nutrients to maintain yields. Nitrogen, phosphorus, and potassium are the major nutrients required in large quantities to cultivate enset (Eyasu Elias, 2003).

Enset is drought tolerant; withstanding droughts that seriously damage cereals (Shigeta, 1990; Zerihun Yemataw *et al*., 2014). Enset is not tolerant to freezing-frost damage on upper leaves is commonly observed above 2800 meters above sea level, and serious stunting is seen above 3,000 meters (Endale Tabogie, 1997; Zerihun Yemataw *et al*., 2014).

2.3. Economic and ecological uses of enset

Enset is a multipurpose crop and all parts of the plant are economically important. Enset is used as food in three forms: *Kocho, Bulla* and *Amicho*. '*Kocho'* is a fermented product obtained from the corm, the pseudostem and inflorescence stalk. '*Bulla'* is made by dehydrating the juice collected during the decortication of the pseudostem and grating of the corm. '*Amicho'* is a boiled enset corm. The most important characteristic feature of

enset is its productivity of food per unit area and its storability for long periods without spoilage (Seifu Gebremariam, 1996). 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 Bezuneh, 1984). The fiber obtained from enset is used to make bags, ropes, cordage and mats. Enset leaves and dried leaf sheath are also used for wrapping materials. Furthermore, the leaves are also used as a plate for serving food. The dried midribs and petiole are used for making mats and rope (in place of nail) in house construction and as fuel. Some enset clones are used as local medication for different illness and damage such as bone fracture, bone breakage and diarrhea for both human beings and animals (Brandt *et al.,* 1997).

Fresh *Kocho* contains 47–62 g moisture per 100 g. Per 100 g dry matter the approximate composition of *Kocho* has 1.1–2.8 g protein, 0.2–0.5 g fat, 95–98 g carbohydrates, 2.3–6.2 g fiber, 1.7 g ash, 60 mg Ca, 68 mg P, 7 mg Fe, 0.06 mg thiamine, 0.08 mg riboflavin, and 0.6 mg niacin (Abraham Bosha *et al*., 2016). *Bulla* has a moisture content ranging from 44 to 55 g per 100 g fresh material. Per 100 g dry matter the approximate composition of *Bulla* is 0.4–0.8 g protein, 0.2–0.4 g fat, 93–98 g carbohydrates, 0.6–0.8 g fiber, 0.2 g ash, 91 mg Ca, 44 mg P, 5.8 mg Fe, 0.02 mg thiamine, and 0.2 mg niacin (Ashagrie Zewdu, 2016). The unprocessed corm of enset is also rich in calcium (Ca), magnesium (Mg), potassium (K), zinc (Zn), and iron (Fe) (Ayalew Debebe *et al*., 2012; Sirawdink Fikreyesus *et al*., 2013). In addition, *Amicho* (boiled corm) has the highest total phenolics content next to teff and corn (Sirawdink Fikreyesus *et al*., 2013).

Starch accounts for more than 90% of *bulla* (on dry weight basis). The starch is composed of moisture (14.0%), ash (0.16%), fat (0.25%), protein (0.35%), and amylose (29.0%). Fat and protein content of enset starch are significantly higher than potato starch but lower than that of maize (Tsige Gebre-Mariam and Schmidt, 1996). Starch that can be used for paper, textile and adhesive industries can be produced from enset (ESTC, 2003). There is also a potential to use enset starch in binding and disintegrating of compressed tablets (Tsige Gebre-Mariam and Nikolayev, 1993). Industrially, enset starch is used for various applications. Physical properties such as firmness, stickiness, adhesiveness and cohesiveness of enset, potato, sago and corn starch gels were determined by (Hirose *et al*., 2010). It was reported that rheological (flow of matter, primarily in a liquid state, but also as "soft solid" state) properties represented by cohesiveness, adhesiveness and firmness of enset starch gel were comparable with those of corn starch. The enset starch paste quickly formed a fragile gel during storage, which was similar to the behavior observed of corn starch. Research results of Tsige Gebre-Mariam and Nikolayev (1993) illustrate that enset starch can be used both as a tablet binder and disintegrate possessing a better binding ability and less disintegrating power than potato starch. It is also used as gelling agent. The cross-linked and acetylated form of enset starch shows the potential use as a novel drug delivery system (Abrham Wondimu *et al*., 2014). In addition, enset *flour*, '*Bulla*', has been found to be a substituting gelling agent for pineapple *in vitro* propagation at different concentration and in mixture with agar. Therefore, it offers new possibilities of using low cost gelling material as agar alternative which will reduce material costs considerably and will help in popularizing plant tissue culture techniques.

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 (Taye Bezuneh and Asrat Feleke, 1966; Shigeta, 1990). The energy yield of enset is by far the highest compared to several cereals. A mature enset plant could yield 20 x10^6 cal / ha/ year which is 20 times higher than that of barley (Olmstead, 1974; Terefe Belhu, 1991). Enset energy yield was also reported to be the highest compared to potato, sweet potato and banana (Pijls *et al.,* 1995). As reported by Agren and Gibbson (1968), the main feature of Enset foods is their high energy values (1410–1950 kJ/100 g dry matter of Kocho, 1580–1850 kJ/100 g dry matter of *Bulla*), derived almost entirely from carbohydrate. Pijls *et al*. (2006) reported that Bulla is more energy rich (850 kJ/100 g) than Kocho (650 kJ/100 g). Since 45-65% of energy requirement of adult person per day should be from carbohydrates, *Kocho* and *Bulla* would be ideal products for nutritional security. Adequate amount of soluble fiber obtained from Enset is important for normal functioning of the intestinal tract, reducing cholesterol level and preventing constipation (William and Hu, 2004; Ashagrie Zewdu, 2016; Solomon Workneh and Neela, 2019). This shows that cultivation of enset can significantly improve food security at household and at national level.

Owing to the leaf architecture (‘funnel-like’ leaves) and the perennial canopy of enset intercepts heavy rains (Tadesse Kippie, 2001) and provides shade (reduces soil temperature) and thereby, protects the soil against erosion (Tessema Chekun, 1998), decreases organic matter decomposition and reduces leaching of plant nutrients (Admasu Tsegaye, 2002). 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; Lee and Girma Zawdie, 1997). Enset contributes to the local environment by improving the nutrient balance in soil (Eyasu Elias, 1998), providing shadow, thus moderating temperature, and being part of farming systems with high biodiversity (Bizuayehu Tesfaye, 2008). A large leaf area, closure of canopy and litter from enset contribute to the maintenance of soil fertility under enset cultivation. Therefore, enset contributes to sustainable agriculture and food security.

2.4. Propagation and cultivation of enset

Although both sexual and asexual way of propagation can be used for enset multiplication, it is commonly propagated vegetatively (Tripathi *et al*., 2017). Cultivated enset produces seeds which are similar to seed producing banana (Mulugeta Diro *et al*., 2002; Zerihun Yemataw *et al*., 2018). Even though germination is uncertain and low, enset seedlings can be obtained from seeds. Low seed germination is due to seed dormancy (Taye Buke *et al*., 2016; Zerihun Yemataw *et al*., 2018). The production of viable seed is also low due to the fact that enset utilizes its stored carbohydrate during fruiting and eventually dies and it is harvested before or shortly after flowering (Mulugeta Diro and Endale Tabogie, 1994). Therefore, propagation by seed is not a common practice for enset. Hence, the plant is usually propagated vegetatively and grown as clones to initiate suckering (Kefale Alemu and Sandford, 1991; Mulugeta Diro and Endale Tabogie, 1994).

Taye Bezuneh and Asrat Feleke (1966) noted that to propagate enset, strong hardy second stage transplants of four to six years old are preferred. To initiate suckering, a mother plant is cut out and its pseudostem is cut off at about 20-30 cm above the corm. Then, the apical meristem is removed. The purpose of removing the apical meristem is to eliminate the apical dominance and allow the development of suckers or side-shoots on the corm (Teketel Makiso, 1996; Brandt *et al*., 1997; Deckers *et al*., 2001; Mulugeta Diro *et al*., 2002; Mulugeta Diro and van Staden, 2005).

After the removal of the central growing point, trimming the leaf sheath base and old roots very close to the corm is planted, cut side up, in a shallow hole dug at a fertile spot and covered with dirt and trash. Removal of the apical dominance causes proliferation of shoots. The mother corm to a large extent disintegrates after serving as energy and nutrient source for regeneration and as a growth medium. After a couple of months, shoots emanating from the buried corm begin to emerge more or less in rings, forming a cluster of sprouts. This is because all of the growth potential is concentrated in the shoots which grow from the cut leaf sheath base surface (Mulugeta Diro *et al*., 1996).

Propagation of suckers is carried out in December or January (e.g. in Kambata and Gurage) or March (e.g. in Sidama). The age of suckers before separation from the mother corm and transplantation to another site, depend on the altitude. In altitudes between 1600 and 3000 meters it takes one and one and half years while above 3000 meters two or more years (Westphal, 1975). Once the suckers are ready for transplanting, they will be separated from the degenerating corm and the small leaves and roots are trimmed and transplanted to a

different plot usually with narrow spacing. The vegetative propagated planting material undergoes several stages of nursery until it finally planted in the main field where it attains maturity. Usually, the suckers are transplanted one to four times (Seifu Gebremariam, 1996; Admasu Tsegaye, 2002).

During transplanting, not all plants within a farm or a field receive the same transplanting approach. Farmers sort out the suckers into size groups. Undersized suckers are replanted in the nursery and maintained for extended periods before being ready for transplanting. Replanting the undersized suckers in the nursery lengthens the time to maturity and curtails production. Therefore, growers need more vigorous suckers of enset for cultivation. After the last transplanting, enset is intercropped with food crops (vegetables, maize, and beans) and cash crops (chat, coffee) for two years (Teketel Makiso, 1996; Brandt *et al*., 1997; Deckers *et al*., 2001).

Not all plants within a farm or a field may receive the same harvesting practices. Some plants may be harvested at a younger age (two to three years) for amicho and some may be harvested at older age for *kocho*. This variation in transplanting and harvest management seems to be a function of ethnic group, household needs, and available resources (such as land, labor, capital, and other food crops in the system) (Brandt *et al*., 1997).

Cultivation of enset also involves regular weeding and application of manure. Weeding is done by hand using local tools. Another management activity in enset cultivation is application of manure. The quantity of manure applied varies with plant age and manure

availability (Asnakech Woldetensae, 1997). Farmers grow the enset crop closest to homestead, where they can easily fertilize it with animal manure (mainly cow-dung) and house refuse, while they grow cereals, and root and tuber crops further away from the homestead (Admasu Tsegaye ,2002). Farmers usually practice heavy application of manure during the wettest months (June to August) by broadcasting on the surface and incorporated into the soil later during the period of weeding (Ferew Kebede, 2012). Young enset plants are intercropped with annual crop (such as maize, common bean, cabbage, taro and Irish potato and with other perennials (such as avocado, coffee, and citrus) (Admasu Tsegaye and struik, 2000). There is also clear gender division of labour in enset cultivation practice. Men are involved in propagating, planting and transplanting activities. Women are involved in manuring, hand weeding, and thinning and land race selection. In addition, the tedious work of harvesting and processing is exclusively left to women (Shack, 1966).

2.5. Harvesting and processing of enset

Harvesting includes cutting the leaf sheaths of the pseudostem into pieces, scraping the leaf sheaths pulp (parenchymatous tissue) from the cut pieces. The optimum harvesting time for enset is shortly after flowering. At this stage, it has maximum storage of food in the pseudostem (Kefale Alemu and Sandford, 1991). Age of flowering depends on climatic condition, clone and management practices. Hence, the flowering time varies from 3 to 15 years but is optimally around 6 or 7 years (Brandt *et al*., 1997). However, in some cases enset is harvested in its premature stage especially when there is lack of food. Kelbessa Urga *et al*. (1996) have reported that premature harvest reduced starch content.

Processing is pulvering the corm, mixing the pulverized corm with the scraped leaf sheaths pulp and fermenting the mixture for a certain period of time. The total time period for this fermentation to occur ranges from a few months to several years (Andeta *et al*., 2018). The product is considered ready for consumption after 90 days from the initial processing day, but can also be kept for one or more years (Kelbessa Urga *et al*., 1996; Deckers *et al*., 2001; Abraham Bosha *et al*., 2016). The main food product obtained by fermenting the mixture is locally known as 'Kocho' part of the starch liquid called 'bulla' obtained by squeezing the mixture can also be consumed after it is allowed to settle for some days. The fleshly cooked corm is locally called 'amicho' and can be consumed in a similar way as Irish potato. The quality of *Kocho* depends on the age of the harvested enset plant, the type of clone (variety), and the harvesting season. Moreover, within one plant, the quality is influenced by the part of leaf sheath and corm processed. The preferred type is white in color and is obtained from the innermost leaf sheaths and inner part of the corm, while the lowest grade is blackish and is obtained from the outer leaf sheath and corm (Zerihun Yemataw *et al.,* 2014). In majority of ethnic groups, harvesting is done between November and January (Teketel Makiso, 1996) and mostly carried out by women.

In addition to the gender division of labor, there are gender issues concerning varieties selected for planting and time of harvesting. Both women and men farmers categorize the varieties of enset into two categories, each with different characteristics, and they distinguish each clone in terms of its "maleness" or "femaleness" (Kefale Alemu and Sandford, 1996; Tibebu Habte-Wold *et al.*, 1996; Spring, 1996). This categorization has nothing to do with the biological or reproductive parts of the plant, but with a set of qualities

and characteristics related to desirability, time of harvesting, fiber and food content, softness and hardness, palatability, length of fermentation period, size, growth rates, and resistance to disease and pests. The so-called "male clones" mature later, and are harder but give a larger yield, while the "female clones" mature earlier, are softer, less fibrous, and more delicious. Men have a preference for the "male" enset, because they say "there is less temptation for the women to harvest the plant before maturity for the sake of eating the delicious boiled corm," (*amicho*) as in the case of "female" plants (Kefale Alemu and Sandford, 1991). However, in some areas farmers plant more "female" than "male" plants. Whether or not there are gender-specific reasons for these choices or if women manage to prevail in their own preferences needs to be investigated.

Yield of enset varies with the landraces used and with the climate it grows. Determination of yield of enset is difficult due to complicated production and processing procedures (Hiebsch, 1996; Admasu Tsegaye and Sturk, 2001; Beyene Teklu *et al*., 2017). Thus, many aspects such as space used by suckers or transplants at each stage of transplantation, the age of the plants and type of clone need to be considered in yield determination (Hiebsch *et al*., 1997; Admasu Tsegaye, 2002). '*Kocho*' pre-dominates other enset food products (i.e., *Bulla* and *Amicho*) in its quantity of production. Consequently, quantification of enset yield mostly considers the yield of '*kocho*'. Reports on the yield of 'kocho' are variable. According to the nationwide survey on enset production (CSA, 2016), the average yield of *kocho* and *bulla* per mature enset plant is 25 and 1.0 kilograms, respectively. Taye Bezuneh (1984) reported a maximum yield of 11.9 t ha^{-1} $year^{-1}$. Shank and Cherinet Eritro (1996) have reported *kocho* yield of mature enset to vary from 19.7 to 84.6 kilograms per plant,

with the average of 44.2 kilograms at 50% moisture. Admasu Tsegaye (2002) reported a maximum yield of 26.26 t ha^{-1} $year^{-1}$ for a plant spacing of 2.83 m^2. The average annual yield of '*Kocho*' is 15 to 61 tons per hectare and the amount of *kocho* obtained from one enset plant ranges from 15 to 61 kg (Abrham Shumbulo *et al*., 2012).

2.6. Diseases of enset

Enset diseases are the most severe biological problems facing enset production in Ethiopia (Brandt *et al*., 1997). These diseases affect enset at any growth stage such as suckers, seedlings; young plants and mature plants (Brandt *et al*., 1997). There are many diseases caused by fungi, bacteria and nematodes that attack different parts of enset plant (Blomme *et al*., 2017a). Their importance also varies depending on the damage they cause.

According to Quimio and Mesfin Tessera (1996) Enset foliar diseases caused by fungi are numerous and widespread. Leaf spot diseases that commonly affect enset suckers, seedlings, and young plants are caused by *Phyllosticta sp*., *Piricularia sp*. and *Drechslera sp*. Leaf spots due to *Cladosporium sp.* and to some extent *Deightoniella sp*. are frequently encountered in older plants (Quimio and Mesfin Tessera, 1996). Little is known about the fungal diseases affecting enset roots, corm and pseudostem. However, severe cases of *Sclerotium* wilt and root rot caused by *Sclerotium rolfi* on young seedlings and transplants are reported (Quimio and Mesfin Tessera, 1996). Although, there are no systematic studies on the fungal diseases attacking enset roots, corms and pseudostems, severe cases of *Sclerotium* wilt and root rot caused by *Sclerotium rolfsi* on young seedlings and transplants

have been reported. Usually this disease kills young plants while older transplants are severely stunted due to rotting of the roots. When enset is attacked by *S. rolfsi* the outer leaves wilt and turn brown as a result of the rotting of the leaf sheaths at the soil line (Quimio, 1992).

Bacterial corm rot disease was reported in 1991 as important disease affecting enset production in Ethiopia (Quimio and Mesfin Tessera, 1996). It 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 sp*. *Pratylenchus goodeyi* is often found in association with bacterial wilt. Therefore, it is suspected in transmission of enset bacterial wilt disease (Peregrine, 1992). The leaf nematode disease of enset caused by *Aphelechoides sp*. 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 Tessera, 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. The only identified viral disease of enset is the enset BaDNA (Bacilliform DNA) virus (Quimio and Mesfin Tessera, 1996).

According to Terefe Belehu and Endale Tabogie (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 *Gedeo* and *Sidama* zones (Bizuayehu Tesfaye, 2008). These soft bodied insects feed on the corm and roots and the infested enset plants show stunted growth (Brandt *et al*., 1997; Nakato *et al*., 2018).

Nevertheless, 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 system in Ethiopia (Brandt *et al*., 1997). The pathogen is very destructive as it kills the plants at all growth stages and regularly causes total losses (Kidist Bobosha, 2003; Bizuayehu Tesfaye, 2008; Nakato *et al*., 2018). Enset bacterial wilt was first reported by Dagnachew Yirgou and Bradbury (1968) in Ethiopia in 1968 and is currently found in all the enset growing regions and on wild enset plants (Taye Buke *et al*., 2016). Enset bacterial wilt invades the vascular system of enset, causing permanent wilting and eventual death of the plant. A cut made through the petioles of newly infected enset plant reveals browning of the vascular strands and yellowish or grayish masses of bacterial ooze comes out from strands (Tripathi *et al*., 2009). The ooze exudes within a few minutes after cutting the tissue and abundant

quantities may be produced over a period of several hours (Fikre Handoro *et al.*, 2012). Yellow or brown streaks occur in the vascular tissues of infected plants. Eventually, infected plants wither and the plant rots. Cross sections at the bases 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, respectively (Eshetu Wondimagegne, 1981; Dereje Ashagari, 1985). In a more advanced stage of disease development, most of the leaves wilt, break at the petioles and wither. Eventually, the whole plant dies and rots to the ground (Archido, 1992).

2.6.1. Morphology, biology and epidemology of *Xanthomonas campestris pv. musacearum*

2.6.1.1. Characteristics of *Xanthomonas campestris pv. musacearum*

Xanthomonas campestris pv. *musacearum* (Xcm) is a gram-negative, rod shaped bacterium belonging to Xanthomonadaceae and produces typical yellow, circular, mucoid, slimy colonies on nutrient agar and semi-selective medium YTSA-CC (Tripathi *et al*., 2007). Cells are straight rods usually with dimension within the range 0.4 - 0.7 µm X 0.7 – 1.8 µm. The optimum temperature for its growth is usually 25-30 ºC (Bradbury, 1984). The yellow colored colonies of the pathogen are due to the abundant production of extracellular polysaccharide, called xanthan gum, which contributes to significant blockage of vessels in infected plant tissues (Biruma *et al*., 2007). Xcm is known to systemically invade all tissues of enset and banana after infection. This may involve the upward movement of

bacteria through the vascular tissues if infection occurs in the lower parts of the plants (rhizome or pseudostem) or the downward movement of bacteria if infection occurs through the inflorescence (Ssekiwoko *et al*., 2006; Blomme *et al*., 2008). The systematic nature of the bacterium is a highly significant factor in understanding the mechanisms of spread.

2.6.1.2. Host range and mode of transmission

The main known natural host plants to *Xanthomonas campestris* pv. *musacearum* are Banana (*Musa* spp.) and cultivated enset (*Ensete ventricosum*) both of which belong to the *Musaceae* family and order zingiberales (Dagnachew Yirgou and Bradbury, 1968; 1974). Screening trial on 45 banana cultivars for resistance to enset bacterial wilt disease revealed the susceptibility of all cultivars (Awassa Agricultural Research Center Progress Report, 2000). However, the host range of this pathogen appears rather controversial. Dagnachew Yirgou and Bradbury (1974) inoculated aubergine, barley, bean, broadbean, castor oil, lettuce, lucerne, maize, peanut, pelargonium, potato, sorghum, sunflower, sweet pepper, tobacco, tomato, and wheat with Xcm and confirmed them as non-hosts. Dereje Ashagari (1985) inoculated plants commonly found in enset growing regions such as *Chenopodium album, Colocasia antiquorium, Commelina* sp., *Guizotia scabra*, *Kalanchoe quartinia*, *Snowdenia polystachya*, *Solanum nigrum,* and *Tagetes minuta* confirmed them as non-hosts. In addition, he reported that Enset ventricosum; Musa paradisiacal subsp. sapientum and Canna orchoides are hosts to the pathogen (Tripathi, 2009). Studies showed that there are several hosts to the pathogen including maize, sorghum, Napier grass, common beans,

cassava, taro and tobacco (Mwangi *et al*. ,2006). several authors reported that *Xanthomonas* species have been found in sweet potato, sugar cane, maize, common beans and sorghum (De Cleene, 2008; Todorovic *et al*., 2008). Aritua *et al*. (2008) reported that Xcm may have the potential to infect maize, sugarcane and sorghum; therefore, these plants may act as alternative hosts and reservoirs for infection. While Ssekiwoko *et al*. (2006) reported Xcm as being able to infect only monocots that belong to the families Musaceae and Cannaceae.

The transmission mechanisms and survival of Bacterial wilt pathogen in different substances has been studied and reported by various authors (Dagnachew Yirgou and Bradbury, 1974; Kidist Bobosha, 2003; Gizachew Wolde-Michael *et al*., 2008; Tripathi *et al*., 2009; Temesgen Addis *et al*., 2010). According to these authors transmission of the disease is aided through: i) farm tools such as machetes, pangas and pruning knives. Contaminated tools transmit the bacteria through injuries on roots and aerial parts when farming. ii) Movement of infected plant materials (suckers, bunches, leaves) iii) Contamination of body parts (hands and feet) iv) Insects as they look for nectar in flowers, v) Animals as they browse from infected to clean plants vi) Water when it moves around infected soil and vii) Rain splash and wind. Rain is believed to aggravate the spread of the disease within a plantation during the rainy season.

According to Quimio and Mesfin (1996) Xcm can survive in the soil for about 3 months, in arid conditions where decomposition of the debris is slow. A study revealed that the survival of Xcm was lower in non-sterile soil as compared to sterile soil (Mwebaze *et al*.,

2006) this implies limited ability of Xcm to survive saprophytically in soil in the presence of other competing microorganisms. Mwebaze *et al*. (2006) also revealed that the pathogen can survive longer (two times) in high soil moisture condition (28%) than in low soil moisture conditions. The pathogen was also found to survive on the surface of contaminated knife for up to 3 and 4 days under dry and humid conditions, respectively (Dereje Ashagari, 1985).

2.6.1.3. Damage and distribution

Enset bacterial wilt is known to cause severe damage, as it attacks and kills the plants at any growth stage, including fully matured plants ready to harvest (Brandt *et al.*, 1997). Maximum yield loss can be observed when the pathogen attacks the plants at late maturity stage and when whole plant systems is affected (Gizachew Weldemichael *et al*., 2008). Dereje Ashagari (1985) reported a serious outbreak of the disease with losses up to 70 %. The results obtained from bacterial wilt disease assessment made in some enset fields of the southern nations, nationalities and people's region (SNNPR), showed losses up to 100% under severe damage condition (Awassa Agricultural Center, 2008). Up to 80% of enset farms in Ethiopia are currently infected with enset Xanthomonas wilt (Mcknight CCRP, 2013). The disease has forced farmers to abandon enset production, resulting in critical food shortage in the densely populated areas of southern Ethiopia (Anita *et al*., 1996; Million Tadesse *et al*., 2003). This disease directly affects the livelihood of more than 20% of farmers in Ethiopia.

Depending on various factors such as attitudes, clonal diversity, farmers' knowledge/awareness and perception towards its management practices, the prevalence, severity and distribution levels of Xcm disease vary from one enset growing area to the other, depending on various conditions most possibly, farmers' knowledge/awareness, attitudes, clonal diversity and perception towards its management practices (Fikre Handoro, 2014) The prevalence of the causal agent of Xcm was first reported in Ethiopia by Dagnachew Yirgou and Bradbury (1968) in very limited enset fields. In the beginning the disease did not draw any attention as the incidence was not as serious as at present. Lack of knowledge/perception about the nature of pathogen survival, mode of transmission etc. at community level likely contributes to the Xcm disease incidence and distributions increasing.

Surveys conducted in the major enset growing zones of Ethiopia revealed the occurrence of enset bacterial wilt in all zones with different degree of incidence (Awassa Agricultural Research Center Progress report, 2000). Forty years after its initial discovery in Ethiopia, Xcm was reported in central Uganda in 2001 (Tushemereirwe *et al*., 2003), and thereafter the disease rapidly spread and developed into a full-blown epidemic on banana, spreading to neighboring countries, including Tanzania (Mgenzi *et al*., 2006), the Democratic Republic of Congo (Ndungo *et al*., 2005), Rwanda (Biruma *et al*., 2007), and Kenya (Aritua *et al*., 2008) where it reportedly caused 80–100% crop loss, especially in beer bananas (ABB genome). Xcm is now recognized as a national problem and spread into most enset and banana growing agro-ecology zones of the country.

2.6.1.4. Effect of altitude on prevalence and incidence of enset bacterial wilt

Altitude is considered as one of the major factors influencing plant pathogen distribution (Smith *et al*., 2008). Previous research results showed that there is variation in disease prevalence and incidence across different altitudinal ranges (Mengistu Oli *et al*., 2014; Mekuria Wolde *et al.,* 2016a). For example, Mekuria Wolde *et al.* (2016a) reported maximum mean incidence of enset bacterial wilt between 2000 and 2500 m.a.s.l. while minimum mean incidence was recorded below 2000 m.a.s.l. Similarly, Mania *et al*. (2006) reported that the disease prevalence in banana plant was higher at midland compared to highland and lowland areas. The association of incidence and prevalence to altitude could be attributed to suitable moisture, temperature and soil conditions for the growth and development of *Xanthomonas* (Dereje Ashagri, 1985; Maina *et al*., 2006; Smith *et al*., 2008). Contrary to these findings, higher disease incidence and prevalence were reported in lower altitude areas compared with high altitude areas (Mengistu Oli *et al*., 2014). Such inconsistent results imply that, in addition to altitude other factors such as soil condition, moisture, humidity and temperature are essential for the development of *Xanthomonas (*Harris, 1976; Martin and French, 1985).

2.7. Host-pathogen interaction

Plant-pathogen interaction is a multifaceted process, mediated by the pathogen and plant-derived molecules that mainly include proteins, sugars and lipopolysaccharides (Boyd *et al*., 2013). The most important factors which determine pathogenicity and allow their successful colonization inside the host is secreted molecules which are derived from the

pathogens. Moreover, plant derived molecules are used in identifying these pathogens to stimulate the defense response of plants (Gupta *et al*., 2015). The plant is able to recognize and defend itself against a potential pathogen landing on its surface and the pathogen manipulates the biology of the plant to create a suitable environment for its growth and reproduction (Boyd *et al*., 2013). Both plant and pathogen have evolved a suite of genes that enable this communication (Gupta *et al*., 2015).

Pathogens use different strategies to invade a plant, feed on and reproduce in the plant. They are usually divided into different groups depending on their lifestyle. The roughest method of attack means killing the plant and feed of dead plant tissue. This lifestyle is termed necrotrophic and is a major source of post-harvest crop loss (Laluk and Tesfaye Mengiste, 2010). Such organisms release enzymes which degrade cell wall and toxic metabolites to invade the plant host´s cell death machinery, overcome plant defenses and kill host cells (Govrin and Levine, 2000; Tesfaye Mengiste, 2012). The opposite strategy is represented by biotrophic pathogens, which are dependent on living hosts to sustain life. Biotrophic pathogens do not kill their plant host under the infection process and are dependent on living plant cells to utilize its nutrients. Hemibiotrophic pathogens include biotrophic and necrotrophic stages. In the initial stage (biotrophic), the pathogen must evade the recognition from the host. While in the necrotrophic stage, toxins can be secreted by the pathogen to induce host cell death (Lee and Rose, 2010; Koeck *et al*., 2011; Vleeshouwers and Oliver, 2014). *Xanthomonas campestris* pv. *musacearum* is hemibiotrophic (Endah *et al*., 2010) that infection phase during which the pathogen spreads

in host tissue followed by a necrotrophic phase during which host cell death is induced (Thomma *et al*., 2001; Dodds and Rathjen, 2010).

In principle, plants are in permanent contact with a variety of microbial and plants must recognize the invaders and activate fast and effective defense mechanisms that arrest the pathogen (Bittel and Robatzek 2007). Plant cells are capable of sensing evolutionarily conserved microbial molecular signatures, collectively named pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) using plant pattern recognition receptors (PRRs) (Ausubel,2005; Boller and Felix,2009). MAMPs are molecules that are essential for microbe fitness and survival and are conserved between different species, resulting in an efficient form to sense the presence of pathogens by the plant. Perception of PAMPs by PRRs activates an immune response, referred to as PAMP-triggered immunity (PTI), which provides protection against non-host pathogens and limits disease caused by virulent pathogens (Jones and Dangl, 2006). Pathogens adapted to their host plants can deliver virulence effector proteins into plant cells, which target key PTI components and inhibit plant defense (Abramovitch *et al*., 2006; Grant *et al*., 2006; Zhou and Chai, 2008; Boller and He, 2009; Cui *et al*., 2010). Plants have changed their resistance (R) proteins in order to detect the effector proteins and trigger disease resistance effector-triggered immunity (ETI) which is often accompanied by the hypersensitive response (HR) and systemic acquired resistance (SAR), (Leon and Montesano, 2013).

The hypersensitive response is a rapid localized a form of cell death that occur at the point of pathogen infection. Hypersensitive response occurs at incompatible and sometimes in compatible plant-pathogen interactions (Morel and Dangl, 1997). When the growth of pathogens is very much reduced, the interaction is said to be incompatible interaction. While during compatible interactions, however, the growth of pathogen is highly manifested. A rapid and localized death of tissues at the site of infection is the main characteristics of HR (Gilchrist, 1998; Heath, 2000; Shirasu and Schulze-Lefert, 2000). During this infection, plants circle the invading pathogen with a layer or a ring of dead plant cells to hinder the growth of the pathogen by killing infected and non-infected cells as well as producing a physical wall. A localized infection can also lead to resistance against a subsequent infection at the site of primary inoculation and in the tissues which are remotely located from the initial site (Sticher *et al*., 1997). This form of induced resistance is called systemic acquired resistance (SAR) (Sticher *et al*., 1997).

Plant reactions to pathogen induce biochemical and physiological changes following MAMP- or effector-triggered immunity. Plants can respond with panoply of defense responses to halt pathogen growth. These responses include physical changes (e.g., cell wall thickening, callose deposition, formation of cork layers, or the formation of tyloses in xylem vessels) and biochemical responses (e.g., production of reactive oxygen species [ROS] or signaling compounds such as salicylic acid (SA), jasmonic acid, abscisic acid, and ethylene) that perturb infection (Chisholm *et al*., 2006; Jones and Dangl, 2006). In addition, de novo production of various defense-related proteins and secondary metabolites

such as phytoalexins and various phenolics can accumulate both locally and systemically (Hammerschmidt, 1999; Van Loon *et al*., 2006).

2.8. Bacterial wilt of enset management strategies

Although, enset bacterial wilt is widely distributed and important disease, intensive research has not been conducted to control it. When enset is affected by the disease, it is difficult to control due to lack of an effective controlling methods (Karamura *et al*., 2005). Moreover, limited knowledge on the biology and epidemiology of the pathogen as well as the perennial nature of the plant contributed for the lack of effective control measures. Consequently, management options have focused on methods that reduce the initial inoculum and subsequent spread of the pathogen (Fikre Handoro *et al*., 2012).

Dagnachew Yirgou and Bradbury (1974) and Million Tadesse *et al.* (2003) reported that, applying sanitary control measures and cultural practices help in reducing the inoculums load of the pathogen. Sanitation has been recommended for enset bacterial wilt by different authors (e.g. Dagnachew Yirgou and Bradbury, 1974; Dereje Ashagari, 1985; Quimio, 1992; 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 been exposed to diseased plants and rotation of crops if the damage is severe. Such measures should be taken in a manner of campaign and as regular practice in all enset growing areas. However, some farmers also uproot and throw away infected plants on the road or near the enset farm, which further spread the disease (Million Tadesse

et al., 1999). Among various traditional practices applied by farmers for controlling the disease include i) smoking bones and tires, ii) burning porcupine body, and iii) use of local spiritual believes such as '*Dua'* prayer ceremony and slaughtering black goat (Million Tadesse *et al*., 1999).

Biological control method is an alternative method of controlling plant diseases. Monteiro *et al.* (2005) reported that the plant disease which is caused by *Xanthomonas campestris* strains could be controlled by using antagonistic microorganisms. Abayneh Tunasha (2010) reported that, some fungal and bacterial antagonistic isolates reduced the disease severity of enset. Even though biological control of bacterial diseases using microbial antagonists are known to be effective (Priou *et al*., 2006), this option has not yet been tried so far in the management of bacterial wilt of enset (Fikre Handoro *et al*., 2012). Although controlling plant diseases using chemical method is common, some plant diseases such as enset wilt disease lack effective chemical control method. However, some chemicals have been reported that work against *Xanthomonas campestris* strains. Various *in vitro* trials were done on antibiotics and plant extracts against *Xanthomonas campestris* pathovars that cause diseases in different crops. It was reported that streptomycin was effective against black rot pathogen of cauliflower, *Xanthomonas campestris* pv. *campestris*, followed by oxytetracycline *in vitro* test (Lenka and Ram, 1997). Currently *in vitro* trial was done on antibiotics effect against *Xanthomonas campestris* pv. *musacearum.* The highest anti Xanthomonas effect was shown by streptomycin sulphate followed by Amoxicilin (Getahun Yemata and Masresha Fetene, 2017) and Mekuria Wolde *et al*. (2016a) reported

that, Amoxacillin and tetracycline antibiotics are the most effective antibiotics in inhibiting Xcm bacterium.

Several studies have also indicated the potential of plant extracts in the control of diseases caused by *X. campestris* in several important crop plants. According to Akhtar *et al.* (1997) diffusates from various parts of *Phyllanthus emblica, Acacia nilotica, Sapindus mukorossis* and *Terminalia chebula* showed antimicrobial effects against *Xanthomonas campestris* pv. *citri*. Extracts from *Acacia arabica*, *Achras zapota*, and other 6 higher plants were also found inhibitory to various pathovars of *Xanthomonas campestris* (Satish *et al*., 1999). According to Getahun Yemata (2016), *Agarista salicifolia* and *Pycnostachys abyssinica* plant extracts showed antimicrobial effects against *Xanthomonas campestris* pv. *musacearum.*

Use of resistant/tolerant enset clones (Dereje Ashagari, 1985; Quimio 1992; Fikre Handoro and Gizachew Weldemichael, 2007) is one of the best approaches in the management of Xcm, cheaper to farmers and safer to environment. 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 *Yesherakinkye* in Gurage, *Ado* and *Genticha* in Sidama, *Siskela* and *Gimbo* in Hadya and *Mezia* in Wolaita have relatively high tolerance against bacterial wilt. The development of effective disease control measures and identification of tolerant clones require continuous and intense evaluation of enset clones under different management practices (Anita *et al*., 1996). Enset clones such as Abate,

Arkya, Heila, Mezya and Sorpie are identified as tolerant clones to enset bacterial wilt (Gizachew Welde-Michael *et al*., 2008a). Integrated disease management involves a mixture of approaches combining regular sanitation, use of tolerant enset clones, uses of biological and chemical controlling techniques.

2.8.1. Inorganic fertilizers as disease management strategy

Application of adequate plant nutrients are important for growth and development of plants and also microorganisms and hence increases disease control (Agrios, 2005). According to Muchovej *et al*. (1980) nutrition affects the rate of growth and the state of readiness of plants to defend themselves against pathogenic attack. This is might be due to the fact that application of proper plant nutrition improves the physiology and biochemistry of the plant host, which in turn reduces infection of plants by pathogens (Agrios, 2005). Most vigorously growing plants often offset the most damaging effects of some diseases, since a balanced nutrient supply optimal for plant growth is usually optimal for plant resistance as well (Agrios, 2005; Dordas, 2008). Averting nutrient deficiencies using fertilizers is one way of controlling some of the most important plant diseases in an integrated pest management system (Atkinson and McKinlay, 1997; Oborn *et al*., 2003). The result about the effects of fertilizers on plant growth and disease development is inconsistent and the debate continues. There is no general rule that a given plant nutrient can decrease the severity of a disease (Huber, 1980; Graham and Webb, 1991; Marschner, 1995). For example, a given plant nutrient may decrease the severity of one disease but it can have completely opposite effect on another disease (Bueschbell and Hoffmann, 1992; Hoffland

et al., 2000). In addition, certain nutrients may have direct and greater impact on controlling plant pathogens while others may have indirect and minimum or no effect (Graham and Webb, 1991; Huber and Graham, 1999). Cooke (1972) cited in Kehinde *et al*. 2011) reported that the major nutrients required by the crop are Nitrogen (N), Phosphorus (P) and Potassium (K). Inadequate supply of any of these nutrients during crop growth is known to have negative impact on the reproductive capability, growth and yield of the plant (Vine, 1953; Solubo, 1972).

Nitrogen (N) is considered as one of the essential macronutrients required in large quantities for the growth and development of plants (Singh *et al*., 2003). Therefore, its deficiency symptoms are common in crops (Tisdale *et al*., 1993; Mengel & Kirkby, 2001). Plants take up nitrogen as NH_4^+ and NO_3^- ions from organic matter, inorganic materials and fixation of free nitrogen by microorganisms (Pierce, 1987). Nitrogen plays a major role in protein formation and is a component of chlorophyll. Chlorophyll is required for light energy absorption by the process of photosynthesis. Therefore, adequate N supply will enhance the amount of chlorophyll as a result increase photosynthesis (More, 2006). Although, nitrogen is one of the most important plant nutrient for the growth and development of plants, deficiency and excess use of Nitrogen has difference consequences. On the one hand, nitrogen deficiency resulted in stunted growth and chlorotic leaves which is caused by poor assimilate formation and resulted in premature flowering and then shortening of the growth cycle. On the other hand, the presence of excess promotes development of the above ground organs with abundant dark green (high chlorophyll) tissues of soft consistency and relatively poor root growth. This increases the risk of

lodging and reduces the plants resistance to harsh climatic conditions and to foliar diseases (Lincoln and Edvardo, 2006). In general, the effect of nitrogen on the control of plant disease is inconsistent due to differences in rate of application, time of application, form of nitrogen, and soil conditions (Katan, 2009).

Phosphorus (P) is the second most important plant nutrient which can be applied to most crops. It is part of many organic molecules of the cell (deoxyribonucleic acid (DNA), ribonucleic acid (RNA), adenosine triphosphate (ATP) and phospholipids) and it involves in many metabolic processes of plants as well as pathogen. However, the effect of P in controlling plant disease is highly variable and inconsistent (Kiraly, 1976). P has been shown to be most beneficial when it is applied to control seedlings and fungal diseases where vigorous root development permits plants to escape disease (Huber and Graham, 1999). There are reports indicating a reduction in disease incidence by phosphorus with the opposite also being found, although it appears that phosphorus has a predominantly beneficial effect (Katan, 2009). For most plant species, the total P content of healthy leaf tissue is not high, usually comprising only 0.2 to 0.4% of the dry matter (Brady and Weil, 2002). Plants absorb phosphorus in the form of HPO_4^{-2} and $H_2PO_4^{-}$ (Tisdale and Nelson, 1995). Phosphorus deficiency is one of the largest constraints to crop production in many tropical soils, owing to low native content and high P fixation capacity of the soil (Barber, 1995; Fairhurst *et al.,* 1999). P is essential for root development and when the availability is limited, plant growth is usually reduced.

Potassium (K) is a basic nutrient for plant life and plays many essential roles in plant nutrition. Potassium has significant contribution in photosynthesis, enzyme activation, cell turgor maintenance and ion homeostasis (Marschner, 1995). Potassium is critical in water relations and in transport and accumulation of sugars in the plant (Mengel, 1997). Inside plant, K is found in ionic form only; it is co-factor of many enzymes. Major role of K in plant is osmotic adjustment (Afzal *et al*., 2015). Usually, K is mobile plant nutrient in the plant and moves to the newly growing parts. When there is insufficient amount of K, deficiency is manifested in the older leaves of the plant, showed by an orange-yellow chlorosis symptom with brown patches. When plants have severe K deficiency symptom, the midrib of the leaf curls, the tip of the leaf points to the base of the plant and then eventual death of the leaf tissue (Lahav, 1972; Murray, 1960). Studies showed that K decreases the susceptibility of host plants and contributed for the control of plant disease (Huber and Graham, 1999). The high susceptibility of the K-deficient plant to parasitic disease is due to the metabolic functions of K in plants. Synthesis of high molecular weight compounds such as proteins, starch and cellulose are impaired when K deficient in the plant. Application of K prevents plants from disease attack through promoting the development of thicker outer walls in epidermal cells and influencing plant metabolism (Linus *et al*., 2004) It has been argued that potassium-deficient plants might be predisposed to diseases (Prabhu *et al.*, 2007) and indeed in many cases, potassium application has been shown to reduce the incidence of both foliar and soil-borne diseases, while in a few cases the opposite has been found to be true (Katan,2009).

2.8.2. Biofumigation of brassica plants as a disease management strategy

Biofumigation is an approach to manage soilborne pests and pathogens that involves the use of volatile chemicals (allelochemicals) which are, released from decomposing *Brassica* tissues through incorporation of *Brassicaceae* plants as green manure. It is used as an environmentally friendly alternative to chemical control measure (Kumar *et al*., 2005). The term biofumigation was first coined by Kirkegaard *et al.* (1993) who specifically described using glucosinolate hydrolysis products, notably isothiocyanates, to control soil borne pests and pathogens in horticulture and agriculture. Biofumigation works on the principle of exploiting the natural biocide compounds from glucosinolate containing plants (Kirkegaard *et al.,* 1998; 1999; 2000; Matthiessen and Shackleton, 2005) to suppress soil microorganisms, such as fungal, bacterial pathogens and nematodes (Angus *et al.,* 1994; Brown and Morra, 1997; Sarwar *et al.,* 1998; Bianco *et al.,* 2000; Smolinska *et al.,* 2003).

Most glucosinolate containing genera are clustered within the Brassicaceae, Capparaceae and Caricaceae families (Rodman, 1981). The glucosinolates (GSL) concentration in the cells of the various plants in the families differs substantially. Therefore, it is crucial to identify species that will be effective in suppressing soil-borne pests and diseases, including nematodes. Most plant species mainly family Brassicaceae which include *Brassica oleracea* (broccoli, cabbage, cauliflower, kale), *Brassica rapa* (turnip), *Raphanus sativus* (radish), *Brassica napus* (canola, rapeseed), *Sinapis alba* (white mustard) and *Brassica juncea* (Indian mustard) (Sarwar *et al*., 1998; Ploeg, 2007) are used for biofumigation. However, many cruciferous species of Brassicaceae produce significant

levels of glucosinolates (GSLs), which are held in plant cells separately from the enzyme myrosinase and are not fungitoxic by themselves (Manici *et al*., 1997). However, when plant cells are ruptured the GSLs and myrosinase come into contact and are hydrolysed in the presence of water to release various products, including isothiocyanates (ITCs) (Fig 2.2). Isothiocyanates have a wide range of biocidal characteristics and are acutely toxic to a variety of pests and pathogens (Chew, 1988).

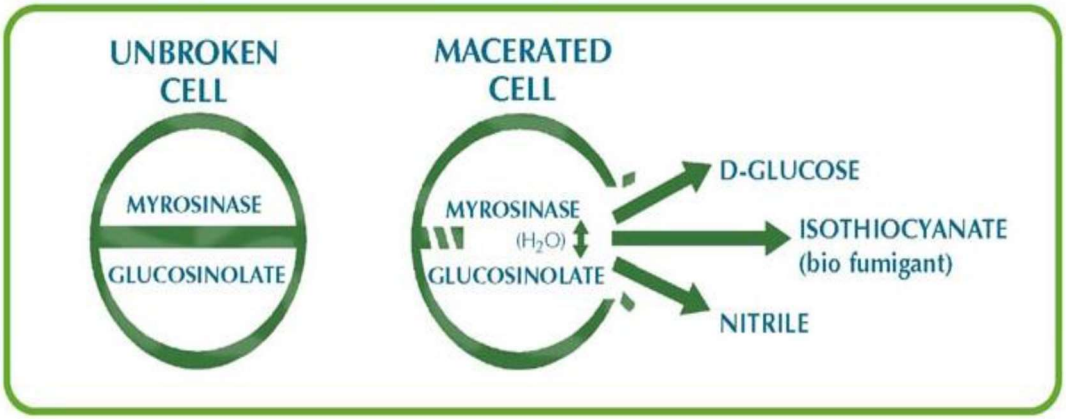

Figure 2. 2. Hydrolysis of glucosinolates. (Source or image from http://serve-ag.com.au/services/seed-salesproduction/biofumigation-seed/)

Based on the basis of their organic chemical structure, glucosinolates in brassica tissues can be classified into three different types, namely aliphatic, aromatic and indolyl (Fig 2.3) (Wittstock and Halkier, 2002). Matthiessen and Shackleton (2005) demonstrated that the ITCs derived from aliphatic GSLs (allyl-ITCs) are more active in the soil than those from aromatic GSLs (2- phenylethyl-ITCs). It was also reported that the green manures of *Brassicas* with more short-chain aliphatic ITCs are more efficient in pest suppression and those with long-chain aromatic ITCs have a low biofumigation capacity (Matthiessen and Shackleton, 2005).

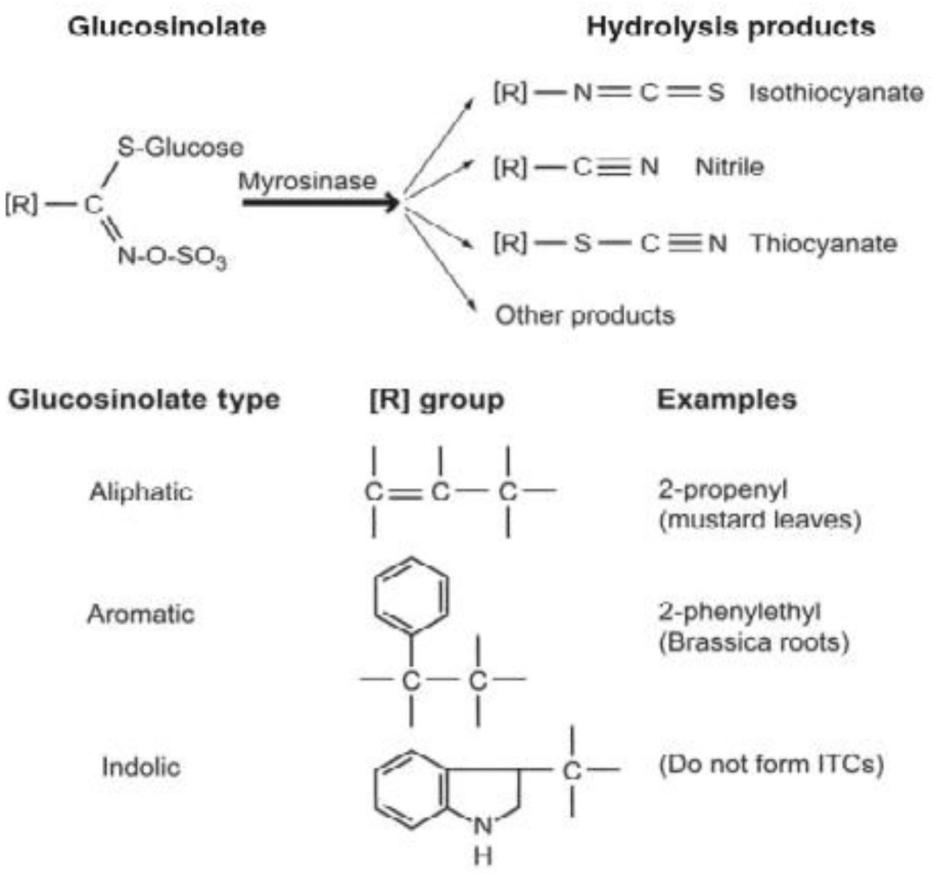

Figure 2. 3.Glucosinolate structure and products of hydrolysis. Source: Kirkegaard (2009).

Biofumigation as an approach to control multiple soil-borne pathogens using *Brassica* spp. as green manure or as seed meal amendment or as rotation has been receiving increased attention (Kirkegaard and Sarwar, 1998; Xiao *et al*., 1998; Smolinska, 2000; Matthiessen and Kirkegaard, 2003). Defatted seed meal produced after the processing of brassica seeds for oil (e.g. in mustard crops) also offer a convenient source of high GSL material for soil amendment (Brown and Morra, 1997). In addition, *Brassica*-cover crops are increasingly used as catch crops and/ or green manure crops with in rotations to provide a number of agronomic benefits such as control of nitrogen leaching, increase soil organic matter, improve plant nutrient and improve soil structure (Kuo and Jellum, 2002).

Generally, it can be understood from the literature review that studies on the effect of altitude on the prevalence and incidence of *Xanthomonas campestris* pv. *musacearum* in Ethiopia were limited. In addition, the *in vitro* antimicrobial activities and *in vivo* biofumigation effects of Brassica plants extracts against *Xanthomonas campestris* pv. *musacearum* have not been reported in Ethiopia. Furthermore, information on the effect of inorganic fertilizer application and Brassica plants green manuring on *Xanthomonas campestris* pv. *musacearum* control is scarce and inconsistent. Hence, it is important to investigate the role of green manure and inorganic fertilizers in controlling bacterial wilt enset in the aforementioned areas. Therefore, the main aim of the study was to assess the prevalence and incidence of *Xanthomonas campestris* pv. *musacearum* across different altitude and evaluate the effects of inorganic fertilizers and Brassica plants green manure on the control of *Xanthomonas campestris* pv. *musacearum*.

CHAPTER 3

3. Materials and Methods

3.1. Assessments of Bacterial wilt of enset

3.1.1. Description of study areas

A disease survey of bacterial wilt of enset caused by *Xanthomonas campestris* pv. *musacearum* was conducted in major enset growing areas of Gurage and Silte zones of the Southern Nations, Nationalities and Peoples' Regional State (SNNPRS) (Fig.3.1). Gurage Zone is bordered by Hadiya zone and Yem special district/woreda/ in the southeast, by the Oromia Regional state in the west, north and east, and by Silte zone in the southeast (CSA, 2008). Welkete town, which is 158 km from Addis Ababa, is the capital of the zone. Gurage zone contains districts such as, Abeshge, Butajira (town), Cheha, Endegagn, Enemorina Eaner, Ezha, Geta ,Gumer, Kebena, Gedebano Gutazer Welene, Mareko, Meskane, Muhor Na Aklil, Soddo and Welkete (town). In Gurage zone, Welkete town and Cheha districts were selected for this study.

Silte zone is also one of the zones of SNNPR and it is the second site selected for this study. Current districts of Silte zone are Alicho Werero, Dalocha, Lanfro, Mirab Azerenet Berbere, Misraq Azerenet Berbere, Sankurra and Wulbareg (CSA, 2008). Mirab Azernet Berbere district was selected for this study. The survey was conducted in 2014/2015 growing season at low, mid and high altitudes. The two administrative zones, namely

Gurage and Silte were selected purposively by their potential to enset production. Districts were stratified into three altitudinal range and one district was selected in each altitudinal category. In Gurage zone two districts were taken (representing low and mid altitude) and whereas Silte zone one district (representing high altitude) were selected. The first site was *Welkete town district* (*Gurage* zone) located about 169 km south-west of Addis Ababa and representing low altitude which has an altitude range of 1896-1928m.The second site was *Cheha district* (*Gurage* zone), located about 200 km south-west of Addis Ababa and representing mid altitude (2445-2540 m). The third site was *Mirab Azernet Berbere district* (Silte zone) located 250 km south-west of Addis Ababa and representing high altitude area (2950-2968 m).

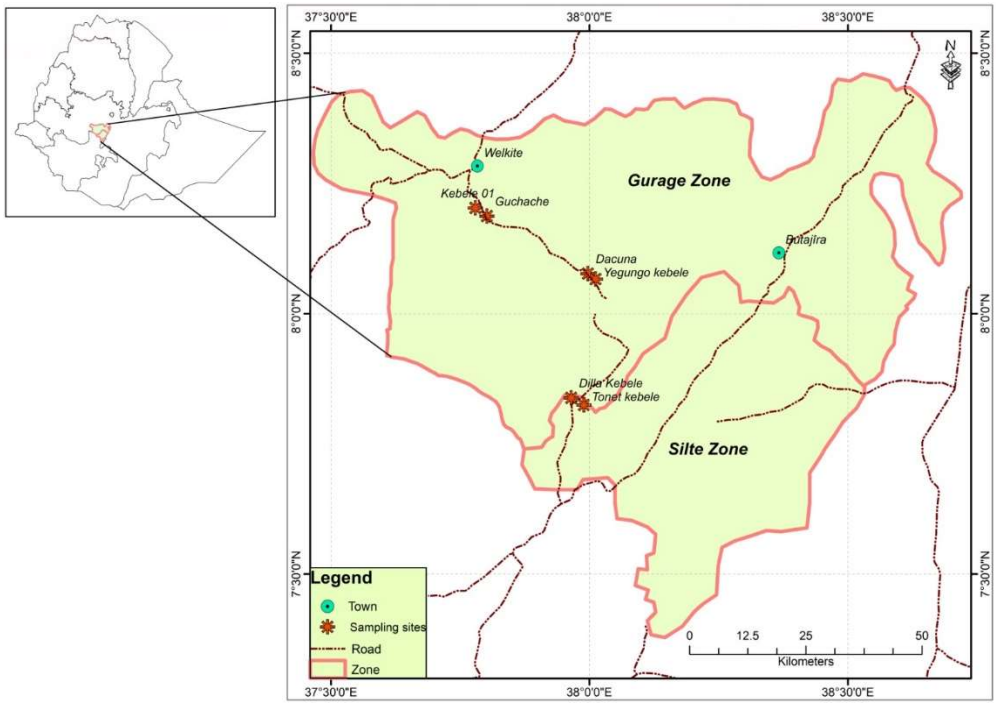

Figure 3. 1. Map showing locations of the study sites in Gurage and Silte zones of Ethiopia

3.1.2. Sampling procedures

To determine the incidence and distribution of bacterial wilt of enset in the study areas a reconnaissance survey was made from enset growing farmers' fields. In each districts two representative kebele (localities) were selected and ten enset farms from each kebele were assessed. Selection of kebeles was based on systematic sampling by considering enset production potential. Accordingly, the study kebeles include Kebele 01 (Gubre) and Guchache (Welkete town district); Dacuna and Yegungo kebeles in Cheha district, and Tonet and Dilla kebeles in Mirab Azernet Berbere district were selected. A total of 60 enset fields (10 farms in each kebele) were assessed in the survey. Surveys were conducted at four season of the year including winter (Dec/Jan/Feb), spring (Mar/Apr/May), summer (Jun/Jul/Aug) and autumn (Sep/Oct/Nov) for two years (2014-2015). The mid-month of each season was selected for survey which means winter season represented by January, Spring by April, Summer by July and Autumn by October.

3.1.3. Disease assessments

The assessment was made along two diagonals in an "X" fashion sampling method (Mengistu Oli *et al.*, 2014). From each kebele ten enset fields were inspected randomly and the incidence and prevalence of the disease was recorded. Interview with 60 farmers was employed using questionnaires to address different issues such as enset clones grown, cultural practices, status of bacterial wilt, farmer awareness about the spread of disease, management practices including indigenous knowledge, and any other issues relevant to

the disease spread and management (Appendix 1 and 2). The altitude, latitude and longitude of the survey fields were recorded using Global Positioning System (GPS).

In each field, plants within the area were counted and recorded as disease and healthy and the incidence of enset bacterial wilt was calculated as follows: Prevalence of the disease was calculated using the number of fields affected divided by the total number of fields assessed and expressed in percentage.

$$\text{Disease prevalence, DP (\%)} = \frac{Total\ No, of\ field\ with\ disease\ symptom}{Total\ No. of\ field\ observed} x100 \ldots\ldots (Eq. 3.1)$$

Disease incidence was calculated using the number of infected plants and expressed as percentage of total number of plants assessed.

$$\text{Disease incidence, DI (\%)} = \frac{Total\ No. of\ symptomatic\ plants}{Total\ No. of\ plants} x100 \ldots\ldots (Eq. 3.2)$$

3.2. Evaluation of the antibacterial activity of *Brassica* species leaf extracts against *Xanthomonas campestris* pv. *musacearum*

3.2.1. Brassica species *plant materials preparation and extraction*

Antibacterial activity test of Brassica plants was made for screening effective extracts of *Brassica* species *in order to* control of *Xanthomonas campestris pv. musacearum, based on the result of antibacterial activity, Brassica* species with better antibacterial activity were selected for field experiment as a green manure. Plants of Brassicaceae family tested

for antibacterial activity were Ethiopian mustard (*B. carinata A.Br*.), Tekur gomen (*Brassica oleracea var acepala*), White gomen (*Brassica oleracea var acepala*), Cabbage (*Brassica oleracea var capitata*), Cauli flower (*Brassica oleracea var botrytis)*, Broccoli (*Brassica oleracea var italica),* Black mustard (*Brassica nigra (L.)* and Radish *(Raphanus sativious L.).* In addition to these *Brassica* species leaf extracts*, Brassica carinata* seed extract residue was used for antibacterial test. The study species were selected on the basis of follow-up of antimicrobial activity reports of (Brown and Morra, 1997; 2005). Those Brassica plants for antibacterial activity were collected from vegetable farms at Akaki in Addis Ababa, Ethiopia (Fig 3.2).

Figure 3. 2. Sample of different types of Brassica plants used for extract preparation

Then 50-day old leaves of Brassica plants were collected from the site and washed with tap water to remove surface impurities. Then they were left to dry under shade for two days until some of its water content evaporates. Then they were oven dried at 40^0 C for three days. The dried plant materials were then blended into powder using an electric blender and sieved through 0.6 mm wide mesh. Then the powder obtained was used for extraction purposes.

Extraction of crude leaf extracts was performed using maceration method. Leaf powder and 80% methanol solvent were added into conical flasks for each species separately in the ratio of 1:10 (W/V), then soaked by putting it on an orbital shaker (250 RAM). Then, the extract was filtered first by four layers of cheesecloth and cotton followed by Whatman's No. 1 filter paper. After filtration, the extracts were dried and concentrated by evaporating methanol using rotary evaporator. Then dried extract was collected in an airtight container and stored at 4^0C till further analysis. The extract obtained was then used for testing in-vitro antibacterial activity (Zamir, 2013).

In addition, *Brassica carinata* seed extract residue was brought from Ethiopian spice extraction factory located at Akaki to the laboratory. Then extracts were preserved at 4^0C till analysis.

Extracts yield (%) of different Brassica plants was also calculated by taking dry crude extract obtained after extraction process and leaf powdered weight taken for extraction (Eq.3.3). The extract yield (%) was determined (Parekh and Chanda, 2007) as follows:

$$\text{Extract yield (\%)} = \frac{\text{dry extract weight}}{\text{dry material weight}} \times 100 \ldots\ldots \text{(Eq.3.3).}$$

3.2.2. Infected enset material collection and isolation of *Xanthomonas campestris pv. musacearum*

Samples of diseased specimen were collected from farmer's home garden from Dacuna kebele in Gurage zone. Disease samples were taken from fresh infected enset leaf petioles,

which showed early stage of the disease symptom to avoid some saprobic microorganisms that grew in tissues killed by the primary pathogen (Quimio, 1992). Bacterial ooze that exuded from infected plants was taken from the field and placed in ice box to transport to Ecophysiology laboratory, Addis Ababa University.

In the laboratory, a small portion of the diseased sample tissues (2-5 mm^2) were cut using sterile scalpel and surface disinfected by dipping in 5% Sodium hypochlorite solution for one minute and immediately immersed three times in distilled water to remove the disinfectant. Then the cut portions were placed in test tube containing distilled water and crushed with sterile glass rod. It was allowed to stand for five minutes until the bacteria diffuse out of the cut tissue into the water. One Loopful of bacterial suspension was taken from the sample and transferred to sterile Petri dishes containing growth media (Fig 3.3). The growth media composed of 5 g Yeast extract; 10 g Peptone;20 g Sucrose; 15 g Agar, in 1 liter distilled water and autoclaved at 121^{o}C for 15 min following Tripathi *et al*. (2007).

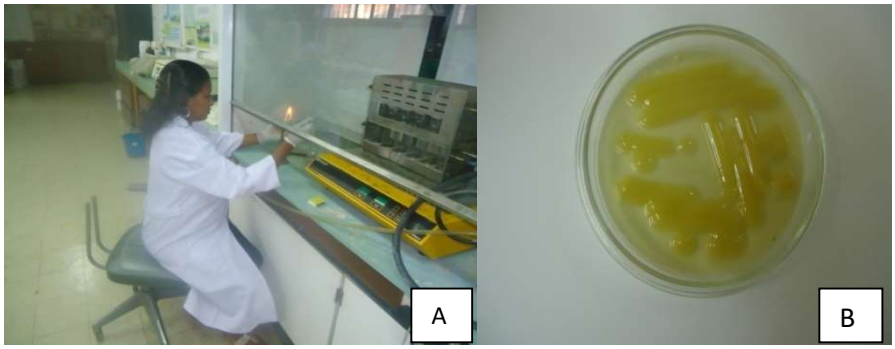

Figure 3. 3. Streaking of bacterial suspension on prepared media (A) and Pure cultures of Xanthomonas campestris pv. musacearum on YPSA growth media(B).

The Petri dishes were incubated in an inverted position at 28 ºC for 72 hours according to Schaad and Stall (1988). Then isolated colonies were selected and streaked on newly prepared yeast peptone sucrose agar (YPSA) growth medium. This sub-culturing was made several times until uniform yellow colored colonies of *Xanthomonas campestris* were produced. Incubation colonies showing light yellow mucoid growth typical of *Xanthomonas campestris* was transferred to individual YPSA slant and maintained at 4^0C in refrigerator for further studies (Schaad and Stall, 1988). A pure culture bacterium showed mucoid growth with light yellow colony color. The colonies were also dome shaped and had shiny appearance that conform with *Xanthomonas campestris* pv. *musacearum* (Fig 3.3). Preserved culture was adjusted to optimal inoculums amount 1.5x 10^8 colonies cfu/ml as a source of inoculum.

3.2.3. Hypersensitivity test

In order to check whether bacteria isolate is pathogenic or non-pathogenic, hypersensitivity test was conducted on tobacco (*Nicotiana tabacum*) plants according to Quimio (1992) and Kidist Bobosha (2003). The plants were grown in pot under greenhouse condition. The inoculum was prepared by suspending bacterial cells from 48 hours old cultures into sterilized distilled water (SDW) at a density of $1.5x10^8$ CFU/mL (0.5 McFarland standard) CFU/mL (using spectrophotometer). An aliquot of 10ml of bacterial suspension ($1.5x10^8$ cfu/ml) was injected using a sterilized hypodermic syringe into the intercellular spaces of expanded leaves of a two-month tobacco plant (*Nicotiana tabacum* var. white burley) (Quimio,1992). Injection of sterilized distilled water was used as negative control. Tobacco

plant inoculated with bacterial suspension of *Xanthomonas campestris pv. musacearum* pathogen showed brown spot or brown necrosis around the injection point (Fig. 3.4) were considered as positive for the test and identified as pathogenic isolate.

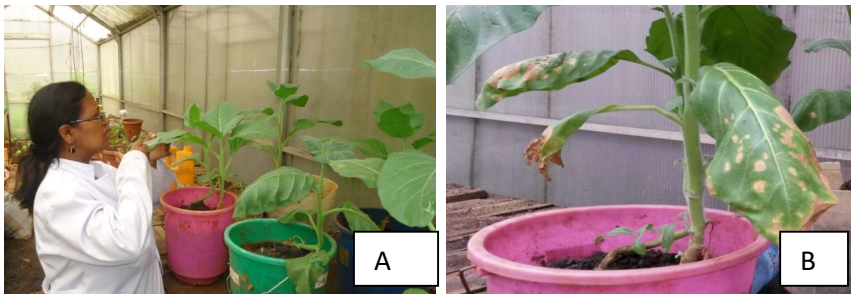

Figure 3. 4. Inoculating tobacco plant (A) and Hypersensitivity test result (B)

3.2.4. Pathogenicity test

Bacterial isolate that induced hypersensitivity on tobacco plants were subjected to pathogenicity test on susceptible enset clone. One-year-old susceptible enset suckers of Amaratie clone were collected from Dacuna area in Gurage zone and brought to Addis Ababa University. These enset suckers were grown in a greenhouse condition for two months. The inoculum was adjusted to a concentration of $1.5x10^8$ CFU/mL (0.5 McFarland standards) using spectrophotometer. An aliquot of 10 ml of the bacterial suspension were inoculated using a hypodermic sterile syringe to the second innermost leaf petiole (Dereje Ashagri, 1985). The control plants were inoculated with the same amount of sterilized distilled water at the same location (Fig 3.5) and the experiment was conducted with three replications. The reaction of the plants was observed every week for 3 months. The inoculated leaves of the enset suckers showed dark brown necrosis around the inoculated area of the leaves and no necrosis was observed in control suckers inoculated with sterilized water (Fig 3.5).

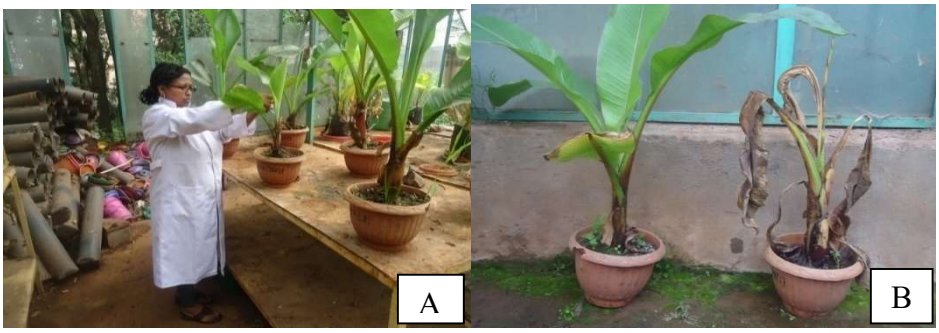

Figure 3. 5. Inoculating enset plant (A) and pathogenicity test result (B)

3.2. 5. Biochemical characteristics of *Xanthomonas campestris* pv. *musacearum*

i) 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 the solution was viscous enough to stick to the loop causing a thin strand of slime, then the test was recorded as KOH soluble positive or not (Fahy and Hayward, 1983).

ii) Catalase test

The presence of Catalase activity was checked by flooding a drop of 3% hydrogen peroxide on pure colonies of 24 hr cultures over the slide, immediate effervescence of gas bubble was recorded as a positive result (Harisha, 2007).

iii) Salt Tolerance

The salt tolerance of the bacteria to different salinity level was tested on YPSA medium plates. The media supplemented with NaCl at concentration of 1% ,2%, 3%, 4%, 5% (w/v).

The colony growth was recorded after 3 days of incubation at 28 ± 2ºC (Abdel-Latif *et al*., 2006).

3.2.6. Antibacterial test of *Brassica* species leaf extracts and *Brassica carinata* seed extract residue

The antibacterial potential of crude leaf extracts against Xcm was evaluated by disc diffusion method (EUCAST, 2012). For the preparation of dilutions of crude extracts for antibacterial assay, serially diluted extracts of different brassica plants were done by dissolving 1.2, 0.9, 0.6, 0.3, 0.15 g of each species extract separately in 3ml of 80% methanol solvents and obtained 400, 200, 100, 50 and 25mg/mL serial concentrations, respectively (El-Mahmood *et al*., 2008). Then antibiotic assay discs with 5 mm size were prepared by cutting Whatman's No 1 filter paper using paper perforator and sterilized in an autoclave. Then the discs were impregnated in to 3ml of each pure extract and allowed to rinse over night for the control 3ml of 80% methanol solvents used. Then they were left to dry by putting them inside an incubator at 28ºC. For *Brassica carinata* seed extract residue to prepare extracts for antibacterial assay, pure concentration of their extract taken as 100% and 2ml of extract (100%) was diluted using 2 ml methanol to obtain 50 % concentration. The above process was repeated several times to obtain other dilutions: 25%, 12.5% and 6.25%) concentrations by serial dilution methods. Simultaneously isolate was grown on nutrient broth for 72 hrs at 28ºC. The inoculum was prepared using direct colony suspension method and the bacterial suspension was standardized to the density of a 0.5 McFarland standard (Sutton, 2011). The standardization was done by measuring the

absorbance of the suspension with a spectrophotometer (NV202, Sunny) at 600 nm. The absorbance was adjusted to 0.132 which is approximately equal to 1.5 x 10^8 CFU/mL.

For inoculation, cotton swab was dipped into the standardized bacterial suspension and the inoculum was spread evenly over the entire surface of new YPSA (yeast peptone sucrose agar) petri dishes and dry disc were placed on the petri dishes. Then the petri dishes were immediately sealed with Para film. The cultures were maintained in an incubator with a temperature at 28^0C. Radial growth of the pathogen was measured after 72 h of incubation periods. Three replicates were used for each treatment. Zones of complete inhibition were measured using transparent ruler at the widest possible diameter including the disc (CLSI, 2018). The experiments were conducted three times with randomized complete block design with three replications.

3.2.7. Determination of minimum inhibitory and bactericidal concentrations of leaf extracts

The minimum inhibitory concentrations (MICs) of *Brassica* leaf extracts were determined using agar dilution method as described in EUCAST (2000). One ml from each serial dilution of each species leaf extract was thoroughly mixed with 19 ml of agar medium and poured into a growth Petri dishes. Then the Petri dishes with agar medium were then allowed to solidify at room temperature. The bacterial suspension was adjusted to 0.5 McFarland standard using spectrophotometer and 0.3 ml of this suspension was inoculated at four spots in each Petri-dishes. Petri dishes were incubated at 28^0C for 72 hr. Control

Petri dishes without leaf extracts were used and result was compared against them. After 72 hr the growth of the bacterium was observed.

The minimum bactericidal concentrations (MBCs) of leaf extracts were determined as described in Njinga *et al*. (2014). Agar medium was poured into sterile Petri dishes and allowed to cool and solidify. The contents of the MIC Petri dishes that did not show growth were sub-cultured onto the prepared Petri dishes. The Petri dishes were then incubated at 28°C for 72 h. Then after the Petri-dishes were observed for growth. The Petri dishes without growth represent the minimum bactericidal concentration (MBC). After 72 h, the results were recorded and taken as MBC (Njinga *et al*., 2014).

3.2.8. Phytochemical analysis of plant extracts and *Brassica carinata* seed extract residue

3.2.8.1. Qualitative screening

Qualitative screening of different Brassica plants was conducted to understand the presence or absence of phytochemical in the methanol crude leaf extract of *Brassica* species. The screening was carried out according to standard methods and procedures as described below:

Test for phenols

Ferric chloride test: - 0.5 g of plant leaf powder was boiled with 10ml of distilled water and filter using filter paper. Then 2 ml of filtered extracts were taken and 3-4 drops of 10%

ferric chloride solution added. The formation of bluish black color gave an indication of the presence of phenols (Harborne, 1998; Nawaz *et al.*, 2018).

Test for alkaloids

Wagner's test: - About 20 mg of extract was taken and dissolved in 5 ml 2% HCl and filtered. Then Wagner's reagent was prepared by dissolving 1.27 g of iodine and 2 g of potassium iodide (PI) in 100 mL of distilled water and 2ml of prepared reagent mixed with 1ml of filtrate and the formation of a reddish-brown precipitate indicates the presence of alkaloids (Harborne, 1998; Sasidharan *et al*., 2010).

Test for flavonoids

Ammonium test: 0.5 g of extract added in test tube and 10 ml distilled water added and filtered. Diluted ammonia solution (10%) 5 ml added in proportion of 2 ml of extract followed by addition of 1ml of concentrated H_2 SO_4. The formation of yellow colored precipitate showed the presence of flavonoids (Harborne, 1998; Hossain *et al*., 2013)

Test for terpenoids

Salkowski test: An amount of 0.8 g of plant sample was taken in a test tube, and then poured 10 ml of methanol in it, shaken well and filtered to take 5 ml of extracts of plant sample. Then 2 ml of chloroform were mixed in extract of selected plant sample and 3ml of sulphuric acid were added in selected sample extract. Formation of reddish-brown color indicated the presence of terpenoids in the selected plants (Harborne, 1998; Talreja and Moon, 2014).

Test for saponins

Foam test: One gram of each extract was boiled with 5 ml of distilled water and filtered. To filtrate 3 ml distilled water was added and shaken vigorously about 5 minutes. If foam produced persists for 10 minutes with 1 cm layer this was taken as indication for the presence of saponins (Harborne, 1998; Kar, 2007).

Test for tannins

Ferric chloride test: Crude leaf extracts were taken separately in test tubes and 5ml of distilled water was added and warmed and filtrated. To 1ml of the filtrate, 2 ml of 5% (w/v) ferric chloride solution prepared in 90% alcohol was added. Appearance of a dark green or blue color indicates for the presence of tannins (Harborne, 1998; Tiwari *et al*., 2011).

3.2.8.2. Quantitative estimation of total phenol

Quantitative estimation of total phenolics content was done on different types of Brassica plants. The amount of total phenolics in extracts was determined according to the Folin-ciocalteu method (Singleton *et al*., 1999). Extracts of 0.5 g of Brassica plant leaf sample was mixed with 10 ml of 80% (v/v) methanol and the homogenate was centrifuged at 10,000 rpm for 10 minutes and the supernatant was used for total phenolics content analysis. An aliquot (0.2 ml) of the supernatant was taken and diluted with 3 ml distilled water. Consecutively, 0.25 ml of Folin Ciocalteau reagent was added. After 3 minutes, 1 ml of 20% (w/v) sodium carbonate was added and thoroughly mixed. The tubes were

placed in boiling water for one minute and cooled. A blue color was developed in each test tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in Folin Ciocalteau reagent in alkaline medium. This blue colored complex was molybdenum blue. The absorbance was measured at 650 nm against a reagent blank using a spectrophotometer (NV202 spectrophotometer, Sunny). The blank was composed of 3 ml of distilled water, 0.25 ml of Folin Ciocalteau and 1 ml of 20% sodium carbonate. The absorbance of the blank was subtracted from each reading. Catecol was used to prepare the standard calibration curve from which the amount of total phenols in a given sample was calculated. The amount of total phenols was expressed in mg catechol equivalent of phenol/g of sample (Zieslin and Ben-zaken, 1993).

A calibration curve was prepared by dissolving 0.5 g of dry catechol in 100 ml of distilled water as a stock solution (Fig 3.6). From the catechol stock solution zero (0), 1, 2, 3, 5, and 10 ml were taken and added in to 100 ml separate volumetric flasks, and then diluted to volume with distilled water. These solutions had 0, 0.05, 0.1, 0.15 and 0.25mg/ml of catechol. From each calibration solution 0.2ml was taken into separate test tubes and to each 2.8 ml of distilled water and 0.25µL Folin Ciocalteau reagent was added and mixed thoroughly. After 3 minutes, 1ml of 20% sodium carbonate solution was added, well mixed and warmed in a boiling water for one minute, cooled and absorbance was determined against the blank at 650 nm.

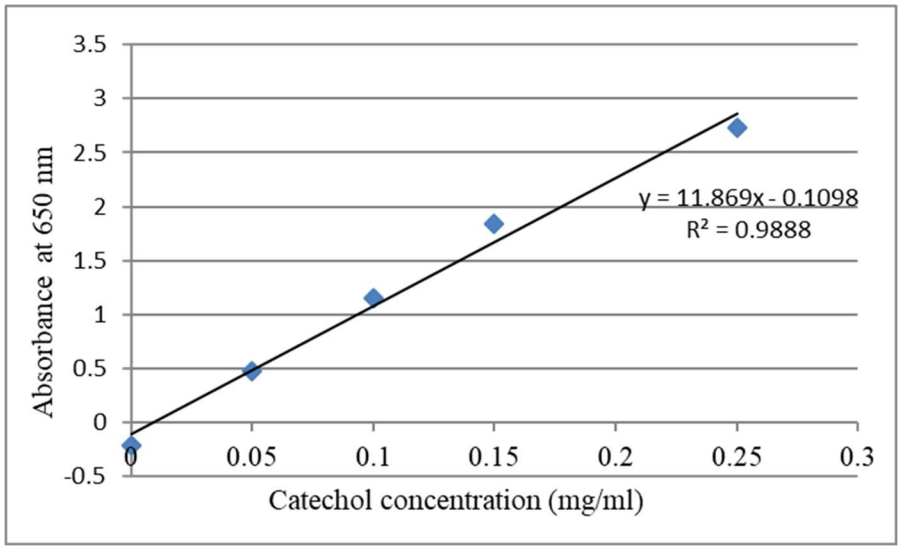

Figure 3. 6. Catechol calibration curve for total phenol determination

3.3. Effects of inorganic fertilizers and *Brassica* species as green manure and seed extract residue against *Xanthomonas campestris* pv. *musacearum*

3.3.1. Study site

Field experiment was conducted in 'Yefereze' site which is located in Cheha district of Gurage Zone (Fig 3.7). The experimental site is one of the research sites of Wolkete University, located about 190 km south of Addis Ababa. The experimental site is located at the elevation of 2046 m.a.s.l which geographically lies between 08°08'55.2"N and 037°55'09.1"E. The main rainy season which accounts for 70-90% of the total annual rainfall occurs from June to September (Fig 3.8).

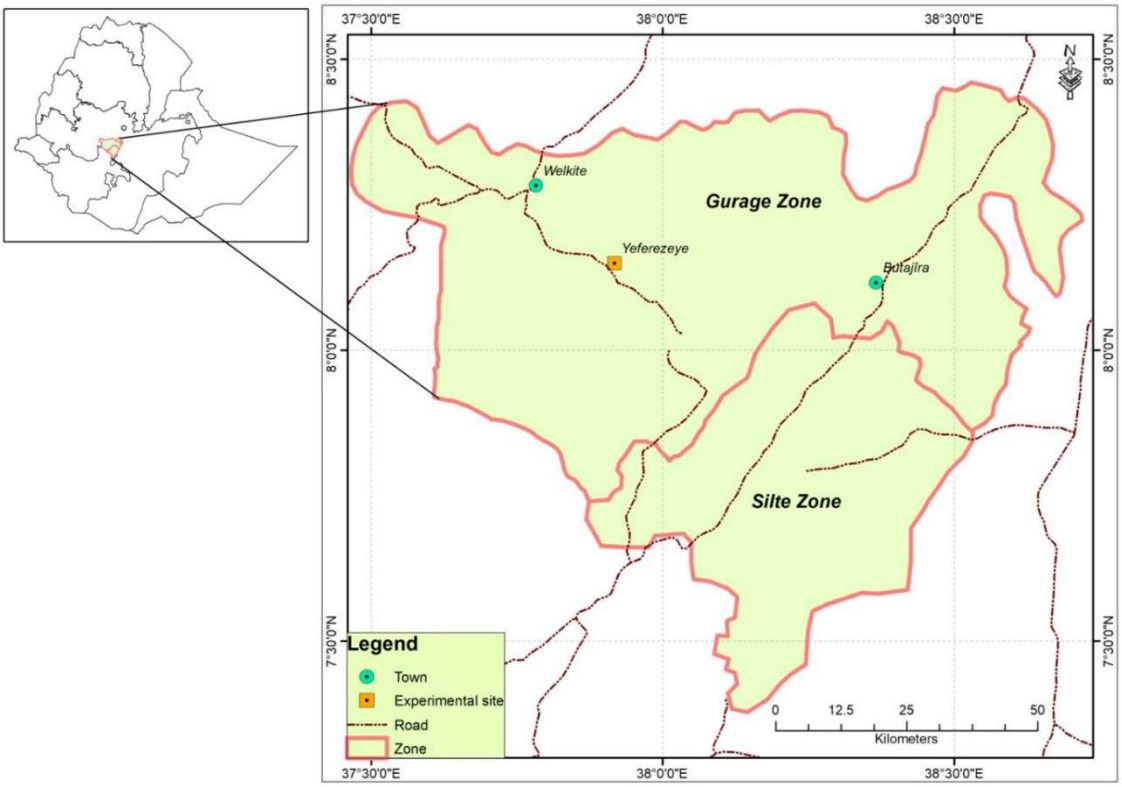

Figure 3. 7. Map showing the location of study sites in Gurage zone of Ethiopia.

The slope steepness of Yefereze sites are characterized by nearly level (Teshome Yitbarek *et al*., 2018). Based on the Ethiopian agroecological classification Yefereze site is located in the Woyena Dega zone (Hurni, 1998). According to Teshome Yitbarek *et al.* (2018), soil type of research site is classified as Vertic Alisols (Hyperdysric), which is correlated with Ultisols (Typic Haplustults) in USDA classification. The clay proportion was highest (66%) as a result the textural class of the soils of the research site is clay (Table 3.1). The soil is acidic (pH value of 4.9). The organic carbon content of the soil was 2.6% which is equivalent to 4.5% organic matter.

Table 3. 1. Selected soil properties of the experimental site (0-28 cm depth). Source: Teshome Yitbarek et al. (2018)

Soil properties	Values
Soil particle distribution (%)	
Sand	8
Silt	26
Clay	66
pH	4.9
Organic carbon (%)	2.6
Total Nitrogen (%)	0.26
Available phosphorus (mg kg^{-1})	1.27
Exchangeable Potassium (cmol kg^{-1})	0.84

Two main distinct seasons, dry and wet seasons are recognized in the area. The dry season starts from November to February, while the wet season covers the remaining part of the year, when most of the rainfall takes place. Rain usually starts in March, but the effective rainy season is from June to September with the peak in July, receiving a monthly mean of 333 mm. Three years' data from EMSA showed that the mean temperature ranged from 19^0C in August to 22^0C in April (Fig. 3.8). In addition, rainfall and temperature data of the experimental site were obtained from the Ethiopian Meteorological Service Agency (EMSA). The mean monthly rainfall and mean daily temperature between 2013 and 2015 are presented in Fig 3.8. As shown in the figure, the significant proportion of the rainfall was concentrated in June, July and August. Similarly, the lowest average temperature was observed in July and August.

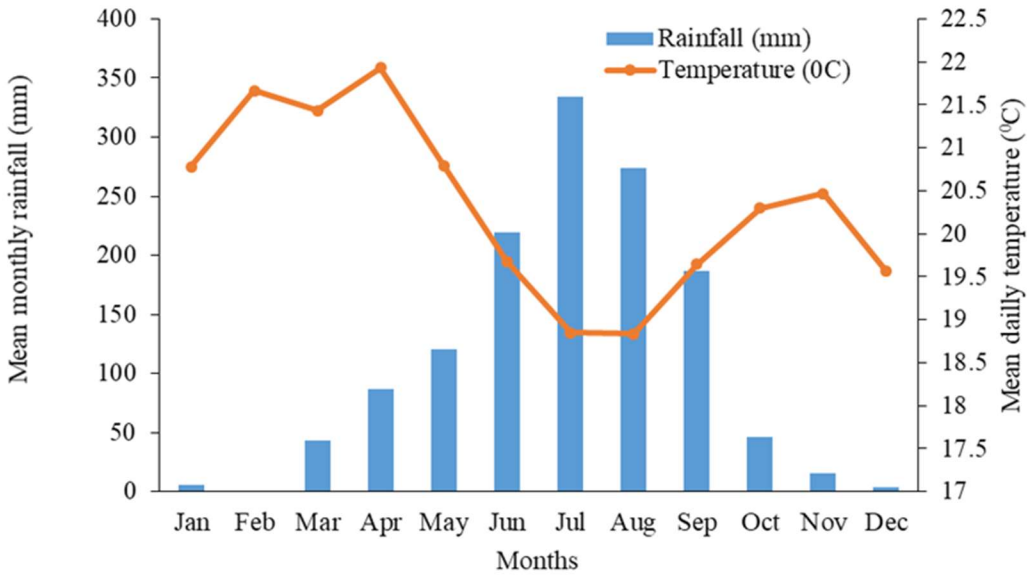

Figure 3. 8. Mean monthly total rainfall (mm) and mean daily temperature (^{0}C) of the experimental site.

3.3.2. Selection of enset clones

The enset clones for this study were selected based on research reports about their sensitivity to Xcm, farmers' knowledge and farmers' preference for their food quality and yield. Two types of enset clones which are tolerant clone (Yesherakinkye) and susceptible clone (Ameratye) variety (Anita *et al.* 1996) were selected from enset growing area in Gurage Zone. From each clone type, one-year-old suckers of equal size were selected to minimize any variation in height that might be created during establishment. Enset clones were taken from a single mother plant to minimize genetic difference between them.

3.3.3. Experimental design and treatments

Field experiment was done for two independent experiments: i) influence of inorganic fertilizer (NPK) on *Xanthomonas campestris* pv. *musacearum*, and ii) influence of Brassica green manure on *Xanthomonas campestris* pv. *musacearum*.

3.3.3.1. Inorganic fertilizer treatments

Experimental field plots were prepared using tractors and manual digging. The experimental land was plowed two times using tractors and then refined by manual digging so as to make suitable for planting. In order to prevent interference of the experiment by animals such as porcupine, deep trench was dug surrounding the experimental plots.

Inorganic fertilizer experiment had 22 combined treatments and the experiment was conducted on an area of 1904.5 m^2. Experimental land was then divided into three blocks (Appendix 5). Each block was divided into 22 plots. Plots were 3m by 4.75 m with 2 m spacing between adjacent plots and between blocks for each experiment. In each plot, six holes with 25 cm deep and 25 cm wide with 1.5 m spacing were prepared for enset sucker plantation. Each hole was 0.5 m far from the edge. Like green manure experiment, inorganic fertilizer treatments were arranged in randomized complete block design (RCBD) with three replications. Treatments in each block were assigned using lottery methods.

Inorganic fertilizers applied for this experiment include Nitrogen (N), Phosphors (P) and Potassium (K) to understand their effect on the control of bacterial wilt of enset. After three weeks of planting enset clones (Yesherakinkye representing tolerant clone and Ameratye representing susceptible clone), different rates of inorganic fertilizer application were applied. Inorganic fertilizers applied were $N_{1/2}P_0K_0$ (50% recommended N); $N_0P_{1/2}K_0$ (50% recommended P); $N_0P_0K_{1/2}$ (50% recommended K); $N_1P_0K_0$ (Recommended N); $N_0P_1K_0$ (Recommended P); $N_0P_0K_1$ (Recommended K); NPK (Recommended NPK); $N_{1/2}P_{1/2}K_{1/2}$ (50% recommended NPK) and $N_{3/2}P_{3/2}K_{3/2}$ (50% higher than recommended NPK). In total experiments contain twenty-two treatments including positive and negative control. According to Abay Ayalew and Mikias Yeshitila (2011) and Ganeshamurthy (2011) the recommended rates for enset were Nitrogen 138 kg/ha; Phosphorus 46 kg/ha and Potassium 200 Kg/ha per year (Table 3.2). Each fertilizer was applied in different forms, nitrogen in the form of urea, phosphors in the form of triple super phosphate and potassium in the form of murate of potash. For urea and potash application split type of application was used. Based on the rates, application of fertilizers amount per plans was calculated. After weighting the amount of fertilizers for each plant field application took place. In addition, the experiment contained two types of control. Positive control with *Xanthomonas campestris pv. musacearum* and negative control without *Xanthomonas campestris pv. musacearum.* Combining fertilizer rates and two enset clones, a total of twenty-two treatments were used in this experiment (Appendix 5).

Table 3. 2. Description of inorganic fertilizer treatments.

Fertilizer treatment	Description
C_1	Positive control (No fertilizer)
C_2	Negative control (No fertilizer)
$N_{1/2}P_0K_0$	50% recommended N (69 kg/ha)
$N_0P_{1/2}K_0$	50% recommended P (46kg/ha)
$N_0P_0K_{1/2}$	50% recommended K (100 kg/ha)
N	Recommended N (138 kg/ha)
P	Recommended p (46 kg/ha)
K	Recommended K (200 kg/ha)
NPK	Recommended NPK (138 kg N + 46kg P+200kg K)
$N_{1/2}P_{1/2}K_{1/2}$	50% recommended NPK (69 kg N+ 23 Kg P + 100 Kg K)/ha
$N_{3/2}P_{3/2}K_{3/2}$	50% higher than recommended NPK (207 Kg N + 69kg P + 300kg K)/ha

After one and half years following the application of inorganic fertilizer, aliquot of 10 ml of the bacterial suspension were inoculated using a hypodermic sterile syringe to the second innermost leaf petiole of enset plants and the negative control was inoculated with the same amount of distilled water using a hypodermic sterile syringe (Dereje Ashagri, 1985).

3.3.3.2. *Brassica* species as green manure and *Brassica carinata* seed extract residue treatments

Green manure experiment had 10 treatments (Table 3.3) and the experiment was conducted on an area of 851.5 m^2. Experimental land was divided into three blocks. Again each block was divided in to 10 plots. Plots were 3 m by 4.75 m with 2 m spacing between adjacent plots and between blocks for each experiment. In each plot, six holes with 25 cm deep and 25 cm width with 1.5 m spacing were prepared for enset sucker plantation. Each hole was 0.5 m far from the edge. The treatments were arranged in randomized complete block

design (RCBD) with three replications. Treatments in each block were assigned using lottery methods (Appendix 6).

Table 3. 3. Description of treatments used for green manure field experiment.

Treatments	Description
G_1V_1	Cabbage (*Brassica oleracea var capitata*) leaf extract + *Yesherakinkye* variety
G_2V_1	Tekur gomen (*Brassica oleracea var acepala*) leaf extract + *Yesherakinkye* variety
G_3V_1	Ethiopian Mustard (*B. carinata*) seed extract residue + *Yesherakinkye* variety
C_1V_1	Positive control + *Yesherakinkye* variety
C_2V_1	Negative control + *Yesherakinkye* variety
G_1V_2	Cabbage (*Brassica oleracea var capitata*) leaf extract + *Ameratye*
G_2V_2	Tekur gomen (*Brassica oleracea var acepala*) leaf extract + *Ameratye* variety
G_3V_2	Ethiopian Mustard (*B. carinata* seed extract residue) + *Ameratye* variety
C_1V_2	Positive control + *Ameratye* variety
C_2V_2	Negative control + *Ameratye* variety

Green manure experiment included two types of *Brassica* species and *Brassica carinata* seed extract residue (by products of Ethiopian Spice Extraction Factory). Five weeks old *Brassica* species namely, *Brassica oleracea var capitata* (Cabbage*)* and *Brassica oleracea var acepala* (Tekur gomen) which showed better antibacterial efficiency during laboratory test were selected for field application. Accordingly, *Brassica carinata* seed extract residue with minimum inhibitory concentration was taken for field applications. In addition, the experiment contained two types of control, positive control with *Xanthomonas campestris* pv. *musacearum* and negative control without *Xanthomonas campestris* pv. *musacearum*

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For experimental soil infestation, inoculum was prepared using direct colony suspension method and the bacterial suspension was standardized to the density of a 0.5 McFarland

standard. The standardization was done by measuring the absorbance of the suspension with a spectrophotometer. The absorbance was adjusted to 0.132 which is approximately equal to 1.5 x 10^8 CFU/mL. The prepared *Xanthomonas campestris* bacterial suspension was used for experimental plot infestation.

After six-month growth of the selected enset clones namely Yesherakinkye (tolerant) and Ameratye (susceptible), the soil was infested with *Xanthomonas campestris* by applying prepared 1litter of inoculums in each hole by mixing it with four kilogram of soil along with plant debris (Gizachew Weldemicael *et al.*, 2008). The control had two forms: positive control (with *Xanthomonas*) and negative control (without *Xanthomonas*). Manual weed control and watering were carried out on regular basis throughout the experiment.

After one week of soil infestation, 225 gm of five-weeks old *Brassica* species namely, *Brassica oleracea var capitata* (Cabbage*)* and *Brassica oleracea var acepala* (Tekur gomen) *incorporated* in the respective plants (Assefa Sintayehu *et al*., 2014). For treatments applying *Brassica carinata* seed extract residue, 1 liter of extracts with minimum inhibitory concentration were applied (Brown and Morra, 2005; Merkuz Abera *et al*., 2011). All plots received treatments except for the negative control which was without green manure treatment and *Xanthomonas campestris pv. musacearum* infestation. Then soil was mulched for two weeks and watered regularly to keep available soil moisture.

3.3.3.3. Data collection

i) Growth data measurements

Four enset plants per treatment/plot was randomly picked and tagged at the beginning of the experiment for monitoring the time course of growth parameters. Growth parameters were monitored until the end of the experiment. Plant height, pseudostem girth, the number of green leaves, and leaf area were recorded (Fig.3.9). Data measurements were taken three times. The first measurement was taken before inoculation with Xanthomonas campestris pv. musacearum (after 18 months of planting for inorganic fertilizer and six months for green manure), second measurement was taken four weeks after inoculation and the last measurements was taken eight weeks after inoculation

Plant height (m): Plant height of four randomly selected plants per treatment was measured from the ground to the tip of the lamina of a recently expanded leaf.

Pseudostem girth (m): was measured at the widest point (10 cm above the ground) along the lower length of the pseudostem.

The green leaf number: The leaf number of four randomly selected plants per treatment was counted and recorded.

Total leaf area: Total leaf area of Enset was determined according to Kumar *et al.* (2002) used for banana by counting the total number of leaves (N), measuring the length (L) and breadth (B) of the third leaf from the top and calculating the total leaf area (TLA) as:

$$TLA = L \times B \times 0.83 \times N \times 0.662 \ldots\ldots \text{(Eq. 3.9)}$$

Where 0.83 is a correction factor for leaf shape differences and 0.662 is a correction factor for leaf size differences among Enset plant leaves.

Leaf area index: Leaf area index (LAI) is the total area of green leaf per unit area of land and this was measured as follows:

$$\text{LAI} = \frac{TLA}{Ag} \ldots\ldots \text{(Eq. 3.10)}$$

Where LAI is leaf area index, TLA is total leaf area and A_g is the ground area occupied by each plant which was computed from the spacing between individual plants.

Plant height was measured as the pseudostem height from ground level to the tip of the second youngest leaf while plant girth was the circumference of the pseudostem at 15cm above ground level (Fig. 3.9). The estimated total leaf area was calculated based on information of biometric measurements of the third leaf taken from each plant using the formula TLA = L × B × 0.8 × N × 0.662 (Kumar *et al*., 2002) whereby: TLA is the total leaf area of the plant, L and B are the length and breadth of third leaf, N is the number of leaves on the plant, 0.80 is a correction factor for leaf shape differences and 0.662 is a correction factor for leaf size differences among enset plant leaves.

Figure 3. 9. Growth parameters taken before Xanthomonas inoculation

The relative growth promotion efficacy (GPE) of biological control agents was assessed according to Algam *et al*. (2010) as:

$$\text{GPE (\%)} = \frac{(\text{plant parameter of treated } - \text{plant parameter of control})}{\text{Plant parameter of control}} X100 \ldots Eq.3.11$$

ii) Physiological data measurement

Leaf gas exchange: Leaf gas exchange measurement was made on fully expanded 2^{nd} and 3^{rd} order pair of leaves of enset plants using LCPro^{+} portable photosynthesis system (ADC, Bioscientific, Ltd. UK). This measurement includes net assimilation rate (A), stomatal conductance (gs), intercellular CO_2 concentration (Ci) and transpiration rate (E). Measurements were taken from 3-4 plants between 9:00 and 11:30 AM. Five recordings were taken from each plant at different positions when the CO_2 uptake was steady.

Water use efficiency: Water use efficiency (WUE) defined as mmol of CO_2 uptake per mole of water transpired was calculated by dividing instantaneous values of A by E.

$$\text{WUE } = \frac{A}{E} \ldots (Eq.3.12)$$

Relative water content: For midday leaf relative water content (RWC) determination, four leaf pieces (each 9cm^2) were punched out from the lamina of individual leaves using a modified leaf cutter. These pieces were immediately weighed to obtain fresh weight (FW) and the pieces will be then floated in distilled water for 24 hours and re-weighed to determine turgid weight (TW). Finally, dry weight (DW) of leaf pieces was determined after drying samples to constant weight at 80^0C for 24 hours. Relative water content of the leaf was calculated as follows based on Turner (1986) and Yamasak and Dillenbur (1999).

$$\text{RWC} = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100 \ldots\ldots \text{(Eq. 3.13)}$$

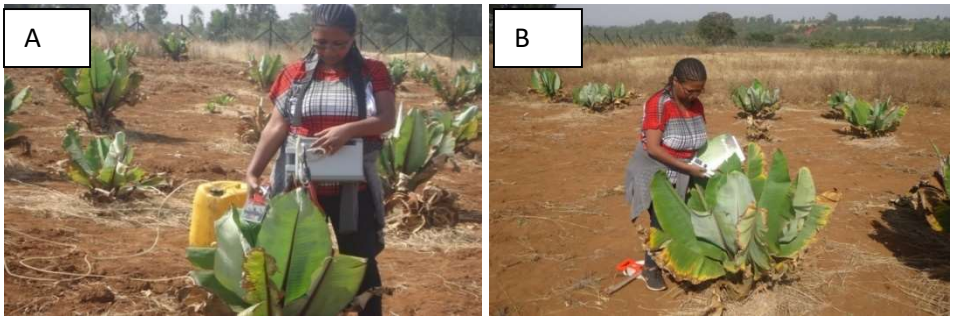

Figure 3. 10. Measurements of gas exchange parameters using LCPro+ (A) and Measurements of Chlorophyll content using SPAD-502 on the 3rd leaf (B)

Chlorophyll content: Chlorophyll content was measured in leaves with Chlorophyll meter, SPAD-502 (Minolta Co. Ltd, Japan) (Fig. 3.10). The SPAD values were taken at fully expanded 2^{nd} and 3^{rd} pair of leaves. These values provide an indication of the relative amount of total chlorophyll present in plant leaves. Higher SPAD value represent higher total chlorophyll content. The average of the nine measurements represented for individual plants.

iii) Disease incidence and severity measurements

The status of enset bacterial wilt of experimental field sites was assessed and recorded through direct field observations. The result showed that symptoms of enset bacterial wilt were observed from 20 to 30 days of after inoculation. Data on disease incidence and severity were taken within a week for two months. For the assessments of effect of green manure and inorganic fertilizers disease incidence, severity and area under disease progress curve (AUDPC) measurements were taken.

Disease incidence

Disease incidence is the percentage of diseased plants or parts of the sample or population of plants. It can be the proportion or percentage of diseased leaves in a plant, diseased stalks or a tiller or diseased seedlings in a field. Disease incidence is calculated according to Mekuria Wolde *et al*. (2016b) using the formula as follows:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plant assessed}} \times 100 \ldots\ldots \text{(Eq. 3.14)}$$

The disease incidence record was started after two weeks of Xanthomonas infestation and after that it was assessed at weekly interval.

Disease severity

Disease severity is the percentage of relevant host tissues or organ covered by symptom or lesion or damaged by the disease. Severity results from the number and size of the lesions. Disease severity was carried out by scoring diseased plants using a 0-5 scale as described by Winstead and Kelman (1952). Xanthomonas wilt severity was rated visually using the following scale: 0 = no wilt symptoms; 1 = 1 leaf wilted; 2 = 2 – 3 leaves wilted; 3 = 4 leaves wilted; 4 = all leaves wilted; and 5 = plant dead (Winstead and Kelman, 1952 and Ssekiwoko *et al*., 2006). Complete or partially wilted plants were tagged to avoid double counting in subsequent assessments and also to avoid the possibility of missing out those plants that died early during the experiment. Then disease severity (DS) was calculated using eq. 3.14.

$$\text{DS (\%)} = \frac{[(0 \times \text{a}) + (1 \times \text{b}) + (2 \times \text{c}) + (3 \times \text{d}) + (4 \times \text{e}) + (5 \times \text{f})]}{(n \times 5)} \times 100 \ldots Eq.\ 3.15)$$

Where: letters a-f represents how many times each scale occurs and n represents the maximum number of disease severity assessed plants.

Area under disease progress curve (AUDPC)

In addition, percentage of wilted plants at each assessment period was used to calculate AUDPC using the following formula (Shaner and Finney, 1977):

$$AUDPC = \sum_{i=1}^{n} \left[\left(\frac{Di_{i+1}+Di_i}{2}\right) \times (t_{i+1} - t_i)\right] \ldots\ldots \text{(Eq. 3.16)}$$

Where Di_i = percentage of wilted plants at the i^{th} observation, t_i = time (days) at the i^{th} observation, n = total number of observations.

Efficacy

It is percentage disease reduction. This was determined according to Algam *et al*. (2010) and Xue *et al*. (2015) as:

$$S\ (\%) = \frac{(A-B)}{A} 100 \ldots.. \text{(Eq.3.17)}$$

Where; S = Efficacy, A = Disease incidence of the control, B = Disease incidence of treated group

3.4 Data analysis

The data collected in the laboratory and field experiments were subjected to different analysis. Data on the assessments of incidence and prevalence of enset bacterial wilt (section 4.1) was analyzed using descriptive statistics such as mean and percentages. In this section, disease prevalence (%) and disease incidence (%) were the most important response variables. The results of the analysis were plotted in bar/line graphs.

In antibacterial activity study (section 4.2), the effect of Brassica plant leaf and seed extracts on response variables such as inhibition zone (mm), extract yield (%) and total phenolic content were analyzed using analysis of variance (ANOVA). Similarly, in the inorganic fertilizer study (section 4.3), ANOVA was employed to analyses the response variables. The response variables in this study include plant height (m), pseudostem (m), green leaf number, leaf length (mm), leaf width (mm), leaf area (m^2) and leaf area index, disease incidence (%), disease severity (%) and area under disease progress curve (AUDPC).

In green manure study (section 4.4), the major response variable were plant height (m), pseudostem (m), green leaf number, leaf length (mm), leaf width (mm), leaf area (m^2) and leaf area index, disease incidence (%), disease severity (%), AUDPC, assimilation rate (µmol CO_2 m^{-2} s^{-1}), relative water content (%), chlorophyll content, transpiration (mmol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (µmol CO_2 mol^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}) and water use efficiency (µmol of CO_2/ mmol of H_2O). All data collected were

subjected to analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences, version 23). ANOVA was done after carrying out of a test of homogeneity and the normal distribution for each measured parameter. Disease incidence and severity data were transformed using Arcsine (Angular) transformation as data was collected in percentage. Then, significant difference of severity and incidence among treatment means was tested using Tukey's Honestly Significant Difference (HSD) at 5 % probability.

CHAPTER 4

4. Results

4.1. Assessments of prevalence and incidence of enset bacterial wilt in Gurage and Silte zones.

4.1.1. Disease prevalence and incidence of enset bacterial wilt

Disease prevalence and incidence of enset bacterial wilt (EBW) in three altitudinal categories including altitudes of 1896-1928 m (category 1), 2445-2540 m (category 2) and 2950-2968 m (category 3) are presented in Fig 4.1. Altitudinal based survey result showed that, in the years 2014 and 2015, no disease prevalence and incidence was observed in Welkete town district which represented lower altitude category (Fig. 4.1), relatively. Mid altitude represented by Cheha district was an area having relatively the highest disease prevalence in 2014 (55%) and 2015 (43.8%). Likewise, the highest disease incidence (12.2%) was observed in 2014 and (8.2%) 2015 in Cheha district. The highest altitude area represented by Mirab Azernet Berbere district had disease prevalence of 35% (2014) and 32.5% (2015). In Mirab Azernet Berbere district, disease incidence of 10.21% and 8.14% was observed in 2014 and 2015, respectively. This also implies that the disease incidence in 2015 was reduced by 4% compared with that of 2014 in Cheha district. However, in the highest altitude area represented by Mirab Azernet Berbere district, the incidence in 2015 was reduced by only 2.5% compared to 2014. The computed disease incidence in the surveyed farms ranges from 0-12.21% and 0-8.2% with mean of 7.5% and 5.4% in the year

2014 and 2015, respectively. Generally, there was a reduction in the severity of the disease from first year to the second year.

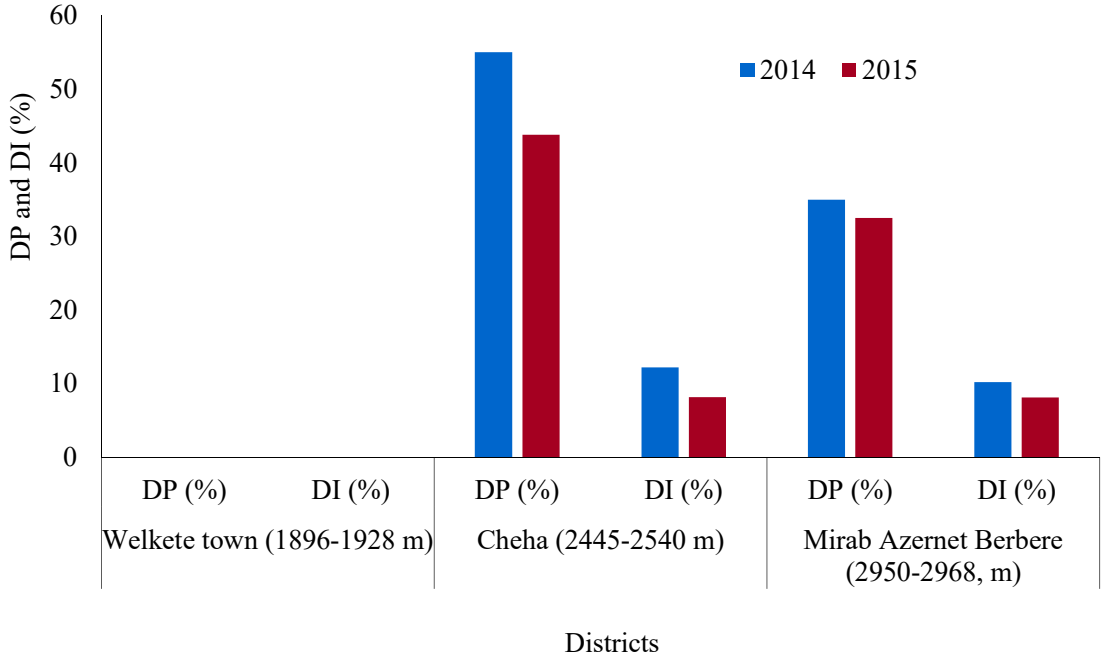

Figure 4. 1. Survey on enset bacteria wilt in three districts during 2014 and 2015. DP: Disease Prevalence, DI= and Disease Incidence.

The result of seasonal based survey showed that the cumulative disease prevalence of EBW reached at maximum in July during 2014 and 2015 (Fig. 4.2). However, the result indicated that disease incidence of EBW reached at maximum in October during 2014 and 2015 (Fig. 4.2). Over all, disease prevalence of EBW was 35% and 31.7% in 2014 and 2015, respectively. The maximum disease incidence was 13% and 9.3% in 2014 and 2015, respectively. The maximum change in disease prevalence (10%) was recorded between January and April in 2014. In 2015, disease prevalence increased by 8.3% between January and April and 8.4% between April and July, indicating that there were seasonal variations in EBW prevalence and incidence in the study areas.

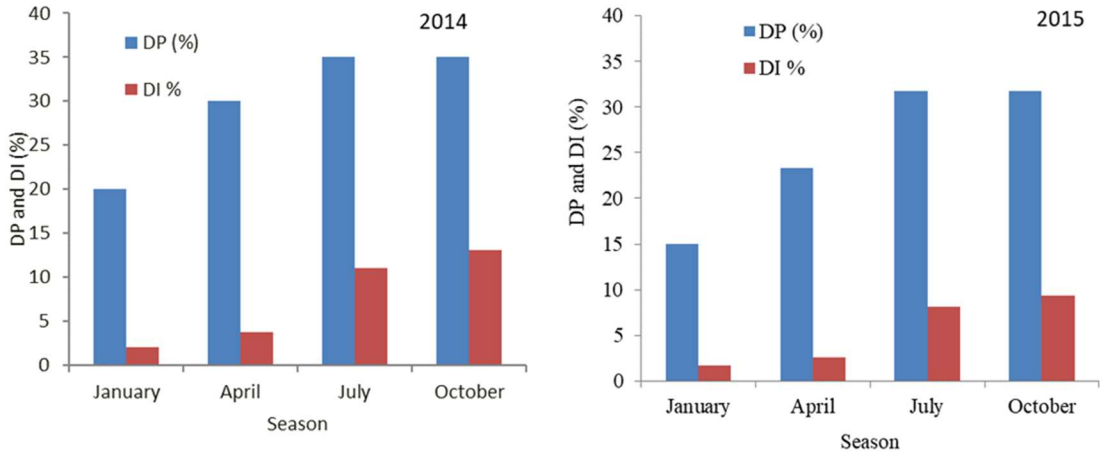

Figure 4. 2. Survey on enset bacteria wilt in 2014 and 2015. DP: Disease Prevalence, DI= and Disease Incidence.

Out of 60 enset cultivated fields assessed, 40 (66.7%) were affected by different levels of disease prevalence and incidence. The disease prevalence of EBW was recorded highest in Dacuna kebele with a mean prevalence of 65% and the lowest incidence was recorded in Gubre and Guchache kebele with 0% (Table 4.1). The incidence of EBW was recorded highest in Dacuna kebele with a mean incidence of 19.15% and the lowest incidence was recorded in Gubre and Guchache kebele with 0%. However, disease prevalence and incidence varied among kebeles and between years (Table 4.1). Accordingly, disease prevalence varied from no prevalence (0%) in Gubre and Guchache kebeles to 70% prevalence in Dakuna Kebele. Similarly, the disease incidence also varies from no incidence (0%) in Gubre and Guchache kebeles to 23% in Dakuna. In 2014, the highest EBW prevalence were 70% and 60% which were observed in Dacuna and Yegungo kebele (Cheha districts), respectively. Similarly, in 2015, the highest prevalence of EBW were observed in Dacuna (60%) and Yegungo (50%) kebeles. Moreover, in some areas enset

fields were completely destroyed due to EBW and farmers were forced to replace with other crops (Figure 4.3)

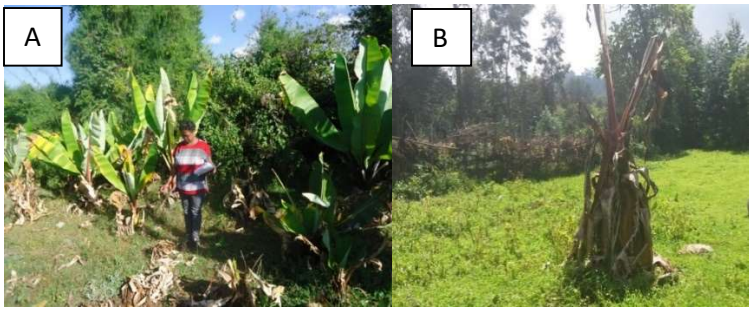

Figure 4. 3. During the assessment of EBW disease distribution (A) Enset field replaced by other crops due to EBW disease (B).

Table 4.1. Disease prevalence and incidence of enset bacterial wilt in different kebeles with in three districts of Gurage and Silte zones

Districts	Localities(Kebeles)	Number of fields			Prevalence	Incidence
		OB*	DF*	DS*	(%)	(%)
2014						
Welkete town	Gubre	10	10	0	0	0
	Guchache	10	10	0	0	0
Cheha	Dacuna	10	4	7	70	23.2
	Yegungo	10	4	6	60	18.8
Mirab Azernet Berbere	Dilla	10	6	4	40	21.2
	Tonet	10	6	4	40	15.1
	Average				35.0	13.1
2015						
Welkete town	Gubre	10	10	0	0	0
	Guchache	10	10	0	0	0
Cheha	Dacuna	10	4	6	60	15.1
	Yegungo	10	5	5	50	13.0
Mirab Azernet Berbere	Dilla	10	6	4	40	16.1
	Tonet	10	6	4	40	11.7
	Average				31.7	9.3

*OB: observed; *Df: Disease free; * DS= Diseased

4.1.2. Farmers' views on enset clones, spreading mechanisms and controlling cultural practices of enset bacterial wilt

4.1.2.1. Farmers' views on enset clones

Farmers were interviewed to list out at least one most important tolerant enset clone in the study areas. Accordingly, farmers identified 5 tolerant enset clones (Table 4.2). Majority of the farmers (56%) reported that Yeshrakinkye is a tolerant enset clone to bacterial wilt (BW) whereas about one-fourth (23.3%) of the farmers perceived Nechewe as tolerant enset clone to BW. Again, Anikefye, Astara, and Badedate were also mentioned as tolerant enset clone by 10%, 7% and 3% of the respondents, respectively.

Farmers' views regarding susceptible enset clones to Xcm are also presented in Table 4.2. Accordingly, Yeregye and Ameratey were reported as susceptible enset clones to BW by 45% and 35% of the respondents. Ginbwe, Kenbat and Agade were also reported by 8%, 7% and 5% respondents, respectively as susceptible enset clone to EBW.

Table 4. 2. Portions of respondents (%) on tolerant and susceptible enset clone to enset bacterial wilt (n=60)

No	Enset clone	Number of respondents	Percentage (%)
	Tolerant clone		
1	Yesherakinkye	34	56.7
2	Nechewe	14	23.3
3	Anikefye	6	10.0
4	Astara	4	6.7
5	Badedate	2	3.3
	Susceptible clone		

1	Yeregye	27	45.0
2	Ameratey	21	35.0
3	Ginbwe	5	8.3
4	Kenbat	4	6.7
5	Agade	3	5.0

4.1.2.2. Farmers cultural practices that increase the spread of enset bacterial wilt

The interviewed farmers identified three cultural practices that spread enset bacterial wilt (Table 4.3). According to respondents, the most important cultural practices responsible for the spreading of bacterial wilt were use of contaminated tools (47%) and planting diseased material (28%). Grazing cattle in the infected field was also mentioned as disease spreading mechanism by 8.3% of the respondents. However, about 17% of the respondents didn't know the spreading mechanisms of enset bacterial wilt.

Table 4 3. Farmers' views on the spreading mechanisms of enset bacterial wilt (n=60)

No	Cultural practice	Number of respondents	Percentage (%)
1	Use of contaminated tools	28	46.7
2	Use of diseased planting materials	17	28.3
3	No information	10	16.7
4	Grazing cattle in the infected field	5	8.3

4.1.2.3. Farmers cultural control practice for enset bacterial wilt

Farmers identified four cultural practices to control enset bacterial wilt (Table 4.4). Data on the control options used by the farmers indicated that removal of infected plants together with burning after observation of symptom was the most commonly practiced control

option. Thus, 40% of enset farmers practiced this option, while 20% practiced removal of infected plant and 15% of the farmers practiced removal of infected plant together with burying. Some 12% of farmers separated infected enset plants from non-infected ones. About 13% of the farmers didn't practice any control measures against enset bacterial wilt.

Table 4. 4. Proportions of farmers on the cultural control practices for enset bacterial wilt (n=60).

No	Cultural practice	Number of respondents	Percentage (%)
1	Removal of infected plants together with burning	24	40
2	Removal of infected plant	12	20
3	Removal of infected plant together with burying infected plants	9	15
4	Use of tolerant enset clone	7	11.7
5	No action	8	13.3

4.2. Evaluation of the antibacterial activity of *Brassica* species against *Xanthomonas campestris* pv. *Musacearum*

4.2.1. Biochemical and physiological characteristics of *Xanthomonas campestris* pv. *musacearum* isolate.

Biochemical test done on bacterial isolates were KOH solubility test and Catalase test. In addition, salt tolerance test was also conducted.

KOH solubility test: Result showed that, the isolated bacterium dissolve in 3% KOH solution. When the mixed bacterial culture in the solution was lifted it sticks to the loop

causing a thin strand of slime which is similar to characteristics of Xanthomonas campestris pv. musacearum (Fig 4.4).

Figure 4. 4. KOH solubility test

Catalase test: Result showed that, the addition of Hydrogen peroxide to culture release bubbles, which is similar to characteristics of *Xanthomonas campestris* pv. *musacearum* (Fig 4.5).

Figure 4. 5. Catalase test

Physiological characteristics: For NaCl tolerance test, Bacterial isolate tolerated 1% and 2% NaCl but not tolerant to 3-5% NaCl.

4.2.2. Extract yield of different Brassica plants

The result of the present study showed that there is wide variation in extract yield of Brassica plant species. The highest extract yield was obtained from the leaves of *Brassica oleracea (NG)* (48.6%) followed by *Brassica carinata* (46.2 %), while the lowest was obtained from *Raphanus sativus* (Raddish) (18.2%) and *Brassica nigra* (Black mustard) (20.9%) (Fig 4.6).

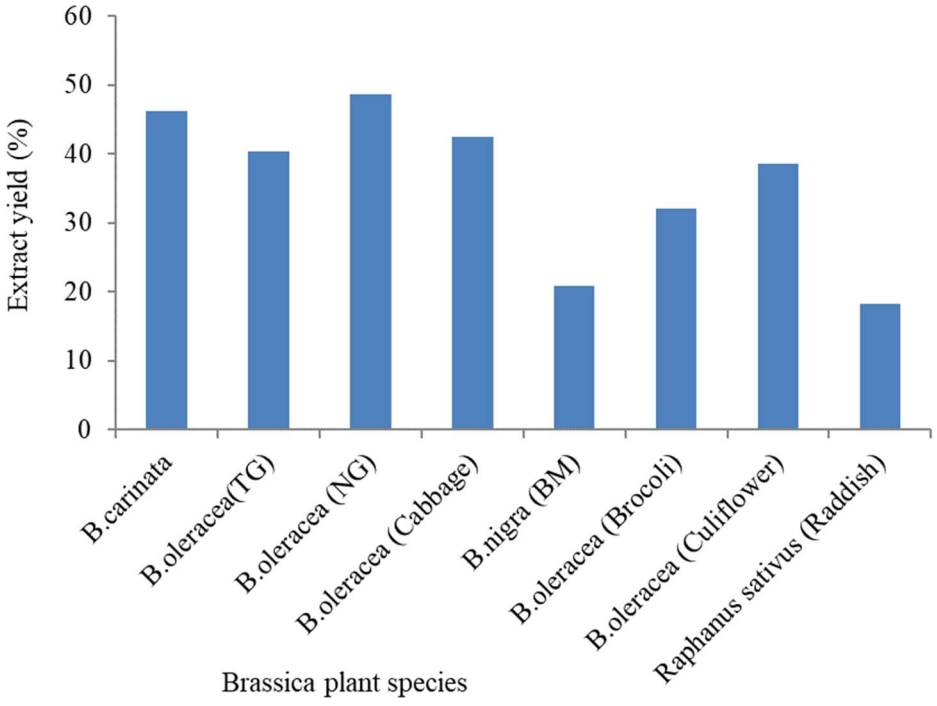

Figure 4. 6. Extract yield percentage of some Brassica plants

4.2.3. Antibacterial tests of different *Brassica* species leaf extracts and *Brassica carinata* seed extract residue

4.2.3.1. Antibacterial tests of different Brassica plants

The antibacterial activities of leaf extracts of eight Brassicaceae species including *B. carinata A. Braun* (Ethiopia mustard), *Brassica oleracea var acepala* (Tekur gomen), *Brassica oleracea var acepala* (Nech gomen), *Brassica oleracea var capitata* (Cabbage), *Brassica oleracea var botrytis* (Cauliflower), *Brassica oleracea var italic* (Broccoli), *Brassica nigra (L.)* (Black mustard) and *Raphanus sativious L.* (Radish) were tested under *in vitro* against Xcm. The test was carried out at 400, 200, 100, 50 and 25 mg/mL concentrations. The extracts showed a wide variation in mean inhibition zone diameter of the test Brassicaceae species (Fig 4.7). The extracts of *Brassica oleracea var capitata* (Cabbage) and *Brassica oleracea var acepala* (Tekur gomen) created the widest bacterial growth inhibition zone at (400 and 200 mg/mL) compared to other concentration. The inhibition zone decreased with decreasing concentrations. This study reveals that, the leaf extract of *Brassica oleracea var acepala* (Tekur gomen) showed significantly widest bacterial growth inhibition zone at all test concentrations. Extract of this species produced a significantly wider inhibition zone at all test concentrations as compared to other species ($P < 0.05$) except with *Brassica oleracea var capitata* (Cabbage), *Brassica carinata A. Braun* (Ethiopia mustard) and Cauli flower (*Brassica oleracea var botrytis)*. In addition, the least inhibition zone is produced by leaf extracts of *Brassica oleracea var italic* (Broccoli) and *Brassica oleracea var acepala* (Nech gomen) at all tested concentrations.

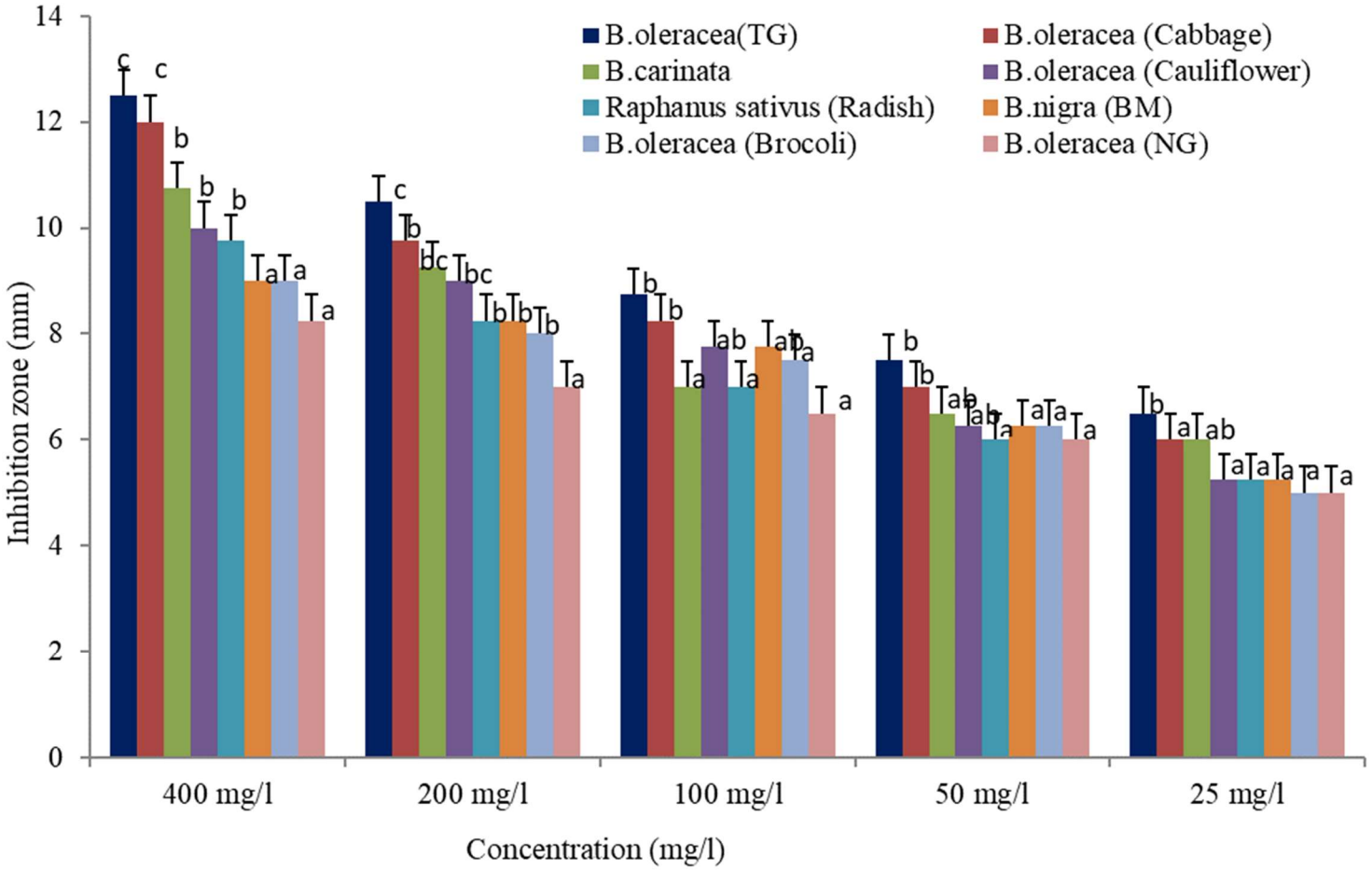

Figure 4. 7. Inhibition zone (mm) of different Brassica plant at different concentration. Bars at each test concentration followed by different letters are significantly different at $p < 0.05$.

4.2.3.2. Antibacterial tests of *Brassica carinata* seed extract residue

Brassica carinata seed extract residue was tested under *in vitro* against Xcm and showed inhibition at different concentration tested. The inhibition zone increased with increasing concentration of *Brassica carinata* seed extract residue (Fig. 4.8). The result revealed that *Brassica carinata* seed extract residue has significantly difference among different concentrations tested ($P < 0.05$).

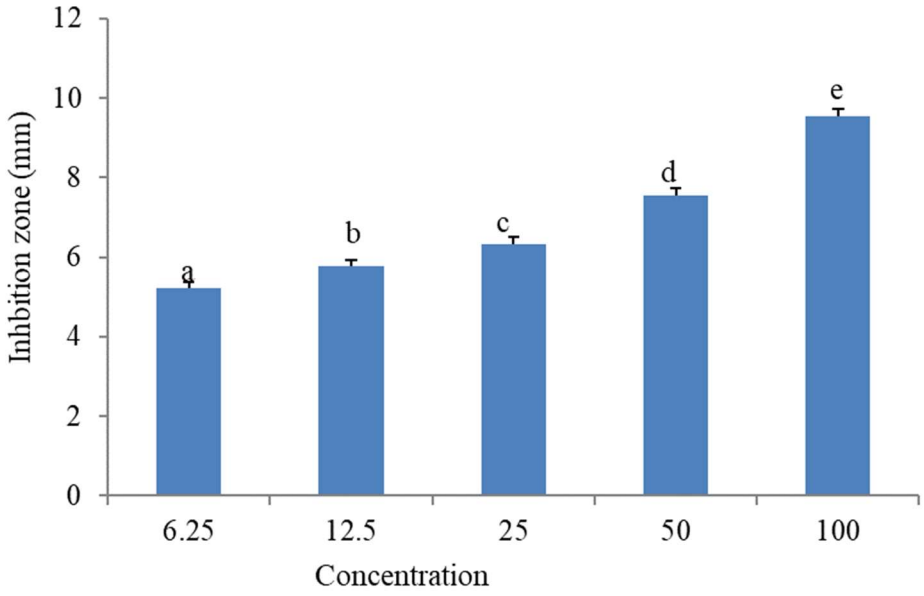

Figure 4. 8. Inhibition zone of enset bacterial wilt in different concentration of Brassica carinata seed residue. Bars at each test concentration followed by different letters are significantly different at $p< 0.05$.

4.2.4. Minimum inhibitory and bactericidal concentration of *Brassica* species leaf extracts and *Brassica carinata* seed extract residue

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values indicated that the effect of extracts on the growth of Xcm was variable among species. The MIC and MBC values of extracts ranged from 25 mg/ml to100 mg/ml (Fig 4.9). The highest MIC and MBC values (100 mg/ml) were recorded by extracts of *Brassica oleracea var acepala* (Nech gomen), *Brassica nigra L.* (Black mustard) and *Raphanus sativious L. (*Radish). The lowest MIC and MBC values were recorded by extracts of *Brassica carinata seed residue* (25 mg/ml) followed by *B. carinata A. Braun* (Ethiopia mustard), *Brassica oleracea var acepala* (Tekur gomen), *Brassica oleracea var capitata* (Cabbage), *Brassica oleracea var italic* (Broccoli) and *Brassica oleracea var botrytis* (Cauliflower) with (50 mg/ml). These extracts with lowest MIC and MBC values were potent that inhibited the growth of the test bacterium even at low concentrations as compared to others.

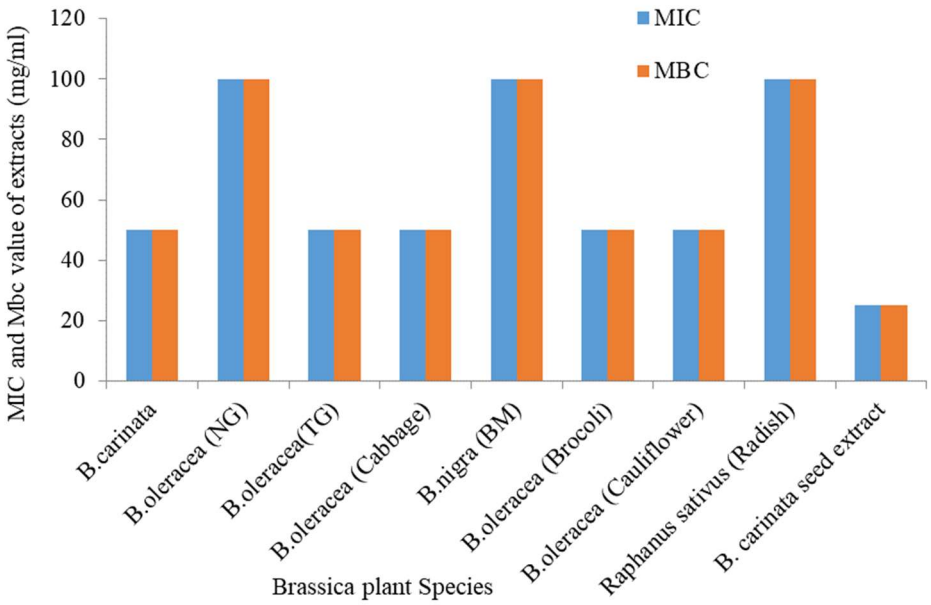

Figure 4. 9. Minimum inhibitory and bactericidal concentrations of extracts of different Brassica plant. MIC= Minimum inhibitory concentration, MBC=Minimum bactericidal concentration.

4.2.5. Phytochemical analysis of *Brassica* species leaf extracts and *Brassica carinata* seed extract residue

4.2.5.1. Qualitative screening

Qualitative phytochemical screening results revealed that the chemical constituents of extracts vary between *Brassica* species (Table 4.5). Brassica plant species contain abundant amount of the tested secondary metabolites except *Brassica carinata and B. nigra* (Black mustard). However, saponins were absent in the extract of *Brassica oleracea (*Cabbage*)* and *Brassica carinata* seed extract residue.

Table 4. 5. Qualitative phytochemical analysis of different *Brassica* plants

S.No	Plant species	Phenol	Alkaloid	Flavonoids	Trepenoids	Saponin	Tanins
1	*Brassica carinata*	++	+	++	+	+++	++
2	*Brassica oleracea* (Cabbage)	+++	+++	++	+++	_	++
3	*Brassica oleracea* (NG)	++	+	+++	+++	+++	++
4	*Brassica oleracea* (TG)	+++	+++	+++	+	+++	+++
5	*Brassica oleracea* (Brocoli)	+++	+++	+++	+++	+++	+++
6	*Brassica oleracea* (Cauliflower)	+++	+++	+++	++	+++	+++
7	*Brassica nigra* (BM)	++	++	++	++	++	++
8	*Raphanus sativus* (Radish)	+++	+++	+++	++	+++	+++
9	*B.carinata s*eed extract residue	+++	+++	+++	+++	_	+++

Note that: -: absent; +: present; ++: moderate; +++: abundant, NG: Nech Gomen, TG: Tikur Gomen, BM: Black Mustard.

4.2.5.2. Quantitative determination of total phenol

The result of the quantitative chemical analysis of extracts showed that total phenolic content was significantly different between the studied *Brassica* species (Fig 4.10). Total phenolic contents recorded by the extract of *Brassica carinata* seed extract residue, *Brassica oleracea* (Tekur gomen) and *Brassica oleracea* (Cabbage) were equal and had significantly highest values ($p < 0.05$) compared to other *Brassica* species tested. similarly, total phenolic contents recorded by the leaf extracts of *Brassica nigra* (Black mustard) and *Brassica oleracea* (Nech gomen) were equal and significantly the lowest ($p < 0.05$ as compared to *Brassica* species tested (Fig. 4.10). After in vitro screening tests, two types of brassica species namely *Brassica oleracea* (Tekur gomen) and *Brassica oleracea (*Cabbage*)* were selected for field green manure application.

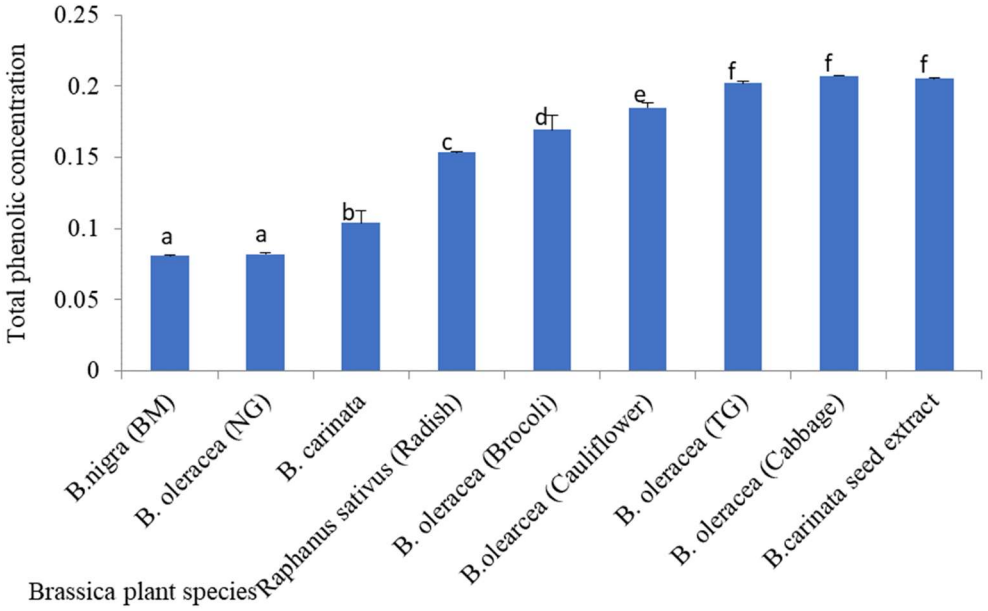

Figure 4. 10. Total phenolic content (mg of catechol equivalent of phenol/g of FW) of Brassica plant leaf extracts and Brassica carinata seed extracts. Bars at each test concentration followed by different letters are significant at $P < 0.05$.

4.3. Effects of Inorganic fertilizer (NPK) against *Xanthomonas campestris pv. musacearum*

4.3.1. Effect of inorganic fertilizers on growth parameters of enset clones infected with *Xanthomonas campestris pv. musacearum*

The growth parameters measured include plant height (PH), pseudostem girth (PSG), green leaf number (GLN), leaf length (LL), leaf width (LW), leaf area (LA) and leaf area index

(LAI). The effects of nine levels of nitrogen (N), phosphorus (P) and potassium (K) on the performance of these parameters are presented in Table 4.6.

4.3.1.1. Effects of inorganic fertilizer on growth parameters of enset before inoculation

The effects of inorganic fertilizers on the growth parameters of two enset clones after 18 months of planting and before inoculation with *Xanthomonas campestris pv. musacearum* are presented in Table 4.6.

Plant height (PH)

The mean height of enset after 18 months of planting ranged from 0.34 m to 1.08 m (Table 4.6). The result shows that the mean heights of the two enset clones (V1 and V2) after 18 months of planting were not statistically different at $p<0.05\%$ (Table 4.6). However, application of inorganic fertilizer significantly influenced the height of both enset clones. As shown in Table 4.6, height of enset was superior with $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ for both clones. In clone V_1, height of enset increased by 126%, 140% and 177% compared with positive control due to application of $N_{1/2}P_{1/2}K_{1/2}$ (0.88 m), NPK (0.937 m), and $N_{3/2}P_{3/2}K_{3/2}$ (1.08 m), respectively. Similarly, in susceptible clone, application of $N_{1/2}P_{1/2}K_{1/2}$ (0.837 m), NPK (0.953 m), and $N_{3/2}P_{3/2}K_{3/2}$ (0.990 m) increased plant height of V_2 by 127%, 159% and 170%, respectively as compared to positive control.

Pseudostem girth (PSG)

Mean pseudostem girth (PSG) of enset varied from 0.198 m in the control treatment of V_2 to 0.743 m in $N_{3/2}P_{3/2}K_{3/2}$ of V_1(1.5 times the recommended NPK). As shown in Table 4.6, the pseudostem girth (PSG) of enset varied significantly ($p<0.05$) due to application of

inorganic fertilizer (Table 4.6). Like plant height, the highest value of PSG were observed in $N_{3/2}P_{3/2}K_{3/2}$ followed by NPK and $N_{1/2}P_{1/2}K_{1/2,}$ and for both clones. In tolerant enset clone, application of $N_{1/2}P_{1/2}K_{1/2}$ (0.607 m), NPK (0.680 m) and $N_{3/2}P_{3/2}K_{3/2}$ (0.743 m) increase PSG of enset by 161%, 192% and 219%, respectively. Similarly, in susceptible enset clone, application of $N_{1/2}P_{1/2}K_{1/2}$ (0.547 m), NPK (0.617 m) and $N_{3/2}P_{3/2}K_{3/2}$ (0.717 m) increased PSG of enset by 176%, 212% and 262%, respectively as compared to positive control.

Green leaf number (GLN)

The mean green leaf number (GLN) per plant ranged from 4.7 (susceptible clone without fertilizer, CV_2) to 10.7 (under tolerant clone with $N_{3/2}P_{3/2}K_{3/2}$). The mean GLN per plant was significantly influenced by the application of NPK fertilizers for both clones (Table 4.6). Highest GLN per plant were recorded in $N_{3/2}P_{3/2}K_{3/2}$ followed by $_{NPK}$ and $N_{1/2}P_{1/2}K_{1/2}$ for both clones. In the tolerant clone, the GLN per plant for $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ were 9.133, 9.533 and 10.667, respectively. This means application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased GLN by 100%, 109% and 134% compared with CV_1, respectively. Similarly, for susceptible clone, application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased GLN by 59%, 84%, 116% compared to the positive control, respectively. In general, the relatively the highest GLN were recorded in tolerant clone compared with susceptible clone in almost all fertilizer treatments (Table 4.6).

Leaf length (LL)

The leaf length (LL) of enset plant across the different treatments ranged from 0.266 m to 0.617 m. The lowest leaf length (LL) was recorded in susceptible clones while highest leaf

length (LL) values were recorded in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ (Table 4.6) in both clones. In tolerant clone, the average leaf length in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 0.493, 0.570, 0.617 m, respectively. Similarly, in susceptible clone, the average leaf length in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 0.570, 0.517 and 0.613 m, respectively. This shows that the combined NPK fertilizer treatments resulted in the highest number of leaves as compared to other fertilizer treatment. This means application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased GLN by 53%, 79% and 91% compared with CV_1, respectively. Similarly, for susceptible clone, application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased GLN by 114%, 94%, 130% compared to the positive control, respectively.

Leaf width (LW)

The effect of inorganic fertilizer on leaf width (LW) followed similar trend with other growth parameters such as PH, PSG, GLN and LL. Accordingly, application of $N_{3/2}P_{3/2}K_{3/2}$ was recorded the highest leaf width of 0.32 m in tolerant enset clone and 0.34 m in susceptible clones (Table 4.6). The lowest leaf width of enset across the treatments was 0.16 m in both tolerant and susceptible clones. In tolerant clone, the average leaf width in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 0.31, 0.33, 0.32 m, respectively. Similarly, in susceptible clone, the average leaf width in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 0.33, 0.323 and 0.337 m, respectively. This shows that the combined NPK fertilizer treatments resulted in the highest leaf width as compared to other fertilizer treatment. This means application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased LW by 90%, 104% and 96% compared with CV_1, respectively. Similarly, for susceptible clone,

application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased LW by 106%, 102%, 111%, respectively compared to the positive control.

Leaf area (LA) and leaf area index (LAI)

Like other growth parameters, total leaf area (LA) and leaf area index (LAI) were the highest in NPK combined fertilizer as compared to other treatments in both clones (Table 4.6). Application of $N_{3/2}P_{3/2}K_{3/2}$ fertilizer recorded the highest leaf area of 1.2 m^2 and 1.16 m^2 recorded for tolerant and susceptible clones respectively while control treatments recorded the lowest leaf area of 0.16 m^2 and 0.12 m^2 in tolerant and susceptible clones respectively. Similarly, application of $N_{3/2}P_{3/2}K_{3/2}$ fertilizer recorded the highest leaf area index of 0.54 and 0.51 in tolerant and susceptible clones, respectively. The result also showed that control treatments recorded the lowest leaf area index of 0.073 and 0.05 in tolerant and susceptible clones, respectively. In tolerant clone, the average leaf area in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 0.812, 1.037, 1.203 m^2, respectively. Similarly, in susceptible clone, the average leaf area in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 0.788, 0.798 and 1.157 m^2, respectively. This shows that the combined NPK fertilizer treatments resulted in the highest leaf area as compared to other fertilizer treatment. This means application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased LA by 398%, 536% and 638% compared with CV_1, respectively. Similarly, for susceptible clone, application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased LA by 557%, 565%, 864%, respectively compared to the positive control.

In tolerant clone, the average leaf area index in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 0.361, 0.461, and 0.535 respectively. Similarly, in susceptible clone, the

average leaf area index in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 0.35, 0.354 and 0.514, respectively. This shows that the combined NPK fertilizer treatments resulted in the highest leaf area index as compared to other fertilizer treatment. This means application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased LAI by 395%, 532% and 633% compared with CV_1, respectively. Similarly, for susceptible clone, application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased LAI by 560%, 568%, 870% compared to the positive control, respectively.

In general, from field observations, visible differences were seen in plant height, pseudostem girth, leaf length, width, total leaf area and leaf area index between plants of the two enset clones before inoculation. The measured data also indicated that in all morphological parameters, the control before *Xanthomonas* inoculation was significantly different at ($P < 0.05$) with the interaction between the three nutrients including $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ (Table 4.6).

Table 4. 6. The effect of inorganic fertilizers on enset growth parameters (mean ± standard error) before inoculation.

Treatment	PH (m)	PSG (m)	GLN	LL (m)	LW (m)	LA (m^2)	LAI
$N_{1/2}V_1$	0.510±0.102^{ab}	0.267± 0.043^{ab}	5.133± 0.297^{abc}	0.303± 0.017^{a}	0.193± 0.041^{ab}	0.182± 0.044^{a}	0.081± 0.020^{a}
$P_{1/2}V_1$	0.4367±0.045^{ab}	0.250±0.032^{ab}	5.433±0.133^{abc}	0.360±0.042^{ab}	0.210±0.021^{ab}	0.246±0.053^{a}	0.109±0.024^{a}
$K_{1/2}V_1$	0.400±0.020^{a}	0.247±0.035^{ab}	5.767±0.393^{abcd}	0.317±0.026^{a}	0.187±0.015^{ab}	0.227±0.040^{a}	0.101±0.018^{a}
NV_1	0.660± 0.050^{bc}	0.383± 0.020^{b}	6.800± 0.493^{cd}	0.470± 0.010^{bcd}	0.223± 0.015^{ab}	0.412± 0.053^{a}	0.183± 0.024^{a}
PV_1	0.433± 0.094^{ab}	0.300± 0.052^{ab}	5.567± 0.941^{abc}	0.300± 0.066^{a}	0.183± 0.047^{ab}	0.290± 0.138^{a}	0.129±0.061^{a}
KV_1	0.460± 0.055^{ab}	0.263± 0.019^{ab}	6.100± 0.100^{abcd}	0.360± 0.017^{ab}	0.213± 0.013^{ab}	0.261± 0.043^{a}	0.116± 0.019^{a}
$N_{3/2}P_{3/2}K_{3/2}V_1$	0.880± 0.055^{cd}	0.607± 0.062^{cd}	9.133± 0.433^{fg}	0.493± 0.061^{cde}	0.310± 0.045^{c}	0.812± 0.265^{b}	0.361± 0.118^{b}
$NPKV_1$	0.937± 0.139^{de}	0.680± 0.06^{cd}	9.533± 0.623^{fg}	0.570± 0.047^{de}	0.333± 0.032^{c}	1.037± 0.235^{bc}	0.461± 0.105^{bc}
$N_{3/2}P_{3/2}K_{3/2}V_1$	1.08± 0.163^{e}	0.743± 0.120^{e}	10.667± 0.49^{g}	0.617± 0.048^{e}	0.320± 0.038^{c}	1.203± 0.252^{c}	0.535± 0.112^{c}
CV_1	0.390± 0.029^{a}	0.233± 0.009^{ab}	4.567± 0.133^{a}	0.323± 0.012^{a}	0.163± 0.009^{a}	0.163± 0.026^{a}	0.073± 0.011^{a}
$N_{1/2}V_2$	0.48±0.072^{ab}	0.33±0.074^{ab}	6.43±0.868^{bcd}	0.36±0.060^{ab}	0.227±0.03756^{ab}	0.333±0.143^{a}	0.148±0.064^{a}
$P_{1/2}V_2$	0.337± 0.033^{a}	0.257± 0.024^{ab}	5.367± 0.333^{abc}	0.313± 0.037^{a}	0.180± 0.021^{ab}	0.181± 0.052^{a}	0.081± 0.023^{a}
$K_{1/2}V_2$	0.380± 0.020^{a}	0.237± 0.035^{ab}	5.433± 0.433^{abc}	0.297± 0.015^{a}	0.180± 0.015^{ab}	0.198± 0.039^{a}	0.088±0.017^{a}
NV_2	0.490± 0.032^{ab}	0.300± 0.025^{ab}	6.300± 0.000^{abcd}	0.393± 0.029^{abc}	0.267± 0.003^{bc}	0.357± 0.034^{a}	0.1589± 0.015^{a}
PV_2	0.400± 0.085^{a}	0.233± 0.068^{ab}	6.033± 0.882^{abcd}	0.337± 0.063^{a}	0.200± 0.042^{ab}	0.271± 0.144^{a}	0.121±0.064^{a}
KV_2	0.440 ± 0.017^{ab}	0.293± 0.052^{ab}	6.100± 0.667^{abcd}	0.327± 0.042^{a}	0.187± 0.012^{ab}	0.218± 0.069^{a}	0.097± 0.031^{a}
$N_{1/2}P_{1/2}K_{1/2}V_2$	0.837± 0.107^{cd}	0.547± 0.052^{c}	7.433± 0.809^{de}	0.570± 0.015^{de}	0.330± 0.020^{c}	0.788± 0.137^{b}	0.350± 0.061^{b}
$NPKV_2$	0.953± 0.08^{de}	0.617± 0.084^{cd}	8.567± 0.433^{ef}	0.517± 0.050^{cde}	0.323± 0.013^{c}	0.798± 0.141^{b}	0.354± 0.063^{b}
$N_{3/2}P_{3/2}K_{3/2}V_2$	0.990± 0.064^{de}	0.717± 0.053^{cd}	10.100± 0.416^{fg}	0.613± 0.013^{e}	0.337± 0.018^{c}	1.157± 0.083^{bc}	0.514± 0.037^{bc}
CV_2	0.368± 0.034^{a}	0.198± 0.014^{a}	4.667± 0.000^{ab}	0.266± 0.023^{a}	0.160± 0.010^{a}	0.120± 0.018^{a}	0.053± 0.008^{a}

PH = Plant height, PSG = Pseudostem girth, GLN = Green leaf number, LL = leaf length, LW = leaf width, LA = leaf area and LAI = Leaf area index. *Means with different superscripts within the same column are significantly different at $p<0.05$

4.3.1.2. Effects of inorganic fertilizer on growth parameters of enset four weeks after inoculation

The effects of inorganic fertilizers on the growth parameters of two enset clones after 4 weeks of infection with *Xanthomonas campestris pv. musacearum* are presented (Table 4.7), The growth parameters measured include PH, PSG, GLN, LL, LW, LA and LAI. The effects of nine different levels of nitrogen (N), phosphorus (P) and potassium (K) on the performance of these parameters after four weeks of inoculation are described below.

Plant height (PH)

The mean height of enset after four weeks of inoculation ranged from 0.34 m to 1.08 m (Table 4.7). The result of this study shows that the mean heights of tolerant and susceptible enset clones after four weeks of inoculation were not statistically different at $p<0.05\%$ (Table 4.7). However, application of inorganic fertilizer significantly influenced the height of both enset clones. As shown in the table, height of enset was superior in $N_{1/2}P_{1/2}K_{1/2}$, NPK, and $N_{3/2}P_{3/2}K_{3/2}$ for both clones. Accordingly, in tolerant clone, height of enset was increased by 138.8%, 132% and 175% due to application of $N_{1/2}P_{1/2}K_{1/2}$ (0.94 m), NPK, (0.913 m) and $N_{3/2}P_{3/2}K_{3/2}$ (1.082 m) compared to the positive control, respectively. Similarly, in susceptible enset clone, application of $N_{1/2}P_{1/2}K_{1/2}$ (0.84 m), NPK (0.87 m), and $N_{3/2}P_{3/2}K_{3/2}$ (0.89 m) increased the height of susceptible enset clone by 129%, 138.3% and 144.6% compared to the positive control, respectively. The result also shows that application of $K_{1/2}$ did not affect the height of enset in both clones.

Pseudostem girth (PSG)

Mean pseudostem girth (PSG) of enset varied from 0.205 m in the control treatment (C1V2) to 0.743 m in $N_{3/2}P_{3/2}K_{3/2}$ (1.5 times the recommended NPK). As shown in Table 4.7, the pseudostem girth (PSG) of enset varied significantly ($p<0.05$) due to application of inorganic fertilizer (Table 4.7). Like plant height, the highest values of PSG were observed in $N_{1/2}P_{1/2}K_{1/2,}$ NPK, and $N_{3/2}P_{3/2}K_{3/2}$ for both clones. In tolerant enset clone, application of $N_{1/2}P_{1/2}K_{1/2}$ (0.59 m), NPK (0.60 m) and $N_{3/2}P_{3/2}K_{3/2}$ (0.73 m) increased PSG of enset by 135.6%, 140.9% and 193.9% compared to the positive control, respectively. Similarly, in susceptible enset clone, application of $N_{1/2}P_{1/2}K_{1/2}$ (0.56 m), NPK (0.64 m) and $N_{3/2}P_{3/2}K_{3/2}$ (0.65 m) increase the PSG by 173.4%, 210.2% and 217.7%, respectively.

Green leaf number (GLN)

The mean green leaf number (GLN) per plant ranged from 4.7 (susceptible clone with KV_2) to 11.7 (under susceptible clone with $N_{3/2}P_{3/2}K_{3/2}$). The mean GLN per plant was significantly influenced by the application of NPK fertilizers for both clones (Table 4.7). Highest GLN per plant were recorded in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ for both clones. In the tolerant clone, the GLN per plant for $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ were 9.33, 10 and 10, respectively. This means application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased GLN by 96.2%, 110.2% and 110.2% compared with CV_1, respectively. Similarly, for susceptible clone, GLN per plant for $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ were 7.7, 8 and 11.7, respectively. In general, the relatively the highest GLN were recorded in tolerant clone compared with susceptible clone in almost all fertilizer treatments (Table 4.7).

Leaf length (LL)

As shown in Table 4.7, the lowest leaf length (LL) was recorded in susceptible clones while the highest leaf length (LL) values were recorded in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ (Table 4.7) in both clones. In tolerant clone, the average leaf length in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 0.67, 0.68, 0.81 m, respectively. This means $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments increased LL of enset by 108%, 111.7% and 150% compared to the positive control, respectively. Similarly, in susceptible clone, the average leaf length in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 0.61, 0.63 and 0.65 m, respectively. In general, the combined NPK fertilizer treatments resulted in the highest leaf length as compared to other fertilizer treatment.

Leaf width (LW)

The lowest leaf width of enset across the treatments was 0.17 m in both tolerant and susceptible clones while the highest LW were recorded in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ for both clones. The effect of inorganic fertilizer on leaf width (LW) followed similar trend with other growth parameters such as PH, PSG, GLN and LL. In the tolerant clone, the LW per plant for $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ were 0.33, 0.35 and 0.397, respectively. This means application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased LW by 96.3%, 106.3% and 133.9% compared with CV_1, respectively. Similarly, for susceptible clone, LW per plant for $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ were 0.35, 0.34 and 0.35, respectively increased LW by 103.9%, 101.8%, 107.6% compared to the positive control, respectively.

Leaf area (LA) and leaf area index (LAI)

Similar to other growth parameters, total leaf area (LA) and leaf area index (LAI) were the highest in NPK combined fertilizer as compared to other treatments in both clones (Table 4.7). Application of $N_{3/2}P_{3/2}K_{3/2}$ fertilizer recorded the highest leaf area of 1.88 m^2 and 1.58 m^2 recorded for tolerant and susceptible clones, respectively, while control treatments recorded the lowest leaf area of 0.182 m^2 and 0.156 m^2 in tolerant and susceptible clones, respectively. Similarly, application of $N_{3/2}P_{3/2}K_{3/2}$ fertilizer recorded the highest leaf area index of 0.84 and 0.704 in tolerant and susceptible clones, respectively. The result also showed that control treatments recorded the lowest LAI of 0.081 and 0.069 in tolerant and susceptible clones, respectively.

In general, from field observations, significant differences were recorded in plant height, pseudostem girth, leaf length, width, total leaf area and leaf area index between treatments of the two enset clones four weeks after inoculation. The measured data also indicated that application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ after four weeks of inoculation significantly influenced all growth parameters for both clones (Table 4.7).

Table 4. 7. The effect of inorganic fertilizers on enset growth parameters four weeks after inoculation, Mean ± standard error.

Treatment	PH (m)	PSG (m)	GLN	LL (m)	LW (m)	LA (m^2)	LAI
$N_{1/2}V1$	0.532± 0.111^{a}	0.352± 0.014^{a}	6.447±0.777^{abcd}	0.488±0.049^{abcd}	0.209± 0.022^{a}	0.386± 0.112^{b}	0.187±0.061^{ab}
$P_{1/2}V1$	0.434±0.093^{ab}	0.329±0.054^{a}	5.555± 0.949^{ab}	0.301±0.066^{ab}	0.186±0.047^{a}	0.290±0.138^{ab}	0.129± 0.051^{ab}
$K_{1/2}V1$	0.382±0.048^{a}	0.262±0.019^{a}	5.667±0.192^{abc}	0.312±0.040^{ab}	0.196±0.016^{a}	0.222±0.074^{ab}	0.098±0.033^{ab}
NV1	0.645±0.065^{abcd}	0.357±0.008^{a}	6.000±0.693^{abcd}	0.428±0.077^{abc}	0.268±0.023^{abcd}	0.357±0.044^{ab}	0.158±0.019^{ab}
PV1	0.544±0.029^{ab}	0.246±0.033^{a}	5.667±04000^{abs}	0.352±0.006^{ab}	0.218±0.004^{ab}	0.240±0.021^{ab}	0.107±0.009^{ab}
KV1	0.481±0.071^{a}	0.329±0.075^{a}	6.444±0.867^{abcd}	0.359±0.059^{ab}	0.225±0.039^{ab}	0.332±0.143^{ab}	0.148±0.064^{ab}
$N_{1/2}P_{1/2}K_{1/2}V_1$	0.940±0.025^{de}	0.589±0.066^{c}	9.333±0.881^{ef}	0.673± 0.003^{de}	0.334±0.050^{bcde}	1.171±0.309^{cde}	0.520±0.138^{cde}
$NPKV_1$	0.913±0.130^{de}	0.602±0.076^{c}	10.000±1.000^{fg}	0.684±0.084^{de}	0.351±0.033^{de}	1.400±0.392^{def}	0.622±0.174^{def}
$N_{3/2}P_{3/2}K_{3/2}V_1$	1.082±0.162^{e}	0.735±0.116^{c}	10.000±0.577^{fg}	0.809±0.105^{e}	0.398±0.044^{e}	1.880±0.464^{ef}	0.834±0.2062^{f}
C_1V_1	0.394±0.023^{a}	0.250±0.016^{a}	5.333±0.333^{ab}	0.323±0.011^{abc}	0.170±0.004^{a}	0.183±0.008^{ab}	0.081±0.004^{ab}
C_2V_1	0.587±0.182^{ab}	0.340±0.127^{a}	6.310±1.000^{abcd}	0.379±0.130^{ab}	0.179±0.062^{a}	0.257±0.375^{ab}	0.114±0.167^{ab}
$N_{1/2}V_2$	0.455± 0.046^{a}	0.273± 0.045^{a}	7.000±0.000^{bcd}	0.522± 0.163^{bcd}	0.266± 0.079^{abcd}	0.624±0.351^{abc}	0.277±0.156^{abc}
$P_{1/2}V_2$	0.399± 0.085^{a}	0.233± 0.068^{a}	6.333±0.882^{abcd}	0.336± 0.062^{ab}	0.199± 0.041^{a}	0.272± 0.143^{ab}	0.121±0.064^{ab}
$K_{1/2}V_2$	0.335± 0.033^{a}	0.294± 0.054^{a}	6.000±0.577^{abcd}	0.314± 0.036^{ab}	0.231± 0.036^{abc}	0.275± 0.089^{ab}	0.122±0.040^{ab}
NV_2	0.674±0.220^{abcd}	0.382±0.106^{b}	5.667±0.333^{abc}	0.363± 0.046^{ab}	0.176±0.022^{a}	0.199±0.013^{ab}	0.088±0.006^{ab}
PV_2	0.509±0.102^{a}	0.269±0.043^{a}	5.333±0.333^{ab}	0.304±0.016^{ab}	0.192±0.039^{a}	0.183±0.044^{ab}	0.081±0.020^{ab}
KV_2	0.397± 0.020^{a}	0.309±0.018^{a}	4.667±0.333^{a}	0.318±0.025^{ab}	0.186±0.015^{a}	0.183±0.034^{ab}	0.081±0.015^{ab}
$N_{1/2}P_{1/2}K_{1/2}V_2$	0.839±0.107^{bcde}	0.562±0.014^{bc}	7.667±0.333^{bcde}	0.605± 0.089^{cde}	0.348± 0.005^{cde}	0.895±0.132^{bcd}	0.398±0.059^{bcd}
$NPKV_2$	0.871±0.127^{cde}	0.638±0.089^{c}	8.000± 0.578^{de}	0.637± 0.085^{cde}	0.344± 0.042^{cde}	1.081±0.321^{cde}	0.481±0.143^{cde}
$N_{3/2}P_{3/2}K_{3/2}V_2$	0.894±0.068^{cde}	0.653±0.016^{c}	11.667± 0.333^{g}	0.652± 0.021^{cde}	0.354± 0.02^{de}	1.585± 0.040^{f}	0.704± 0.017^{ef}
C_1V_2	0.366±0.032^{a}	0.206±0.017^{a}	5.000±0.333^{ab}	0.281± 0.035^{a}	0.171± 0.020^{a}	0.157± 0.048^{a}	0.069± 0.021^{a}
C_2V_2	0.422±0.065^{a}	0.283±0.037^{a}	6.222±0.294^{abcd}	0.347± 0.049^{ab}	0.192± 0.015^{a}	0.293± 0.096^{ab}	0.130± 0.042^{ab}

* Means with different superscripts within the same column are significantly different at $p<0.05$. PH = Plant height, PSG = Pseudostem girth, GLN = Green leaf number, LL = leaf length, LW = leaf width, LA = leaf area and LAI = Leaf area index.

4.3.1.3. Effects of inorganic fertilizer on growth parameters of enset eight weeks after inoculation

The effects of inorganic fertilizers on the growth parameters of two enset clones after eight weeks of *Xanthomonas* inoculation are presented in Table 4.8. The effects of different levels of nitrogen (N), phosphorus (P) and potassium (K) fertilizers on the performance of these parameters after eight weeks of inoculation are described (Table 4.8).

Plant height (PH)

The mean height of enset after eight weeks of inoculation ranged from 0.535 m to 1.28 m (Table 4.8). The result of this study shows that the mean heights of tolerant (V_1) and susceptible (V_2) enset clones after eight weeks of inoculation were not statistically different at $p<0.05\%$. However, application of inorganic fertilizer significantly influenced the height of both enset clones. Accordingly, in tolerant clone, height of enset increased by 87.3%, 97.3% and 122.3% due to application of $N_{1/2}P_{1/2}K_{1/2}$ (1.08 m), NPK, (1.14 m) and $N_{3/2}P_{3/2}K_{3/2}$ (1.28 m) compared to the positive control, respectively. Similarly, in susceptible enset clone, application of $N_{1/2}P_{1/2}K_{1/2}$ (1.04m), NPK (1.15 m), and $N_{3/2}P_{3/2}K_{3/2}$ (1.19 m) increased the height of enset clone by 87.7%, 108.3% and 114.7%, compared with positive control, respectively.

Pseudostem girth (PSG)

Pseudostem girth (PSG) of enset varied from 0.297 m in the control treatment (C_1V_2) to 0.84 m in $N_{3/2}P_{3/2}K_{3/2}$ (1.5 times the recommended NPK). As shown in Table 4.8, the mean PSG of enset varied significantly ($p<0.05$) due to application of inorganic fertilizer (Table

4.8). Similar to plant height, significantly the highest values of PSG were recorded for $N_{1/2}P_{1/2}K_{1/2,}$ NPK, and $N_{3/2}P_{3/2}K_{3/2}$ for both clones. In tolerant enset clone, application of $N_{1/2}P_{1/2}K_{1/2}$ (0.71m), NPK (0.78 m) and $N_{3/2}P_{3/2}K_{3/2}$ (0.841 m) increased PSG of enset by 112%, 134% and 152%, respectively. Similarly, in susceptible enset clone, application of $N_{1/2}P_{1/2}K_{1/2}$ (0.65 m), NPK (0.72 m) and $N_{3/2}P_{3/2}K_{3/2}$ (0.82 m) increased PSG of enset by 116.9%, 140.8% and 173.6%, respectively.

Green leaf number (GLN)

The mean green leaf number (GLN) per plant ranged from 4.9 (susceptible clone with C_1V_2) to 11.7 (under susceptible clone with $N_{3/2}P_{3/2}K_{3/2}$). The mean GLN per plant was significantly influenced by the application of NPK fertilizers for both clones (Table 4.8). The highest GLN per plant were recorded in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ for both clones. In the tolerant clone, the GLN per plant for $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ were 9.3, 9.8 and 10.9, respectively. This means application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increased GLN by 74.6%, 82.9% and 103.8% compared with C_1V_1, respectively. Similarly, for susceptible clone, GLN per plant in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 7.6, 8.8 and 10.3, respectively. This shows that the use of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ fertilizer rate increased GLN of susceptible clone by 56.9%, 79.8%, 111.7%, respectively. In general, relatively the highest GLN were recorded in tolerant clone compared with susceptible clone in almost all fertilizer treatments (Table 4.8).

Leaf length (LL)

The leaf length (LL) of enset plant across the different treatments ranged from 0.27 m to 0.615 m. The highest leaf length (LL) values were recorded in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ (Table 4.8) in both clones. In tolerant clone, the average leaf length in

$N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 0.49, 0.57, 0.616 m, respectively. Similarly, in susceptible clone, the average leaf length in $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments were 0.57, 0.51 and 0.613 m, respectively. This means $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ increase LL of enset by 115.3%, 93.7% and 130.9%, respectively. This shows that the combined NPK fertilizer treatments resulted in the highest leaf length as compared with other fertilizer treatment.

Leaf width (LW)

The effect of inorganic fertilizer on leaf width (LW) followed similar trend with other growth parameters such as PH, PSG, GLN and LL. Accordingly, application of $N_{3/2}P_{3/2}K_{3/2}$ was recorded with the highest leaf width of 0.32 m in tolerant enset clone and 0.34 m in susceptible clones (Table 4.8). The lowest leaf width of enset across the treatments was 0.16 m in both tolerant and susceptible clones.

Leaf area (LA) and leaf area index (LAI)

Like other growth parameters, total leaf area (LA) and leaf area index (LAI) were the highest in NPK combined fertilizer as compared to other treatments in both clones (Table 4.8). Application of $N_{3/2}P_{3/2}K_{3/2}$ fertilizer recorded the highest leaf area of 1.196 m^2 and 1.160 m^2 recorded for tolerant and susceptible clones, respectively while control treatments recorded the lowest leaf area of 0.16 m^2 in C_1V_1 and 0.114 m^2 in C_1V_2. Similarly, application of $N_{3/2}P_{3/2}K_{3/2}$ fertilizer recorded the highest leaf area index of 0.532 and 0.516 in tolerant and susceptible clones, respectively. The result also showed that control treatments recorded the lowest leaf area index of 0.07 and 0.05 in tolerant and susceptible clones, respectively.

Table 4. 8. The effect of inorganic fertilizers on enset growth parameters eight weeks after inoculation, Mean ± standard error.

Treatments	PH (m)	PSG (m)	GLN	LL (m)	LW (m)	LA (m^2)	LAI
$N_{1/2}V_1$	0.681±0.071^{ab}	0.430±0.075^{a}	6.643±0.868^{bcd}	0.360±0.059^{ab}	0.225±0.039^{ab}	0.331±0.150^{a}	0.147±0.067^{a}
$P_{1/2}V_1$	0.634±0.094^{ab}	0.353± 0.053^{a}	5.757± 0.949^{abc}	0.335±0.063^{a}	0.208±0.047^{ab}	0.233±0.094^{a}	0.104±0.041^{a}
$K_{1/2}V_1$	0.599±0.088^{a}	0.345±0.068^{a}	5.976±0.882^{abc}	0.318±0.066^{ab}	0.186±0.041^{ab}	0.197±0.140^{a}	0.087± 0.062^{a}
NV_1	0.860±0.049^{bc}	0.482± 0.019^{a}	6.980± 0.485^{cd}	0.471±0.001^{bcd}	0.224± 0.014^{ab}	0.405±0.060^{a}	0.179± 0.027^{a}
PV_1	0.635±0.050^{ab}	0.353± 0.032^{a}	5.757± 0.113^{abc}	0.301± 0.041^{a}	0.186± 0.020^{ab}	0.217±0.050^{a}	0.097± 0.022^{a}
KV_1	0.660±0.055^{ab}	0.400± 0.019^{a}	6.310± 0.110^{abcd}	0.360± 0.017^{ab}	0.215± 0.012^{ab}	0.267±0.033^{a}	0.119± 0.014^{a}
$N_{1/2}P_{1/2}K_{1/2}V_1$	1.080±0.054^{cde}	0.707±0.062^{bc}	9.313± 0.443^{fg}	0.490±0.060^{cde}	0.309± 0.047^{c}	0.815± 0.274^{b}	0.362± 0.122^{b}
NPK	1.138±0.140^{de}	0.780±0.061^{bc}	9.753± 0.619^{fg}	0.571± 0.047^{de}	0.330± 0.031^{c}	1.028±0.223^{bc}	0.457±0.099^{bc}
$N_{3/2}P_{3/2}K_{3/2}V_1$	1.282±0.162^{e}	0.841± 0.120^{c}	10.867± 0.507^{g}	0.616± 0.048^{e}	0.319± 0.038^{c}	1.196± 0.260^{c}	0.532± 0.115^{c}
C_1V_1	0.577± 0.082^{a}	0.333± 0.008^{a}	5.333± 0.113^{abc}	0.340± 0.072^{ab}	0.162± 0.007^{a}	0.160± 0.014^{a}	0.071± 0.006^{a}
C_2V_1	0.587±0.029^{a}	0.3870±0.070^{a}	6.310± 0.780^{abcd}	0.433±0.013^{abc}	0.249± 0.044^{abc}	0.645±0.134^{abc}	0.286±0.060^{abc}
$N_{1/2}V_2$	0.597± 0.020^{a}	0.346± 0.034^{a}	6.20± 0.400^{abcd}	0.335± 0.025^{a}	0.199± 0.015^{ab}	0.268± 0.038^{a}	0.119± 0.017^{a}
$P_{1/2}V_2$	0.578±0.020^{a}	0.338± 0.033^{a}	5.643± 0.443^{abc}	0.299± 0.014^{a}	0.177± 0.015^{ab}	0.167±0.035^{a}	0.075± 0.016^{a}
$K_{1/2}V_2$	0.535±0.033^{a}	0.356± 0.023^{a}	5.537± 0.333^{abc}	0.314± 0.037^{a}	0.176± 0.022^{ab}	0.176± 0.046^{a}	0.078± 0.021^{a}
NV_2	0.709±0.102^{ab}	0.368± 0.043^{a}	5.313± 0.294^{abc}	0.303± 0.015^{a}	0.192± 0.039^{ab}	0.176± 0.052^{a}	0.078± 0.023^{a}
PV_2	0.692±0.032^{ab}	0.400± 0.026^{a}	6.530±0.000^{abcd}	0.392±0.028^{abc}	0.265± 0.004^{bc}	0.368± 0.032^{a}	0.164± 0.014^{a}
KV_2	0.641±0.016^{ab}	0.394± 0.054^{a}	6.310± 0.675^{abcd}	0.327± 0.042^{a}	0.185± 0.010^{ab}	0.217± 0.064^{a}	0.098± 0.029^{a}
$N_{1/2}P_{1/2}K_{1/2}V_2$	1.039±0.107^{cd}	0.646± 0.052^{b}	7.643± 0.802^{de}	0.572± 0.014^{de}	0.330± 0.019^{c}	0.798± 0.153^{b}	0.355± 0.068^{b}
$NPKV_2$	1.152±0.080^{de}	0.717±0.084^{bc}	8.757± 0.443^{ef}	0.515±0.051^{cde}	0.321± 0.015^{c}	0.798± 0.142^{b}	0.355± 0.063^{b}
$N_{3/2}P_{3/2}K_{3/2}V2$	1.188 ±0.063^{de}	0.815±0.052^{bc}	10.310± 0.402^{fg}	0.613± 0.013^{e}	0.337± 0.015^{c}	1.160± 0.095^{bc}	0.516± 0.042^{bc}
C_1V_2	0.553±0.024^{a}	0.298± 0.014^{a}	4.870±0.000^{ab}	0.266± 0.023^{a}	0.160± 0.010^{a}	0.114± 0.017^{a}	0.050± 0.008^{a}
C_2V_2	0.568±0.033^{a}	0.364± 0.041^{a}	6.203± 0.333^{abcd}	0.293± 0.027^{a}	0.178± 0.011^{ab}	0.183± 0.040^{a}	0.081± 0.017^{a}

*Means with different superscripts within the same column are significantly different at $p<0.05$. PH = Plant height, PSG = Pseudostem girth, GLN = Green leaf number, LL = leaf length, LW = leaf width, LA = leaf area and LAI = Leaf area index.

4.3.2. Effects of inorganic fertilizers on disease incidence, severity and AUPDC of enset against *Xanthomonas campestris pv. musacearum*

4.3.2.1. Disease incidence

The effect of inorganic fertilizers on disease incidence of Xcm was observed every seven days. In most of the treatments, disease symptom was observed between 14 and 42 days after inoculation (Fig 4.11). This means between 14 and 42 days after inoculation, all inoculated plants had exhibited typical disease symptoms such as chlorosis, necrosis and wilting of leaves. The average disease incidence of enset inoculated with *Xanthomonas* ranged from 64% to 90%. The result showed that as compared to positive control, application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ reduced the disease incidence of tolerant and susceptible enset clone. Accordingly, tolerant enset clones treated with $N_{1/2}P_{1/2}K_{1/2,}$ NPK and $N_{3/2}P_{3/2}K_{3/2}$ fertilizers had 68.9%, 68.3%, 63.7% disease incidence, respectively which is lower than the positive control (73.9%). Similarly, susceptible enset clones treated with $N_{1/2}P_{1/2}K_{1/2,}$ NPK and $N_{3/2}P_{3/2}K_{3/2}$ fertilizers had 70%, 65%, 60% disease incidence, respectively while the positive control had 90% disease incidence. This means, compared to the positive control, $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ decreased the disease incidence of tolerant enset clones by 6.8%, 7.7% and 13.8%, respectively compared with the positive control. In the same way, application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments decreased the disease incidence of susceptible enset clones by 22.2%, 27.8%, and 33.1%, respectively compared with the positive control.

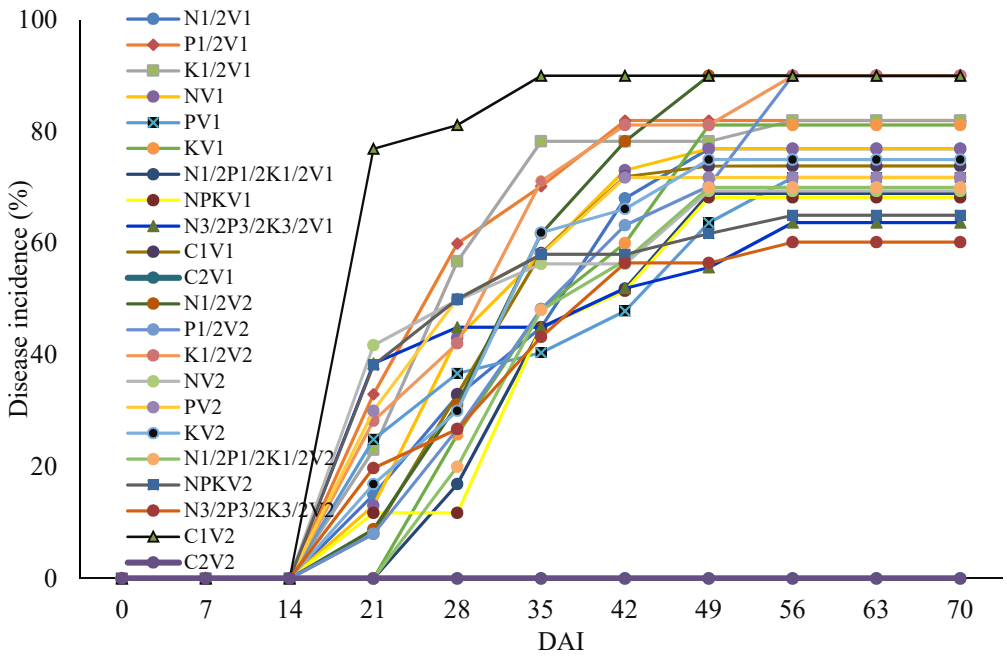

Figure 4. 11. Effects of inorganic fertilizer on Xanthomonas campestris pv. musacearum disease incidence of enset.

4.3.2.2. Disease Severity and Area Under Disease Progress Curve (AUDPC)

The effect of inorganic fertilizers on disease severity of Xcm was observed every seven days. (Fig 4.12). Similar to disease incidence, disease severity was observed between 14 and 42 days after inoculation (Fig 4.12). As shown in Table 4.9, the average disease severity of enset inoculated with *Xanthomonas* ranged from 27% to 44.4% after 70 days of inoculation and treated with different levels of fertilizer. The result showed that application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ reduced the disease severity of tolerant and susceptible enset clone. Accordingly, tolerant enset clones treated with $N_{1/2}P_{1/2}K_{1/2,}$ NPK and $N_{3/2}P_{3/2}K_{3/2}$ fertilizers had 36.6%, 34.6%, 27.1% disease severity, respectively which is lower than the positive control (41.8%). Similarly, susceptible enset clones treated with

$N_{1/2}P_{1/2}K_{1/2,}$ NPK and $N_{3/2}P_{3/2}K_{3/2}$ fertilizers had 39.9%, 37.4%, 36.4% disease severity, respectively while the positive control had 44.4% disease incidence. This indicates that disease severity of tolerant enset clone with $N_{1/2}P_{1/2}K_{1/2,}$ NPK and $N_{3/2}P_{3/2}K_{3/2}$ application decreased severity by 12.4%, 17.3% and 35.2%, respectively as compared to positive control. Similarly, application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ treatments decreased the disease severity of susceptible enset clones by 10.1%, 15.7%, and 17.9%, respectively.

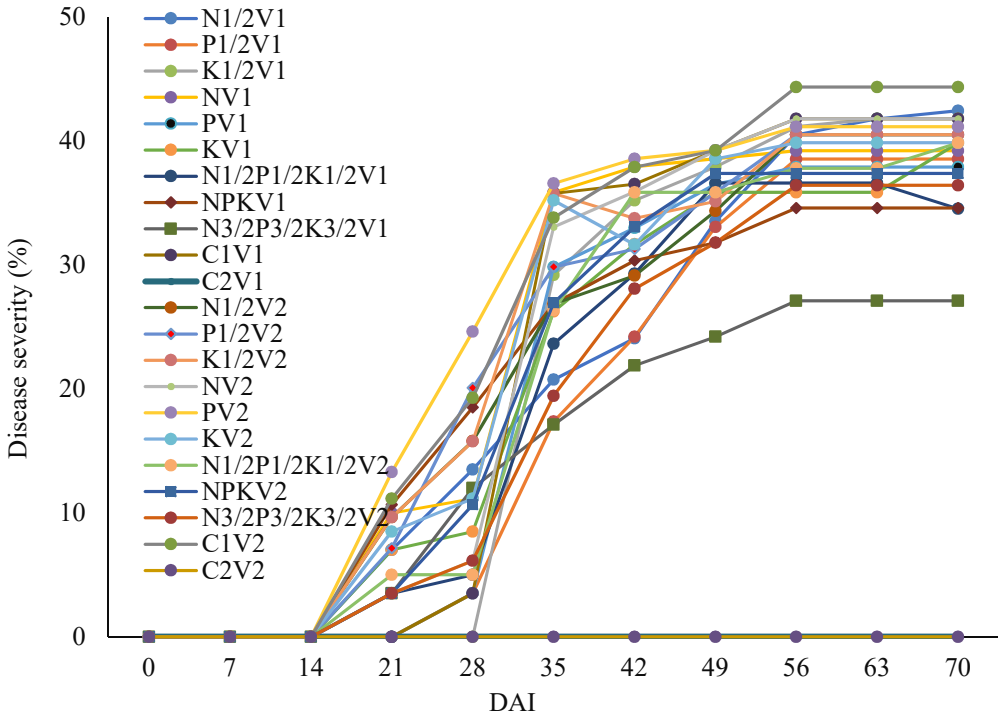

Figure 4. 12. Effects of inorganic fertilizer on Xanthomonas campestris pv. Musacearum disease severity of enset.

Area under disease progress curve (AUDPC) of the two enset clones is presented in Table 4.9. Generally, AUDPC of susceptible enset clone is the highest than tolerant enset clones. The highest AUDPC value (1455) was recorded in susceptible clone with positive control while the lowest AUDPC value (623) was recorded on tolerant clone treated with

$N_{3/2}P_{3/2}K_{3/2}$ fertilizers (Table 4.9). In the tolerant clone, the AUDPC value with $N_{3/2}P_{3/2}K_{3/2}$ application was significantly lower than the AUDPC in the positive control (C_1V_1). For susceptible clone $N_{1/2}P_{1/2}K_{1/2}$ AUPDC value is significantly lower than the AUDPC in the positive control (C_1V_2).

Table 4. 9. Arcsine transformed disease severity and area under disease progress curve (AUDPC) of enset clones infected with Xcm and treated with inorganic fertilizer treatments.

Treatments	Average disease severity (%) DAI											
	0	7	14	21	28	35	42	49	56	63	70	AUDPC
$N_{1/2}V_1$	0	0	0	7.01	13.48	20.76	24.09	33.53	42.44	42.44	42.44±1.70^{de}	1211.133±2.246^{cd}
$P_{1/2}V_1$	0	0	0	0	3.51	17.38	24.21	33.07	38.54	38.54	38.54±2.35^{cde}	951.783±95.408^{bcd}
$K_{1/2}V_1$	0	0	0	0	0	29.19	35.21	37.92	41.15	41.8	41.80±1.29^{de}	1150.630±50.774^{bcd}
NV_1	0	0	0	9.98	11.13	35.86	37.92	38.58	39.22	39.22	39.22±1.13^{cde}	989.133±226.259^{bcd}
PV_1	0	0	0	0	3.51	29.84	33	36.52	37.91	37.91	37.91±1.32^{cde}	751.983±170.030^{bc}
KV_1	0	0	0	7.01	8.49	26.24	31.68	39.86	39.86	39.86	39.86±1.71^{cde}	890.560±115.777^{bcd}
$N_{1/2}P_{1/2}K_{1/2}V_1$	0	0	0	3.51	4.99	23.65	29.31	36.61	36.61	36.61	36.61±0.67^{cd}	896.803±147.087^{bcd}
$NPKV_1$	0	0	0	10.65	18.51	26.87	30.36	31.8	34.58	34.58	34.58±0.68^{c}	1030.553±74.095^{bcd}
$N_{3/2}P_{3/2}K_{3/2}V_1$	0	0	0	3.51	12	17.11	21.87	24.21	27.11	27.11	27.11±6.19^{b}	623.330±241.500^{b}
C_1V_1	0	0	0	0	3.51	35.78	36.52	39.23	41.8	41.8	41.80±1.29^{de}	969.580±279.289^{bcd}
C_2V_1	0	0	0	0	0	0	0	0	0	0	0.00±0.00^{a}	0.000±0.000^{a}
$N_{1/2}V_2$	0	0	0	9.65	15.8	26.87	29.14	34.35	40.52	40.52	40.52±1.29^{cde}	1236.103±171.103^{cd}
$P_{1/2}V_2$	0	0	0	7.14	20.09	29.84	31.29	35.86	40.5	40.5	40.50±2.32^{cde}	1207.850±258.257^{cd}
$K_{1/2}V_2$	0	0	0	9.65	15.77	35.78	33.78	35.13	40.5	40.5	40.50±2.32^{cde}	1249.063±23.774^{cd}
NV_2	0	0	0	3.51	6.14	33.07	35.9	39.23	41.8	41.8	41.80±1.29^{de}	1172.373±182.8^{bcd}
PV_2	0	0	0	13.28	24.62	36.59	38.58	39.23	41.16	41.16	41.16±1.11^{cde}	1132.110±290.36^{bcd}
KV_2	0	0	0	8.49	11.13	35.21	31.64	38.58	39.86	39.86	39.86±1.71^{cde}	941.520±210.685^{bcd}
$N_{1/2}P_{1/2}K_{1/2}V_2$	0	0	0	4.99	4.99	26.36	35.86	35.86	37.8	37.8	39.87±1.71^{cde}	733.667±200.997^{bc}
$NPKV_2$	0	0	0	3.51	10.65	26.95	33.07	37.39	37.39	37.39	37.39±1.03^{cd}	911.763±136.579^{bcd}
$N_{3/2}P_{3/2}K_{3/2}V_2$	0	0	0	3.51	6.14	19.43	28.07	31.80	36.43	36.43	36.43±1.52^{cd}	1005.180±95.114^{bcd}
C_1V_2	0	0	0	11.13	19.28	33.80	37.91	39.23	44.36	44.36	44.36±0.64^{e}	1455.317±96.732^{d}
C_2V_2	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00±0.00^{a}	0.000±0.000^{a}

*Means with different superscripts within the same column are significantly different at $p<0.05$. DAI= Days after inoculation, AUDPC= Area under disease progress curve.

4.4. Biofumigation of *Brassica* species against *Xanthomonas campestris pv. musacearum*

4.4.1. Effect of *Brassica* species as a green manures and *Brassica carinata* seed extract residue on growth and physiological parameters of *enset* clones

4.4.1.1. Effect on growth of enset clones

The effects of *Brassica* species as green manure and *Brassica carinata* seed extract residue on the growth parameters of two enset clones infected with *Xanthomonas campestris pv. musacearum* are presented in Table 4.11. The growth responses of the tolerant (V_1) and susceptible enset clones (V_2) treated with *Brassica oleracea var capitata* (Cabbage) represented by (G_1) and *Brassica oleracea var acepala* (Tekur gomen) represented by (G_2) as green manure and *Brassica carinata* seed extract residue represented by (G_3), positive control *represented* by (C_1) and negative control represented by (C_2) on the control of bacterial wilt of enset under field condition were evaluated in three rounds. The first measurement was taken before inoculation with *Xanthomonas campestris pv. musacearum* (after six months of planting), while the second and third measurements were taken four and eight weeks after inoculation, respectively. The growth parameters measured include plant height (PH), pseudostem girth (PSG), green leaf number (GLN), leaf length (LL), leaf width (LW), leaf area (LA) and leaf area index (LAI). The effects of *Brassica* species (*Brassica oleracea var capitata* (Cabbage), *Brassica oleracea var acepala* (Tekur gomen)

as green manure and *Brassica carinata* seed extract residue on the performance of these parameters are presented below.

4.4.1.1.1. Growth parameters of two enset clones before inoculation

At the beginning of the experiment, growth parameters (plant height, pseudostem girth, green leaf number, leaf length, leaf area and leaf area index) were measured directly for all enset clones and a mean value of these parameters was calculated (Table 4.10). Fifteen enset clones in each plot were selected as experimental plants. The two groups were not statistically different at $p<0.05\%$ (Table 4.10). However, tolerant clones showed better performance in all growth parameters

Table 4. 10. Growth parameters (mean± standad error)of two enset clones before inoculation (after 18 months of planting).

Enset clones	PH(m)	PSG(m)	GLN	LL(m)	LW(m)	LA(m^2)	LAI
V_1	0.609±0.339	0.360±0.018	5.951±0.204	0.447±0.028	0.235±0.012	0.355±0.039	0.158±0.017
V_2	0.476±0.365	0.310±0.027	5.514±0.349	0.411±0.030	0.212±0.013	0.273±0.032	0.121±0.014

* V_1 and V_2 represent Yeshrakinkye and Ameratye clones, respectively.

4.4.1.1.2. Effects of *Brassica* species green manure and seed extract residue on growth parameters of enset four weeks after inoculation

Plant height (PH)

The mean height of enset after four weeks of treatment application ranged from 0.50 m to 0.81 m (Table 4.11). The mean heights of two enset clones (V_1 and V_2) four weeks after treatment application were not statistically different at $p<0.05\%$ (Table 4.11). However, application of *Brassica* species as a green manures and *Brassica carinata* seed extract residue affected the height of both enset clones. As shown in Table 4.11, height of enset was superior under G_2 application in the tolerant clone (0.81 m). In tolerant clone, height of enset increased by 13.5% due to application of G_2 (0.81 m) but decreased by 8.68% and 5.04% due to application of G_1 (0.65 m) and G_3 (0.68 m) as compared to positive control, respectively. Similarly, susceptible clone, application of G_1 (0.51 m), G_2 (0.67 m) and G_3 (0.53 m) increased the height by 1.4%, 32.87% and 5.2%, respectively as compared to positive control.

Pseudostem girth (PSG)

Mean pseudostem girth (PSG) of enset varied from 0.28 m in the control treatment (CV_2) to 0.49 m in G_2V_2. As shown in Table 4.11, PSG of enset was not significantly different at ($p<0.05$) due to application of *Brassica* species as a green manure and *Brassica carinata* seed extract residue (Table 4.11). The highest values of PSG were observed in G_2V_2 (0.49 m) for susceptible clones. In tolerant enset clone, application of G_1 (0.42 m), G_2 (0.42 m) and G3 (0.42 m) increased PSG of enset by 4.1%, 3.6% and 4.8% compared to positive

control, respectively. Similarly, in susceptible enset clone, application of G_1(0.38 m), G_2 (0.49 m) and G_3 (0.36 m) increased PSG of enset by 36.2%, 74.9% and 28.7%, respectively compared to positive control.

Green leaf number (GLN)

The mean green leaf number (GLN) per plant ranged from 5.89 (susceptible clone without green manure, CV_2) to 7.55 (under tolerant clone with G_2). The mean GLN per plant was not significantly influenced by the application of green manure for both clones (Table 4.11). In the tolerant clone, the GLN per plant under G_1, G_2 and G_3 treatments were 7.33, 7.55 and 6.78, respectively. This means application of G_1, G_2 and G_3 increased GLN by 11.8%, 15.2% and 3.4%, respectively compared with positive control. Similarly, for susceptible clone, application of G_1 and G_3 increased GLN by 6.8% and 1.7% compared to positive control, respectively. Application of G_2 decreased GLN only by 10.1% as compared to positive control. In general, the relatively the highest GLN were recorded in tolerant clone compared with susceptible clone in almost all green manure treatments and *Brassica carinata* seed extract residue treatment (Table 4.11).

Leaf length (LL)

The leaf length (LL) of enset plant across the different treatments ranged from 0.41m to 0.65 m. The lowest leaf length (LL) was recorded in susceptible clones while the highest leaf length (LL) values were recorded in G_1, followed by G_2 (Table 4.11) in tolerant clones. In tolerant clone, the average leaf length in G_1, G_2 and G_3 treatments were 0.65, 0.55, 0.53 m, respectively. Similarly, in susceptible clone, the average leaf length in G_1V_2, G_2V_2 and

G_3V_2 treatments were 0.53, 0.55 and 0.53 m, respectively. This shows that the use of G_1 green manure treatments resulted in the highest number of leaves as compared to other green manure treatment. This means application of G_1, G_2 and G_3 increased LL by 43.7%, 21.8% and 17.8%, respectively compared with the positive control. Similarly, for susceptible clone, application of G_1, G_2 and G_3 increased LL by 29.8%, 34.9%, 30.3%, respectively compared with the positive control.

Leaf width (LW)

The effect of *Brassica* species as green manure and *Brassica carinata* seed extract residue on leaf width (LW) followed similar trend with other growth parameters such as PH, PSG, GLN and LL. Accordingly, application of G_2 resulted in the highest leaf width of 0.31 m in tolerant enset clone (Table 4.11). The lowest LW of enset across the treatments was 0.21 m in the susceptible clones. In tolerant clone, the average LW in G_1, G_2 and G_3 treatments were 0.31, 0.33, 0.32 m, respectively compared with positive control. This implies that G_2V_1 treatment had the highest LW as compared to other green manure treatments. Hence application of G_1, G_2 and G_3 increased LW by 5.81, 19.77 and 4.65% compared with C_1V_1, respectively. Similarly, in susceptible clone, average LW of 0.27, 0.309 and 0.27 m were recorded in G_1, G_2 and G_3 treatments, respectively. Again, in susceptible clone, application of G_1, G_2 and G_3 increased LW by 41.06, 33.82, 13.04% compared with C_1V_2, respectively.

Leaf area (LA) and leaf area index (LAI)

Similar to other growth parameters, total leaf area (LA) and leaf area index (LAI) were the highest in G_1 followed by G_2 green manure treatments as compared to other treatments in

both clones (Table 4.11). Application of G_2 green manure treatments recorded the highest leaf area of 0.71 m^2 for tolerant clones and G_1 green manure treatments recorded 0.59 m^2 for susceptible clones while control treatments recorded the lowest leaf area of 0.39 m^2 and 0.31 m^2 in tolerant and susceptible clones, respectively. Similarly, application of G_2 green manure treatments recorded the highest leaf area index of 0.32 and 0.26 in tolerant and susceptible clones, respectively. The result also showed that control treatments recorded the lowest leaf area index of 0.18 and 0.14 in tolerant and susceptible clones, respectively. In tolerant clone, the average leaf area in G_1, G_2 and G_3 treatments were 0.69, 0.71, 0.56 m^2, respectively. Similarly, in susceptible clone, the average leaf area in G_1, G_2 and G_3 treatments were 0.59, 0.49 and 0.45 m^2, respectively. This shows that the G_2V_1 and G_1V_2 treatments resulted in higher leaf area as compared to other green manure treatments. This means application of G_1, G_2 and G_3 increased LA by 74.06%, 79.35% and 41.06% compared with C_1V_1, respectively. Similarly, for susceptible clone, application of G_1, G_2 and G_3 increased LA by 89.9%, 60.4%, 44.8%, respectively compared to positive control.

In tolerant clone, the average leaf area index in G_1, G_2 and G_3 treatments were 0.31, 0.32, and 0.25, respectively. This shows that G_2 and G_{11} treatments resulted in higher leaf area index as compared to G_2 green manure treatment. Application of G_1, G_2 and G_3 in tolerant clones increased LAI by 74.4%, 80.11% and 41.48% compared with C_1V_1, respectively. Similarly, in susceptible clone, the average leaf area index in G_1, G_2 and G_3 treatments were 0.26, 0.22 and 0.198, respectively. And, application of G_1, G_2 and G_3 in susceptible clone increased LAI by 89.8%, 60.6%, 44.5%, respectively compared with positive control.

In general, the results show that even though growth parameters 4 weeks after Xanthomonas inoculation were not significantly different at ($P < 0.05$), there was better performance of all treatments in all growth parameters compared to the positive control.

Table 4. 11. Growth parameters of enset taken four weeks after Xanthomonas inoculation

Treatments	PH (m)	PSG (m)	GLN	LL (m)	LW (m)	LA (m^2)	LAI
G_1V_1	0.652±0.139	0.422±0.091	7.333±0.837	0.645±0.072	0.273±0.050	0.691±0.131	0.307±0.058
G_2V_1	0.810±0.078	0.424±0.036	7.553±0.400	0.547±0.038	0.309±0.024	0.712±0.129	0.317±0.057
G_3V_1	0.678±0.076	0.419±0.044	6.780±0.588	0.529±0.062	0.270±0.025	0.560±0.162	0.249±0.072
C_1V_1	0.714±0.082	0.440±0.054	6.557±0.483	0.449±0.085	0.258±0.044	0.397±0.149	0.176±0.066
C_2V_1	0.730±0.102	0.409±0.041	6.780±0.485	0.461±0.089	0.272±0.022	0.492±0.156	0.219±0.069
G_1V_2	0.509±0.102	0.380±0.027	6.997±0.882	0.527±0.111	0.292±0.031	0.585±0.142	0.260±0.063
G_2V_2	0.667±0.042	0.488±0.043	5.890±1.126	0.548±0.067	0.277±0.019	0.494±0.135	0.220±0.060
G_3V_2	0.528±0.131	0.359±0.096	6.667±0.384	0.529±0.060	0.234±0.033	0.446±0.065	0.198±0.029
C_1V_2	0.502±0.114	0.279±0.082	6.553±0.619	0.406±0.085	0.207±0.043	0.308±0.092	0.137±0.041
C_2V_2	0.592±0.111	0.342±0.077	6.330±1.732	0.408±0.080	0.234±0.034	0.422±0.166	0.188±0.074
Values	0.449	0.588	0.961	0.533	0.636	0.552	0.552

*none of the parameters is significant among treatments. PH = Plant height, PSG = Pseudostem girth, GLN = Green leaf number, LL = leaf length, LW = leaf width, LA = leaf area and LAI = Leaf area index.

4.4.1.1.3. Effect of *Brassica* species on growth parameters of enset eight weeks after inoculation

Plant height (PH)

The mean height of enset after four weeks of treatment application ranged from 0.66 m to 0.89 m (Table. 4.12). The result shows that the mean heights of two enset clones (V_1 and V_2) eight weeks after treatment application were not statistically different at $p<0.05\%$ (Table 4.12). However, application of *Brassica* species as green manure and *Brassica carinata* seed extract residue affected the height of both enset clones. As shown in the Table, height of enset was superior in G_2 in both clones, $_1$ 0.89 m and 0.76 m in V1 and V2, respectively. Accordingly, in clone V_1, height of enset increased by 9.58%, 23.19% and 10.28 % due to application of G_1 (0.79 m), G_2 (0.89 m) and G_3 (0.79 m), respectively compared to positive control. Similarly, in susceptible clone V_2, application of G_2 (0.76 m) and G_3 (0.75 m) increased the height of V_2 by 11.89% and 9.84%, respectively compared to positive control. However, application of G_1 (0.66) decreased the height of susceptible clone by 2.94%.

Pseudostem girth (PSG)

Mean pseudostem girth (PSG) of enset varied from 0.42 m in the control treatment (CV_2) to 0.57 m in G_2V_1. As shown in Table 4.12, the pseudostem girth (PSG) of enset was not significantly different at ($p<0.05$) due to application of *Brassica* species as a green manure and *Brassica carinata* seed extract residue (Table 4.12). The highest values of PSG were observed in G_2 (0.57 m) for tolerant clones. In tolerant enset clone, application of G_1 (0.54

m), G_2 (0.57 m) and G_3 (0.46 m) increased PSG of enset by 22.99%, 31.49% and 5.29%, respectively compared to positive control. Similarly, in susceptible enset clone, application of G_1 (0.47 m), G_2 (0.47 m) and G_3 (0.49 m) increased PSG of enset by 12.65%, 11.93% and 16.95%, respectively compared to positive control.

Green leaf number (GLN)

The mean green leaf number (GLN) per plant ranged from 6.7 (susceptible clone without green manure, CV_2) to 8.4 (under tolerant clone with G_2). The mean GLN per plant was not significantly influenced by the application of green manure for both clones (Table 4.12). Highest GLN per plant were recorded in G_2 for tolerant clones and G_1 for susceptible clone. In the tolerant clone, the GLN per plant for G_1, G_2 and G_3 application were 7.9, 8.4 and 8, respectively. This means in susceptible clone, application of G_1, G_2 and G_3 increased GLN by 5.98%, 13.4% and 7.5% compared with CV_1, respectively. Similarly, for susceptible clone, application of G_1, G_2 and G_3 increased GLN by 18.3%, 8.3% and 4.9%, respectively compared with CV_2. In general, the relatively higher GLN were recorded in tolerant clone compared with susceptible clone in almost all green manure treatments and *Brassica carinata* seed extract residue treatments (Table 4.12).

Leaf length (LL)

The leaf length (LL) of enset plant across the different treatments ranged from 0.49 m to 0.59 m. In tolerant clone, the average LL in G_1, G_2 and G_3 treatments were 0.59, 0.59, 0.52 m, respectively. This shows application of G_1 and G_2 increased LL of V_1 by 12.7% and 11.6% respectively while, G_3 decreased LL by 1.3% compared with C_1V_1. Similarly, in

susceptible clone, on average 0.49, 0.60 and 0.57 m LL of enset were recorded in G_1, G_2 and G_3 treatments, respectively. Application of G_1, G_2 and G_3 decreased LL of susceptible clone by 18.03%, 0.5%, 5.01%, respectively compared to positive control.

Leaf width (LW)

The effect of *Brassica* species as a green manures and *Brassica carinata* seed extract residue on leaf width (LW) followed similar trend with other growth parameters such as PH, PSG, GLN and LL. Accordingly, application of G_2 was recorded the highest leaf width of 0.38 m in tolerant enset clone (Table 4.12). The lowest leaf width of enset across the treatments was 0.25 m in both tolerant and susceptible clones. In tolerant clone, the average leaf width in G_1, G_{21} and G_3 treatments were 0.29, 0.38, 0.29 m, respectively. This shows that G_2 treatments resulted in higher leaf width as compared to other green manure treatments. This means application of G_2 increased LW by 19.8%, while application of G_1 and G_3 decreased LW by 7.35% and 7.03% compared with C_1V_1, respectively. Similarly, in susceptible clone, average LW of 0.28, 0.29 and 0.35 m were recorded in G_1, G_2 and G_3 treatments, respectively. This shows that use of G_1, G_2 and G_3 increased LW of susceptible clone by 11.51%, 16.7%, 39.7%, respectively.

Leaf area (LA) and leaf area index (LAI)

Although the mean values were not statistically significant ($P<0.05$), total leaf area (LA) and leaf area index (LAI) were the highest in G_1 and G_2 green manure treatments for tolerant clones and G_2 and G_3 for susceptible clone as compared to other treatments in both clones (Table 4.12). Application of G_2 green manure treatments recorded the highest leaf

area of 1.02 m^2 for tolerant clones and G_3 green manure treatments recorded 0.78 m^2 for susceptible clones, respectively. Similarly, application of G_2 green manure treatments for tolerant clones and G_3 for susceptible clones recorded the highest leaf area index of 0.45 and 0.35, respectively. The result also showed that positive control treatments recorded the lowest leaf area index of 0.27 and 0.24 in tolerant and susceptible clones, respectively. In tolerant clone, the average leaf area in $G_{1,}$ G_{21} and G_3 treatments were 0.69, 1.02, 0.66 m^2, respectively. Similarly, in susceptible clone, the average leaf area in $G_{1,}$ G_2 and G_3 treatments were 0.59, 0.69 and 0.78 m^2, respectively. This shows that the G_2 and G_3 treatments resulted in higher leaf area as compared to other green manure treatments. Application of G_2 increased LA by 42.3%, while application of G_1 and G_3 treatments decreased LA by 2.7% and 8.8% compared with C_1V_1, respectively. Similarly, for susceptible clone, application of $G_{1,}$ G_2 and G_3 increased LA by 6.75%, 27.2%, 42.7%, respectively compared with C_2V_2.

In tolerant clone, the average leaf area index in $G_{1,}$ G_2 and G_3 treatments were 0.31, 0.45, and 0.29 respectively. Similarly, in susceptible clone, the average leaf area index in $G_{1,}$ G_2 and G_3 treatments were 0.26, 0.31 and 0.35, respectively. This shows that G_2 and G_3 treatments resulted in higher leaf area index as compared to other green manure treatment. Application of G_2 increased LAI by 42.3, while application of G_1 and G_3 treatments decreased LAI by 2.5% and 8.8% compared with C_1V_1, respectively. Similarly, for susceptible clone, application of $G_{1,}$ G_2 and G_3 increased LAI by 6.6%, 27.1%, 42.2%, respectively.

Table 4. 12. Growth parameters taken eight weeks after Xanthomonas inoculation

Treatments	PH (m)	PSG (m)	GLN	LL (m)	LW (m)	LA (m^2)	LAI
G1V1	0.789±0.217	0.535±0.068	7.889±1.094	0.593±0.135	0.290±0.011	0.699±0.095	0.311±0.042
G2V1	0.887±0.085	0.572±0.041	8.444±0.484	0.587±0.059	0.375±0.034	1.022±0.154	0.454±0.069
G3V1	0.794±0.071	0.458±0.107	8.000±0.694	0.519±0.063	0.291±0.037	0.655±0.102	0.291±0.045
C1V1	0.720±0.130	0.435±0.082	7.444±1.725	0.526±0.081	0.313±0.031	0.718±0.279	0.319±0.124
C2V1	0.859±0.152	0.488±0.070	7.778±0.401	0.519±0.083	0.265±0.034	0.612±0.177	0.272±0.079
G1V2	0.661±0.122	0.472±0.121	7.889±1.310	0.491±0.092	0.281±0.054	0.585±0.175	0.260±0.078
G2V2	0.762±0.097	0.469±0.057	7.222±0.294	0.596±0.076	0.294±0.025	0.697±0.108	0.310±0.048
G3V2	0.748±0.100	0.490±0.040	7.000±0.192	0.569±0.048	0.352±0.019	0.782±0.128	0.347±0.057
C1V2	0.681±0.118	0.419±0.072	6.667±0.694	0.599±0.104	0.252±0.025	0.548±0.117	0.244±0.052
C2V2	0.711±0.119	0.400±0.073	6.889±0.401	0.520±0.082	0.261±0.023	0.541±0.164	0.240±0.073
P-values	0.955	0.89	0.903	0.987	0.19	0.624	0.625

*None of the parameters is significant among treatments. PH = Plant height, PSG = Pseudostem girth, GLN = Green leaf number, LL = leaf length, LW = leaf width, LA = leaf area and LAI = Leaf area index.

In general, the data showed that there are no visible differences in plant height, pseudostem girth, leaf length, width, total leaf area and leaf area index between plants of the two enset clones before and after treatment application. The result indicated that in all morphological parameters *Brassica* species as a green manures and *Brassica carinata* seed extract residue treatments are not significantly different at ($P < 0.05$) compared to the positive control. Although they were not statically significant ($P < 0.05$) the treatments had better growth compared to the positive control. In addition, tolerant clone had better growth performance compared to susceptible clone.

4.4.1.2. Effects of *Brassica* species green manure and seed extract residue on physiological parameters of enset clones

In this section, the effects of *Brassica* species as green manure and *Brassica carinata* seed extract residue on the physiological parameters of two enset clones infected with *Xanthomonas campestris pv. musacearum* is presented (Table 4.13). The physiological responses of the tolerant(V_1) and susceptible enset clones (V_2) treated with *Brassica oleracea var capitata* (Cabbage*)* represented by (G_1) and *Brassica oleracea var acepala* (Tekur gomen) represented by (G_2) and *Brassica carinata* seed extract residue represented by (G_3) positive control represented by (C_1) and negative control represented by (C_2) on the control of bacterial wilt of enset under field condition were evaluated twice, the first measurement was taken four weeks after inoculation with *Xanthomonas campestris pv.musacearum* and the second measurement was taken eight weeks after inoculation. The physiological parameters measured include Relative water content (RWC), Assimilation rate (A), transpiration rate (E), intercellular CO_2 concentration (Ci), stomatal conductance (gas), Water use efficiency (WUE) and Chlorophyll contents.

4.4.1.2.1. Effects green manures and *Brassica carinata* seed extract residue on relative water contents (RWC)

The relative water content (RWC) of enset four weeks after inoculation ranged from 75.3% to 81.9% (Fig. 4.13). RWC of the two enset clones (V_1 and V_2) four weeks after treatment were not statistically different at $p<0.05\%$ (Fig 4.13). There was no significant difference

also at p<0.05% between treatments and positive control. However, RWC of treatments were higher than that of positive control. In tolerant clone, RWC of enset increased by 1.5%, 1.5% and 2.2% due to application of G_1 (80.1%), G_2 (80.1%) and G_3 (80.7%), respectively compared with positive control. Similarly, in susceptible clone, application of G_1 (75.3%) decreased RWC by 1.9%. However, use of G_2 (78.3%) and G_3 (81.9%) increased the RWC of susceptible clone by 2.1% and 6.8%, respectively compared with positive control.

After 8 weeks of inoculation, the relative water content (RWC) ranged from 60.6% to 86.7% (Fig 4.13). RWC of the two enset clones (V_1 and V_2) eight weeks after treatment were not statistically different at p<0.05% (Fig 4.13). Generally, the RWC of treatments was higher than positive control even if, there was no significant difference between the values. Accordingly, in tolerant clone (V_1), RWC of enset increased by 14.8%, 12.2% and 26.7% due to application of G_1 (69.9%), G_2 (68.3%) and G_3 (77.1%), respectively. Similarly, in susceptible clone, application of G_1 (70.6%), G_2 (69.1%) and G_3 (75.4%) increased RWC of susceptible clone by 16.5%, 14.2% and 24.5%, respectively. In general, the RWC of enset eight weeks after inoculation was lower compared to the RWC of four weeks after inoculation.

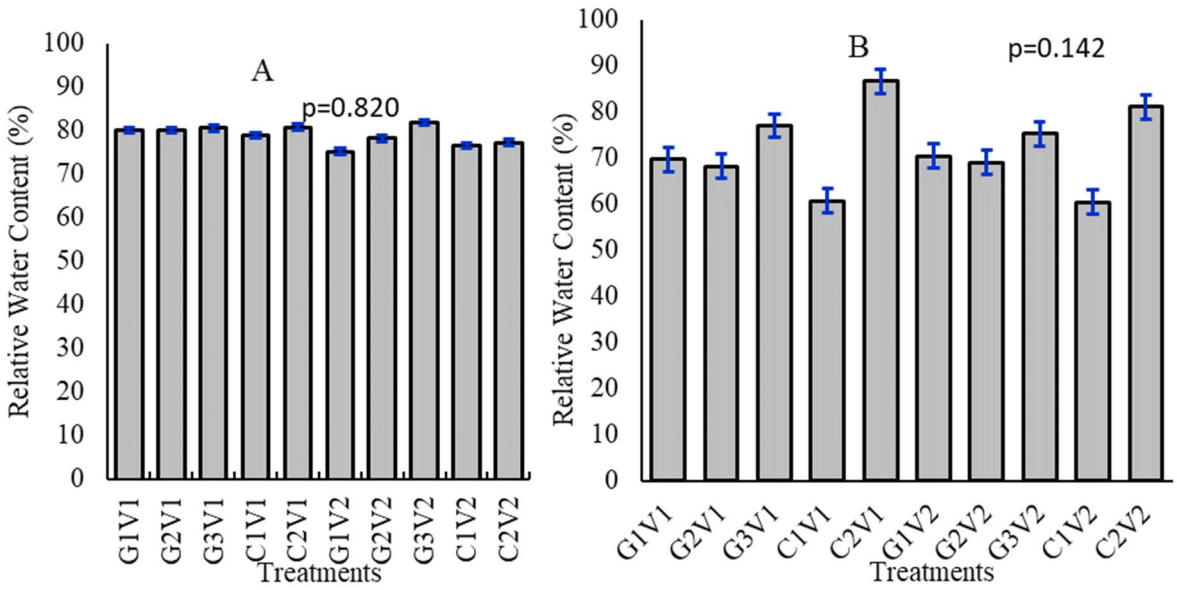

Figure 4. 13. Effect of green manures and Brassica carinata seed extract residue on relative water content (%) of enset. Error bars are standard errors of the means. A: Measurement 4 weeks after inoculation, and B: Measurement 8 weeks after inoculation.

4.4.1.2.2. Effects of green manures and *Brassica carinata* seed extract residue on Assimilation rate

The net assimilation rate of enset after 4 weeks of inoculation ranged from 0.55 to 1.29 µmol of CO_2 $m^{-2}s^{-1}$ (Fig 4.14). Net assimilation rate of the two enset clones (V_1 and V_2) four weeks after treatment application were statistically different at $p<0.05\%$ (Fig 4.14). In addition, net assimilation rate of G_1 green manure showed significantly ($P < 0.05$) higher assimilation rate compared to positive control. In susceptible clone, assimilation rate of enset increased by 134.2%, 60.9% and 65.9% due to application of G_1 (1.28 µmol of CO_2 $m^{-2}s^{-1}$), G_2V (0.88 µmol of CO_2 $m^{-2}s^{-1}$) and G_3 (0.91µmol of CO_2 $m^{-2}s^{-1}$) respectively compared with positive control. Similarly, in susceptible clone, application of G_1 (0.72 µmol of CO_2 $m^{-2}s^{-1}$), G_2 (0.62 µmol of CO_2 $m^{-2}s^{-1}$) and G_3 (0.63 µmol of CO_2 $m^{-2}s^{-1}$)

increased the assimilation rate of by 21.3%, 4.5% and 4.8% respectively compared with positive control.

Assimilation rate after eight weeks of inoculation ranged from 0.34 m to 1.39 µmol of CO_2 $m^{-2}s^{-1}$ (Fig. 4.14). Net assimilation rate of the two enset clones eight weeks after treatment application were statistically different at $p<0.05\%$ (Fig 4.14). In addition, G_3 in susceptible enset clone was the only treatments significantly different at $p<0.05\%$ compared to the positive control. In other treatments, assimilation rates were higher than infected control even if it was not statically significant. Accordingly, tolerant clone, assimilation rate of enset increased by 9.86%, 6.1% and 6.6% due to application of G_1 (0.78 µmol of CO_2 $m^{-2}s^{-1}$), G_2 (0.75 µmol of CO_2 $m^{-2}s^{-1}$) and G_3 (0.76 µmol of CO_2 $m^{-2}s^{-1}$), respectively as compared to positive control. Similarly, in susceptible clone, application of G_1 (0.44 µmol of CO_2 $m^{-2}s^{-1}$), G_2 (0.38 µmol of CO_2 $m^{-2}s^{-1}$) and G_3 (0.74 µmol of CO_2 $m^{-2}s^{-1}$) increased the assimilation rate of V_2 by 28.4%, 12.7% and 117.7%, respectively. In general, Assimilation rate of the enset after 8 weeks inoculation was lower compared to assimilation rate of enset after 4 weeks of inoculation.

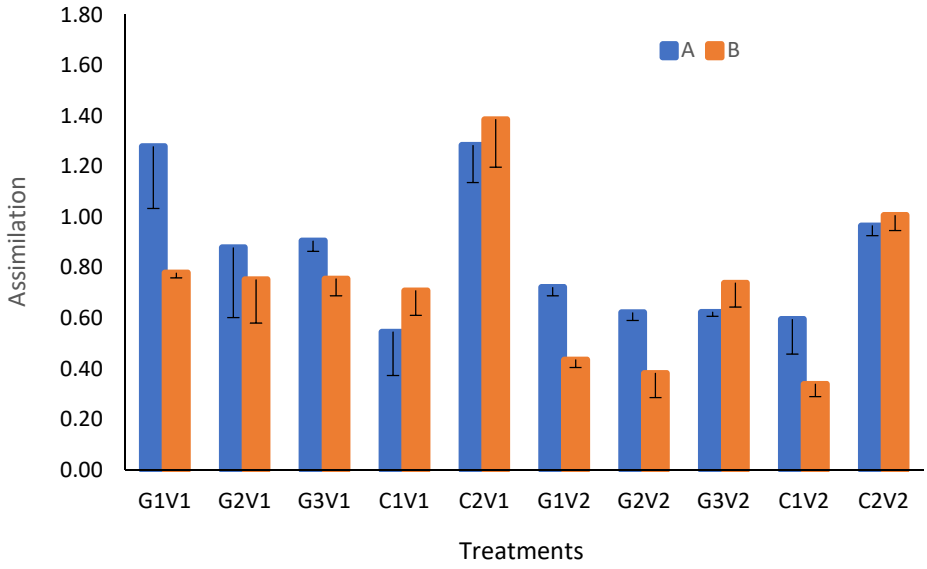

Figure 4. 14. Effect of Brassica species green manures and Brassica carinata seed extract residue on assimilation rate. A: 4 weeks after inoculation and B: eight weeks after inoculation.

4.4.1.2.3. Effects of *Brassica* species green manures and *Brassica carinata* seed extract residue on Transpiration (E), intercellular CO_2 concentration (Ci), stomatal conductance (gs) and water use efficiency (WUE)

Transpiration (E)

After 4 weeks of inoculation measurement, transpiration rate (E) ranged from 1.8 to 3.2 mmol H_2O m^{-2} s^{-1} (Table. 4.13). The result of this study shows that transpiration rate of two enset clones (V_1 and V_2) were not statistically different at $p<0.05\%$ (Table. 4.13). Transpiration rate of positive control was not significantly different ($p<0.05$) with all treatments for both resistant and susceptible clones. Accordingly, in tolerant clone, transpiration rate of enset increased by 29.5%, 10.2% and 12.1% due to application of G_1 (2.89 mmol H_2O m^{-2} s^{-1}), G_2 (2.5 mmol H_2O m^{-2} s^{-1}) and G_3 (2.5 mmol H_2O m^{-2} s^{-1}),

respectively. Similarly, in susceptible clone, application of G_1 (3.1 mmol H_2O m^{-2} s^{-1}) and G_3 (3.2 mmol H_2O m^{-2} s^{-1}) increased transpiration by 7.8% and 12.7%, respectively. While, G_2 (2.8 mmol H_2O m^{-2} s^{-1}) decreased transpiration rate of susceptible clone by 1.8%.

Eight weeks after inoculation, transpiration rate (E) ranged from 1.77 to 2.95 mmol H_2O m^{-2} s^{-1} (Table. 4.13). The transpiration rate of the two enset clones (V_1 and V_2) were not statistically different at $p<0.05$% (Table. 4.13). The transpiration rate of positive control was not significantly different ($p<0.05$) with all treatments for both resistant and susceptible clones. In tolerant clone, transpiration rate of enset increased by 66.6%, 56.9% and 23.9% due to application of G_1 (2.95 mmol H_2O m^{-2} s^{-1}), G_2 (2.78 mmol H_2O m^{-2} s^{-1}) and G_3 (2.19 mmol H_2O m^{-2} s^{-1}), respectively as compared to positive control. Similarly, in susceptible $_{clone}$, application of G_1 (2.37 mmol H_2O m^{-2} s^{-1}), G_2 (2.64 mmol H_2O m^{-2} s^{-1}) and G_3 (2.91 mmol H_2O m^{-2} s^{-1}) increased transpiration rate of enset by 17.6%, 30.9% and 44.4 %, respectively compared with positive control. In general, transpiration rate of enset after 8 weeks of inoculation was lower than transpiration rate of enset after 4 weeks of inoculation.

Intercellular CO_2 concentration (Ci)

After four weeks of inoculation, intercellular CO_2 concentration (Ci) ranged from 381.0 to 409.7 µmol CO_2 mol^{-1} (Table. 4.13). The intercellular CO_2 concentration of two enset clones (V_1 and V_2) was not statistically different at $p<0.05$% (Table. 4.13). The intercellular CO_2 concentration of positive control was also not significantly different ($p<0.05$) with all treatments for both resistant and susceptible clones. Accordingly, in tolerant clone,

intercellular CO_2 concentration of enset decreased by 6.3%, 5.3% and 1.5% due to application of G_1 (384 µmol CO_2 mol^{-1}), G_2 (388.1 µmol CO_2 mol^{-1}) and G_3 (403.6 µmol CO_2 mol^{-1}) respectively compared with positive control. Similarly, susceptible clone, application of G_1 (386.9) decreased intercellular CO_2 concentration of enset by 2.1%., while application of G_2 (395.9 µmol CO_2 mol^{-1}) and G_3 (409.4 µmol CO_2 mol^{-1}) increased intercellular CO_2 concentration by 0.22% and 3.6%, respectively compared with the control.

After 8 weeks of inoculation, intercellular CO_2 concentration (Ci) ranged from 318.1 to 403.9 µmol CO_2 mol^{-1} (Table. 4.13). The intercellular CO_2 concentration of the two enset clones (V_1 and V_2) was not statistically different at $p<0.05$% (Table. 4.14). The intercellular CO_2 concentration of positive control was also not significantly different ($p<0.05$) with all treatments for both resistant and susceptible clones. Accordingly, tolerant clone$_1$, intercellular CO_2 concentration of enset was increased by 10.9% and 5.6% due to application of G_1 (366.3 µmol CO_2 mol^{-1}) and G_2 (348.4 µmol CO_2 mol^{-1}) while, intercellular CO_2 concentration of enset was decreased by G_3 (328.7) respectively compared to the control. Similarly, in susceptible, application of G_1 (341 µmol CO_2 mol^{-1}) and G_3 (318.1 µmol CO_2 mol^{-1}) decreased intercellular CO_2 concentration by 0.53% and 7.2%, respectively compared with the positive control. In general, intercellular CO_2 concentration measurement eight weeks after inoculation was lower compared with measurement four weeks after inoculation.

Stomatal conductance (gs)

After four weeks of inoculation, stomatal conductance (gs) ranged from 0.13 mol H_2O m^{-2} s^{-1} to 0.24 mol H_2O m^{-2} s^{-1} (Table 4.13). The result of this study shows that the Stomatal conductance of two enset clones (V_1 and V_2) were not statistically different at $p<0.05\%$ (Table. 4.13). Stomatal conductance of positive control was not significantly different ($p<0.05$) with all treatments for both resistant and susceptible clones. Accordingly, in tolerant clone, stomatal conductance was increased by 24.7%, 11.3% and 3.3% due to application of G_1 (0.19 mol H_2O m^{-2} s^{-1}), G_2 (0.17 mol H_2O m^{-2} s^{-1}) and G_3 (0.16 mol H_2O m^{-2} s^{-1}), respectively compared with the positive control. Similarly, susceptible clone, application of G_1 (0.17 mol H_2O m^{-2} s^{-1}), G_2 (0.16 mol H_2O m^{-2} s^{-1}) and G_3 (0.24 mol H_2O m^{-2} s^{-1}) increased transpiration rate by 15.3%,8.7%and 60%, respectively compared with the positive control.

After 8 weeks of inoculation, stomatal conductance (gs) ranged from 0.037-0.224 mol H_2O m^{-2} s^{-1} (Table 4.13). The result of this study shows that the Stomatal conductance of two enset clones (V_1 and V_2) were not statistically different at $p<0.05\%$ (Table. 4.13). Stomatal conductance of positive control was not also significantly different ($p<0.05$) with all treatments for both resistant and susceptible clones. Accordingly, in tolerant clone, Stomatal conductance of enset was increased by 126.2%, 117.9% and 63.5% due to application of G_1 (0.083 mol H_2O m^{-2} s^{-1}), G_2 (0.08 mol H_2O m^{-2} s^{-1}) and G_3 (0.06 mol H_2O m^{-2} s^{-1}), respectively compared with positive control. Similarly, in susceptible clone, application of G_1 (0.053 mol H_2O m^{-2} s^{-1}), G_2 (0.063 mol H_2O m^{-2} s^{-1}) and G_3 (0.074 mol

H_2O m^{-2} s^{-1}) increased transpiration rate by 44.4%,71.7%and 101.6%, respectively compared with positive control.

Water use efficiency (WUE)

Four weeks after inoculation, water use efficiency (WUE) ranged from 0.213 to 0.78 µmol of CO_2/ mmol of H_2O (Table. 4.13). Water use efficiency of positive control was not significantly different ($p<0.05$) with all treatments for both resistant and susceptible clones (Table 4.13). In tolerant clone, WUE increased by 75.8%, 47.3% and 46.2% due to application of G_1 (0.46 µmol of CO_2/ mmol of H_2O), G_2 (0.38 µmol of CO_2/ mmol of H_2O) and G_3 (0.38 µmol of CO_2/ mmol of H_2O), respectively compared with positive control. While, in susceptible clone, application of G_1 (0.233 µmol of CO_2/ mmol of H_2O), G_2 (0.223 µmol of CO_2/ mmol of H_2O) and G_3 (0.213 µmol of CO_2/ mmol of H_2O) decreased Water use efficiency by 10.4%, 14.2% and 18.1%, respectively compared with positive control.

After 8 weeks of inoculation, WUE ranged from 0.143 to 0.68 µmol of CO_2/ mmol of H_2O (Table. 4.13). WUE of positive control was not significantly different ($p<0.05$) from all treatments in both tolerant and susceptible clones. In tolerant clone, WUE decreased by 39.7%, 34.2% and 13.5% due to application of G_1 (0.26 µmol of CO_2/ mmol of H_2O), G_2 (0.29 µmol of CO_2/ mmol of H_2O) and G_3 (0.38 µmol of CO_2/ mmol of H_2O), respectively. Similarly, susceptible clone, application of G_1 (0.19 µmol of CO_2/ mmol of H_2O), G_2 (0.14 µmol of CO_2/ mmol of H_2O) and G_3 (0.27 µmol of CO_2/ mmol of H_2O) decreased WUE

by 55.7%, 67.2% and 38.2%, respectively compared with positive control. In general, WUE after 8 weeks of inoculation was lower than WUE after 4 week of inoculation.

The photosynthetic assimilation rate, transpiration rate, intercellular CO_2 concentration (Ci), stomatal conductance (gs) and water use efficiency of the two enset clones are presented in Table 4.13. Even if transpiration rate, stomatal conductance and water use efficiency of all treatments were greater than infected control, there were no significant differences in both resistance and susceptible enset clones (Table 4.13). Intercellular CO_2 concentration of all treatments was lower than positive control even if the values were not statically significant (Table. 4.13).

Table 4. 13. Effect of green manures and *Brassica carinata* seed extract residue on Transpiration (E), intercellular CO_2 concentration (Ci), stomatal conductance (gs) and water use efficiency (WUE).

Treatments	E (mmol H_2O m^{-2} s^{-1})	Ci (µmol CO_2 mol^{-1})	g_s (mol H_2O m^{-2} s^{-1})	WUE (µmol of CO_2/ mmol of H_2O)
4 weeks after inoculation				
G1V1	2.887±0.311	384.000±28.457	0.187±0.026	0.457±0,102^{a}
G2V1	2.457±0.413	388.133±20.938	0.167±0.067	0.383±0.120^{a}
G3V1	2.500±0.408	403.600±13.436	0.155±0.012	0.380±0.061^{a}
C1V1	2.230±0.219	409.667±6.742	0.150±0.029	0.260±0.090^{a}
C2V1	1.827±0.439	381.007±0.696	0.125±0.040	0.780±0.175^{b}
G1V2	3.077±0.144	386.940±7.643	0.173±0.022	0.233±0.007^{a}
G2V2	2.803±0.189	395.940±9.425	0.163±0.013	0.223±0.003^{a}
G3V2	3.217±0.778	409.407±13.297	0.240±0.035	0.213±0.043^{a}
C1V2	2.855±0.372	395.080±19.397	0.164±0.022	0.227±0,076^{a}
C2V2	2.531±0.438	403.267±6.839	0.207±0.023	0.413±0.088^{a}
p-value	0.447	0.861	0.502	0.007
8 weeks after inoculations				
G1V1	2.953±0.102	366.267±5.348^{bc}	0.083±0.009^{a}	0.263±0.007^{ab}
G2V1	2.783±0.712	348.400±7.905^{ab}	0.080±0.020^{a}	0.287±0.045^{ab}
G3V1	2.197±0.330	328.733±13.269^{ab}	0.060±0.006^{a}	0.377±0.102^{ab}
C1V1	1.773±0.509	330.000±7.095^{ab}	0.0367±0.012^{a}	0.436±0.061^{b}
C2V1	2.323±0.566	397.000±15.475^{cd}	0.146±0.020^{b}	0.680±0.171^{c}
G1V2	2.370±0.263	341.000±2.107^{ab}	0.053±0.009^{a}	0.193±0.035^{ab}
G2V2	2.640±0.571	343.333±6.47^{ab}	0.063±0.019^{a}	0.143±0.007^{a}
G3V2	2.912±0.621	318.133±17.391^{a}	0.074±0.004^{a}	0.270±0.050^{ab}
C1V2	2.016±0.463	342.803±8.956^{ab}	0.043±0.015^{a}	0.183±0.039^{ab}
C2V2	2.792±0.549	403.900±16.894^{d}	0.224±0.015^{c}	0.397±0.087^{ab}
P-values	0.745	0.001	0.000	0.004

*Means with different superscripts within the same column are significantly different at $p<0.05$.

4.4.1.2.4. Effects on chlorophyll content

Measurement of leaf chlorophyll content measured using chlorophyll meter (SPAD) after 4 weeks of inoculation ranged from 31.4 to 48.9 (Fig 4.15). The SPAD value of the two enset clones (V_1 and V_2) were not statistically different at $p<0.05\%$ (Fig. 4.15). In tolerant clone, the SPAD value of positive control was significantly lower ($p<0.05$) than the SPAD values of enset under G1, G2 and G3 treatments. But in susceptible clones, G_1 and G_2 were the only treatments which were significantly different ($p<0.05$) from positive control. In tolerant clone, SPAD value of enset was increased by 43.7%, 47.6% and 39.6% due to application of G_1 (47.6), G_2 (48.9) and G_3 (46.2) respectively as compared to positive control. Similarly, in susceptible clone, application of G_1 (48.6), G_2 (47.7) and G_3 (38.5) increased the SPAD value by 46.8% and 43.95% and 16.31%, respectively compared with positive control.

After 8 weeks of inoculation, in tolerant clone, the SPAD value of positive control was significantly different ($p<0.05$) from SPAD value under G_1 and G_2 treatments. In susceptible clone, the SPAD value of positive control was significantly different ($p<0.05$) from SPAD values under G_1 and G_3 treatments. In tolerant clone, SPAD value of enset increased by 33.9%, 23.9% and 17.8% due to application of G_1 (40.5), G_2 (37.4) and G_3 (35.6), respectively compared to positive control. Similarly, in susceptible clone, application of G_1 (38.0) and G_3 (31.8) increased SPAD value by 25.8% and 5.36%, respectively compared with positive control. While, G_2 (29.9) decreased SPAD value of susceptible clone by 1.14%.

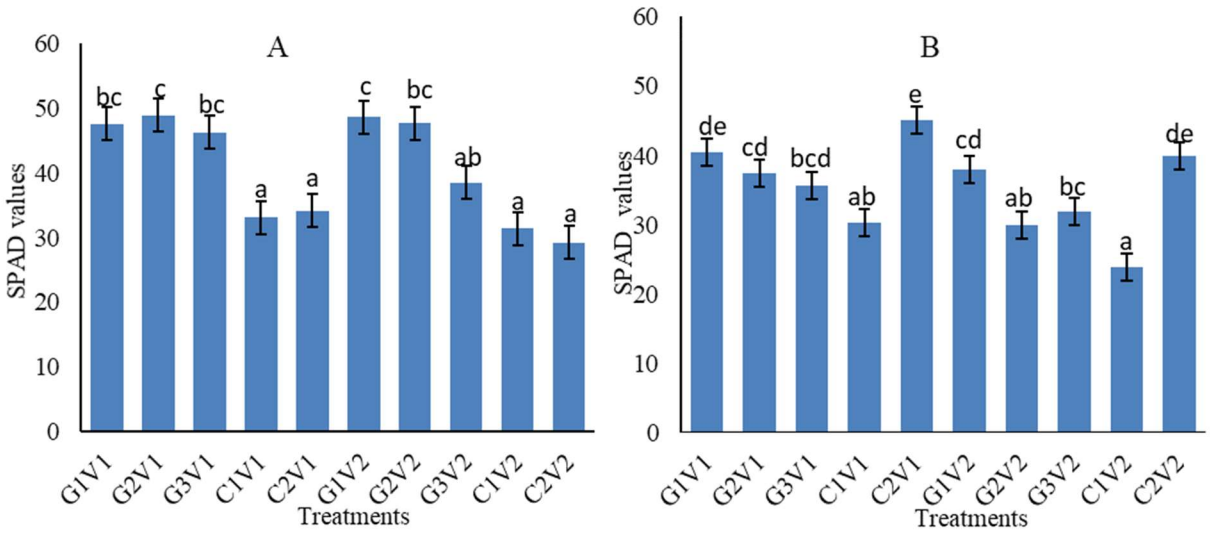

Figure 4. 15. Effect of green manures and Brassica carinata seed extract residue on chlorophyll content measured by SPAD. Means with different letters are significantly different at $p<0.05$.

4.4.2. Effect of *Brassica* species green manure and seed extract on disease incidence, severity and AUDPC of *Xanthomonas campestris* pv. *musacearum* on enset clones

4.4.2.1. Disease incidence

The effect of *Brassica* species as green manures and *Brassica carinata* seed extract residue on disease incidence of Xcm was observed for 84 days by weekly interval. In most of the treatments, disease symptom was observed between 14 and 49 days after inoculation (Fig 4.16). This means between 14 and 49 days after inoculation, all inoculated plants had exhibited typical disease symptoms such as chlorosis, necrosis and wilting of leaves. The average disease incidence of enset inoculated with *Xanthomonas* ranged from 60% to 82.4%. The result showed that as compared to positive control, application of G_1, G_2 and G_3 reduced the disease incidence of tolerant enset clone and for susceptible enset clone G_2

and G_3 treatments have lower disease incidence. Accordingly, tolerant enset clones treated with G_1, G_2 and G_3 *Brassica* species as green manures and *Brassica carinata* seed extract residue had 63.1%, 60%, 60.2% disease incidence, respectively which is lower than the positive control (78.3%). Similarly, susceptible enset clones treated with G_1, G_2 and G_3 *Brassica* species as green manures and *Brassica carinata* seed extract residue had 76.9%, 72.3%, 62.2% disease incidence, respectively while the positive control had 82.4% disease incidence. This means, compared to the positive control, G_1, G_2 and G_3 decreased the disease incidence of tolerant enset clones by 19.4%, 23.3% and 23.1%, respectively. In the same way, application of G_1, G_2 and G_3 treatments decreased the disease incidence of susceptible enset clones by 6.7 %, 12.3 %, and 4.6%, respectively.

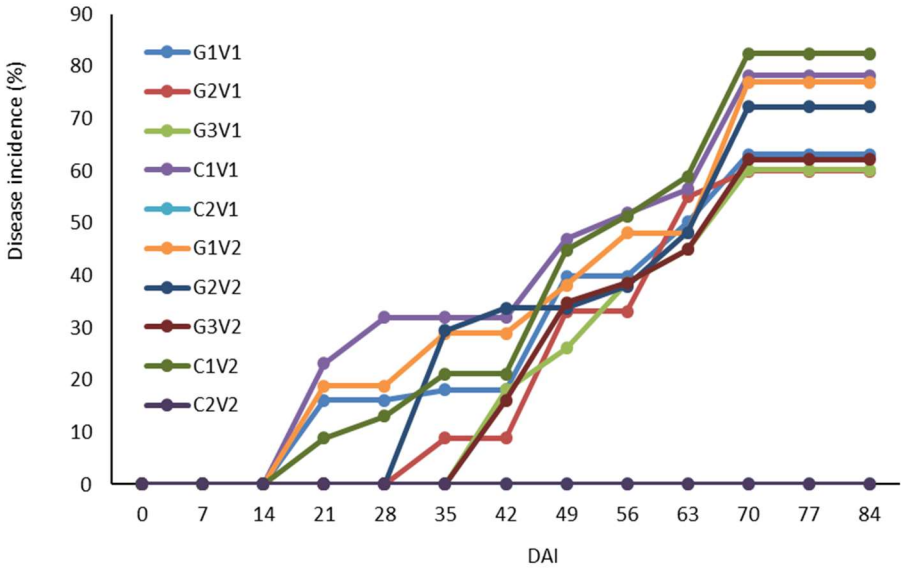

Figure 4. 16. Effects of Brassica species as green manures and Brassica carinata seed extract residue on Xanthomonas campestris pv. musacearum disease incidence of enset.

4.4.2.2. Disease severity and area under disease progress curve (AUDPC)

The effect of *Brassica* species as green manure and *Brassica carinata* seed extract residue on disease severity of Xcm was observed for 84 days by weekly interval (Fig 4.17). Similar to disease incidence, disease severity was observed between 14 and 49 days after inoculation (Fig 4.17). As shown in Table 4.14, the average disease severity of enset inoculated with Xanthomonas ranged from 39.2 % to 47.9 %. The result showed that application of G_1, G_2 and G_3 reduced the disease severity of tolerant and susceptible enset clone. Accordingly, tolerant enset clones treated with G_1, G_2 and G_3 *Brassica* species as green manure and *Brassica carinata* seed extract residue had 41.9%, 40.8 %, 39.2% disease severity, respectively which is lower than the positive control (44.3 %). Similarly, susceptible enset clones treated with G_1, G_2 and G_3 *Brassica* species as green manure and *Brassica carinata* seed extract residue had 42.4%, 43.7%, 43.1% disease severity, respectively while the positive control had 47.9% disease severity. This indicates that disease severity of tolerant enset clone with G_1, G_2 and G_3 application decreased severity by 5.3 %, 7.8% and 11.4%, respectively as compared to positive control. Similarly, application of G_1, G_2 and G_3 treatments decreased the disease severity of susceptible enset clones by 11.4 %, 8.6 %, and 10 %, respectively.

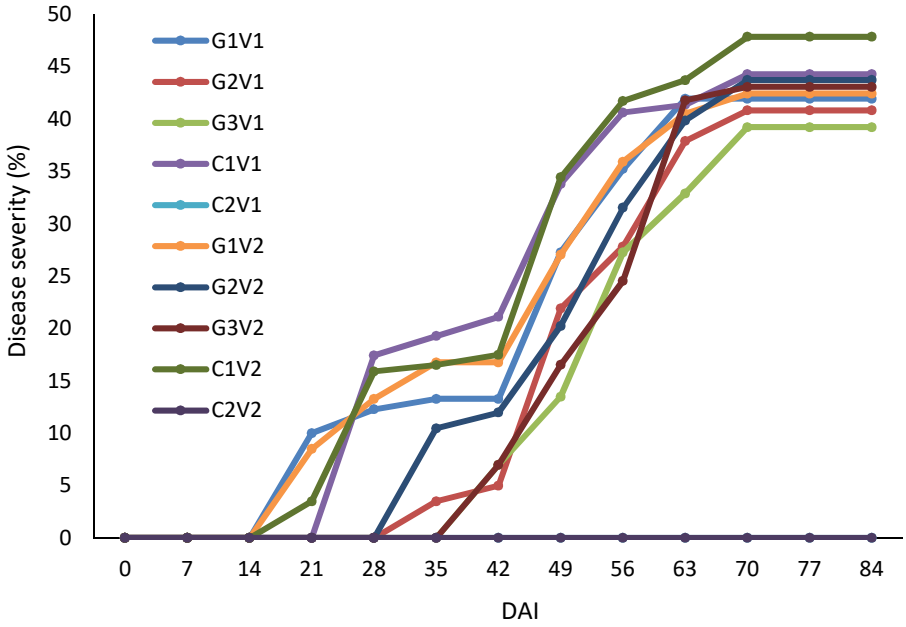

Figure 4. 17. Effects of Brassica species as green manures and Brassica carinata seed extract residue on Xanthomonas campestris pv. musacearum disease severity of enset.

Area under disease progress curve (AUDPC) of the two enset clones is presented in Table 4.14. Generally, AUDPC of susceptible enset clone is higher than tolerant enset clones. The highest AUDPC value (1828.9) was recorded in susceptible clone with positive control while the lowest AUDPC value (976.5) was recorded on tolerant treated with G3 treated with *Brassica carinata* seed extract residue which minimized the AUPDC value by 45.3% compared to positive control (Table 4.14). The AUDPC value was significantly different ($p< 0.005$) between positive control and treatments for both tolerant and susceptible clones. In the tolerant clone, the AUDPC value with G_3 application was significantly lower than the AUDPC in the positive control (C_1V_1). Disease progress was rapid on susceptible clone, whereas it was relatively slow progress was recorded on tolerant enset clones (Table 4.14).

Table 4. 14. Arcsine transformed disease severity and area under disease progress curve (AUDPC) of enset clones infected with Xcm and treated with *Brassica* species as green manure and *Brassica carinata* seed extract residue.

Treatments	Disease severity (%) DAI													AUDPC
	0	7	14	21	28	35	42	49	56	63	70	77	84	
G_1V_1	0	0	0	10.000	12.290	13.274	13.274	27.281	35.244	41.957	41.957	41.957	41.957±0.333^{cd}	1220.110±182.197^{b}
G_2V_1	0	0	0	0.000	0.000	3.489	5.000	21.911	27.828	37.894	40.837	40.837	40.837±0.018^{bc}	1101.642±121.243^{b}
G_3V_1	0	0	0	0.000	0.000	0.000	6.977	13.489	27.281	32.898	39.232	39.232	39.232±0.00^{b}	976.459±45.385^{b}
C_1V_1	0	0	0	13.444	24.874	28.780	29.891	35.971	40.667	41.381	44.293	44.293	44.293±0.038^{d}	1784.009±77.935^{c}
C_2V_1	0	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000±0.000^{a}	0.000±0.000^{a}
G_1V_2	0	0	0	8.489	13.275	16.763	16.763	27.055	35.925	40.511	42.433	42.433	42.433±1.282^{cd}	1364.980±182.276^{bc}
G_2V_2	0	0	0	0.000	0.000	10.466	11.978	20.239	31.536	39.871	43.739	43.739	43.739±0.063^{d}	1257.889±58.282^{b}
G_3V_2	0	0	0	0.000	0.000	0.000	6.977	16.529	24.556	41.794	43.077	43.077	43.077±1.922^{cd}	1094.767±249.205^{b}
C_1V_2	0	0	0	3.489	8.040	16.516	17.501	34.446	41.742	43.719	47.870	47.870	47.870±0.384^{e}	1828.993±275.455^{c}
C_2V_2	0	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000±0.000^{a}	0.000±0.000^{a}

*Means with different superscripts within the same column are significantly different at $p<0.05$. DAI: Days after inoculation, AUDPC: Area under disease progress curve.

CHAPTER 5

5. Discussion

5.1. Assessment of disease prevalence and incidence of enset bacterial wilt in the study area

Bacterial wilt of enset (EBW) was widespread and destructive in all the surveyed areas although the extent varied across altitude and seasons, the disease being most prevalent in mid and high altitude compared to low altitude. The association of wilt incidence to altitude could be attributed to the characteristics of *Xanthomonas campestris pv. musacearum* (Xcm) in its moisture and temperature requirement. Similarly, high temperature and high soil moisture promote survival, reproduction, infectivity, and spread of the bacterium, and hence disease development (Harris, 1976; Martin and French, 1985; Smith *et al.*, 2008). Dereje Ashagri (1985) and Maina *et al*. (2006) also have reported that the pathogen requires humid condition for survival. High moisture, whether in the form of rain, dew, or high humidity, is the dominant factor for the development of most epidemics diseases caused by bacteria (Agrios, 2005). Agrions (2005) also explained that moisture not only promotes new succulent and susceptible growth in the host, but, more importantly, it increases multiplication of bacteria. Moreover, moisture facilitates the oozing of bacteria to the host surface, and it enables spores to infect the plant by bacterial pathogen to colonize with in the host.

The increase in disease prevalence and incidence at mid altitude in this study may be attributed to existence of suitable moisture, temperature and soil conditions for EBW growth and development. The result is in agreement with Brandt *et al.* (1997) who found out that BW was severe at highland altitude than lowland altitude. Similarly, Mania *et al*. (2006) also reported that the disease was severe at midland in banana. The results are in agreements with the report of Mekuria Wolde *et al.* (2016a) where a maximum mean incidence was recorded in the altitude of 2000-2500 m.a.s.l. and minimum mean incidence was recorded in an altitude of less than 2000 m.a.s.l. The high EBW incidence in Cheha districts representing mid altitude might be attributed to management practices, environmental factors and awareness of the farmer for transmission and management. Similarly, the association of EBW incidence with enset growing areas (administrative zones and districts) could be attributed to enset production and management system, type of clone grown and environmental effect. Moreover, in some areas of Mirab Azert district representing high altitude sites, fields were completely destroyed due to the disease and farmers were forced to replace the field with other crops. In line with this, in some study areas, where the severity of the disease and loss was high, farmers were obliged to abandon the whole field and replace it with other crops such as barley, maize, wheat, as well as developing enset corm again to replace the damage of enset due to high incidence of the disease (Arehaido, 1992 cited in Mengistu Oli *et al*., 2014). In contrast to the present result, greater disease incidence and prevalence was reported in lower altitude areas compared with high altitude areas. For example, a study was conducted by Mengistu Oli *et al*. (2014) in Tikur Inchini and Jibat districts of Ethiopia with annual rainfall range of 1000-1900 mm and temperature range of 6- 24^0C. The result showed 32, 31 and 18% disease incidence at

lower (2300-2500 m a.s.l.), middle (2500-2700 m a s l) and higher (2700-2900 m a.s.l.) altitude areas, respectively. The same study reported 88, 89, 75% disease prevalence at lower, middle and higher altitude areas, respectively (Mengistu Oli *et al*., 2014). Generally, such inconsistent results imply that in addition to altitude other factors such as soil condition, moisture, humidity and temperature are essential for the development of EBW.

The result of seasonal based survey showed that cumulative disease incidence of enset bacterial wilt reached maximum in July. Moreover, the maximum change in disease prevalence was recorded between January and April. This is mainly because months between January and April have high temperature and suitable moisture from the small rains in this season. The study also showed that there was a reduction in the severity of the disease from first year (2014) to the second year (2015). This may be due to the increase in the awareness of farmers regarding EBW management over time. This is in agreement to Desalegn Regasa and Addis Shiferaw (2015) who reported that there was a reduction in the severity of the disease from first year to the second year due to the awareness given to the farmers during the first year in the management of the disease such as rouging out the diseased plant from the farm.

According to farmers, Yeshrakinkye, Nechewe, Anikefye, Astra and Badedate enset clones were believed to be tolerant to Xcm. This farmers' view is in line with Anita *et al.* (1996) that Yeshrakenkye was categorized as tolerant clone. Gizachew Welde-Michael (2000) also reported that certain enset clones such as Yeshirekinke in Gurage, Mezia in Wolaita, Ado and Genticha in Sidama, Siskela and Gimbo in Hadya and Nobo in Keficho have

relatively high tolerance against bacterial wilt. However, Mekuria Wolde *et al.* (2016b) reported Yeshrakinkye and Badedat as moderately tolerant to Xcm. Anikefye and Nechwe were categorized as relatively tolerant to Xcm (Gizachew Welde-Michael *et al.*, 2008b). These variations may have been caused by a variation in Xcm isolates used for inoculation. The variations among isolates were observed in preliminary laboratory and field experiments (Kidist Bobosaha, 2003). Farmers in the study area also identified Yeregye, Ameratey, Ginbwe, Kenbat and Agade as susceptible to enset bacterial wilt by farmers. Similarly, Dereje Ashagri (1985) and Mekuria Wolde *et al.* (2016b) reported that Agade was susceptible to Xcm.

The survey result showed that there were various cultural practices that increase the spread of EBW. These include the use of contaminated tools and planting diseased material, grazing cattle in the infected field and lack of information about the spreading mechanisms of enset bacterial wilt. It is clearly showed that many farmers (47%) knew the contaminated farm tools contribute to the rapid spread of Xcm. According to Dereje Ashagri (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. The latent nature of Xcm especially in the early stages may lead farmers to plant infected suckers and this may increase the spread of the disease across farms and regions. Hayward (2006) reported that suckers are an important means of spreading for systemic bacterial diseases. Farmers also believe that animals such as cattle moving through infected fields, could also contribute to the spread of enset bacterial wilt. The finding is in agreement with Befekadu Haile (2012), that the most important factors responsible for spreading disease of bacterial wilt were use

of contaminated tools (46%), grazing cattle in the infected field (26%) and use of diseased planting material (15%). Similarly, Zerihun Yemataw *et al*. (2017) reported that majority of respondents (70–80%) identified contaminated tools, diseased plant debris, insects and animals as principal means of EBW wilt transmission.

Although there are several cultural practices for spreading EBW, farmers employed different strategies to control the disease. Removal of infected plants together with burning after observation of symptom was the most commonly practiced option by 40% of the interviewed farmers to control EBW in the study areas. Moreover, removal of infected plants and removal of infected plant together with burying were also important practices by famers. This result is in agreement with reports of Million Tadesse *et al.* (2003) and Mekuria Wolde *et al* (2016b) who found out that enset farmer practiced removal of infected plants, buried or discarded of infected suckers and crop rotation to manage the disease. In this connection, early detection and destruction of the diseased plants is a key step in preventing bacterial disease spread (Karamura *et al*., 2005).

5.2. Antibacterial Activity of *Brassica* species against *Xanthomonas campestris pv. musacearum*

Brassica species have long been known for their antimicrobial activity against various microorganisms, including Gram-positive and Gram-negative bacteria and fungi (Jaiswal *et al*., 2011). Recently, biofumigation is used as an approach to control multiple bacterial and fungal soil-borne pathogens using *Brassica* spp. as green manure or as seed meal amendment has been receiving increased attention (Kirkegaard and Sarwar, 1998; Xiao *et*

al., 1998; Smolinska, 2000; Matthiessen and Kirkegaard, 2003). In addition, crude extracts of *Brassica* spp are effective in inhibiting the growth of pathogens (Matthiessen and Kirkegaard, 2003). The result of the present study showed that there was wide variation in extract yield of *Brassica* species using aqueous methanol. The highest extract yield was obtained from the leaves of *Brassica oleracea* (NG) (48.6%) followed by *Brassica carinata* (46.2%), while the lowest was obtained from *Raphanus sativus* (Radish) (18.2%) and *Brassica nigra* (Black mustard) (20.9%). Thus variation in extract yield might be resulting from varied chemical composition of plants (Sultana *et al*., 2009). In addition, the type and concentration of solvents affect the extract yield of the plant (Chatha *et al*., 2006 and Sultana *et al.,* 2009). Similarly, Anwar, *et al.* (2013) reported that cauliflower (*Brassica oleracea* L.) with 80% methanol gave 30% extract yield. Mustard (*Brassica juncea*) with 70% methanol gave 38.7% extract yield (Huang *et al*., 2012). Similarly, Chatha *et al*. (2006); Chan *et al*. (2009); and Katsube *et al*. (2009) reported that methanol and ethanol with some water content (typically 20 - 40%) have been found to be superior in extracting antioxidant compounds from a wide range of plants. Effects of extracting solvent on the extract yield of different medicinal plants have reported by different authors. Chatha *et al*. (2006) and Sultana *et al.,* (2009) reported that aqueous methanol (methanol: water, 80:20 v/v) gave the highest extract yield compared to absolute methanol, absolute ethanol and aqueous ethanol.

The extracts of *Brassica oleracea var capitata* (Cabbage) and *Brassica oleracea var acepala* (Tekur gomen) formed significantly the widest bacterial growth inhibition zone at (400 and 200 mg/mL) compared to other lower concentration. The presence of antibacterial

compounds in the extracts is evident from the results of the phytochemical screening that shows the presence of different secondary metabolites in the leaf extracts of *Brassica oleracea var capitata* (Cabbage) and *Brassica oleracea var acepala* (Tekur gomen). Similarly, Zamir *et al*. (2013) reported that, the methanol extract of *Brassica oleracae* exhibited distinct zones of inhibition towards bacterial strains including for *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Escherichia coli and Proteus* against the methanol control which did not show any growth inhibitory zone. Moreover, Lewis and Papavizas (1970) demonstrated *in vitro* studies that volatile products from decomposing cabbage tissues inhibit hyphal growth of *Aphanomyces euteiches*. Ozusaglam and Karakoca (2013) reported that methanol and ethanol extracts of *Brassica oleracea* var. *gongylodes* showed better antibacterial activities against *Vibrio alginolyticus* and *Aeromonas hydrophila*. Agrawal *et al*. (2013) reported that antibacterial activity of *Brassica campestris* showed antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus epidermidis*. Further, aqueous extracts of mustard (*Brassica campestris*) inhibited *Verticillium chlamydosporium* (Owino *et al.,* 1993) and those of cabbage inhibited *Glomus mosseae* spore germination (Vierheilig and Ocampo, 1990).

In addition to *Brassica* leaf extracts, *Brassica carinata* seed extract residue showed significantly different inhibition zone at different concentration. The antimicrobial activity of plants may be due to their ability to synthesize several secondary metabolites of relatively complex structures having antimicrobial properties (Pavithra *et al*., 2010). The presence of antibacterial compounds in the extracts is evident from the results of the

phytochemical screening that shows the presence of different secondary metabolites in the *Brassica carinata* seed extract residue. The results are in conformity with those of Noble *et al.* (2002) who reported Seed meal of *Brassica* species suppresses the growth of *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium sambucinum.* Similarly*,* Chung *et al.* (2002), Robert and Griffin (2007) and Goud *et al*. (2013) reported that Indian mustard resulted in nearly complete inhibition (80-100%) of growth of soil borne pathogens of potato, including *Rhizoctonia solani*, *Phytophthora erythrospetica*, *Pythium ultimum*, *Sclerotinia sclerotiorum* and *Fusarium sambucinum*. Another *Brassica* species (*Brassica napus)* seed meal extracts slightly enhanced growth of a *Propionibacterium Petersonii and Propionibacterium pshermanii* (Smolinska *et al*.*,* 1997).

The minimum inhibitory concentration (MIC) refers to the lowest concentration of an antimicrobial that will inhibit visible growth of a microorganism after overnight incubation while minimum bactericidal concentration (MBC) refers to lowest concentration of an antimicrobial that will prevent the growth of an organism after subculture on to antibiotic free media (i.e. concentration that will kill the microorganism). The present study showed that, the MIC and MBC values of extracts ranged from 25 mg/ml to100 mg/ml. The highest MIC and MBC values (100 mg/ml) were recorded by extracts of *Brassica oleracea var acepala* (Nech gomen), *Brassica nigra (L.)* (Black mustard) and *Raphanus sativious L.* (Radish). The lowest MIC and MBC value (25 mg/ml) was recorded from *Brassica carinata* seed residue while 50 mg/ml (MIC and MBC) was recorded from *B. carinata A. Braun* (Ethiopia mustard), *Brassica oleracea var acepala* (Tekur gomen), *Brassica oleracea var capitata* (Cabbage), *Brassica oleracea var italic* (Broccoli) and *Brassica*

oleracea var botrytis (Cauliflower). Those Brassica plant extracts having the lowest MIC and MBC were those having better antibacterial activity which is expected because the determination of Minimal Inhibitory Concentration (MIC) is sufficient to indicate the ability of a compound to inhibit microbial replication (Hernandes *et al*., 2013). The results are similar to the report of Ozusaglam and Karakoca (2013) that the extracts of *Brassica oleracea* var. *gongylodes* showed a higher antimicrobial activity with low MBC values in the range of 12-45 mg/ml. In general, antibacterial activity together with MIC and MBC results provided evidence that the *Brassica oleracea var capitata* (Cabbage), *Brassica oleracea var acepala* (Tekur gomen) and *Brassica carinata* seed extract residue extract is highly effective as antimicrobial agent against Xcm.

Plants have ability to synthesize aromatic secondary metabolites including phenols, phenolic acids, quinines, flavones, flavonoids, flavonols, tannins and coumarins (Cowan, 1999; Gurjar *et al*., 2012). These groups of compounds showed antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms (Das, 2010; Gurgar *et al*., 2012). In the present study, extract of *Brassica oleracea var capitata* (Cabbage), *Brassica oleracea var acepala* (Tekur gomen) and *Brassica carinata* seed extract contain abundant amount of alkaloids, flavonoids, phenols, tannins, saponins and terpenoids and they are also extracts with high antibacterial activity against Xcm. Similar to the current result, Nawaz *et al*. (2018) reported that the bioactive phytochemical compounds commonly found in most of the *Brassica* species include polyphenols, phenolic acids, flavonoids, carotenoid, alkaloids, tannins and saponins. According to Nawaz *et al*. (2018), *Brassica oleracea*, *Brassica oleracea*, *Brassica oleracea, Brassica juncea*,

Brassica rapa and *Brassica nigra* contain a treasure of phytochemical compounds of medicinal and pharmaceutical importance. Due to the presence of these compounds, *Brassica* plants showed biological activities against various diseases. Similarly, Ozusaglam and Karakoca (2013) reported that, *Brassica oleracea* like cauliflower, broccoli and cabbage are rich in a number of biologically active compounds such as phenolic acids, flavonoids and glucosinolates which have antibacterial activity. (Alagesaboopathi *et al.*, 2011; Kevit *et al*., 2012). These secondary metabolites exert antimicrobial action through different mechanism. Accordingly, alkaloids intercalate into cell wall and hinder its formation (Gurjar *et al*., 2012). Flavonoids have the ability to bind and complex with bacterial cell walls and with extracellular proteins (Tiwari *et al*., 2011). Tannins cause inhibition in the cell wall synthesis by forming irreversible complexes with propene rich protein (Mamtha *et al*., 2004). Terpenoids are responsible for dissolution of the cell wall of microorganism by weakening the membranous tissue (Hernandez *et al*., 2000). The saponins have the ability to cause leakage of proteins and certain enzymes from the cell (Zablotowicz *et al*., 1996).

The result of the quantitative chemical analysis of extracts showed that total phenolic content was significantly different between the studied Brassica plant species. Significantly the highest ($P < 0.05$) total phenolic contents were recorded by the extract of *Brassica* seed extracts residue, *Brassica oleracea* (Tekur gomen) and *Brassica oleracea* (Cabbage). These extracts also had the highest antibacterial potential against the test bacterium (Xcm). Lowest total phenolic contents were recorded from the leaf extracts of *Brassica nigra* (Black mustard), *Brassica oleracea* (White Gomen) and *Brassica carinata.* This shows that there was correlation between total phenolics content and antibacterial potential. In

agreement to this result, several studies have reported the presence of phenolic compounds in different *Brassica* species such as Brocoli (Vallejo *et al*., 2004); Cabbage (Ferreres *et al*., 2005); Cauliflower (Liorach *et al*., 2003) and Rape seed (Velasco *et al*., 2010).

5.3. Effects of Inorganic fertilizer (NPK) on the control of enset bacterial wilt

Nutrition affects the rate of growth and the state of readiness of plants to defend them against pathogenic attack (Muchovej *et al*., 1980). Averting nutrient deficiencies using fertilizers is one way of controlling some of the most important plant diseases in an integrated pest management system (Atkinson and Mckinlay, 1997; Oborn *et al*., 2003). Appropriate management which takes into account fertilizer type and rates has the potential to achieve high crop productivity while reducing the incidence of diseases, or at least avoiding their increase (Katan, 2009). Nitrogen acts directly on vegetative growth and it is an integral component of many compounds that are essential for growth and development (Mkhabela *et al*., 2001 and Nomura *et al.,* 2017). Waddell *et al*. (1999) and Babaji *et al.* (2007) reported that Phosphorus is responsible for energy transfer necessary for metabolic processes within the plant and increasing the number of leaves in the early stages of plant growth. Moreover, Mengel and Kirkby, (1987) and Wang *et al*. (2013) reported that potassium plays a particularly critical role in plant growth and metabolism, and it contributes greatly to the survival of plants that are under various biotic and abiotic stresses. The present study revealed that the interactions of nitrogen (N), phosphorus (P) and potassium (K) fertilizers at different levels ($N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{2/3}P_{2/3}K_{2/3}$) significantly ($p<0.05$) increased the growth parameters throughout the study period.

Growth parameters that increased due to NPK fertilizer include plant height, pseudostem girth, leaf length, width, total leaf area and leaf area index in enset plants compared to control without fertilizer. The current findings are in agreement with previous studies such as Firew Kebede (1999) who reported that an increase in concentrations of nitrogen and phosphorous increases pseudostem girth and leaf area of enset. Yohannis Uloro and Mengel (1994) also reported that combination of NPK application increased enset yield. In addition, the interaction of N and P significantly increased enset production (Kelsa Kena, 1996; Abay Ayalew and Mikias Yeshitila, 2011; Sharma *et al*., 2014).

Moreover, previous studies showed that the effect of inorganic fertilizers significantly influenced the growth parameters of plants other than enset. For instance, Bhalerao *et al*. (2009) reported that combined application of NPK increased the growth and yield attributes of banana. In addition, increasing application of nitrogen and phosphorus influence growth, yield component and quality parameters of potato (Asmare Zelalem *et al.,* 2009; Birtukan Belachew, 2016).

Pathogens that destroy part of the photosynthetic area of plants and cause significantly reduced photosynthetic output often result in smaller growth of these plants and smaller yields. Similarly, pathogens that destroy part of the roots of a plant or clog their xylem or phloem elements, thereby severely interfering with the translocation of water and inorganic or organic nutrients in these plants, often cause a reduction in size and yields of plants (Agrios, 2005). In the present study, the results of growth parameters revealed that all inorganic fertilizer treatments, positive control and negative control plants kept growing as

the experiment progressed which was supported by the report of Getahun Yemata, (2016) and Yan *et al.,* (2013). However, plant height, pseudostem diameter, leaf number, length, width and the consequent total leaf area and leaf area index were highly affected by the infection of Xcm. The effect of infection was described by a clear reduction in plant height, pseudostem girth, leaf number, leaf length, leaf width, total leaf area and leaf area index in infected control plants as compared to negative control and inorganic fertilizer treated plants of both clones. One of the explanations for reduced growth parameters in plants infected with pathogens is reductions in the amount of available assimilate (Ayres, 1992). When a plant is infected by a pathogen its physiology is impaired, especially nutrient uptake, assimilation, translocation from the root to the shoot and also utilization (Marschner, 1995). Pathogens trigger physical and biochemical defenses in infected plants, diverting resources from plant growth to defense, and reduce the living leaf areas and photosynthetic capacity (Aldea *et al,* 2006; Nabity *et al*., 2009; Maharachchikumbura *et al*, 2011). Thus plant growth and biomass are subsequently decreased (Spiering *et al*, 2006). In agreement with the present work Getahun Yemata and Masresha Fetene (2018) reported that *xantomonas campestris* affected growth of enset plants. They reported that infected plants showed lowest value in all growth parameters like plant height, pseudostem girth, total leaf area and leaf area index.

Although it was not statistically significant, the study showed that tolerant clone had slightly higher growth parameter than susceptible clones. This result is in agreement to Frew Kebede (2012) who reported that the number of green leaves maintained by the enset clone Ado (tolerant clone) was significantly higher than that of Ganticha (susceptible

clone) which may imply the existence of clonal differences in performance despite exposure to similar rate of fertilizer supply. Similarly, Getahun Yemata, (2016) reported that enset plant height was significantly reduced in infected control plants of the susceptible clone than the tolerant clone in all time of measurements of different morphological parameters.

Plants receiving a balanced nutrition, in which all required elements are supplied in appropriate amounts, are more capable of protecting themselves from new infections and limiting existing infections than when one or more nutrients are supplied in excessive or deficient amounts (Agrios, 2005). In the present study, result showed that application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ decreased the disease incidence and severity of enset clones compared to the positive control or infected control, even if, it was not statistically significant (p<0.05). Application of proper plant nutrition might influence the physiology and biochemistry of the host, which in turn affects the microclimate and finally might reduce the infection of plants by pathogens (Agrios, 2005). Most vigorously growing plants often offset the most damaging effect of some diseases, since a balanced nutrient supply optimal for plant growth is usually optimal for plant resistance as well (Agrios, 2005; Dordas, 2008). These findings are in line with Atim *et al*., (2013) that nitrogen and potassium reduced susceptibility to *Xanthomonas* wilt in banana plant. Similarly, Abolusoro *et al*., (2013) reported that NPK fertilizers decreased incidence of root-knot of nematode infected Ethiopian eggplant (*Solanum aethiopicum).* Adejumo (2010) reported that application of nitrogen and phosphorus decreased the disease incidence of banana plant. Berga Lemaga *et al*. (2001) reported bacterial wilt incidence of potato decreased by

application of NP and PK compared to the control. According to Chase (1989) nitrogen and potassium fertilizers decreased severity of Xanthomonas blight of white butterfly plant. However, in others report high N levels increased diseases such as bacterial blight severity of rice (*X. oryzae*) (Reddy *et al*., 1979) and leaf blight of onion (*X. axonopodis* pv. *allii*) (Gent *et al*., 2005). Similarly, application of Phosphate may increase the severity of diseases caused by *Sclerotinia* in many garden plants, *Bremia* in lettuce and flag smut in wheat (Huber, 1980). Nevertheless, Williams and Smith (2001) also reported that increased K fertilizer significantly reduced the disease incidence of stem rot and aggregate sheath spot. These inconsistent results show that there is no general rule, as a particular nutrient can decrease the severity of a disease but can also increase the severity of the disease incidence of other diseases or have a completely opposite effect in a different environment (Huber, 1980; Graham and Webb, 1991; Marschner, 1995).

5.4. Effect of green manures and *Brassica carinata* seed extract residue on the control of enset bacterial wilt

Green manures are the crops which are returned into the soil in order to improve the growth of subsequent crops and they offer considerable potential as a source of plant nutrients and organic matter (Singh *et al.,* 1991). The incorporation of green manures into the soil can also contribute to the improvement of soil texture, soil infiltration and nutrient values (Cherr *et al.,* 2006). Moreover, the application of green manures to the soil produced an improvement in the soil biological properties as well as in the nutrition, production and quality of the plants (Tejada *et al*., 2008). It is known that improvement of soils physical

condition by adding green manure crops into the soil create the potential for crop growth (Mahmoody *et al.,* 2014). The application of green manures to soil is considered a good management practice in any agricultural production system because it stimulates soil microbial growth and activity, with subsequent mineralization of plant nutrients (Eriksen, 2005), and therefore increase soil fertility and quality (Doran *et al*., 1988). In the present study, application of *Brassica* species as a green manure and *Brassica carinata* seed extract residue on growth parameters under the different treatments was relatively stable throughout the assessment period. However, the difference in growth parameters between treatments was not statistically significant across all data collection periods. Although not significant, there was better performance for all treatments in all growth parameters compared to the positive control. This performance might be due to disease control nature of brassica plants and additional nutrients supplemented from the decomposition of the applied plant materials in the soil. This result is similar with a report of Assefa Sintayehu *et al.* (2014) which showed that Ethiopian mustard and rapeseed green manures increased plant height and bulb weight of shallot (*Allium cepa* L. var. *ascalonicum)* compared to positive control. According to Rostami *et al*. (2013) the use of *Brassica napus* as green manure is one of the ways of increasing stolen number and tuber yield of potato plant. Lehrsch and Gallian (2010) found that planting radish as a green manure lead to yield improvement in potato and sugar beet crops. Tomato plant growth was increased by using cabbage plant as a green manure (Youssef and Lashein, 2003). Similar to the effect of inorganic fertilizer, this study also showed that tolerant clone had slightly higher growth parameter than susceptible clones, although no statistically significant difference was observed.

Phytopathogen infection leads to changes in secondary metabolism as a result of the induction of defense programmes as well as changes in primary metabolism which affect growth and development of the plant. The results led to the proposal that plants switch off photosynthesis and other assimilatory metabolism to initiate respiration and other processes required for defense (Berger *et al*., 2007). Pathogens affect the water balance of plants in one or more ways. Some interfere with the function of the roots; others with the translocation of water through the stem by growing in the xylem vessel and some others with the water economy of the plant by causing excessive transpiration through their effects on leaves and stomata (Agrios, 2005). In the present study, the relative water content of eight weeks after inoculation was lower than four weeks after inoculation measurements even if it was not statically significant. In addition, the relative water content of green manure treatments was higher than positive control. In agreement with the current result, Agamy *et al*., 2013; Pazarlar *et al*., (2013) and Wang *et al*., (2015) reported that decreased leaf relative water contents were recorded in infected plants of pepper, tomato and cucumber, respectively. In contrast to the present study, Solomon Tadesse *et al.* (2012) on grapevines and Getahun Yemata (2016) on enset reported that relative water content was not affected by disease. Where there was a reduction in relative water content it was suggested to be related to an increased rate of respiration and membrane injury (Orcutt and Nilsen, 2000). The membrane injury of infected plant leaves may result in uncontrolled water loss from damaged cells, and the leaf water balance could be affected (Wang *et al*., 2012). Moreover, non-stomatal water loss induced by leaf cell membrane injury leads to uncontrolled water loss from damaged cells to the apoplast (Wang *et al*., 2015). In addition, decrease in leaf relative water content occurs due to reduced hydraulic conductance caused

by clogging of the vessels (McElrone *et al.*, 2003) and loss of xylem water transporting function caused by a decrease in stem-specific hydraulic conductivity and the presence of tyloses in the lumens of obstructed water conduits (Perez-Donoso *et al.,* 2007). But for green manure treated plants relative water content was higher than positive control. This may be due to decrease in incidence of Xcm in green manure plants resulting better relative water content compared to positive control.

Pathogens trigger physical and biochemical defenses in infected plants, diverting resources from plant growth to defense, and reduce the living leaf areas and photosynthetic capacity (Aldae *et al.,* 2006; Nabity *et al*., 2009 and Maharachchikumbura *et al.*, 2011). Pathogens that destroy part of the photosynthetic area of plants and cause significantly reduced photosynthetic output (Agrios, 2005). In the present study, assimilation rate (A), transpiration rate (E), intercellular CO_2 concentration (C_i), stomatal conductance (gs) and water use efficiency (WUE) showed decreasing pattern from four weeks after inoculation to eight weeks after inoculation. However, these physiological parameters showed better performance following brassica green manure and seed extract residue treatment compared to positive control in both clones. These physiological parameters were lower in the susceptible clone than the tolerant clones. Reduction in photosynthesis of infected plants is in agreement with the research report of Getahun Yemata (2016); Karkanis *et al*. (2007); Ponmurugan *et al*. (2007); Silveira *et al*. (2015); Dallagnol *et al*. (2011); Petit *et al*. (2006) on enset, tobaco, tea cultivars, tomato, rice and grapevine, respectively. Moreover, for green manure treated plants Karkanis *et al*. (2007) reported that physiological characteristics of tobacco plants were significantly influenced by using Vetch and red clover as green manures. The report showed that, the lowest stomatal conductance,

photosynthetic rate and transpiration rate were found in control plots of tobacco plants. Similarly, Islam *et al*. (2019) showed that the net assimilation rate (NAR) of rice was significantly influenced by the integration of *Sesbania aculeata* and Crotalaria juncea plant as green manures. The reduction in photosynthesis of infected plants was related to water stress, caused by decreased sap flow rates (Machado *et al*., 1994). The reduced in transpiration rate of infected plants is caused by reduced water flow that results in leaf water deficits leading to stomatal closure (Tyree and Sperry, 1989; Saeed *et al*., 1999). The reduction in net photosynthetic rate in infected plants was probably caused by stomatal closure, since infected plants was accompanied, especially later stage of infection, by lower Gs (Guo *et al*., 2005). Decreased net photosynthetic rate has been attributed to stomatal closure induced by pathogen infection and a disruption in the metabolic pathways of photosynthesis, such as a reduction in the mesophyll conductance and the Rubisco activity (Lorenzini *et al.*, 1997; Saeed *et al.*, 1999; Santos *et al.*, 2000 and Guo *et al*., 2005). Taking the plant as whole, the overall decreased photosynthetic productivity in response to the disease also results from a decreased amount of healthy leaf area. Previous result has suggested that reduced rate of photosynthesis may be due to the declined content of chlorophyll per unit leaf area in infected leaves (Funayama *et al*., 1997; Sayed, 2003).

In the present study, the chlorophyll value of enset eight weeks after inoculation was lower than the chlorophyll value of enset after four weeks of inoculation. In addition, the chlorophyll values of green manure treated plants were significantly higher than positive control plants for both clones. Rajalakshmi and Ramarethinam (2000) found reduction in contents of chlorophyll due to infection of leaves by blister blight. Similarly, research

report of Guo *et al*. (2005) and Hooks *et al*. (2008), Palanisamy *et al.* (2009) and Pazarlar *et al*. (2013) showed infected plants have lower chlorophyll contents in turnip mosaic virus (TMV) infected banana, in bunchy top virus (BBTV) infected banana plants, in yellow vein mosaic virus infected genotypes of *Abelmoschus esculentus* leaves and in tobacco mosaic virus (TMV) infected varieties of pepper respectively. The low chlorophyll content in infected plants might also be due to inhibition of chlorophyll synthesis (Kariola *et al*., 2005). According to Roca and Minguez-Mosquera (2003) and Kariola *et al*. (2005) decreases in chlorophyll content of infected plants might be caused by increased chlorophyllase activity that participates in shaping the plant defense response by causing a rise in reactive oxygen species that result in programmed cell death (Kariola *et al*., 2005; Agamy *et al*., 2013). Higher chlorophyll value of green manure treated plants was also reported by Islam *et al*. (2019) who showed that incorporation of *Sesbania aculeata* and *Crotalaria juncea* increased the SPAD reading at different growth stages of rice. Similarly, Xie *et al.* (2017) found that Chinese milk vetch as a green manure significantly increased the chlorophyll content in rice. The reason for green manure treated plants having higher SPAD value may be due to their leaf greenness compared to positive control. This is supported by research report of (Rorie *et al*., 2011; Hakim *et al*., 2015) who stated that SPAD meter reading fairly correlate with leaf greenness and N contents in crop and thus, can be used to estimate leaf chlorophyll content.

Biofumigation is the agronomic practice of using volatile chemicals (allelochemicals), released from decomposing plant tissues such as *Brassica* tissues, to suppress soil-borne pests and pathogens through incorporation of *Brassicaceae* plants as green manure (Kumar

at al., 2005). Biofumigation works on the principle of exploiting the natural biocide compounds from glucosinolate containing plants (Kirkegaard *et al.,* 1998; 1999; 2000; Matthiessen and Shackleton, 2005) to suppress soil microorganisms, such as fungal, bacterial pathogens and nematodes, (Angus *et al.,* 1994; Brown & Morra, 1997; Sarwar *et al.,* 1998; Bianco *et al.,* 2000; Smolinska *et al.,* 2003). In the present study, application of G_1, G_2 and G_3 as green manure reduced the disease incidence of both tolerant as well as susceptible enset clone. Moreover, diseases severity was significantly ($p<0.05$) reduced by G_2 and G_3 for tolerant clones and G_1, G_2 and G_3 for susceptible clones compared to positive control. This result is in line with the report of Assefa Sintayehu *et al.* (2014) who showed that, green manure amendments of rapeseed and Ethiopian mustard significantly reduced incidence and disease severity of shallot Fusarium basal rot. Similar reductions in *Fusarium* infection was reported after *Brassica* plant tissue incorporation (Subbarao *et al*., 1999; Smolinska, 2000; Mazzola *et al*., 2001; Cohen *et al*., 2005). Brassica crops including, canola, rapeseed, Indian mustard, yellow mustard and oilseed radish as a green manure reduced soil inoculum levels of *Rhizoctonia solani* in potato plant (Larkin, 2013). Moreover, Tamire Zewde *et al*. (2007) reported that, amendments of infested soil with *Brassica carinata* (Ethiopian mustard) seed meal reduced Garlic white rot incidence down to 0%. Goud, *et al*. (2013) and Chung *et al.* (2002*)* reported that, mustard seed meal proved to be effective for controlling the pathogen. Similarly, Dandurand *et al*. (2000) proved that the volatile hydrolysis products of rapeseed meal (*Brassica napus*) strongly inhibited the soil-borne pathogens *Sclerotinia sclerotiorum* and *Aphanomyces euteiches*. In the contrary, Rahman *et al*. (2010) described that mustard extract has not shown inhibitory activity against *Klebsiella pneumonia*e*, Pseudomonas aeruginosa, Enterococcus faecalis*,

Micrococcus luteus, *Escherichia coli* and *Candida albican*s. In a similar study, the *Fusarium* and *Pythium* populations in *Brassica* amended soils were even significantly higher than those in control (Njoroge *et al*., 2008). It has been reported that after soil amendments with *Brassica juncea* and *Brassica napus*, the populations of both fungi and bacteria including *Fluorescent pseudomonads* increased compared with non-amended soils (Smolinska, 2000). *Brassica* amendments have been successful for soil-borne pathogen control (Subbarao *et al*., 1999; Cohen *et al*., 2005). Matthiessen and Shackleton (2005) suggest that *Brassica* green manure incorporation increases soil organic matter and enhances soil structure and erosion control, which have been associated with reduction of soil-borne pathogens. Cohen *et al.* (2005) found that suppression of *Rhizoctonia solani* by *Brassica napus* seed meal was associated with changes in soil microbial communities. Green manures promote soil suppression of soil-borne pathogens due to a range of several mechanisms such as competition and antagonism by the soil biota associated with microbial diversity and stability (Hoitink and Boehm, 1999). Green manure and mustard seed meals have been proposed as alternative method to chemical control of soil-borne plant pathogens (Janvier *et al*., 2007). These strategies aim to prevent or decrease the development of disease problems caused by soil-borne pathogens, by the provision of soil conditions that are optimal for crop growth and unsuitable for pathogen survival (Litterick *et al.,* 2004).

6. Summary and Recommendation

6.1. Summary

Bacterial wilt of enset, which is caused by *Xanthomonas campestris* pv. *musacearum* (Xcm) is one of the destructive and the most important factor affecting enset production in Ethiopia. This requires integrated disease management strategy to control the disease. This study provides information about the prevalence and incidence of Xcm in relation to altitude variation, antibacterial effect of eight *Brassica* species and *Brassica Carinata* seed extract residue against Xcm. Moreover, the study provided data on the effects of inorganic fertilizers on the growth of enset and the incidence and severity of Xcm. The study also presented the effect of *Brassica* species as a green manure and seed extract residue on growth and physiology of enset plant as well as their effect on the incidence and severity of Xcm. In general, the result of this study could be summarized as follows:

- Mid altitude represented by Cheha district was an area having higher disease prevalence and disease incidence in both 2014 and 2015.
- The extracts of *Brassica oleracea var capitata* and *Brassica oleracea var acepala* created the widest bacterial growth inhibition zone at (400 and 200 mg/mL) compared to other concentrations. This suggests that cabbage and Tekur Gomen could be used to control the growth and development of Xcm of Enset. The lowest minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) values were recorded for extracts of *Brassica carinata seed residue*

followed by *Brassica carinata, A. braun* (Ethiopia mustard), *Brassica oleracea var acepala* (Tekur gomen) and *Brassica oleracea var capitata* (Cabbage). This implies that use of these *Brassica* species in the field is effective to control Xcm. Brassica plant species contained abundant amount of chemicals such as Phenol, Alkaloid, Flavonoids, Trepenoids and Tanins except *Brassica carinata and Brassica nigra*. The highest total phenolic content was also recorded by the extract of *Brassica carinata* seed extracts residue, *Brassica oleracea* (Tekur Gomen) and *Brassica oleracea* (Cabbage).

- Inorganic fertilizers treatments with $N_{1/2}P_{1/2}K_{1/2,}$ NPK and $N_{3/2}P_{3/2}K_{3/2}$ significantly ($p<0.05$) increased all growth parameters of both enset clones including plant height, pseudostem girth, green leaf number, leaf length, leaf width, leaf area and leaf area index at different measuring periods compared to positive controls. Application of $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ decreased the disease incidence of tolerant enset clones by 6.8%, 7.7% and 13.8%, respectively compared to the positive control. In the same way, $N_{1/2}P_{1/2}K_{1/2}$, NPK and $N_{3/2}P_{3/2}K_{3/2}$ decreased the disease incidence of susceptible enset clones by 22.2%, 27.8%, and 33.1 %, respectively. This clearly showed that application of NPK combined fertilizer is effective to control the disease as compared to application of single fertilizer alone. Similarly, disease severity of tolerant enset clone with $N_{1/2}P_{1/2}K_{1/2,}$ NPK and $N_{3/2}P_{3/2}K_{3/2}$ application decreased disease severity by 12.4%, 17.3% and 35.2%, respectively as compared to positive control and decreased disease severity of susceptible enset clones by 10.1%, 15.7%, and 17.9 %, respectively. Moreover, the lowest AUDPC value (623) was recorded on tolerant clones treated with

$N_{3/2}P_{3/2}K_{3/2}$ fertilizers while the highest AUDPC value (1455) was recorded in susceptible clone with positive control.

- Application of *Brassica* species as green manures such as *Brassica oleracea var capitata* (G_1) and *Brassica oleracea var acepala* (G_2) and *Brassica carinata* seed extract residue (G_3) were not significantly different at ($P > 0.05$) in all growth parameters compared to positive controls at different measuring periods. Similarly, application of *Brassica* species as green manures and *Brassica carinata* seed extract residue did not have significant difference ($P<0.05$) on all physiological parameters except Assimilation rate and Chlorophyll content compared to positive control. However, compared to the positive control, G_1, G_2 and G_3 decreased the disease incidence of tolerant enset clones by 19.4%, 23.3% and 23.1%, respectively. In the same way, application of G_1, G_2 and G_3 treatments decreased the disease incidence of susceptible enset clones by 6.7 %, 12.3 %, and 4.6%, respectively. Disease severity of tolerant enset clone with G_1, G_2 and G_3 application decreased severity by 5.3 %, 7.8% and 11.4%, respectively as compared to positive control. Similarly, application of G_1, G_2 and G_3 treatments decreased the disease severity of susceptible enset clones by 11.4 %, 8.6 %, and 10 %, respectively. The lowest AUDPC value (976.5) was recorded on tolerant treated with G_3 while the highest AUDPC value (1828.9) was recorded in susceptible clone with positive control.

From the findings of field assessment, laboratory *in vitro* work and field experiments, it is possible to draw the following major conclusions:

i) In general the prevalence, severity and distribution level of BW of enset diseases tend to vary from one enset growing area to the other; depending on various conditions mainly altitude, type of enset clones, management practices and farmers' attitudes and perceptions towards the spreading and controlling mechanisms of Enset

ii) *Brassica* plants and *Brassica carinata* seed extract residue had antibacterial effect against *Xcm* and induction were effective to control enset bacterial wilt in Ethiopia,

iii) Use of recommended levels of inorganic fertilizers NPK and $N_{3/2}P_{3/2}K_{3/2}$ amount could be used as one of the alternative ways to increase the growth performance of enset and reduce the effect of bacterial wilt on enset clones.

iv) Use of *Brassica oleracea var capitata* and *Brassica oleracea var acepala* as green manure and *Brassica carinata* seed extract residue showed that they are effective to control enset bacterial wilt.

6.2. Recommendations

Introducing an effective enset bacterial wilt controlling method is required to bring back the wide spread cultivation of enset in high bacterial wilt risk areas and contribute to better food security in the study areas and similar agro-ecologies of enset growing parts of the country. The results of the present study can be useful and applied in alleviating the major constraint of enset cultivation. The study has shown that use of inorganic fertilizer and green manuring of *Brassica* species is crucial to control the disease. Therefore, based on farmers' resource endowments, the use of recommended NPK and $N_{2/3}P_{2/3}K_{2/3}$ are crucial

to control enset bacterial wilt. Moreoover, Brassica oleracea var capitata and Brassica oleracea var acepala as green manure and Brassica carinata seed extract residue can be used by farmers to control enset bacterial wilt. However, further research is needed to assess the economic feasibility of different rates of inorganic fertilizer and *Brassica* species as green manure. Although it is beyond the result of this study, it is important to encourage and support the enset farmer in application of sanitary measures in an organized way to minimize the loss incurred by the disease.

Finally, the result of this study suggests the following further researchable issues related to enset bacterial wilt control.

- Since Ethiopia is the center of origin of many plant species, many more screening researches should be conducted on the anti-Xcm activity of different plant extracts to identify plants with high potency characteristics.
- The maximum extract yield, high potency against Xcm and low minimum inhibitory concentration of *Brassica oleracea var capitata, Brassica oleracea var acepala* and *Brassica carinata* seed extract residue could make the species better candidates. However, further isolation and evaluation of the active ingredients is needed.
- Application of *Brassica* species as green manure to control EBW provided valuable information to continue further research on different parts of enset growing areas. However, further researches into these extracts should be conducted to identify the active Glucosinolate compounds responsible for such antibacterial activity and the formulation as well as commercialization of brassica extracts as biocides should be done.

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8. Appendices

Appendix 1. Questioner and survey formats of enset bacterial wilt incidences in Gurge and Silte zone

Part I. Farmers information

1. Name of respondent: ……Woreda…… Kebele ……

Part II. Background information

1. Total Area you have for enset farm ……
2. Number of enset plant in your farm……
3. When do you plant your enset clones? ……
4. Is there enset bacterial wilt infected enset plant in your farm? A= Yes, B= No
5. What do you think the causes of the disease?
   A. Contaminated farm tools
   B. Infected planting materials
   C. By insects
   D. Spiritual
6. What do you think about the disease rate of the previous year compared to the current one? Increase/Decrease
7. In which month of the year disease rate increases? ……
8. Do you traditionally exchange planting material (seeds, suckers, etc..,) with neighbors or relatives? A=Yes, B=No
9. If yes to question 6, who do you exchange with and explain the processes
   A=with neighbors only, B=with relatives or kin, C=with both
   D=with anybody, E= others……
10. Do you apply additional input such as pesticides, fertilizers etc.?
    A=Yes, B=No

11. List the type of enset clones you grow in your farm and the number of plants/clone
……
12. What is the size of the plot used for growing enset? …….

13. Do you grow crops other than enset in your farm? A=Yes B=No
14. If yes to question 13, list the type of crops, why you grow it and the size of plot each occupy? ……

15. What are the major production problems you encounter? (More than one answer is possible)? A=disease B=drought C=premature harvest due to food insecurity D=Porcupine E=any combination of the above …….

16. Which clone (s) of enset is/are more tolerant to bacterial wilt of enset than others

1) …….

2) …….

3) …….

4) …….

Appendix 2. Data recording sheet for field survey of enset bacterial wilt prevalence and severity

Administrative Region: _____Zone_____

Woreda _____locality/kebele_____

Owner of the farm: _____Collecting date: _____

Collected by_____

Local code number	Woreda	Locality	Altitude	Type of enset clone planted	Soil types	Months of the year disease rate increase

Cause of disease	Mechanism used to prevent disease	Other crop planted mixed with enset plant	Manure add to enset plant	Fertilizer used for disease management	Estimation of total no of enset plant

No infected enset plant	Infected plant clone type	Total no infected parts

Appendix 3. Data collection sheet for enset bacterial incidence and severity of the survey area

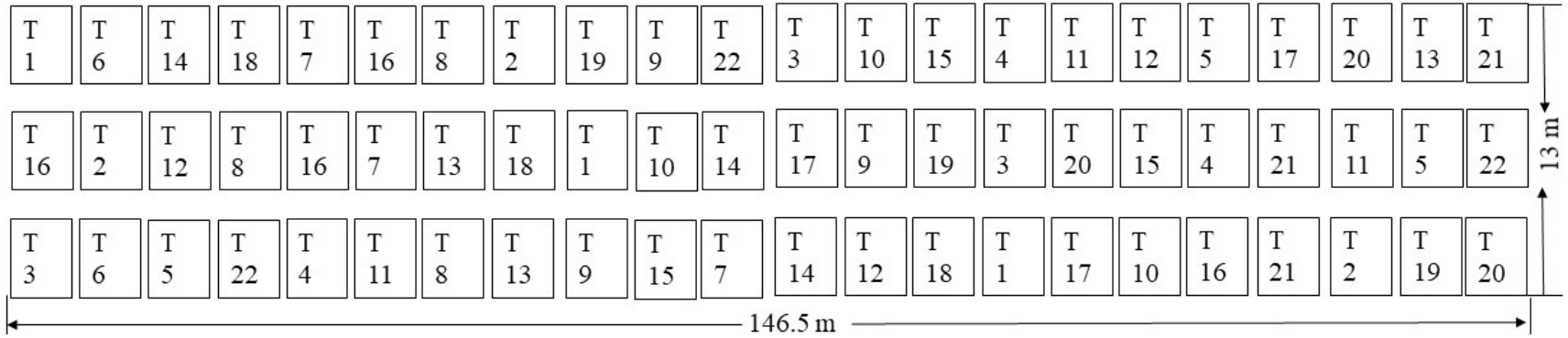
Data collection sheet for Enset bacterial incidence and severity of the survey area. Experiment:Effect of altitudinal and seasonal variation on EBW incidence and severity																			
The name of district and Kebele	Code given to the farm	October					BW Incidence	January					BW Incidence	April					BW Incidence
		Number of plants rated 0, 1, 2, 3 or 4						Number of plants rated 0, 1, 2, 3 or 4						Number of plants rated 0, 1, 2, 3 or 4					
		0	1	2	3	4		0	1	2	3	4		0	1	2	3	4	
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		
	8																		
	9																		
	10																		

Appendix 4. Data collection sheet for enset bacterial wilt incidence and severity

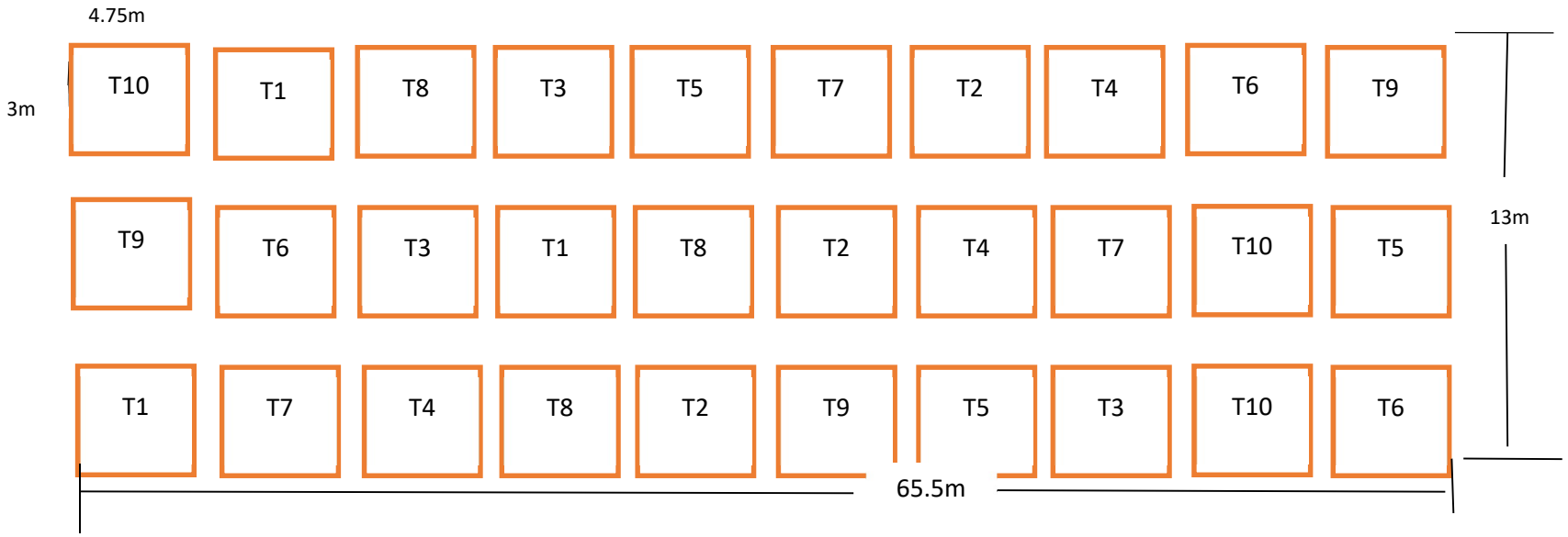
Data collection sheet for Enset bacterial wilt incidence and severity Experiment: Effect of Inorganic fertilizer and green manure on EBW control

Treatments	replication	Date 1: Number of plants rated 0, 1, 2, 3 or 4					BW Incidence	Date 2: Number of plants rated 0, 1, 2, 3 or 4					BW Incidence	Date 3: Number of plants rated 0, 1, 2, 3 or 4					BW Incidence
		0	1	2	3	4		0	1	2	3	4		0	1	2	3	4	
	1																		
	2																		
	3																		
	4																		
	5																		
	1																		
	2																		
	3																		
	4																		
	5																		

Appendix 5. Layout of inorganic fertilizer experimental plot showing the random distribution of treatments



Appendix 6. Field layout for green manure experiment



Appendix 7. ANOVA of Antibacterial test of different brassica plants at different dilutions

Tests of Between-Subjects Effects

Dependent Variable: Inhibition zone

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	556.244[a]	39	14.263	29.637	.000
Intercept	9471.006	1	9471.006	19680.013	.000
Plant Spp	98.844	7	14.121	29.341	.000
Concentration	427.963	4	106.991	222.318	.000
Plant Spp * Concentration	29.438	28	1.051	2.185	.002
Error	57.750	120	.481		
Total	10085.000	160			
Corrected Total	613.994	159			

a. R Squared = .906 (Adjusted R Squared = .875)

Appendix 8. ANOVA and mean comparison of *Brassica carinata* seed extract residue

Inhibition zone

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	112.311	4	28.078	120.333	.000
Within Groups	9.333	40	.233		
Total	121.644	44			

Multiple Comparisons

Dependent Variable: Inhibition zone

Concent_Brassica	Concent_Brassica	Mean Difference	Std. Error	Sig.
6.25	12.50	-.55556(*)	.22771	.019
	25.00	-1.11111(*)	.22771	.000
	50.00	-2.33333(*)	.22771	.000
	100.00	-4.44444(*)	.22771	.000

12.50	6.25	.55556(*)	.22771	.019
	25.00	-.55556(*)	.22771	.019
	50.00	-1.77778(*)	.22771	.000
	100.00	-3.88889(*)	.22771	.000
25.00	6.25	1.11111(*)	.22771	.000
	12.50	.55556(*)	.22771	.019
	50.00	-1.22222(*)	.22771	.000
	100.00	-3.33333(*)	.22771	.000
50.00	6.25	2.33333(*)	.22771	.000
	12.50	1.77778(*)	.22771	.000
	25.00	1.22222(*)	.22771	.000
	100.00	-2.11111(*)	.22771	.000
100.00	6.25	4.44444(*)	.22771	.000
	12.50	3.88889(*)	.22771	.000
	25.00	3.33333(*)	.22771	.000
	50.00	2.11111(*)	.22771	.000

* The mean difference is significant at the .05 level.

Appendix 9. Total phenolics content of Brassica plant extracts and *Brassica carinata* seed extract residue

Concentration

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.066	8	.008	128.870	.000
Within Groups	.001	18	.000		
Total	.067	26			

Appendix 10. ANOVA on morphology before inoculation of inorganic fertilizers

		Sum of Squares	df	Mean Square	F	Sig.
plantheight_before_m	Between Groups	3.563	19	.188	10.777	.000
	Within Groups	.696	40	.017		
	Total	4.259	59			
PsGirth_before_m	Between Groups	1.998	19	.105	12.207	.000
	Within Groups	.345	40	.009		
	Total	2.343	59			
Greenleafno_before	Between Groups	194.663	19	10.245	12.369	.000
	Within Groups	33.133	40	.828		
	Total	227.796	59			
Leaflength_before	Between Groups	.776	19	.041	8.788	.000
	Within Groups	.186	40	.005		
	Total	.962	59			
Leafwidth_before	Between Groups	.237	19	.012	5.763	.000
	Within Groups	.087	40	.002		
	Total	.324	59			
LeaqfArea_before_m^2	Between Groups	7.305	19	.384	8.085	.000
	Within Groups	1.902	40	.048		
	Total	9.207	59			
LAI_before	Between Groups	1.443	19	.076	8.083	.000
	Within Groups	.376	40	.009		
	Total	1.819	59			

Appendix 11. ANOVA on Morphology first round after inoculation of inorganic fertilizers

		Sum of Squares	df	Mean Square	F	Sig.
plantheight_1ST_m	Between Groups	3.232	21	.154	5.193	.000
	Within Groups	1.304	44	.030		
	Total	4.536	65			
PsGirth_1ST_m	Between Groups	1.634	21	.078	6.902	.000
	Within Groups	.496	44	.011		
	Total	2.130	65			
Greenleafno_1ST	Between Groups	222.530	21	10.597	9.408	.000
	Within Groups	49.558	44	1.126		
	Total	272.088	65			
Leaflength_1ST	Between Groups	1.598	21	.076	5.224	.000
	Within Groups	.641	44	.015		
	Total	2.239	65			
Leafwidth_1ST	Between Groups	.334	21	.016	4.046	.000
	Within Groups	.173	44	.004		
	Total	.506	65			
LeaqfArea_1ST_m2	Between Groups	16.869	21	.803	6.181	.000
	Within Groups	5.719	44	.130		
	Total	22.588	65			
LAI_1ST	Between Groups	3.332	21	.159	6.177	.000
	Within Groups	1.130	44	.026		
	Total	4.463	65			

Appendix 12. ANOVA on morphology second round after inoculation of inorganic fertilizers

		Sum of Squares	df	Mean Square	F	Sig.
plantheight_2ND_m	Between Groups	3.862	21	.184	11.029	.000
	Within Groups	.734	44	.017		
	Total	4.596	65			
PsGirth_2ND_m	Between Groups	2.083	21	.099	11.327	.000
	Within Groups	.385	44	.009		
	Total	2.468	65			
Greenleafno_2ND	Between Groups	191.443	21	9.116	10.496	.000
	Within Groups	38.218	44	.869		
	Total	229.661	65			
Leaflength_2ND	Between Groups	.806	21	.038	8.712	.000
	Within Groups	.194	44	.004		
	Total	1.000	65			
Leafwidth_2ND	Between Groups	.254	21	.012	5.399	.000
	Within Groups	.099	44	.002		
	Total	.353	65			
LeaqfArea_2ND_m2	Between Groups	7.835	21	.373	8.490	.000
	Within Groups	1.934	44	.044		
	Total	9.768	65			
LAI_2ND	Between Groups	1.548	21	.074	8.491	.000
	Within Groups	.382	44	.009		
	Total	1.929	65			

Appendix 13. ANOVA on AUDPC of inorganic fertilizers

AUDPC_Severity

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8083070.857	21	384908.136	4.667	.000
Within Groups	3628539.714	44	82466.812		
Total	11711610.571	65			

Appendix 14. ANOVA on morphology first round after inoculation of *Brassica* species as green manure and *Brassica carinata* seed extract residue treatments

		Sum of Squares	df	Mean Square	F	Sig.
PH_1	Between Groups	.287	9	.032	1.032	.449
	Within Groups	.618	20	.031		
	Total	.906	29			
PSEUG_1	Between Groups	.092	9	.010	.842	.588
	Within Groups	.244	20	.012		
	Total	.336	29			
GLN_1	Between Groups	6.141	9	.682	.314	.961
	Within Groups	43.507	20	2.175		
	Total	49.648	29			
LL_1	Between Groups	.147	9	.016	.914	.533
	Within Groups	.358	20	.018		
	Total	.505	29			
LW_1	Between Groups	.024	9	.003	.781	.636
	Within Groups	.069	20	.003		
	Total	.094	29			
LAm2_1	Between Groups	.444	9	.049	.888	.552
	Within Groups	1.110	20	.056		
	Total	1.554	29			
LAI_1	Between Groups	.088	9	.010	.888	.552
	Within Groups	.219	20	.011		
	Total	.307	29			

Appendix 15. ANOVA on morphology second round after inoculation of *Brassica* species as green manure and *Brassica carinata* seed extract residue treatments.

		Sum of Squares	df	Mean Square	F	Sig.
PH_2	Between Groups	.144	9	.016	.330	.955
	Within Groups	.970	20	.048		
	Total	1.114	29			
PSEUG_2	Between Groups	.073	9	.008	.452	.890
	Within Groups	.357	20	.018		
	Total	.429	29			
GLN_2	Between Groups	8.743	9	.971	.430	.903
	Within Groups	45.186	20	2.259		
	Total	53.929	29			
LL_2	Between Groups	.045	9	.005	.225	.987
	Within Groups	.441	20	.022		
	Total	.485	29			
LW_2	Between Groups	.042	9	.005	1.576	.190
	Within Groups	.059	20	.003		
	Total	.101	29			
LAm2_2	Between Groups	.540	9	.060	.797	.624
	Within Groups	1.506	20	.075		
	Total	2.046	29			
LAI_2	Between Groups	.106	9	.012	.794	.625
	Within Groups	.297	20	.015		
	Total	.404	29			

Appendix 16. ANOVA on the physiological parameters analysis first round after inoculation of green manure

		Sum of Squares	df	Mean Square	F	Sig.
A_1	Between Groups	1.999	9	.222	3.441	.010
	Within Groups	1.291	20	.065		
	Total	3.289	29			
E_1	Between Groups	4.654	9	.517	1.035	.447
	Within Groups	9.991	20	.500		
	Total	14.645	29			
GS_1	Between Groups	.027	9	.003	.956	.502
	Within Groups	.064	20	.003		
	Total	.091	29			
Ci_1	Between Groups	2969.484	9	329.943	.495	.861
	Within Groups	13322.507	20	666.125		
	Total	16291.990	29			
WUE_1	Between Groups	.819	9	.091	3.675	.007
	Within Groups	.495	20	.025		
	Total	1.314	29			
RWC_1	Between Groups	119.650	9	13.294	.551	.820
	Within Groups	482.932	20	24.147		
	Total	602.581	29			
Chlorophyl1	Between Groups	1736.852	9	192.984	7.533	.000
	Within Groups	512.340	20	25.617		
	Total	2249.193	29			

Appendix 17. ANOVA on the physiological parameters analysis second round after inoculation of green manure

		Sum of Squares	df	Mean Square	F	Sig.
A_2	Between Groups	2.615	9	.291	9.123	.000
	Within Groups	.637	20	.032		
	Total	3.252	29			
E_2	Between Groups	4.370	9	.486	.646	.745
	Within Groups	15.026	20	.751		
	Total	19.395	29			
GS_2	Between Groups	.088	9	.010	16.722	.000
	Within Groups	.012	20	.001		
	Total	.100	29			
Ci_2	Between Groups	22164.398	9	2462.711	6.457	.000
	Within Groups	7628.107	20	381.405		
	Total	29792.505	29			
WUE_2	Between Groups	.671	9	.075	4.214	.004
	Within Groups	.354	20	.018		
	Total	1.025	29			
RWC_2	Between Groups	1868.215	9	207.579	1.754	.142
	Within Groups	2367.544	20	118.377		
	Total	4235.759	29			
Chlorophyl 2	Between Groups	1060.848	9	117.872	9.112	.000
	Within Groups	258.721	20	12.936		
	Total	1319.569	29			

Appendix 18. ANOVA on the AUDPC of *Brassica* species as green manure and *Brassica carinata* seed extract residue treatments.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10591157.355	9	1176795.262	17.008	.000
Within Groups	1383778.799	20	69188.940		
Total	11974936.153	29			

Appendix 19. Pictures showing some activities during the research in the laboratory and in the field

Appendix 19. Continued

Appendix 20. Pictures showing effect of infection of enset under inorganic and green manure treatments

Declaration

I, the undersigned, declare that this Thesis is based on my original work and that it has not been presented for a degree in any other universities, colleges or institutes for a degree or other purpose. All sources of materials have been duly acknowledged.

Name: Bruktawit Desta Liben Signature _____Date _____

This Thesis has been submitted for examination with my approval as
Supervisor of the Thesis

Name: Prof. Masresha Fetene Signature_____Date_____

Name: Dr. Tesfaye Alemu Signature_____ Date_____