Effect of variety tolerance, soil amendment and biological control agents in the management of bacterial wilt (*Ralstonia solanacearum*) of potato (*Solanum tuberosum* L.) under field condition in Chencha, Southern Ethiopia

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GRADUATE PROGRAMES

This is to certify that the thesis prepared by Atsede Solomon Retta entitled "Effect of soil amendment, Biocontrol agents and screening of potato genotypes for the management of Bacterial wilt under Natural Infestation Condition in Chencha, Southern Ethiopia" and submitted for the fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Biology and Biodiversity Management complies with the regulations of the Universityand meets the accepted standards with respect to originality and quality.

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Abstract

Effect of variety tolerance, soil amendment and biological control agents in the management of bacterial wilt (*Ralstonia solanacearum*) of potato (*Solanum tuberosum* L.) under field condition in Chencha, Southern Ethiopia

Atsede Solomon, PhD Disertation

Addis Ababa University, 2019

Potato (*solanum tuberosum* L.) is an important food security crop which provides higher carbohydrate per unit area within a short period of time than the major cereals wheat and reice. Its production in Ethiopia is increasing rapidly, as it is grown by more than a million small holder farmers as a food and a cash crop. However, potato yield is low due to many factors among which diseases and insect pests are among the most limiting factors to the production of potato. Bacterial wilt caused by *Ralstonia solanacearum* is the second most important disease next to late blight. The pathogen is soil born and transmitted by seed, irrigation water and contaminated tools making its management a complex issue that require an integrated approach. Three separate experiments were carried out during 2015 and 2016 short rainy seasons under natural infestation condition on farmers' fields at Chencha, Southern Ethiopia. The aim of the first experiment was to screen bacterial wilt tolerant potato genotypes and better yield. In the second experiment, soil amendments compost and manure at the rate of 20 t/ha, lime, and recommended fertilizer (110 kg of N/ha and 96 kg of P_2O_5/ha) and their combinations were studied to identify effective soil amendments which reduce the effect of the disease, improve yield and the soil condition. Thirdly, different biocontrol agents which were found effective in previous laboratory and greenhouse studies were tested for their efficacy on the disease development and improvement of yield. All experiments were laid in RCBD with four replications. Data on disease, yield and yield components were collected and subjected to analysis using SAS 9.3 and minitab softwares. The response of potato genotypes to the wilt disease varied significantly ($p < 0.05$) under natural infestation condition. The genotypes Cruza, Shangai, and CIP clone CIP-392661.18 showed lower DSI and AUDPC combined with a higher marketable yield. The local variety Sula also showed a better performance in terms of disease tolerance and yield which calls for further molecular study. Soil amendements showed better disease control than the control treatment though there was no significant differences between the organic fertilization and their

combinations except for lime only treatment. The biological control agents also generally improved crop performance and delayed the onset of the disease. Treatments also significantly differed in terms of DSI, AUDPC and tuber yield. The lowest AUDPC value (738) and highest tuber yield (34.8 t ha^{-1}) was obtained from Neem gold @ 0.25 ton ha^{-1}. However, Neem gold @ 0.1 ton ha^{-1} showed the highest AUDPC (1565) followed by arbuscular mycorrhizal fungui (AMF) and control, which achieved intermediate and the lowest AUDPC (1545 and 1500, respectively). The lowest yield (28 t ha^{-1}) and the highest percentage of infected tubers (25.3 %) was recorded from the control plot. From these results, it can be recommended that the use of an integrated approach including tolerant varieties, application of soil amendment and biocontrol agents can improve potato productivity and lower the effect of bacterial wilt in the study area although in the presence of pathogen in the soil. This approach can be recommended as a better alternative to current practices as it is user-friendly and environmentally safe. However, the availability and cost of biocontrol agents and soil amendments should be taken in to consideration when making recommendations outside the study area.

Key words: *Ralstonia solanasearum,* integrated management, natural infestation, biocontrol agents. Soil amendment

DEDICATION

This dissertation is dedicated to:

My mother, W/ro Alemitu Hunegnaw, who pays every succrifice to make me reach this level. She is always strong, loving and caring.

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Abbreviations

AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
ATN	Average tuber number
ATW	Average tuber weight
AUDPC	Area under disease progress curve
AVRDC	Asian vegetable research and development center
BCA	Biocontrol Agents
CABI	Centre for Agricultural Bioscience International
CGIAR	Center of Group of International Agricultural Research
CIMMYT	International Maize and Wheat Improvement Center
CIP	International Potato Center
CSA	Central Statistics Agency
CV	Coefficient of variation
DAP	Days after planting
DAS-ELISA	Double antibody sandwich enzyme-linked immune-sorbent assay
DisTN	Diseased tuber number,
DisTW	Diseased tuber weight ton ha-1,
DSI	Disease severity index
EARO	Ethiopian agricultural research organization
EM	Essential Microorganisms
EPPO	European and Mediterranean Plant Protection Organization
EPS	Extracellular polysaccharides
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agricultural Organization Statistics
FYM	Farm yard manure
GCV	Genotypic coefficient of variation
HARC	Holetta agricultural research center
IDM	Integrated disease management
LSD	Least significance difference
NCM-ELISA	Nuclotide
NGO	None governmental organization
PCV	Phenotypic coefficient of variation

Pdis	Percent diseased tuber weight (%),
PGPR	Plant growth promoting rhizobacteria
PlHt	Plant height
PRAPACE	Regional Network for Improvement of potato and sweet potato in Eastern and Central Africa)
PSI	Percentage severity index
QDS	Quality declared seed seed
R^2	Coefficient of determination
SAS	Statistical Analysis System
StNo	Stem number per plant
ToTN	Total tuber number,
ToYlth	Total yield ton ha-1,
TTC	trypheny tetrazolium chloride
UnMrkTN	Unmarketable tuber number,
UnMrkTW	Unmarketable tuber weight ton ha-1,
USA	United States of America
USDA	United States Department of Agriculture

CHAPTER I

1. Introduction

1.1 . Background

Potato (*Solanum tuberosum* L.) is produced in all continents except Antarctica and ranks third in importance as a food crop worldwide (Kahlid and Akhtar, 2014). More than half of all production occurs in developing countries (Devaux *et al*., 2014). It is an economically important staple crop prevailing across the world with successful large-scale production, consumption, and affordability with easy availability in the open market (Kahlid and Akhtar, 2014).

Potato production has increased dramatically in developing countries in the past two decades underlining its growing importance as a staple food crop for increasing human populations (Paul *et al.*, 2012). Potato produces considerably more energy and protein than cereals because of its high harvest index where a large proportion of all dry matter produced is edible (Haverkort *et al.*, 2012). Furthermore, it is an excellent food source in which the tuber provides high energy and quality protein as well as substantial amounts of vitamins and minerals providing basic nutrients such as carbohydrates, dietary fiber, several vitamins, and minerals (potassium, magnesium, iron). Dietary intake of potato, especially colored potato, according to Kahlid and Akhtar (2014), plays an important role in the production of defense systems by providing antioxidants, such as vitamins, β-carotene, polyphenols, and minerals. This may help lower the incidence of wide range of chronic and acute diseases like hypertension, heart diseases, cancer, neurodegenerative diseases.

Potato was first introduced to Ethiopia in 1858 by a German scientist (Pankhurst, 1964). Since then, potato production has increased faster than any other food crop

covering an area of 0.3 million ha with production volume of 36.6 million tons of potato tubers in both *Meher* (June to October) and *Belg* (March to June) seasons (CSA, 2015/16). It is widely grown in the country and it is becoming an important crop for smallholder farmers serving both as a cashcrop and food security crop. It is one of the root crops widely grown in the country with the highest rate of growth due to the increasing demand and emerging markets that provide a great opportunity for resource-poor farmers to generate additional income (Gebremedhin, *et al*., 2012). An added advantage of the crop is that tubers can be eaten long before the crop is fully matured (Haverkort *et al.,* 2012). In Ethiopia, potato can fill the gap in food supply during the hunger months of September-November, just before the harvest of the staple grain crops (Gebremedhin, *et al.,* 2012; Haverkort *et al*., 2012).

Ethiopia has favorable climatic and edaphic conditions that favor the production of ware potato and high quality and virus-free seed potato that can be grown on 70% of the arable land in the country (Solomon; 1989, FAO, 2008). Despite the prevailing suitable conditions, potato productivity has remained low with a national average yield of 12.3 t ha^{-1} (CSA, 2015/16). However, farmers produce between 19 and 38 t ha^{-1} using improved seed and crop management practices (Gebremedhin *et al*., 2008). Paul *et al., (*2012) also stated that potato yields vary considerably across the world, with the lowest being in Sub-Saharan Africa; <75 % of the global average and <30 % of the top producing regions. Many factors contribute to the low yield of potato such as narrow genetic basis of the varieties, lack of appropriate agronomic practices, use of poor quality planting material (seed tuber), low soil fertility, poor storage management for ware and seed potato, and pest and diseases (Gebremedhin *et al*., 2012; Haverkort *et.al*., 2012). Among the diseases affecting potato productivity, late blight caused by (*Phytopthora infestans*), bacterial wilt with a causal agent (*Ralstonia*

solanacearum) and viruses play an important role (Yaynu, 1989; Ketema, 1999; Berga *et al*., 2005; Dereje *et al*., 2013).

Bacterial wilt, caused by *Ralstonia solanacearum* (Yabuuchi *et al.,* 1995), is the second most important potato disease in tropical and sub-tropical regions of the world after potato late blight (*Phytophthora infestans* L.) (Champoiseau *et al*., 2010). It is an important soil borne bacterial pathogen with a worldwide distribution and a large host range of more than 200 species in 50 families particularly, members of solanaceous plants (Hayward, 1994). Globally, the disease has been estimated to affect about 1.7 million hectares of potato in approximately 80 countries, with global damage estimates of over USD 950 million per annum (Champoiseau *et al.,* 2009). It is also a serious disease and a major constraint to the production of many economic crops such as tomato, pepper, eggplant, and many other plant species in the tropical and subtropical countries (Dereje *et al*., 2013).

Bacterial wilt of potato, caused by *R. solanacearum*, was first reported in Ethiopia in 1956 by Stewart as reported by Dereje *et al*., (2013) and is currently becoming the most important potato disease after late blight (Berga *et al*., 2005) although complete information on the yield loss caused by the disease is not yet determined. In mid 1970s, the disease was not considered as a serious threat to potato production in Ethiopia except in very limited areas like Wendogenet and Shashemene (Dereje *et al*; 2013). However, the disease was recorded in many regions in the mid-1980s that include Kafa, Sidamo, Wolega, Welo, Gamogofa, Shewa and Alemaya that eventually changed its status (Dereje, *et al*; 2013). Later in mid 1990s, Bekele and Berga (1993) considered this disease as one of the most important disease constraining potato production in Ethiopia. Currently, bacterial wilt is increasingly becoming a serious

threat to potato production in Ethiopia causing heavy crop losses. Dereje *et al*, (2013) reported that the disease is widely spread in different parts of the country and likely to cause serious damage to potato in many places and many farmers around Shashmene, Chencha (Abdurahman *et al*., 2017) have reached a time when they can't produce potato.

A yield loss of potato due to bacterial wilt has been reported to range from 50–80% in Kenya, Burundi and Uganda (Ajanga, 1993), up to 91% in India (Subeda, 2015), 100% in certain parts of Nepal (Gurung and Vaidya, 1997), approximately 14% annual losses in Bangladesh (Elphinstone, 2005), up to 5% crop loss in South Carolina, USA, and 70% in Australia (Subeda, 2015).

The pathogen survives in both soil and seed, has a wide host range and spreads in many ways including through planting materials, irrigation water, farm implements and vectors, which make management of the disease very complex and demanding. Compared to other ways of transmission, seed tubers with latent infection provide the major path for bacterial wilt dissemination. Seed tubers harvested from infected soils have the highest probability of being infected and thus spread the disease (Bekele, 2013).

1.2. Problem statement

Bacterial wilt is a very difficult disease to control on crops such as potato and tomato (Hayward, 1991 and Saddler, 2005) because the causal agent, *R. solanacearum* is a soil borne pathogen and the use of host resistance in these crops is limited. Moreover, *R. solanacearum* is very widely distributed and has an unusually broad host range (Denny, 2006). In high elevation areas, potato is affected mostly by cool temperature-adapted, restricted host range strains of *R. solanacearum* (race 3/biovar 2A) that are

principally transmitted through latently infected tubers (French *et al*., 1998). In Ethiopia many research reports indicated that the strains of *R. solanacearum* that occurred at higher incidences belong to race 3 of biovar 2 (Yaynu, H. 1989; Ketema, A. 1999; Fikre L., 2007; Abdurahman *et al*., 2017).

R. solanacearum is increasingly becoming a serious threat to potato production in Ethiopia causing heavy crop losses as the majority of farmers use farm-saved seed potato that build-up diseases such as bacterial wilt and viruses from repeated cropping cycles resulting in degeneration of planting material from year to year. Many researchers suggested that the major factors associated with bacterial wilt occurrence are lack of well-developed seed systems that certify and regulate the distribution of good quality seed potato, lack of quality assurance in seed potato farmers, planting of susceptible potato varieties and poor pest management practices (Berga *et al*., 1994; Dereje *et al.*; 2013; Bekele, 2013; Abdrhaman *et al*., 2017). According to Dereje *et al.,* (2013) and Bekele and Abebe, (2013), the absence of a formal seed certification scheme and lack of regional quarantine measures in Ethiopia make bacterial wilt a very serious concern, threatening the potato industry. Moreover, bacterial wilt survives in both soil and seed, has a wide host range and spreads in many ways such as through planting materials, irrigation water, farm implements and vectors, which makes management of the disease very complex and demanding (Abdrahman *et al*., 2017).

Crop protection chemicals are mostly ineffective and expensive. In addition, phytosanitary methods such as quarantine are either expensive or difficult to apply and cultural methods such as crop rotation are also not always effective because the farms are too small to allow effective rotation. In addition, rotation practices recommended for one area may not perform well at other locations in addition to

differences in the strains involved (Prior *et al.*, 1994). Thus, no single strategy is 100% effective to control the disease.

However, in locations where the pathogen is established, some level of bacterial wilt control is possible by using a combination of diverse control methods. The common control measures employed to control the disease include crop sanitation, crop rotation, and use of disease-free planting material, resistant variety, and other cultural practices as single or integrated disease management.

Control through the use of resistant varieties alone has showed a little success because there is no high level of resistance in potato cultivars; the required resistance is strain-specific and liable to break down by virulent and highly polymorphic strains of *R. solanacearum* at an ambient temperature and in nematode infested soil (Prior *et al*., 1994). However, some potato cultivars are less susceptible to bacterial wilt than others and can give high yields in the presence of the disease although these are capable of disseminating the disease through progeny tubers with a high rate of latent infection. The use of the moderate levels of resistance that are available, however, can make a great impact on ware potato production in areas where soils are highly infested if bacterial wilt-free seed can be provided (French, 1994; and Priou *et al*., 1999a).

Many trials have been carried out all over the world to control the disease without much success. No promising control of bacterial wilt was achieved using antibiotics (Habashy *et al*., 1993), soil fumigants (Weingartner and Shumaker, 1988), chemical control (Murakoshi and Takahashi, 1984) or breeding of resistant varieties (Hartman and Elphinstone, 1994; Mendoza, 1994; Fock *et al*., 2001; Lopez and Biosca, 2004).

Yuliar *et al*., (2015) reported that only a few acceptablly tolerant tomato cultivars are available that provides moderate levels of disease control and their efficacy is limited geographically. Chemical control, such as soil fumigation with vapam, methyl bromide, or chloropicrin is of limited efficacy. Additionally, detection of the pathogen can be difficult due to occurrence of latent infections in potato tubers. Consequently, the best protection from losses to race 3 biovar 2 will be achieved mainly through the effective use of sanitation standards, development of effective disease management strategies, and improvement of detection and monitoring tools (Prior *et al*., 1994). Studies on the mechanism of disease control by plant extracts have revealed that the biologically active constituents present in them may have either the direct antimicrobial activity or induce the host plants defense response resulting in the reduction of disease development (Amadioha, 2000).

Similarly in Ethiopia, different control strategies have been employed and the use of resistant variety, crop rotation, selection of disease-free planting material, disinfection of plant materials hs been recommended (Guo *et al*., 2004). These include organic soil amendments (Yadessa *et al*., 2010; Getachew *et al*., 2011), microbial antagonists (Fikre and Zeller 2005), and other cultural practices as single or integrated disease management. However, managing the disease totally and protecting host plants from infection through employing a single means is still a challenge of researchers (Derib *et. al*., 2013).

Therefore, alternative management approaches using all possible methods are strongly considered necessary among which the use of clean seed, disease-free soil, field sanitation and the use of effective crop rotation with non-host plants and the use of tolerant varieties will help in managing the disease. As Ephrem, (2015) suggested that

several control options need to be investigated in such a way that an integrated management strategy may be developed based on local needs. This research is, therefore, initiated and carried out with the objectives of providing effective integrated management options for potato bacterial wilt to minimize the effect of the disease for small holder potato growers in Ethiopia.

1.2.1. Objectives

General objective

The overall objective of this study is to select bacterial wilt tolerant potato genotypes, to identify effective soil amendments and biocontrol agents as integrated management options in controlling bacterial wilt under field condition for the small holder potato growers in Ethiopia.

Specific objectives of the study:

i. To evaluate the varietal responses against bacterial wilt disease caused by *Ralstonia solanacearum* and yield of potato.
ii. To assess the effect of soil amendment on bacterial wilt (*Ralstonia solanacearum*) development on potato.
iii. To determine the effect of soil amendment on growth and yield of potato.
iv. To evaluate the effect of bio-control agents on the development of bacterial wilt *Ralstonia solanacearum* disease on potato under field condition.
v. To assess the effect of bio-control agents on the growth and yield of potato under field condition.

1.2.2. Research hypotheses

a. Genetic variation exists in the potato varieties for resistance/tolerance to bacterial wilt with high yield potential to use in the integrated management of the disease.
b. Application of soil amendments improves the soil and plant conditions and as a result reduces the effect of bacterial wilt of potato and increases tuber yield.
c. Bio control agents can help in controlling bacterial wilt disease in an environmentally friendly manner.
d. Biocontrol agents will give more options to potato growers in controlling bacterial wilt and improve potato yield.

1.2.3 Research Questions

i. Is there any variation in potato germplasms regarding tolerance to bacterial wilt disease?
ii. Does the application of different soil amendments reduce bacterial wilt disease incidence and improve potato tuber yield?
iii. Do soil ammendemnts affect the chemical properties of the soil and reduce the development of wilt disease in potato?
iv. Are there biocontrol agents that are effective in suppressing the disease development of (*R. solanacearum)* on potato?
v. Do bio control agents give more options to potato growers in controlling bacterial wilt and improve potato yield?

CHAPTER II

2. Literature Review

2.1. Economic Importance of Potato

Potato (*Solanum tuberosum* L.), ranked as the third most important food crop following rice and wheat and is consumed by over a billion people throughout the world (Devaux et al., 2014; Haverkort and Struik, 2015). Potato promises higher calorie per unit area production potential than any grain and can be produced, stored, and consumed without major technological inputs. Potato is considered as highly nutritious. Recent trends indicate that potato production in densely populated developing nations is on the rise (Bradeen and Haynes, 2011). According to Devaux *et al*., (2014), half of the total production occurs in developing countries that makes potato the third most important food crop globally (FAO, 2013). Lutaladio & Castaldi (2009) suggested that the high yield potential of potato per hectare of arable land, good nutritive value, and cooking versatility have resulted in a threefold per capita potato consumption in the developing world, from 6 kg capita^{-1} year^{-1} in 1969 to 18 kg capita^{-1} year^{-1} in 2009. The crop's short cropping cycle allows it to serve as a hunger- breaking crop, and makes it suitable for intercropping and double cropping, especially in cereal-based production systems in Africa and Asia (Cromme *et al*., 2010; Gebremedhin *et al*., 2012).

Potato constitutes about 75% water, 21% carbohydrates (of which about 82% is starch), 2.5% protein, and less than 1% fat. Often looked upon as primarily a starchy vegetable, potato is actually highly nutritious which are good source of vitamins C, B6, fiber (26% DV), minerals, and proteins, and a well-balanced and complete source of the essential amino acids necessary in the adult human diet (Bradeen and Haynes,

2011). Moreover, the ability of the potato tuber to be stored for months with minimal technological inputs makes potato an ideal food for both developed and developing nations, and as a result its production significantly increased in the populous developing countries.

Gildemacher *et al*., (2009), reported that potato is a source of food and cash income, playing an important role in the rural livelihood system of the densely populated highlands of sub-Saharan Africa. Ethiopia is one of the major potato producing countries in Africa as 70% of its arable lands in the highlands are suitable for potato production. Potato being cultivated for more than 150 years in Ethiopia, it grows dominantly in the Northern Central and Eastern highlands of the country (Gildemacher *et al*., 2009) and the recent reports of CSA, (2015/16) stated that its production area has reached about 0.3 million ha producing more than 3.66 million tons in both *Meher* and *Belg* seasons. However, potato yields are relatively low in developing countries and a number of yield limiting factors are listed by many researchers throughout the glob among which diseases and pests are considered a major cause of low productivity.

2.2. Major constraints of potato production

Despite, having a huge potential with the availability of suitable arable land for potato production in Ethiopia, the national average yield of potato continues to be below 12 tons ha^{-1} (CSA 2015/16), which is by far lower than the potential yield (25-40 t ha^{-1}) of improved varieties grown under good management. The gap between the yield potential and the current average national yield could be attributed to different factors such as lack of quality seeds, narrow genetic base for developing resistant varieties, loses due to disease and pests, inappropriate cultural practices and low research

extension linkages to transform technologies, postharvest handlings for seed and ware potato. Plant diseases, insects, and weeds decrease the production of crops worldwide by 36%, and diseases alone have been shown to reduce crop yield by 14% (Agrios, 2005).

Among plant diseases, soil-borne diseases are considered to be more limiting than seed-borne or air-borne diseases in the production of many crops and accounted for 10–20% of yield losses annually (USDA, 2003). Mansfield *et al.,* (2012) listed the top ten bacterial species based on their scientific and economic importance in plant diseases and put *R. solanacearum* as the second most important bacterial pathogen causing severe yield losses on different Solanaceous crops in different parts of the globe (Mansfid *et al.,* 2012).

Disease and pests are known to play a major role in hindering potato production. Potato is susceptible to a number of diseases, including late blight caused by (*Phytophthora infestans* L.), bacterial wilt and several viral diseases (Bekel and Abebe, 2013). Bacterial wilt is currently becoming the most important potato disease after late blight (Berga *et al*., 2005). Elphinstone, (2005) and Mansfield *et al.,* (2012) considered the disease as one of the most destructive phyto-bacteria due to its lethality, persistence, wide host range and broad geographical distribution. Yaynu, (1989) and Fikre and Zeller, (2007) reported that bacterial wilt is the most destructive in the mid altitude areas around Shashamane, in the Rift Valley, Bako, Jimma, and many irrigated fields throughout Ethiopia. Resently, Abdurahman *et al*. (2017) reported that bacterial wilt is an emerging threat to potato production in Ethiopia, reaching epidemic proportions in the Chencha district with a prevalence of 97% of potato fields in 2015. The same author also suggested that the recent disease outbreak

in the district corresponded with a significant introduction of seed potato that needs a research intervention before the outbreaks spreads to the rest of the country.

2.3. The Pathogen (*Ralstonia solanacearum)*

Ralstonia solanacearum. Smith Yabuuchi et al., (1995) formerly called *Pseudomonas solanacearum* ranking among the most destructive potato diseases in Africa, Asia, and Central and South America (CABI, 2003). It causes yield losses of up to 75% (Cook and Sequeira, 1994). It is a soil borne bacterium that typically invades plants through the roots and colonizes host xylem vessels where symptoms include leaf yellowing, wilting, necrosis, as well as vascular browning (Swanson *et al*., 2005). Some of its economically important hosts are tomato, potato, tobacco, banana/plantain, cowpea, peanut, cashew, papaya, ginger, *eucalyptus* and olive. There are also weed and asymptomatic hosts that may play a role in the survival and persistence of *R. solanacearum* (Hayward, 1994; Moffett and Hayward, 1980). A phylogenetically and phenotypically homogeneous cluster known as race 3 biovar 2 (R3bv2) contains cool-tolerant strains believed to have originated in the Andes (Fegan *et al*; 1998) causing potato brown rot. It ranks among the most destructive diseases of potato in Africa, Asia, and Central and South America (CABI, 2003).

The pathogen commonly forms latent (asymptomatic) infections in the cool tropical highlands, but when infected seed tubers are planted in warmer lowland fields, the resulting plants quickly wilt and die (Allen *et al*., 2001).

R. solanacearum can survive in water, soil, and among the roots of non-susceptible plant hosts. During bacterial wilt pathogenesis, *R. solanacearum* rapidly and effectively colonizes the host plant xylem tissue (Schell, 2000), which is considered a

nutrient-poor micro-aerophilic habitat, seemingly inhospitable to bacterial colonization (Pegg, 1985 as cited by Brown and Allen, 2004).

Bacterial pathogenesis is the result of dynamic interactions between gene products of both host and pathogen (Brown and Allen, 2004). A pathogen must quickly sense and respond to the host environment by expressing genes that facilitate adaptation to conditions encountered during entry, colonization and exit from the host. And such genes according to Brown and Allen and (2004), might allow the pathogen to avoid recognition, combat plant defense responses, use scarce or unique nutrients and influence the plant host to alter its internal environment to suit better the needs of the invading bacterium.

2.4. Phylogenetic and Ecological Diversity of *Ralstonia solanacearum*

Ralstonia solanacearum is a gram-negative soil-borne b-proteobacterium (Hayward, 1991) that causes lethal vascular wilt diseases of over 450 plant species from more than 50 families including solanaceous vegetable crops, banana, ginger, custard apple, peanut, eucalyptus and many other crop plants (Hayward, 1994; Kelman, 1953). Due to its lethality, persistence, wide host range and very broad geographical distribution, *R. solanacearum* is one of the most devastating bacterial plant pathogens known (Elphinstone, 2005) containing a complex species with large heterogeneous group of related strains (Fegan and Prior, 2005). Following its discovery, *R. solanacearum* was originally classified as a member of the genus 'Bacterium' (Smith, 1896) where the application of DNA-based methods eventually resulted in its transfer to the genus Burkholderia (Yabuuchi et al**.,** 1992) and then to the genus *Ralstonia* (Yabuuchi et al**.,** 1995).

R. solanacearum has been historically divided into five races and six biovars based on phenotypic properties such as the ability of the bacterium to wilt specific plant species and to metabolize three sugar alcohols and disaccharides (Fegan & Prior, 2005; Wicker *et al.*, 2012). A more phylogenetically meaningful system has classified the pathogen into four major genetic groups called phylotypes that reflect the geographical origin and ancestral relationships between strains (Fegan and Prior, 2005). Phylotypes I, II and III are composed of strains mainly from Asia, America and Africa, respectively, and surrounding islands, while phylotype IV is primarily composed of strains from Indonesia and some isolates from Japan, Australia and the Philippines.

And recently, this classification was amended by Safni *et al.* (2014), with the complex being divided into three genospecies. *R. solanacearum* consists of strains of phylotype II and blood disease bacterium; the second genospecie, *R. syzygii*, includes phylotype IV of *R. solanacearum*, whereas *R. pseudosolanacearum* is limited to strains belonging to phylotypes I and III. All three genospecies can infect potatoes, but *R. solanacearum* is the most important in terms of pathogenicity and virulence.

R. solanacearum race 3 biovar 2 (R3b2) belongs to Phylotype II (sequevars 1 and 2) are believed to originate in the Andean highlands where the potato and possibly this race of *R. solanacearum* originate and this near-clonal subgroup is widely distributed in tropical regions throughout the world and some temperate regions such as Europe and northern Asia (Elphinstone, 2005).

Race 3 biovar 2 can also survive in temperate climates including highland tropics, UK, and the Netherlands (Elphinstone, 2005), higher altitudes in other tropical areas and also in the Mediterranean basin (Mesiha, 2006).

In Africa, the pathogen is found in many countries causing significant yield loss (OEPP/EPPO, 2004) where phylotype I and IIA are more predominant in the lowlands, whereas phylotype III and IIB prevail in the cooler highlands (N'Guessan *et al.*, 2012). Studies made on the diversity of the pathogen revealed that race 1 biovar 1 known to infect the Solanaceae family, the potato brown rot strains (race 3 biovar 2) and ginger wilting strains (race 4 biovar 3) were reported to be present in Ethiopia (Yaynu, 1989; Fikre & Zeller, 2007; Kifelew *et al.***,** 2015) where race 3 biovar 2 being the dominant strain causing potato bacterial wilt (Abdurahman *et al,* 2017)

Abdurahman *et al.,* (2017) reported a unique diversity of the pathogen within phylotype IIB sequevar 1 in Chencha district which evolved independently of other strains from other regions of the country being an endemic pathogen in the area. Abdurahman *et al.,* (2017) also elucidated the unique diversity to be resulted from the long tradition of potato growing in the district and multiple introductions of the pathogen, as the district was one of the first locations to receive potato genotypes introduced by CIP and known for introduction of seed potatoes by aid agencies for years.

When plants are infected by *Ralstonia solanacearum*, a soil borne bacterium that typically invades plants through the roots and colonizes the xylem vessels, vascular bundles will be filled with multiplying bacteria that obstruct the transportation of water and nutrients. This wilt results in symptoms that include leaf yellowing, vascular browning, necrosis and ultimately wilting of the plant. Physiological changes, such as the increase of respiration rate and reduction of transpiration and photosynthesis, occur in infected plants (Chiwaki *et al*., 2005), which sometimes show a temporary recovery from wilting while the disease is progressing, or may not show any visible change before a sudden wilting.

Bacterial wilt caused by *Ralstonia solanacearum* is also one of the most economically important devastating plant diseases of solanaceous crops such as potato, tomato and pepper, etc., playing significant role as a source of food, income, improved social and nutrition status and employment opportunities as the majority of the horticultural crops product in Ethiopia comes from smallholder farms and are labour intensive in their management (Haverkort *et al*., 2012; Henok and Getachew, 2016).

Ralstonia solanacearum enters roots through wounds made by transplanting, cultivation, insects, or certain nematodes and through natural wounds where secondary roots emerge (McCarter, 1991). Once inside the host, the bacterium has an affinity for the vascular system, where it multiplies rapidly, filling the xylem with bacterial cells and slime. After infection is established, it also moves up through the vascular system, the xylem, and finally blocks water transportation, which causes wilting (Hayward, 1991; Wang and Lin, 2005).

Typical symptoms of bacterial wilt can be observed few days after infection, such as wilting and later yellowing, dwarfing and finally irreversible, sudden wilting and death of plants, caused by invasion of large quantities of bacterial cells and their exopolysaccharide slime in xylem vessels (Henok and Getachew, 2016).

Symptoms of bacterial wilt represent discoloration of the vascular system from pale yellow to dark brown and droplets of milky bacterial ooze exuding from affected tissues (McCarter, 1991) and death of plant cells due to degradation of vessels and adjacent tissues (Buddenhagen and Kelman, 1964; Hayward, 2000).

This results in free flow of *R. solanacearum* cells into the soil from roots or collapsed stems and spread to roots of adjoining plants or to fulfill the saprophytic part of its life cycle (Denny, 2006). The pathogen can persist for a long time in soil, in infected host

plant debris or by colonizing potato volunteer plants, alternative hosts or even non-host plants (Granada and Sequeira, 1983; Akiew and Trevorrow, 1994). When the disease establishes in temperate zones it can survive for periods between 12 months to 3 years in the absence of a potato crop as shown for New South Wales in Australia (Graham *et al*., 1979).

The pathogen, causing a significant yield loss to potato also stays in the soil for several years and prohibits subsequent production of potato in the same field. This calls for a better understanding of the ecology of the organism and the factors that affect its survival and suppression in different soil types (Mesiha, 2009). The problem is further extrapolated by the fact that many plant species remain symptomless after infection and serve as latent carriers of the pathogen, enabling it to persist in the environment and thus it is important to identify these plants as hosts during planning of a control strategy.

The pathogen can, however, also be introduced into an area by planting infected potato tubers, whence it can be disseminated, and survive in contaminated surface water (Janse, 1996; Persson, 1998; Schans and Steeghs, 1998) and weed hosts such as *Solanum dulcamara, S. nigrum, Portulaca oleracea* in Europe (Elphinstone *et al*., 1998) and *Rumex dentatus* and *Solanum nigrum* in Egypt (Farag *et al*., 2004). Volunteer plants or in colder climates; perennial weeds can be a reservoir, and responsible for transmission of the pathogen through successive seasons (Janse, 1996; Lopez and Biosca, 2004).

Moreover, this pathogen may stay latent without showing any symptom in the field with the consequence of high impact on tuber yield in the upcoming season. Detection of latent infections, therefore, requires sensitive diagnostic methods as described by

Swanson et al; (2005). *R. solanacearum* cells can be distributed unevenly in the stems and leaves of both symptomatic and latently infected plants, making the random leaf sampling unreliable testing method.

Berhanu, *et al.,* (2013) stated that inspection of potato seed tubers to monitor the status of latent infection and its consequences has not been adopted in Ethiopia as the detection of latent infections requires sensitive diagnostic methods and consequently, the disease can easily be transmitted through tubers and cause very high economic losses across wide geographic areas as the crop is propagated with vegetative means

2.5. Economic Importance of Bacterial Wilt of potato

In order to meet the demands of an ever increasing human population, the global crop production should double by 2050; however, current estimates are far below what is needed (Ray *et al.,* 2013). Among different factors affect crop production plant diseases, insects, and weeds take the share worldwide up to 36%, where 14% (Agrios, 2005) of the loss is contributed by diseases which requires a control strategy to increased crop production. USDA (2003) reported that soil-borne diseases are more sever plant diseases affecting the production of many crops causing a yield loss of 10–20% each year.

According to Elphinstone (2005), the substantial economic losses caused by the pathogen attributed to its persistence, wide host range and its broad geographical distribution in tropical, subtropical and some warm temperate regions of the world.

Though there is no general information on its economic impacts worldwide, the pathogen causes considerable yield losses depending on host, cultivar, climate, soil type, cropping practices and pathogen strain (Elphinstone, 2005). An estimated losses

USD 1 billion yearly has been reported (Elphinstone, 2005) worldwide on potato alone although the direct economic impact of *R. solanacearum* is difficult to quantify. Losses of about 75% in potato due to bacterial wilt have been described, whereas in tomato, being one of the most susceptible crops, *R. solanacearum* can result in total destruction of the harvest (Elphinstone, 2005; Hayward, 2000; Hayward, 2005). For instance, 50-100% on potato in Kenya (Muthoni *et al*., 2012), 88% of tomato farms affected by bacterial wilt in Uganda (Katafiire *et al*., 2005), 70% on potato in India and varying degree losses in many potato growing countries of the world (APS, 2005). In addition, bacterial wilt affected 95% of tobacco farm field in some counties of South Carolina and resulting in an estimated total loss of USD40 million in both North and South Carolina in 1998 (Fortnum, 2001).

In Ethiopia, a high percentage of bacterial wilt incidence of 63% (Bekele, 1996) on potato, 55% on tomato (EARO, 2002) and 100% on pepper (Mekonnen *et al*.,2015) were recorded in major potato, tomato and pepper growing areas of Ethiopia although a general yield loss was not quantified (Bekele , 1996). In case of potato, since most wilted potato plants do not give marketable tuber, crop yield losses from the disease could be very high under Ethiopian conditions. The presence of bacterial wilt in Ethiopia was first reported on potato and eggplant in Keffa region (South West Ethiopia) by Stewart (1956). Henok and Getachew, (2016) also cited Stewart and Dagnachew (1967) reporting the occurrence of the bacterial wilt on potato, tomato and eggplant in Keffa and on potato in Showa and Arsi region (Central Ethiopia) .

In mid 1970s, the disease was not considered as a serious threat to potato production in Ethiopia except in very limited areas like Wendogenet and Shashemene. In the mid-1980s, however, the disease was recorded in many regions such as Kefa, Sidamo, Welega, Welo, Gamugofa, Shewa and Alemaya that eventually changed its status.

Latter in mid 1990s, bacterial wilt was considered as one of the most important diseases that limits potato production (Bekele and Berga, 1993; Yaynu 1989) and Fikre and Zeller (2007) also reported bacterial wilt was attacking pepper plant in some areas of Ethiopia. Records on the occurrence of bacterial wilt disease increased with elevated production of potato in the country with time.

In the past, when potato was being grown in small acreage, the distribution of the disease was limited to only areas such as Wendogent and Shashemane (South East Ethiopia). Since Shashemane area was of the major sources of seed potato for many years, bacterial wilt was likely spread through the expansion of seed exchange and repeated potato cultivation in the country. Still, the country has great potential for potato expansion (Gebremedhin *et al*., 2006) and potato acreage is increasing every year with the danger of this disease also rises with this expansion. More recently, Berhanu B., (2011) reported bacterial wilt incidence as high as 25% on potato in Amhara regional state (West Gojam zone and North Gonder administrative zone). This indicates the fast distribution of the pathogen from Southern and Central Ethiopia to the Northern Ethiopia where the pathogen was not common possibly through latently infected potato tuber (Henok and Getachew, 2016).

Generally, Berga *et al*. (2000) stated that the significance of the disease is increasing from time to time affecting the potato production because of latently infected seed potatoes and due to diminishing land holdings that limit crop rotation.

In recent times the emergence of highland strains makes the case of bacterial wilt worse and more economically important and affects the expansion of potato industry in the country and hence there is a need for proper management against this pathogen (Dereje *et al*., 2013).

2.6. Spread and dissemination of the pathogen

Ralstonia solanacearum is primarily a soil-borne and water-borne pathogen and it can also spread through several other means including by contaminated soil, irrigation water, surface water, equipment, or personnel as well as infected plant material including seed potatoes (Janse, 1996). The bacterium can be carried over long distances on vegetative propagating materials surviving about 2-3 years in the soil (Hayward, 1991; Coutinho, 2005).

The pathogen also survives in wet soil, deep soil layers (>75cm) (Van Elsas *et al*., 2001), on wooden materials (several days), metal (several weeks) and rubber (several months), in chicken and cattle manure (2-4 weeks) and in waste from the potato processing industry (1-2 months), as well as in latently infected potato and tomato seeds and infected potato tubers (Hayward, 2000; NAPPO, 2001). Crop residue left in the fields infected by *R. solanacearum* also serves as source of disease inoculum in the field (Wang and Lin, 2005).

In greenhouses, it may also be spread by transplanting infected plants, taking cuttings without disinfecting grafting knives between plants, pinching buds of plants, and especially by irrigating with sub-irrigation or ebb-and-flow systems (Swanson *et al*., 2005).

Ralstonia solanacearum primarily infects host plants through their roots by entering through wounds formed by lateral root emergence or by root damage caused by handling or soil borne organisms like the root-knot nematode. The bacterium can also enter plants by way of stem injuries from insects, handling, or tools. The pathogen does not readily spread from plant-to-plant through the splashing of water, casual contact, or aerially (Swanson *et al*., 2005).

2.7. Management strategies of potato bacterial wilt

Recent upsurge of bacterial wilt is threatning potato production in Sub Seharan Africa (SSA) possibley due to *R. solanacearum* infected seed, poor cultural practices, declining soil fertility and global warmimg (Kakuhenzire et al., 2017). This makes fighting against this disease an imperative to sustain potato production in the tropical highlands of SSA (Kakuhenzire et al., 2017). Management of bacterial wilt of potato is difficult once the pathogen has established in the soil (Hayward, 1991; Martin and French, 1985; Fegan and Prior, 2005). However, knowing the features of the disease is quite useful to analyze conditions that determine disease development and plan sound disease management strategy. Currently, there is no single control method effective against the pathogen (Fikre and Zeller, 2005; Berga et al., 2001) although some level of bacterial wilt control has been possible through the use of a combination of methods. These methods include host resistance, agronomic and cultural practices, biological control, chemical control and integrated disease management (IDM) (Berga, 1996, Henok and Getachew, 2016).

Due to the limited efficacy of current management strategies, bacterial wilt continues to be an economically important disease in potato production in many parts of the world requiring an integrated management program for effective control. High-quality pathogen-free seed and plant materials with an acceptable level of resistance, combined with multi-year rotational cropping schemes, should be effective in limiting pathogen population (Yuliar *et al*., 2015). Fumigants and chemical treatments are still used in some situations but increasing public concerns may limit their use in the future making the use of biological control an alternative. Information on resistance

mechanisms is also essential for breeding cultivars with reliable bacterial wilt resistance.

In addition, knowledge regarding the factors that influence the survival of ***R. solanacearum*** in the soil is important, as it can be used in developing an integrated control management strategy.

2.7.1. Chemical Control

Pesticides such as algicide (3-[3-indolyl] butanoic acid), fumigants (metam sodium, 1, 3-dichloropropene, and chloropicrin), and plant activators generating systemic resistance on tomato (validamycin A and validoxylamine) have been used to control bacterial wilt (Henok and Getachew, 2016). The combination of methylbromide, 1,3-dichloropropene or metam sodium with chloropicrin significantly reduced bacterial wilt in the field from 72% to 100% and increased the yield of tobacco and the tomato (Yuliar *et al*., 2015).

The yield of the pesticide-treated tomato was 1.7-2.5 times higher than that of the untreated control (Fortnum and Martin, 1998, Santos *et al*., 2006). Edwards-Jones, (2008) reported that pesticide offered greater net benefit than other control methods, but this has not always been the case. For instance, if farmers apply pesticides without proper care and knowledge, a percentage of the pesticide may remain in the environment for many years (Gadeva and Dimitrov, 2008), become contaminants in soil and/or groundwater (Acero, *et al*., 2008), and be poisonous to farmers (Dasgupta *et al*.; 2007). The use of chemicals like antibiotics to control plant pathogens has been seriously questioned because of development of resistant strains, and its use bears a hazard to human health and the environment (OEPP/EPPO, 2004). For example,

streptomycin application even increased the incidence of bacterial wilt in Egypt (Farag *et al*., 1986).

Although chemicals show promising results, for example Biocine S2HA, stable bleach powder, plant-resistant inducers (acibenzolar-S-methyl), plant essential oils (thymol) and phosphorous acid on a small scale, they are ineffective in large field-scale applications (Champoiseau *et al***.,** 2009; Kabeil et al., 2008; Subedi, 2015).

2.7.2. Host resistance

Host resistance has been considered as an important component of integrated management of bacterial wilt where resistant cultivars could play an important role in managing the disease (Prior *et al*., 1994, French, *et al*., 1998, Muthoni *et al*., 2013). Developing cultivars that are resistant to bacterial wilt is one of the most economical, environmentally friendly, and effective method of disease control. Breeding for resistance to bacterial wilt has focussed on crops of wide economic importance such as the tomato, potato, tobacco, eggplant, pepper, and peanut (Boshou, 2005).

The stability of resistant varieties is highly influenced by the pathogen strains, temperature, soil moisture, and presence of root-knot nematodes, host pathogen interaction, breeding methodology, and genetic linkage between resistance (Boshou, 2005; Elphinstone 2005; Wang and Lin, 2005). Therefore, increasing varietal resistance in the framework of an integrated approach may be the most suitable approach to control the disease (Denny, 2006). Getachew *et al*. (2009) evaluated the resistance of tomato against the highly aggressive *R. solanacearum* strain originating in Ethiopia and found that six genotypes were resistant, eleven moderately resistant

whereas most genotypes including all tomato cultivars commonly grown in Ethiopia were highly susceptible. Resistant plants infected by *R. solanacearum* showed tolerance to the disease. Nakaho *et al*., (2004) suggested that a limited movement of the pathogens from the protoxylem or primary xylem to other xylem tissues, in the stems of resistant tomato plants resulted in the suppression of the pathogen.

Although disease resistance is an important component of integrated disease management, it is generally agreed that breeding for resistance produces only modest gains and often lacks stability and/or durability (Hayward, 1991; Boucher *et.al*., 1992). The resistance to *P. solanacearum* available in potatoes originated mainly from *Solanum phureja* and is controlled by a few genes. It is seldom expressed as immunity since it is overcome by increasing the level of factors favorable for disease: temperature, soil moisture, damage to root system (Martin and French, 1985). Thus, resistance may mean that fewer plants become infected. This is because such kind of resistance is strain specific and liable to break down by virulent and highly polymorphic strains of *R.solanacearum* at an ambient temperature and in nematode infested soil (Prior *et al*., 1994).

According to Martin and French, (1985), resistance is not general, but pathovar-specific as a pathovar at one location may overcome the resistance effective at another and more than one pathovar may occur in a given field. In addition, since the expression of resistance is pathovar and environment specific, local screening is an essential step in the development of resistant varieties (Martin and French, 1985; Muthoni et al., 2013). The Asian Vegetable Research and Development Center (AVRDC) have developed bacterial wilt resistant cultivars of tomato that are grown in some developing countries. However, their resistance is limited to locations, climate, strains of the pathogen, and soil characteristics.

French *et al*., (1998) also stated that no high level of resistance exists in potato cultivars, but some are less susceptible to bacterial wilt than others and can give high yields in the presence of the disease. Some cultivars such as Cruza 148 and Molinera, do not express wilt in cool conditions, but found to be capable of disseminating the disease through progeny tubers with a high rate of latent infection (French *et al*., 1998) when there is conducive environment for disease development. Despite all these complications, the use of moderately resistance varieties can make an impact on ware potato production in areas where soils are highly infested if bacterial wilt-free seed can be provided (French, 1994; Priou *et al*., 1999b).

As Muthoni *et al*. (2014) suggested, resistant potato clones have recently been identified by CIP scientists, and this resistance needs to be incorporated in the variety screening schemes so that those varieties can reach the small holder farmers by replacing the existing susceptible varieties in order to increase potato productivity in sub-Saharan countries.

2.7.3. Soil amendment

The degradation of organic matter in soil can directly affect the viability and survival of a pathogen by increasing soil microbial activity, thereby enhancing the competition in the soil. Ashes and lime can limit *R. solanacearum* by raising soil pH (Muthoni *et al.*, 2012), which should be less than five or more than eight (Messiha, 2006).

Organic amendments to soil have direct positive impacts on plant health and crop productivity through improving the physical, chemical, and biological properties of soil (Bailey *et al*., 2003).

Soil-borne plant pathogens are frequently suppressed in organically farmed land compared to conventional farming (van Bruggen and Termorshuizen, 2003). For example, survival of *R. solanacearum* was found to be affected by soil texture and organic matter, temperature and moisture content (van Elsas et al., 2000).

Despite reports on suppression of various pathogens in organically managed soils (van Bruggen and Termorshuizen, 2003), it was found that *R. solanacearum* survived least in loamy soil with relatively high organic matter content (4%) whilst survival was highest in soil with lower organic matter content of 2.0-2.5% (van Elsas *et al*., 2000). Yet, it was stated that efficient soil management would be related to composition and/or activity of the soil microbiota by enhancing natural biological control capacity (van Elsas *et al*., 2005).

Mesiha, (2006) reported the reduction of soil inoculum and bacterial wilt development due to the application of soil amendments, which resulted in lower yield losses which is especially important for small farmers in tropical countries, where the disease is endemic. The same author showed that this holds true for race 1 in many (food) crops all over the world, but also for the narrow host range or 'potato' race 3, biovar 2 in many mountainous areas in South America and Asia as well as in the Nile Delta area in Egypt.

Berga *et al*., (2001) also reported soil amendment with organic materials (*Sesbania sesbana* and *Leucaena diversifolia*) either singly or combined with inorganic fertilizer reduced wilt incidence and increased potato tuber yield. The decomposition of organic matter in soil can directly affect the viability and survival of a pathogen by restricting available nutrients and releasing natural chemical substances with varying inhibitory properties and it also increases soil microbial activity thereby enhances the likelihood

of competition effects in the soil (Bailey *et al*., 2003). Organic amendment can also stimulate the activities of microorganisms that are antagonistic to pathogenic microorganisms (Akathar and Malik, 2000).

Getachew *et al*. (2011) have reported that application of silicon fertilizer and sugarcane bagasse (an alternative silicon source) significantly reduced the bacterial population and wilt incidence and increased tomato fruit yield. Silicon amendment reported to significantly reduce bacterial wilt incidence and enhanced host resistance in tomato which was attributed to an induced resistance (Dannon and Wydra, 2004; Diogo and Wydra, 2007). Yadessa *et al*. (2010) also reported that soil amendments with coco peat, farmyard manure (FYM) compost and green manure significantly reduced bacterial wilt incidence by 81% and enhanced tomato yield compared to un-amended soil. This could be due to improvement in soil physicochemical characteristics and microbial activity of the amended soil to the advantage of crop growth.

Bekele, (2017) used effective microorganism (EM) fortified compost for potato to accelerate the decomposition of the material used for composting and found living organisms and substances produced or released from EM inhibited the reproduction rate of the *R. solanacearum* suggesting that soil amendment would be an interesting option to manage the pathogen in the major tomato growing regions of Ethiopia.

However, the effect of soil amendment is largely dependent on the type of soil, environment and the material used for amending the soil which requires specific study for different localities according to the soil type and easily available and economically feasible materials.

2.7.4. Crop rotation

Agronomic practices, if properly used, can also reduce the incidence and severity of bacterial wilt. Crop rotation with non-susceptible crops reduces soil borne populations of the bacterium (Berga, 2001). Crop rotation breaks this detrimental effect of continuous cropping with the same susceptible host plant which may end up in the establishment of specific plant pathogenic populations and results in the reduction of plant diseases caused by soil borne pathogens (Janiver *et al*., 2007) although appropriate rotation period and non-host break crops should be well identified.

Rotating potato cultivation with wheat, sweet potato, maize, millet, carrots, sorghum, or phaseolus beans reduced the incidence of wilt by 64-94% while the yield of potato was 1-3 fold higher than that of mono-cultured potato (Katafire *et al*., 2005). Similarly, the onset of bacterial wilt was delayed by 1-3 weeks and wilt severity was reduced by 20-26% when a susceptible tomato variety was grown after corn, lady's fingers, cowpea, or resistant tomato (Adhikari *et al*., 1998).

2.7.5. Biological control

The rhizosphere is a habitat in which several biologically important processes and interactions takes place which is primarily due to the influx of mineral nutrients from accumulation of plant roots exudates through mass flow and diffusion (Sorensen, 1997; Bias, 2004). Bio-control methods represent a significant complement to other control methods, which are based on prophylactic measures, chemical treatments or genetic approaches. The use of rhizosphere resident microbial antagonist is noted as a promising approach in the control of soil born plant diseases as many diseases affect underground organs, which are usually out of reach of germicidal treatments (Joyce *et al*; 2013).

Currently, interest in biological control of plant disease has increased due to public concerns over the use of chemicals (Whipps, 2001) and the promising characteristics of the biological control agent's such as potentially self-sustaining, spread on their own after initial establishment, reduced input of nonrenewable resources, and long-term disease suppression in an environmentally friendly manner (Whipps *et al*., 2007). A variety of soil microorganisms have demonstrated antagonistic activity in the control of various soil-borne plant pathogens, including bacterial wilt pathogen. Yuliar *et al*. (2015) reviewed research reports from books and journals of different sources between 1984 and 2014 and stated that the most common studies (54%) were on methods regarding the biological control of bacterial wilt, followed by those on cultural practices (21%), chemical methods (8%), physical methods (6%) and 11% on integrated pest management suggesting that many researchers were interested in biological control.

Recent studies have indicated that biological control of bacterial wilt could be possible using various species of antagonistic rhizobacteria such as *Bacillus cereus*, *Pseudomonas putida*, *Bacillus subtilis*, *Paenibacillus macerans*, *Serratia marcescens*, *Bacillus pumilis* and *Pseudomonas fluorescens* which were isolated from soil samples collected from potato and tomato rhizosphere from Ethiopia (Henok *et al*., 2007; Fikre and Zeller, 2007; Naser *et al*., 2008; Henok and Wydra, 2014). Similarly, Ciampi-Panno *et al*. (1989) has proved the use of antagonistic bacteria to control *R. solanacearum* under field condition in South America.

According to Dong *et al*. (1999), avirulent mutant of *R. solanacearum* can also be a potential biological agent used to control bacterial wilt caused by *R. solanacearum.* Among the rhizosphere microorganisms fluorescent pseudomonas strains are often selected for biological control strategies because of their ability to utilize a variety of

substrates under different conditions, short generation time and motility that assist colonization of roots. Moreover, they produce active extracellular compounds such as siderophores responsible for the biological suppression of several soil borne plant pathogens (Bagnasco *et al.*, 1998).

The possible suppression mechanisms of biological control agents are thought to be mainly competition, induction of plant-mediated systemic resistance, antibiosis, siderophore production and production of enzymes that degrade the cell wall (Agrios, 2005; Yuliar et al., 2015).

2.7.6. Arbuscular mycorrhizal fungi

Endophytic bacteria are a group of microbes that live in plant tissue, without plant disease and related plants in mutualism symbiosis living in healthy tissues such as seeds, roots, stems and leaves (Raymond and Harrison, (2019). Plants benefit from the presence of endophytic bacteria because they produce compounds or secondary metabolites and antibiotics that stimulate growth hormones to affect plant growth and increases plant resistance to pathogens (Hundley, 2005, Bandara *et a*l., 2006). Plant Growth promoting rhizobacteria (PGPR) which are known to influence the symbiotic association between plant and other microorganisms including arbuscular mycorrhiza (AMF) reduce pathogenic attacks by producing antibiotics (Whipps et. al., 2001). Where as the most common rhizobacteria are *Pseudomonads*, *Azotobacter* and *Bacillus* (Vessey et. al., 2003).

There are studies on tomato (a related *Solanaceae*) that demonstrated the ability of mycorrhizal fungi to limit the density of the soft-rot pathogen *Pectobacterium*

carotovorum in the rhizosphere, to induce a systemic to *Phytophthora* infection (Garcia-Garrido and Ocampo, 1988; Pozo *et. al*., 2002) and *Glomus mosseae* suppression of *R. solanacearum* (Tahat et. al., 2009).

Mycorrhizal fungi are among the most frequent rhizosphere microorganisms, and they can also influence the growth and health of plants (Buée *et. al*., 2009), but their interactions have however been long been underestimated. Arbuscular Mycorrhiza (AMF) is bio-trophic in nature, surviving within the root system until crop maturity, and hence may give mechanical strength to plant roots against soil-borne plant pathogens (Sharma *et. al*., 1992) by inducing plant defense proteins (Agrawal *et. al*., 2002; Van Loon *et. al*., 2006), increasing competition for infection sites (Vigo *et. al*., 2000), conserve the root system by compensating for the loss of root functional and biomass caused by soil borne pathogens (Cordier *et. al*., 1996), and increasing nutrient uptake resulting in more vigorous plants and hence increasing resistance or tolerance to pathogen attack (Linderman, 1994). It also increases phosphorus uptake when plants are grown in nutrient poor soils (Smith and Read, 2008).

Nogueira, *et al*., (2007) also elucidated the importance of fungi in buffering of toxic heavy metal polluted areas and mentioned the case of excess manganese (Mn) in soil toxic to crops where arbuscular mycorrhiza fungi may alleviate the toxic effects by producing compounds that affect the balance between Mn-reducing and Mn-oxidizing microorganisms in the mycorrhizosphere and thus affect the level of extractable Mn in the soil. Although a number of studies have been conducted on potential effect of bio-control agents on bacterial wilt diseases have been made under laboratory and screen house conditions, there is a gap on the information of this results confirmed under field condition and also commercialized biocontrol compounds which can be easily

available in the market and user friendly are lacking to be used by the potato producers.

2.7.7. Integrated Disease Management

Despite the use of several management strategies to control bacterial wilt, no single method was found effective when applied alone due to the complex nature of the pathogen. Control of *R. solanacearum* has proven to be a very difficult task not only due to its broad distribution and wide host range, but also the limited means of protection measures available (Genin and Boucher, 2004) and up to now there is no single effective control measure against it. Therefore, this calls for an integrated disease management strategy which is sustainable and ecologically friendly.

The main goals of an integrated plant disease control program, which is called as integrated pest management (IPM), are to eliminate or reduce the initial inoculum, reduce the effectiveness of initial inocula, increase the resistance of the host, delay the onset of disease and slow secondary cycles (Agrios, 2005).

According to Yuliar *et al*, (2015), IPM reduced bacterial wilt disease by 20–100% in the field or under laboratory conditions, and typically combines two or three methods including cultural practices and chemical and biological methods. Various integrated management options for controlling bacterial wilt have been developed in Ethiopia, Kenya and Uganda and were being disseminated on-farm in several other PRAPACE (Regional Network for Improvement of potato and sweet potato in Eastern and Central Africa) member countries (Kinyua *et al*., 2001). Though the results show positive trends in the management of the disease, bacterial wilt remains a major challenge to potato production in all Sub Sharan countries showing that management of bacterial wilt requires a multi-disciplinary approach (Tusiime *et al*., 2001) and can

only be effective if backed by systematic and continuous community awareness efforts (Henok and Getachew, 2016).

Generally, it is relevant to select and combine different disease control methods that are practical, economical and also environmentally safe to control disease and improve yields. The need for integrated disease management strategy against the devastating bacterial wilt disease is detrimental in countries like Ethiopia where food insecurity and fast population growth rate are evident. Therefore, this research is envisaged to contribute to widening the integrated management options to reduce the incidence of bacterial wilt around Chencha area and other areas with similar agro ecologies, where the disease is prevalent in Ethiopia.

CHAPTER III

3. Materials and Methods

3.1. Description of the study area

The Chencha district is found in Gamo-Gofa Administrative Zone of the Southern Nations, Nationalities and Peoples Region of Ethiopia, 37 kilometers north of Arbaminch. The area is located at a longitude of 6°14’60.00”N and latitude 37° 39’ 59.99” E and an elevation of 2732 meters above sea level (Fig. 1). Based on the 2007 Cenceus conducted by the CSA, the woreda had a total population of 111,686 of whom 51,310 are male and 60,376 female, out of which 11.91% were urban dwellers. However, a resent data according to 2017 projection (QOTERA.ORG, 2019), estimated the population to be 137,692 where 73,680 (53.51%) are female and 64,012 (46.49%) are male.

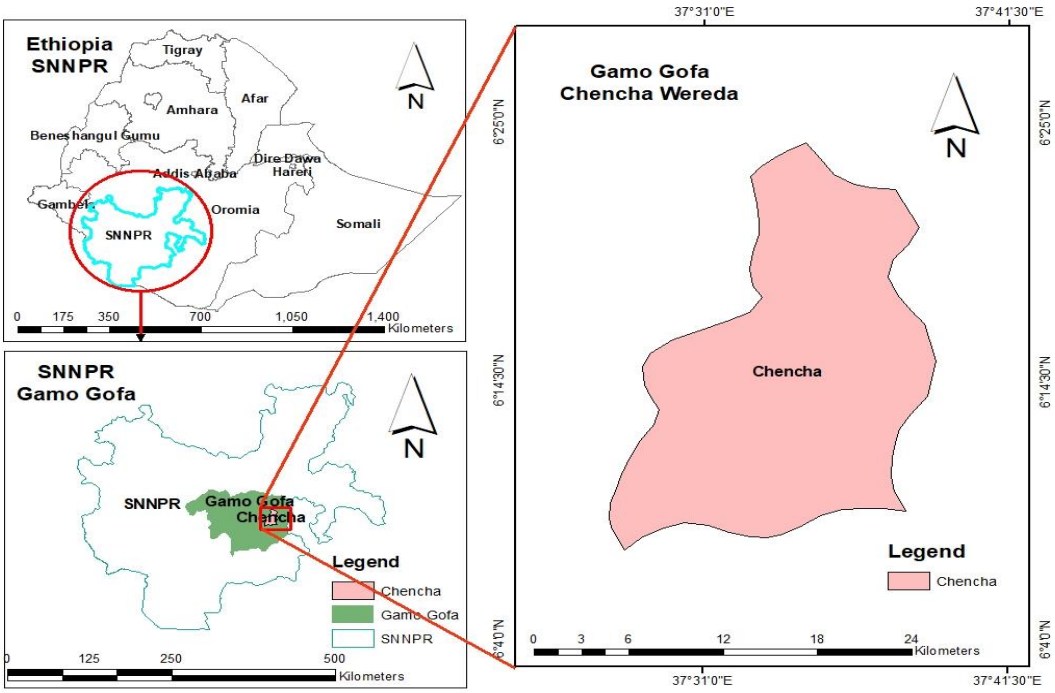


Fig.1. Map of the study area.

Due to its high altitudinal range, the area is characterized by diverse agro-climatic conditions and vegetation cover. The district is divided into two agro-ecological zones, namely, Dega and Woina Dega, which account for about 82 and 18% of the total area, respectively. About 65% of the total land area is mountainous and 3, 17 and 5% comprise plateau, sloppy land and valleys, respectively.

The rainfall regime in Chencha is bimodal; where the first rainy season occurs between March an April and the second round occurs from June to August where the distribution varies from year to year and across seasons. The annual rainfall distribution varies between 900 mm to 1200 mm. The minimum temperature ranges between 11 and 13 degree centigrade, while the maximum temperature is in the range of 18 to 23 degree centigrade https://en.climate-data.org/africa/ethiopia/southern,nations/chencha-55025/. The daily average minimum and maximum temperature, relative humidity and monthly mean rainfall in mm was recorded during the growing period of potato from March to August in the 2015 and 2016 study period (Fig. 2).

The principal soil types of the study site in Chencha district is Nitisols (FAO, 1990). They are well-drained soils with a clayey subsurface horizon that is deeply stretched and has nutty or polyhedric blocky structure elements with shiny ped faces (FAO, 1990).

From the total land area of the Woreda, 27,523.05 ha of land are under cultivation of which 24,420.54 ha are covered by annual plants (wheat, barley, potatoes, beans, peas…etc) while about 3,102.51 ha are covered by permanent plants such as enset and apple https://en.climate-data.org/africa/ethiopia/southern,nations/chencha-55025/. The cropping system in Chencha and particularily at Gendogembela Keble is characterized

by barley, wheat, faba bean and potato as major crops where faba bean is used as a rotation crop for soil amendment and mostly potato follows the fallow land. Potato is produced in two production seasons: the *Belg* season (short rain season: March to June), and the Meher season (long rain season: July to November). The *Belg* season constitutes the bulk of potato production, as the Meher season is conducive for late blight development.

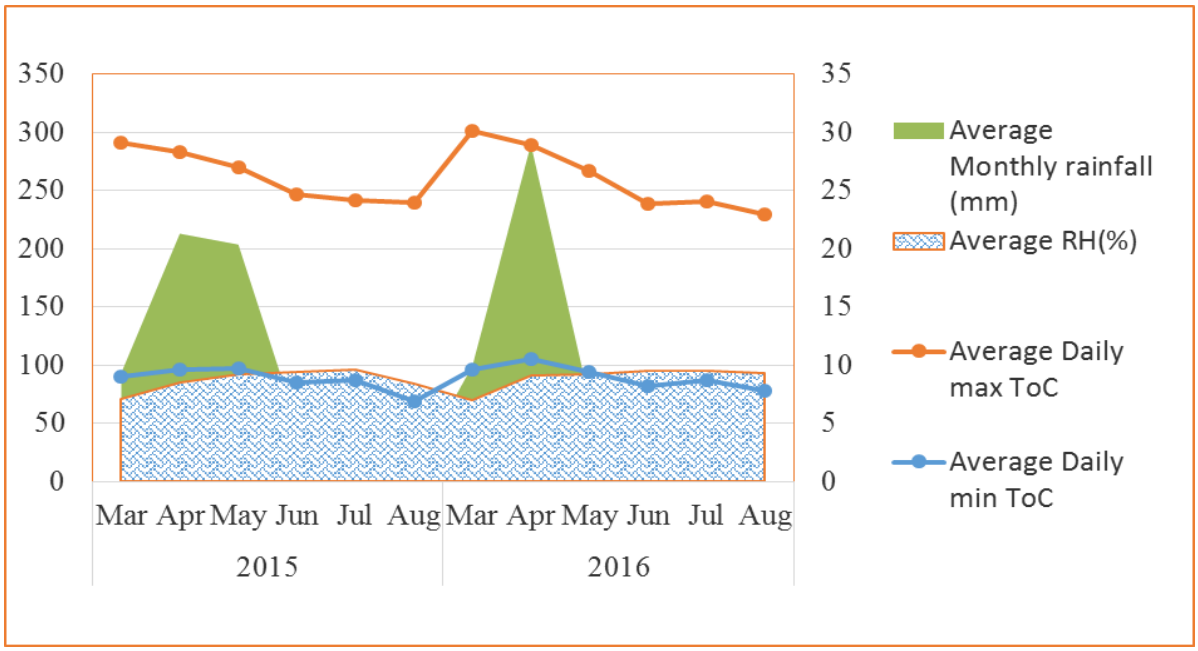


Figure 2. Weather data of Chencha showing daily average minimum and maximum temperature, relative humidity and monthly rainfall in mm during the growing period of potato from March to August in 2015 and 2016.

3.2. General Methodology

The Chencha district was serving as a pilot area for the 'Potato Centre of Excellence' initiative selected by Vita, an Irish NGO, to be a benchmark for best production practices where hundreds of tons of seed tubers of improved varieties have been introduced since 2010 (Abdurahman et al., 2017). However, in the last few years, according to Abdurahman et al., (2017) report, the bacterial wilt disease

has appeared in epidemic proportions in most of the seed potato producers' fields, with increasing trends of both the prevalence and severity of the disease. This was also confirmed by the office of agricultural Bureau and field visits of the district during our preliminary survey and discussion before the development of the research proposal. This issue requires an intervention to develop an integrated management options which can be easily implemented by the potato producers in the area.

To these effects, three different sets of experiment were conducted. The first experiment was designed to screen potato germplasms and varieties for their tolerance to the bacterial wilt disease and their performance regarding adaptability and yield. In the second experiment, different soil amendments which are organic, inorganic and their combinations were tested for their effect on the bacterial wilt disease development and tuber yield of potato, and the third experiment aimed at evaluating five different biocontrol agents to reduce the effect of bacterial wilt and improvement of tuber yield of potato. All experiments were put on farmer's fields under natural infestation of bacterial wilt cauing pathogen *R. solanacearum* where crop losses reported in the previous seasons.

Experimental fields were selected based on the information collected from the staff of agricultural bureau of the district and the farmers them selves and finaly, soil samples were collected from the experimental fields before the start of the experiments, to confirm wether *R. solanacearum* is found in the soil cauing the wilt problem and also to determine the inoculum status of the pathogen in the soil. The test was done in the microbial laboratory of Biotechnology Research Center using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) techhniques using CIP manual as described by Sylvia, (2001). And the results

confirmed that the samples collected from all experimental fields were found to be positive for *R. solanacearum* and the population was estimated to be 10^6 to 10^8 colony forming units (CFU) according to the photometer reading.

Pathogenecity test

The pathogenicity test was done at the green house at Holetta using young succulent potato plants of a susceptible variety Belete grown on a pot. The inoculum of *R. solanacearum* collected from the experimental field and found positive were grown on trypheny tetrazolium chloride (TTC) plates for 48 hours at 30 ^{0}C so that the white or pink, extracellular polysaccharides (EPS)-producing colonies can be differentiated. The Bacterial cultures were washed from the plates and adjusted to approximately 1×10^8 CFU ml^{-1} using sterile deionized water for inoculating. Plants were inoculated using 10 ml syringes and kept under greenhouse condition with temperatures of 25 to 28º C to promote wilt development although there was no wilt development in repeated trial were due to unidentified problem.

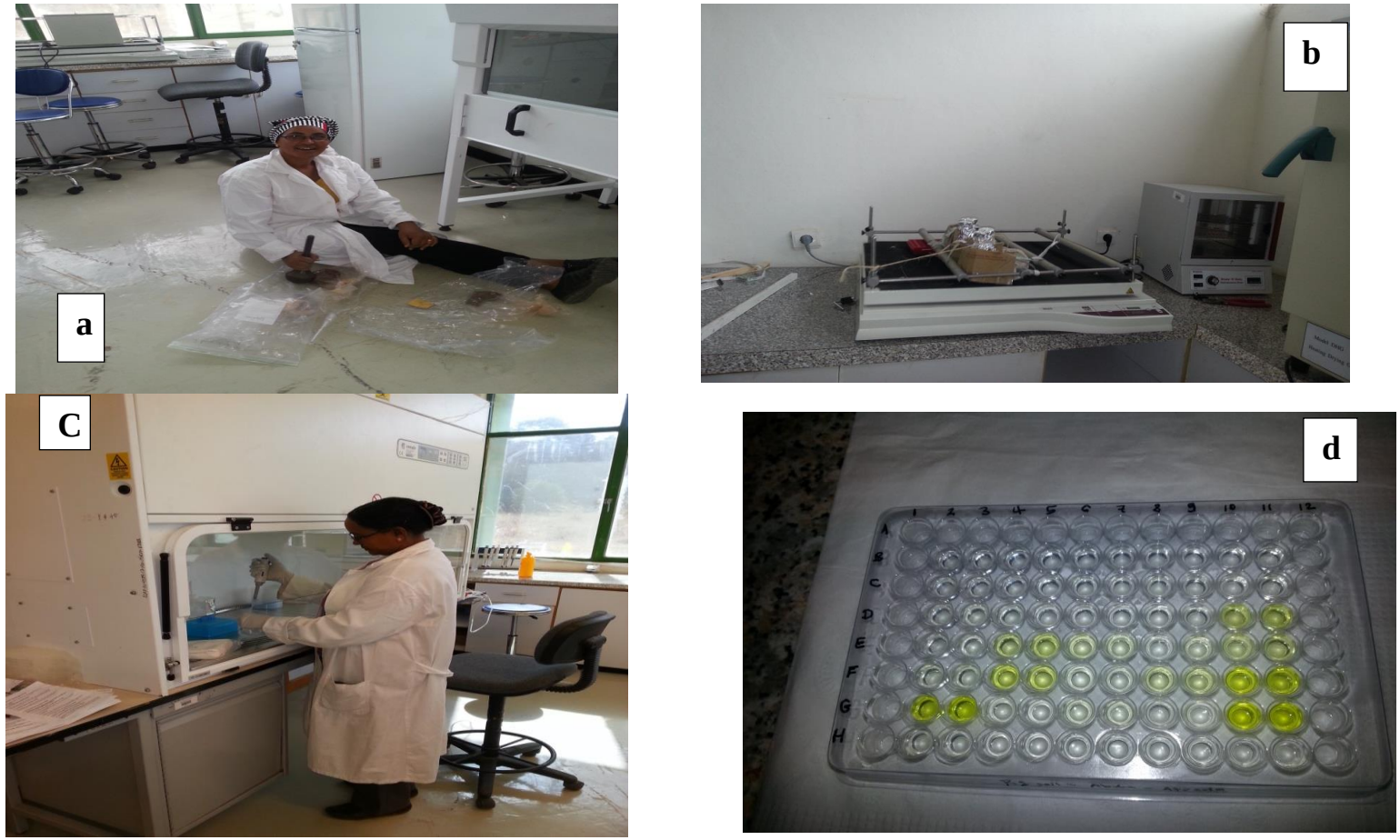


Figure 3. Laboratory analysis of soil samples for determination of the pathogen in the experimental fields using DAS-ELIZA kit wher a = grinding the soil, b = preparing the soil suspension, c = pouring the the suspention in to the plates and d = the development of yellow colour of the samples. The samples that donot develop yellow color are either negative controls or samples free from *Ralstonia solanacearum.*

3.3. Experiment I: Screening of potato genotypes for bacterial wilt (*Ralstonia solanacearum)* tolerance and yield under natural infestation condition.

3.3.1. Trial design and management

A total of fourteen potato genotypes comprised of five released varieties, three from Ethiopia (Jalenei, Gudanei, Belete) released by Holeta Agricultural Research Center (HARC), two from Kenya and Uganda (Cruza and Shangai) released by CIP and eight promising lines obtained from CIP together with a local variety that shows moderate tolerance to bacterial wilt around Chencha area (personal observation) were tested for their tolerance against the bacterial wilt caused by *Ralstonia solanacearum.* A description of potato varieties and clones used in the screening experiment on their source, population and their reaction to some important doseases are given in Table 1.

The experiment was conducted for two *Belg* seasons of 2015 and 2016. The trial was laid out in a randomized complete blocks design with four replications. The plot size was 3 m x 3 m (total plot area 9 m^2) with a spacing of 0.3 m and 0.75 m between plants and rows respectively. This gives space for forty potato seed tubers to be planted per plot. Oxen ploughing were used to reach a maximum level of uniform distribution of the pathogen with in the experimental fields as the experiments were based on field infestation condition.

Table 1. Description of 14 potato genotypes screened for bacterial wilt tolerance under field condition.

Potato genotypes	Pedigree/source	Reaction to diseases		
		Bacterial Wilt	Root Knot Nematode	Late Blight
CIP-391919.3	(69.4 (1043) BW x)	Moderately resistant	Susceptible	Highly Resistant
CIP-392661.18	389743.1 x 390357.4	Moderately resistant	Susceptible	Resistant
Cruza (CIP-720118)	Released variety in Kenya, Uganda	Moderately resistant	not known	Moderately resistant
Shangai	Released variety in Kenya, Uganda	Moderately resistant	not known	Moderately resistant
CIP-393077.159	387348.20 x 389746.2	Moderately Susceptible	Susceptible	Resistant
CIP-394474.16	(4X - 84.1 x 2 X-5.26)	Moderately Susceptible	Susceptible	Resistant
CIP-397006.18	389468.3 (92.119) x 88.052	Moderately Susceptible	Moderately susceptible	Highly susceptible
CIP-392797.22	(387521.3 x APHRODITE)	Moderately Susceptible	Moderately Resistant	Moderatly resistant
CIP-381381.13	Not known	Not known	Not known	Not known
CIP-399062.118	Not known	Not known	Not known	Not known
Belete	Released Variety, Ethiopia	Susceptible	Not known	Resistant
Gudane	Released Variety, Ethiopia	Moderately Susceptible	Not known	Modertely resistant
Jalenie	Released Variety, Ethiopia	Moderately resistant	Not known	Moderately resistant
Chencha local (Sula)	N.A	Moderately resistant (personal observation)		

Planting took place following the onset of rainfall in each year, this being on the 26th of March in 2015 and 6th of March in 2016. All the management practices like fertilizer application, hoeing, weeding, were applied as per the recommended practices for potato production. Hilling was done twice during the growing season before canopy closure. Inorganic fertilizers were applied at the rate of 110 kg ha^{-1} of N in the form of UREA (165 kg ha^{-1}) and 95 kg ha^{-1} of P_2O_5 and DAP (195 kg ha^{-1}) at planting. Data were collected from net plot sizes of 1.5 x 3 m. A maximum of 20 plants were harvested at physiological maturity.

3.3.2. Data collection

All required data on disease incidence, agronomic traits, yield and yield components were collected during the growing period and at harvest.

Data on disease incidence was recorded every two weeks interval after the occurrence of the first symptom based on a 0 - 4 disease scale following Swanson *et al*., (2005), where 0 = no symptoms of wilt, 1 = 1 to 25% leaf area wilted, 2 = 26 to 50% wilted, 3 = 51 to 75% wilted, and 4 = 76 to 100% wilted or dead.

Growth variables including plant emergence, plant vigor, stem number, plant height were recorded throughout the growing season and total tuber yield, total tuber number, marketable yield, diseased and pest infected tubers and tuber deformities were recorded during harvesting.

Yield components like average tuber weight (ATW), average tuber number (ATN) and yield (in t h^{-1}) were calculated from the collected data. Average tuber weight (ATW) was calculated by dividing the weight of the total tubers harvested by the total number of tubers harvested from the plot. This variable can show how big or small the

tubers produced, which determines the marketability of the tuber and size of the seed tuber affecting the total yield of the next crop. Average tuber number (ATN) also determines the seed supply of the potato industry as potato seed market is based on number of tubers than the tuber weight. It was obtained by dividing the total tuber numbers harvested from a single plot by the number of plants (stand count) in the plot.

Harvesting of potato tubers was done at full maturity (when plants reached 75 % senescence). All data were recorded from 20 plants from the 2 middle rows in each plot. The total number of tubers, symptomatic tubers (i.e. showing rotting or bacterial ooze in the tuber eyes or soil adhering to the eyes of the tubers) and total unmarketable tubers were counted from 20 plants in each plot. The weight of the tubers was recorded for each category. Tubers that showed disease symptom were expressed as percentage of the total yields and expressed as a weight in kg/plot and ton/ha, a value which is useful to determine yield loses (t ha^{-1}), and as a number of infected tubers, a value which is used for calculating the rate of infection of tubers.

Table 2. Description of variables measured and derived for the field experiments

Variables	Method of assessment
Emergence	Counting emerged plants from the soil
Plant vigour	Used 1-5 scale where 5 is the most vigorous plant and 1 stands for very poor vigour
Stem number	Five plants were selected from the 2 middle rows randomly and main stems per plant were counted from the base of stem at full flowering stage
Plant height (Cm.)	Five plants were selected from the 2 middle rows randomly and plant height was measured from the ground level to the tip of the center using a plant height meter at full flowering stage
Stand count	Plants in the middle row counted at harvest
Total tuber number	All tubers harvested from each plot (maximum of 20 plants from two middle rows) were counted
Average tuber number (ATN)	All tubers harvested from a plot was divided by the number of plants harvested (maximum of 20 plants)
Total tuber weight (Kg)	All tubers harvested from the two middle row (maximum 20 plants) were Weiged using a portable balance
Average tuber weight (ATW) (g)	calculated by dividing the total tuber weight to the total number of tubers
Total tuber yield per hectare	Calculated from the total tuber weight per net plot area changed in to hectare base and expressed in t ha-1.
Unmarkateble tuber number	It is the sum of all very small sized (< 20mm in diameter), diseased, deformed and insect damaged tubers per plot.
Unmarkateble tuber weight (kg/plot)	It is the sum of all very small sized (< 20mm in diameter), diseased, deformed and insect damaged tubers weights
Percent diseased tubers	It is the proportion of total diseased tuber to the total harvested tuber. Can be expressed in a plot or in a hectare base.

3.3.4. Data Analysis

Data were collected on plot basis from the two middle row except for plant height and stem numbers where five sample plants were randomly selected within the middle row in a plot. The collected data were managed using the EXCEL computer software and subjected to the analysis of variance (ANOVA) using SAS 9.3 (SAS Institute, 2009) and Minitab statistical softwares. The total variability for each trait was quantified using pooled analysis of variance. Tukey's Studentized Range (HSD) Test was used to separate means using 5% probability levels of significance for the characteristics studied after testing for outliers and normality of residuals. Univariate ANOVA was applied following Gomez and Gomez (1984).

The total variability for each trait was quantified using ANOVA according to the following model for each year and combined analyses over two years:

For single year analysis:

The linear model of observations in a complete block design as described by Gomez and Gomez, (1984) is in the form:

$$Y_{ij} = m + G_i + R_j + e_{ij}$$

where m = grand mean; *Yij* denotes the value of the observed trait for *i*-th genotype in the *j*th replication, *Gi* is the fixed effect of the *i*-th genotype (*i = 1,2,…,t*); *Rj* is the effect of the *j*-th replicate (*j = 1,2,…,r*); and *eij* is an experimental error associated with the observation of the *i*-th genotype in the *j*-th replicate.

For combined analysis over years:

$$Y_{ijk} = m + G_i + R_j + L_k + (GxL)_{ik} + e_{ijk}$$

where m = grand mean; Y_{ijk} denotes the value of the observed trait for i^{th} genotype in the j^{th} replicate in the k^{th} year; G_i is the effect of the i^{th} genotype ($i = 1,2,...,t$); R_j is the effect of the j^{th} replicate ($j = 1,2,...,r$); L_k is the effect of the k^{th} year (k=1,2,...,l); $(GxL)_{ik}$ is the interaction effect between genotype and year; and e_{ijk} is an experimental error associated with the observation of the i^{th} genotype in the j^{th} replicate and k^{th} year.

Disease severity and area under disease progress curve (AUDPC)

Four disease assessments on incidence and severity, data were collected at 2 weeks intervals starting from the first symptom around 50 to 95 days after planting until the plants reached senescence. Disease incidence was evaluated by counting the number of plants that showed disease symptom from the total plants assessed in a plot and disease incidence values were converted to percentage severity index (PSI) using the formula:

$$PSI = S_{nr} \times 100/N_{pr} \times M_{sc}$$

Where S_{nr} is the sum of numerical ratings, N_{pr} is the number of plants rated and M_{sc} is the maximum score on the scale given by Swanson et al. (2005). In addition, the area under disease progress curve (AUDPC) was calculated from percentage of disease severity according to the midpoint rule (Garrett and Mundt, 2000) as:

$$AUDPC = \sum_{i=1}^{n-1} [0.5(x_i + x_{i+1})] [t_{i+1} - t_i]$$

Where x_i is the percentage of disease severity at i^{th} assessment, t_i the time of the i^{th} assessment in days from the first assessment and n is the total number of days disease

was assessed. Since severity or incidence (x) was expressed in percent and time (t) in days, AUDPC was expressed in %-days (Campbell and Madden, 1990).

In addition, the data from this experiment were subjected to statistical analysis like bivariate (associations or correlation) and multivariate (cluster and principal component) analyses according to Hardle and Hlavka (2007) using SAS 9.3 (SAS Institute, 2009) and Minitab (2007) computer soft ware. Cluster analysis and principal component byplot were tested using 14 genotypes and 11 yield, disease and yield related traits. It is generally assumed that members within a cluster are more closely related based on the traits under consideration than genotypes within significantly distant clusters.

Partitioning of the total variation into components due to genotype (σ^2_g), environment (years in this case) (σ^2_e) and genotype by year interaction (σ^2ge) was performed using the VARCOMP procedure of SAS which is based on expected mean squares.

Broad sense heritability (H^2b) was estimated based on mean basis for each year and combined over years as suggested by Eckebil *et al*. (1977) as follows:

Single Year: $H^2_b\ \% = \dfrac{\sigma^2_g}{(\sigma^2_g + \sigma^2_e/r)} \times 100$

Combined over Years: $H^2_b\ \% = \dfrac{\sigma^2_g}{(\sigma^2_g + (\sigma^2_{g*}y/y) + (\sigma^2_e/ r*y))} \times 100$

Where H^2b = broad-sense heritability;

σ^2g = genetic variance;

σ^2e = error variance;

r = replication;

y = year;

r*y = replication within year; and

σ^2g*y = variance due to genotype by year interaction.

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were computed according to Singh and Chaudhury (1985) using the following equations:

$$GCV\ (\%) = \frac{\sqrt{\sigma^2_g}}{X} \times 100$$

$$PCV\ (\%) = \frac{\sqrt{\sigma^2_{ph}}}{X} \times 100$$

Where σ^2_g = genotypic variance; σ^2_{ph} = phenotypic variance; and X = sample mean.

Phenotypic and genotypic correlations were estimated using the standard procedure suggested by Miller *et al*. (1958) from the corresponding variance and covariance components using the following equations:

$$r_{pxy} = \frac{\sigma^2 p_{xy}}{\sqrt{\sigma^2 p_x * \sigma^2 p_y}}$$

$$r_{gxy} = \frac{\sigma^2 g_{xy}}{\sqrt{\sigma^2 g_x * \sigma^2 g_y}}$$

Where, r_{pxy} = phenotypic correlation coefficient between characters X and Y and

r_{gxy}= genotypic correlation coefficients between characters X and Y.

3.4. Experiment II: Effects of soil amendments on the development of bacterial wilt (*R. solanacearum*) and potato tuber yield under natural infestation condition.

3.4.1. Trial design and management

Different soil amendments namely compost, manure, lime, inorganic fertilizer and their combinations were used to assess their effects on the development of wilt disease and improvement of tuber yield. The treatments used were: Compost, manure, agricultural lime ($CaCO_3$), fertilizer (recommended) and a combination of each treatment as half compost and half manure, compost and lime, half compost and fertilizer, manure and lime, half manure and fertilizer and lime and fertilizer. A control plot with no soil amendment was also included for comparison. The compost and manure were prepared at Holetta Agricultural Research Center (HARC). The source of composting materials were farm yard manure (FYM), barley and wheat straw, maize and faba bean stalks and other plant materials available at HARC. The compost was prepared in a pit which is already present in the center for the purpose of compost preparation. In addition, well decomposed manure was collected from livestock research department of HARC to be used for this experiment.

A composite soil samples were taken from the experimental fields before the start of the experiment and analyzed to determine the status of major nutrients in the soil including N, P, K, organic C, soil pH and for lime requirement using the methods described by (McLean, 1982). Prior to planting, soil samples were collected from 0 - 20 cm depth at each experimental field and combined to make a bulk sample for each replication, resulting in four composite samples per experimental field for analysis. After manual homogenization, the samples were ground to pass through a 2-mm sieve. Soil samples were analyzed for pH using a ratio of 2.5 ml water to 1 g soil

(McLean, 1982); extractable P using Bray 2 solution as extractant (Bray and Kurtz, 1945). Soil organic carbon was analyzed by wet oxidation using hydrogen peroxide, chromic acid and sulfuric acid (Walkley and Black, 1934). Total N content was determined using Kjeldahl digestion with concentrated H2SO4 and a K2SO4-catalyst mixture (He *et al*., 1990). Exchangeable cations and CEC were determined using ammonium acetate method (Black, 1965), at the soil and plant analysis laboratory of Holetta Agricultural Research Center. Table 11 shows the pre-planting soil chemistry of the trial soil.

Samples from compost and manure were collected randomly from around and within the pile of material to determine N, P, K, organic carbon contents and pH of the amendments before the onset of the trial. The analysis was done using the same analytical procedures that was used for soil analysis. The N content of the compost and manure was used to estimate the fertilizer N rate for each year according to the results of laboratory analysis with the assumption given by Fischer and Glaser (2012) which stated that only up to 20% of the total N content of the compost and manure is mineralized in the first year.

The experiment was arranged in a randomized complete block design with 4 replications. Forty well sprouted tubers of Belete variety were planted in a single experimental plot with a spacing of 30 cm by 75 cm between plants and rows respectively. One-meter border was used between plots and blocks to minimize the overlapping effect of treatments. Following the start of the rains the soil amendments (i.e. compost, manure and lime) were applied manually and evenly to the experimental plots 3 weeks before planting.

Table 3. Physicochemical properties of compost, manure and pre-planting soil from (0-20 cm depth) of the experimental fields for soil amendment trial.

	Soil		Compost		Manure		Test Methods used
	2015	2016	2015	2016	2015	2016	
Chemical properties							
pH (H_2O, HCl??)	4.53	5.15	6.93	7.33	6.95	7.65	1:2,5 H_2O
TN (%)	0.14	0.14	1.17	0.48	1.2	1.21	Kjeldhal bremner, J.M.&C.S. Mulvancy
Available P (ppm)	13.1	5.59	0.65	0.25	0.71	0.7	Bray II
Ex. Acidity (Meq/100g)	0.34	0.3	-	-	-	-	Van reeuwijk,L.p 1N kcl leaching –titration
CEC (Meq/100g)	17.08	22.67	-	-	-	-	Ammonium acetate extraction
OC (%)	1.34	1.17	13.44	15.78	14.33	27.28	Walkley A.&Black,I.A.
K (meq/100g)	0.76	0.86	0.83	0.5	0.92	1.68	Ammonium Acetate Extraction AAS
Ca (meq/100g)	6.36	9.61	-	-	-	-	
Mg (meq/100g)	3.83	4.61	-	-	-	-	
Cu (PPm)	1.69	0.68	-	-	-	-	DTPA Extractionand AAS
Fe (PPm)	254.16	142.6	-	-	-	-	
Zn (PPm)	3.47	0.72	-	-	-	-	
Mn (PPm)	76.89	42.44	-	-	-	-	
Physical properties							
% Clay	47.5	51.9	-	-	-	-	Hydrometer
% Silt	30.3	28.1	-	-	-	-	
% Sand	22.5	20.0	-	-	-	-	

The amendments were thoroughly mixed and incorporated into the soil. Inorganic fertilizers were applied during planting according to the recommended practice for potato production. Compost and manure were applied at the rates of 20 and 10 t ha^{-1} for full and half dose respectively. Inorganic fertilizer N was applied at the rate of 110 kg ha^{-1} as UREA, and P_2O_5 was applied at the rate of 92 kg ha^{-1} as DAP. Lime was applied based on the results of the laboratory analysis for lime requirement of the soil of the experimental using the method described by (McLean, 1982) (Appendix 27).

3.4.2. Data collection and analysis

All the relevant data collected were subjected to a statistical analysis for analysis of variance (ANOVA) and mean separation using SAS version 9.3 (SAS Institute, 2009). Models similar to those described under section 3.3.4 were used for the analysis.

3.5. Experiment III: Evaluation of bio-control agents to control bacterial wilt of potato under field condition in Ethiopia.

3.5.1. Trial design and management

A field experiment was conducted in *R. solanacearum* infected fields for two cropping seasons (2015 and 2016). Bio control agents which are found to be effective from previous works in Kenya were tested for their suppressive action against bacterial wilt (*Ralstonia solanacearum*) under field condition.

Treatments were arbascular micorhizal fungi (AMF), Microbial consortia, Cleanstart, Agrifose-600 and 3 different rates of Neem gold (grounded neem kernel) at 0.1, 0.25 and 1.0 t ha^{-1} used as botanical control and a control plot with no treatment. All biocontrol agents were provided by CIP office Kenya. The description and sources of the biocontrol agents are given in table 4. AMF granules were applied to the soil during planting at the rate of 60 kg ha^{-1} and incorporated to the soil before placing the potato tuber. The white powder microbial consortia were used as seed dressing at the rate of 20 kg tubers dressed per kilogram of the microbial consortia at the time of planting. Agri-fose-600 was a liquid substance sprayed on the field before planting at the rate of 3littres per 900 ml of water per hectare. The different contents of clean start components were mixed in a 20 litters of water and tubers were deeped for 5 minutes just before planting. The cold pressed neem kernel was applied according to each rate of application and incorporated with the soil before planting of the tubers.

The trial was laid out in a randomized complete blocks design with four replications. Potato variety Belete which is susceptible to bacterial wilt was used for testing the agents. Four rows of ten plants were planted in each plot using a spacing of 75c m and 30 cm between rows and plants, respectively, leaving 1 m between the plots and the rows to avoid contamination by biocontrol agents in adjacent treatments.

Before the start of the trial, soil inoculum was determined (Fig. 2) from the experimental field. Soil nutrient analysis on N, P, K, pH, Ca, soil moisture, and organic C were determined each year before applying the biocontrol agents using standard analysis methods as shown in Table 5. A detailed proceedures were given in section *3.4.2*.

Table 4. The product name, source and description of biocontrol agents used in the experiment.

Products	Description
AMF (Arbascular Micorhizal Fungi)	The dominant species was identified in AAU as *Funilinformis mossae* having 628.5 spores/100gm granules. The method used was INVAM-2006/12
Microbial consortia	A white powder consisting of *Trichoderma harzianum* and *Bacillus subtilis* formulated in India with a trade name "*Trichoderma* India".
Clean start (humic acid) Root guard (micro-organisms)	Sourced from CIP office Kenya It is a formulated biological product having effects of nematicide /insecticide, fungicide, nutrients and soil conditioning. It is composed of four products (100 g root guard + 50 mls Phosgard + 40g Humax + 10 mls Natural wet). **Root guard:** Cocktail of plant useful microorganisms formulated with nutrients and enzymes. The microorganisms include *Trichoderma* spp, *Bacillus* spp, *Psudomonas* spp, *Aspergillus* spp, *Chaetomium* spp, *Escccherichia* Spp and *azotobacter* spp. **Phosgard:** Engineered plant nutrient which enhances the plants natural resistance to disease based on phosphoric acid that generates phospite salts when neutralized by potassium hydroxide. **Humax:** soluble powder containing 80% humic acid **Natural Wet:** Biodegradable anti stress wetting agent derived from *Yucca Schidegers* containing 10% sapponins.
Agriphose 600	Consists of P_2O_5, K_2SO_4 obtained from CIP office Kenya
Neem gold	A botanical control from cold press neem cake from neem seed kernel sourced from India and provided by CIP office Kenya using 3 different rates: at 0.1, 0.25 and 1.0 t ha^{-1}.

Figure 4. Photos taken during application of biocontrol agents at planting time from top left =preparation of clean start; top right= Deeping the seed with clean start mixture; below left = measuring Agriphose-600 and below right= planting potato tubers with different treatments in a plot.

3.5.2. Data collection and analysis

Data on disease incidence, agronomic traits and yield data were recorded throughout the growing season and during harvesting as indicated in the previous chapters. The collected data were subjected to ANOVA using SAS 9.3 version (SAS Institute, 2009) and the total variability for each trait was quantified using pooled analysis of variance and Tukey's Studentized Range (HSD) Test was used to separate means using 5 % probability level of significance.

Table. 5. Physicochemical properties of pre-planting soil from of the experimental fields for biocontrol experiment.

	Year 1	Year 2	Test Methods used
Chemical properties			
pH	4.78	4.66	1:2,5 H_2O
TN (%)	0.13	0.15	Kjeldhal bremner,J.M.&C.S. Mulvancy
Avail. P (PPm)	4.72	9.42	Bray II
Ex. acidity (Meq/100g)	0.47	0.19	Van reeuwijk,L.p 1N kcl leaching –titration
CEC (Meq/100g)	31.77	15.19	Ammonium acetate extraction
OC (%)	0.96	1.32	Walkley A.&Black,I.A.
K (meq/100g)	0.22	0.58	Ammonium Acetate Extraction AAS
Ca (meq/100g)	2.82	4.39	
Mg (meq/100g)	8.66	2.62	
Cu (PPm)	0.67	1.49	DTPA Extractionand AAS
Fe (PPm)	139.02	212.59	
Zn (PPm)	1.18	1.79	
Mn (PPm)	45.53	111.45	
Physical properties			
% Clay	58.13	56.56	Hydrometer
% Silt	27.50	23.75	
% Sand	14.38	20.63	

CHAPTER IV

4. Results

4.1. Screening of potato genotypes for bacterial wilt tolerance under natural infestation condition

4.1.1. Disease severity

The separate ANOVA for year 1 and 2 indicated that there were a significant differences ($P < 0.01$) between the genotypes in their disease tolerance interms of disease severity index (DSI) and AUDPC (Appendix 1.). There was a significant difference on disease severity index among potato genotypes starting from the first disease assessment (55 DAP) in the first and second years (Fig. 5a and b).

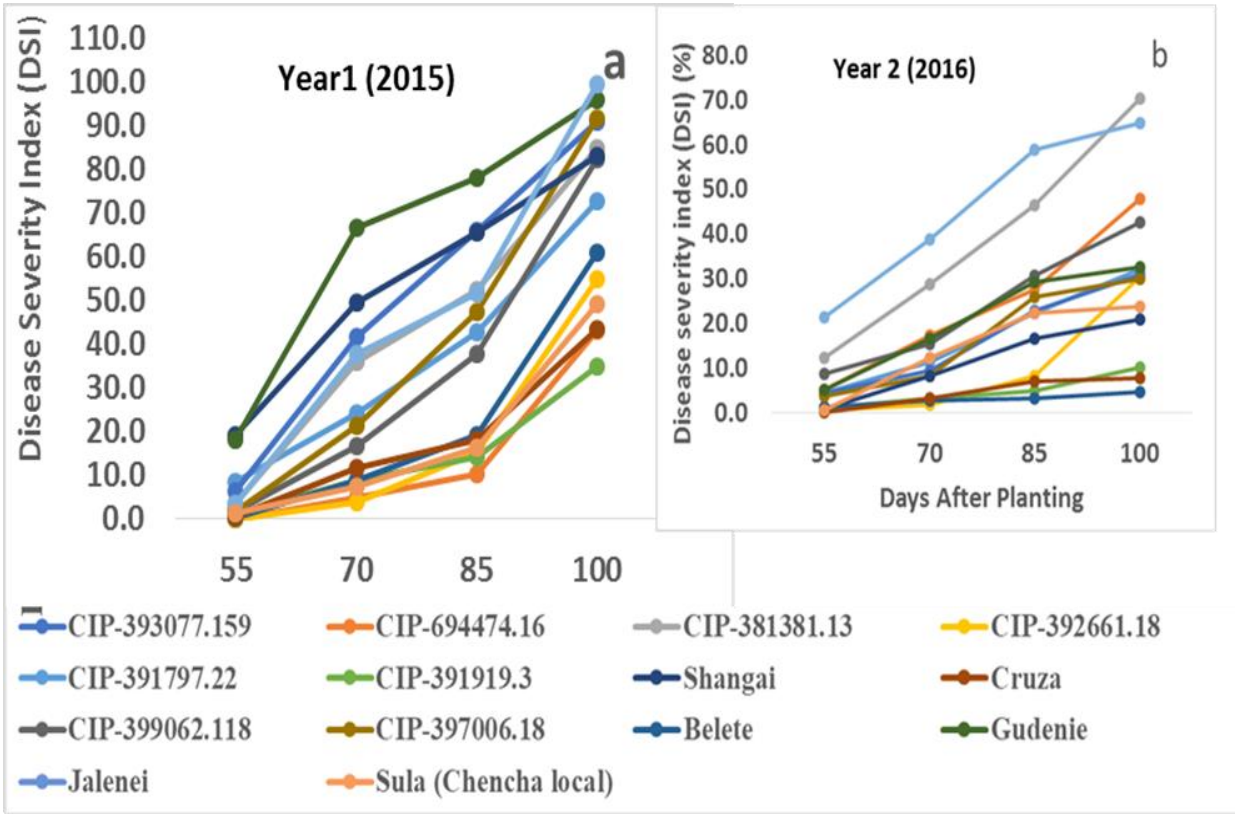


Figure 5. Disease severity index (DSI) recorded at 55, 70, 85 and 100 days after planting (DAP) of 14 potato genotypes tested for bacterial wilt tolerance under natural infestation condition at Chencha **a** = 2015 and **b** = 2016.

For most genotypes, percent wilting increased rapidly from 70 days after planting and levelled off at 90–100 days after planting. The highest DSI at 70 DAP in the first season was for variety Gudanie (66.56%) followed by Blete (49.38 %), CIP-391797.22 (41.56 %) and Jalene (37.81 %) (Fig. 5a and Appendix. 2.). At 85 DAP all the Ethiopian released varieties Gudane, Belete and Jalene had the highest DSI of 78.13, 65.61 and 51.51% respectively and clone CIP-391797.22 also had higher DSI value of 65.94% where as CIP clones CIP-694474.16, CIP-391919.3, CIP-392661.18 and varieties Cruza and Shangai remained to have low DSI that ranged from 10.21-19.13%. The local variety Sula also showed low DSI (16.25%). After 85 DAP Jalene showed a steep rise towards maturity reaching the highest final DSI 99.38% of the plants in a plot was infected with bacterial wilt.

In the second season (Fig. 5b), the disease severity in general was lower than the first year although the trends of disease development and the response of the genotypes showed similarity. At the first disease assessment (55 DAP), Jalenei and CIP-381381.13 had a DSI value of 21.25% and 12.19% respectively whereas most of the genotypes had less than 5% DSI. Jalenei and CIP-381381.13 continued to have the highest DSI values throughout the growing season reaching 64.64 and 70.31% at the final assessment from 85 to 100 DAP (Appendix 3).

In both the seasons, varieties Cruza, Shangai and clone CIP-391,919.3 had the lowest DSI. The local variety Sula also showed a better performance in both the seasons than most of the genotypes.

The combined ANOVA also showed a significant difference among the genotypes, years and their interaction at ($P<0.01$) except at the first disease assessment (55 DAP) where years didn't show a significant difference (Apendix 4.).

The combined means of the two years (Table 6) for final DSI at 100 DAP and area under disease progress curve (AUDPC) showed that the varieties differ significantly in their response to the disease under natural infestation level. Three potato clones CIP-391919.3 and varieties, Cruza and Shangai, gave significantly lower AUDPC value of 410, 495 and 506, respectively. Clones CIP-694474.16, CIP-392661.18 and the local variety Sula also showed moderately lower values of AUDPC ranging from 540 to 810. The highest AUDPC values were recorded from Ethiopian released varieties Jalenie and Gudene followed by a CIP clone CIP-381381.13 with a value 2109, 1997 and 1867, respectively. Table 6 also showed that the final DSI was lower for Cruza, Shangai, CIP-391919.3 and local variety Sula ranging from 22.43 to 36.42% while others had DSI value of 45.55-82.01% the highest being for Jalene.

Table. 6. Combined means final disease severity index (DSI) and AUDPC for 14 potato genotypes screened for BW tolerance in 2015 & 2016 at Chencha

Variety	DSI at 100 DAP	AUDPC
CIP-393077.159	52.43 cd	1196.84 d
CIP-694474.16	45.55 de	809.89 e
CIP-381381.13	77.54 a	1866.58 a
CIP-392661.18	42.67 de	540.58 efg
CIP-391797.22	60.87 bc	1544.05 b
CIP-391919.3	22.43 h	410.10 g
Shangai	32.73 fg	506.05 fg
Cruza	25.61 gh	494.84 fg
CIP-399062.118	62.39 b	1259.78 bcd
CIP-397006.18	60.75 bc	1245.43 cd
Belete	52.00 cd	1512.80 bc
Gudenie	64.19 b	1996.53 a
Jalenei	82.01 a	2108.82 a
Sula (Local)	36.42 ef	716.62 ef

DSI = disease severity index, AUDPC = area under disease progress curve, DAP = Days after planting. Means with different letters within the column are statistically different at ($P < 0.05$) using Tukey's Studentized Range (HSD) Test

4.1.2. Yield and yield component traits

The separate ANOVA for year 2015 and 2016 for yield and yield components (Appendix 6) indicated that there was significant difference at ($P<0.01$ and $P< 0.05$) between the tested genotypes.

Genotypes in this experiment exhibited significant differences ($P < 0.05$) in both the years for all the traits tested (Appendices 7 & 8). Total tuber number (ToTNo), total tuber yield (ToYlth), the proportion of diseased tubers weight (Pdis) as well as average tuber weight (ATW) of the two years results were presented in (Table 7). The local variety Sula gave the highest tuber number followed by Jalene in the first year while in the second year; the highest tuber number was recorded from Cruza followed by Shangai and then the local variety Sula. Similarly, a significantly higher tuber yield in the first year was obtained from the local variety Sula and Jalene. Varieties Cruza, Shangai and CIP clones showed a general lower tuber number, yield and average tuber weight and also with lower disease incidence in the first year when compared to the Ethiopian varieties including the local variety Sula. However, the second year results in the same table showed that variety Cruza gave the highest tuber number and tuber yield followed by Shangai, CIP-392661.18, and the local variety Sula in the order of importance. On average, the second year gave the higher tuber yields (ToYlth) and average tuber weight (ATW) and lower proportion of diseased tuber (Pdis) than the first year while total tuber number (ToTNo) was higher in the first season (Table 7).

Table 7. Means of some traits of tuber yield and yield component of 14 potato genotypes tested for bacterial wilt tolerance at Chencha for two seasons (2015 and 2016).

Variety	Year 1 (2015)					Year 2 (2016)				
	ToTN	ToYlth	Unmrkylth	Pdis	ATW	ToTNo	ToYlth	Unmrkylth	Pdis (%)	ATW
CIP-393077.159	171.8 cde	10.9 bc	3.9 cd	23.73 de	30.0 c	155.3 de	26.9 bc	4.4 bc	8.9 cde	76.3 a
CIP-694474.16	184.5 cd	5.6 de	0.5 g	1.32 h	13.7 def	160.5 d	16.3 d	2.1 fgh	9.5 cd	44.8 efg
CIP-381381.13	143.5 def	5.5 de	1.4 fg	18.12 def	17.4 de	130.3efg	13.6 d	2.8 efg	14.1 c	46.2 defg
CIP-392661.18	179.0 cde	6.3 d	0.5 g	3.62 gh	15.4 de	229.8 bc	26.8 bc	3.0 def	5.8 defg	51.8 bcdef
CIP-391797.22	119.5 fg	11.5 b	6.7 b	57.08 a	42.7 b	150.8 def	24.9 bc	3.6 cde	6.0 def	63.6 ab
CIP-391919.3	187.8 c	6.7 cd	0.4 g	1.56 h	15.3 ed	32.8 k	5.9 e	0.9 i	0.0 g	27.1 h
Shangai	139.8 ef	3.1 de	1.0 g	28.42 d	9.9 ef	244.5 b	30.7 ab	2.8 efg	1.5 fg	40.8 fg
Cruza	207.5 c	6.4 cd	0.6 g	4.98 gh	14.4 def	326.5 a	33.9 a	2.9 def	3.3 efg	44.4 efg
CIP-399062.118	90.3 g	1.4 e	0.4 g	8.77 fgh	7.1 f	90.0 ij	10.4 de	1.2 hi	5.4 defg	48.0 cdef
CIP-397006.18	110.3 fg	5.4 de	3.2 de	53.83 ab	18.9 d	72.0 j	13.3 d	5.3 b	35.0 b	34.0 gh
Belete	90.0 g	11.5 b	4.8 c	42.43 bc	52.9 a	100.3 hi	15.8 d	3.9 cd	11.2 cd	60.7 bc
Gudenie	84.5 g	5.6 de	2.6 ef	44.05 bc	30.0 c	110.0 hij	14.9d	1.8 ghi	6.2 def	55. 8 bcde
Jalenei	265.8 b	22.6 a	9.4 a	41.17 c	38.1 b	127.3 fgh	15.3 d	9.1 a	55.8 a	58.4 bcd
Sula (Ch. local)	322.5 a	25.1 a	3.6 cde	13.2 efg	35.0 bc	205.5 c	23.6 c	2.6 efg	3.2 efg	51.5 bcdef

ToTNo= total tuber number per plot, ToYlth= Total yield ton ha^{-1}, UnMrkylth= unmarketable yield ton ha^{-1}, Pdis= percent diseased yield (%), and ATW= Average tuber weight (g). Note: Means with similar letters within the column are not statistically different.

A significant difference (P<0.01) among the genotypes and years for all the traits tested were observed in the combined analysis of variance of the two years (Appendix 9) also clearly showed except average tuber number (ATN) and plant height which are significant only at (P < 0.05). The interaction between genotype and year also showed a highly significant difference (P< 0.01).

Out of fourteen genotypes tested, seven genotypes gave tuber yield higher than 16 ton ha^{-1} (Fig. 6 and Appendix 10.). From these seven, four genotypes: Cruza, Shangai, CIP-392661.18 and the local variety Sula gave a higher yield combined with lower AUDPC values while the other three (Jalene, CIP-391797.22 and CIP-393077.159) found to give higher yield which is more than 18 tons ha-1 but higher AUDPC values of 2109, 1544 and 1197 (Fig. 6). The CIP clone CIP-391919.3 is the only genotype that had lowest AUDPC (410) and the second lowest total tuber yield (6.3 ton ha^{-1}) exceeding only CIP-399062.118 with a tuber yield 5.9 ton ha^{-1}.

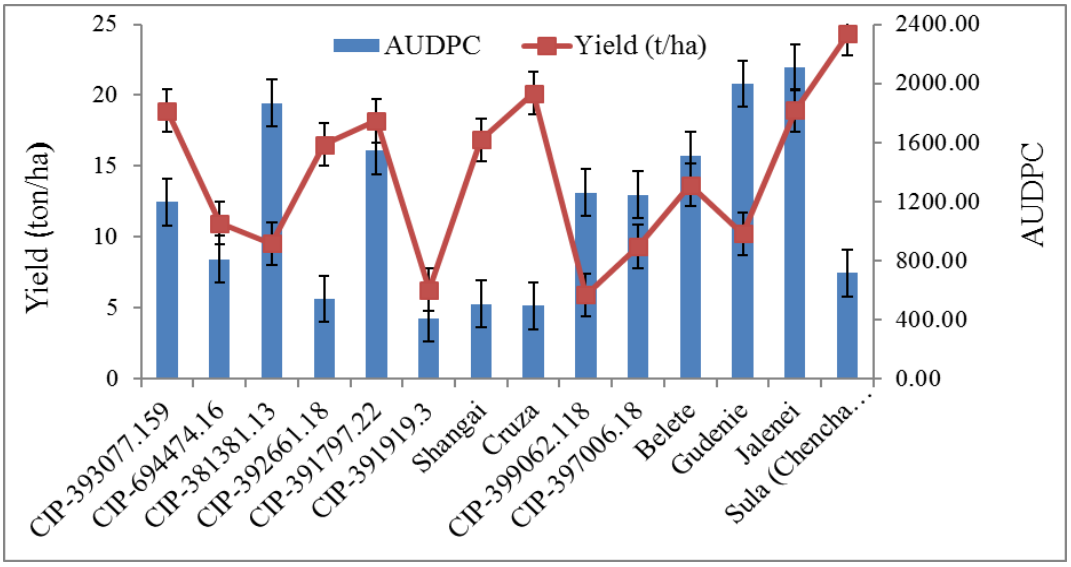


Figure 6. Combined means of tuber yield and AUDPC of 14 potato genotypes tested for bacterial wilt tolerance under natural infestation condition in 2015 & 2016 at Chencha.

The proportion of diseased tuber weight for each genotype was calculated and expressed in percentage (Fig. 7) indicated that Jalene had the highest percent of diseased tuber weight (48.5%) followed by CIP-397006.18 (44.4%) and CIP-391797.22 (31.5%) while Cruza, CIP-392661.18, Sula (local variety) and Shangai had lower proportion of diseased tuber weight 4.1%., 4.7%, 8.2% and 15%, respectively.

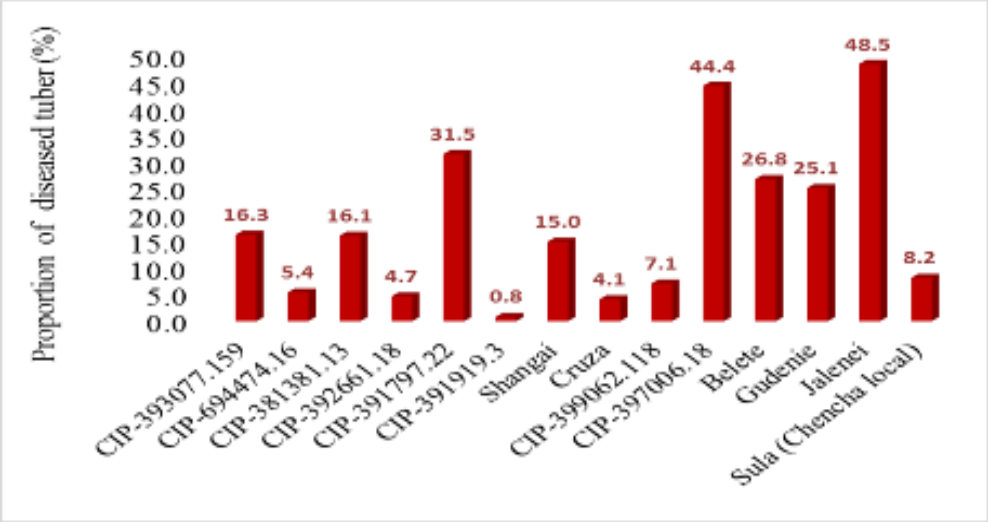


Figure 7. Proportion of diseased tuber weight from the total tuber yield of 14 genotypes under test for bacterial wilt tolerance expressed as percentage

The results of the combined analysis of the two years data for 11 traits on yield, yield components and agronomic characteristics are shown in Table 8. The results revealed that Cruza and the local variety Sula gave significantly highest total tuber number 267 and 264 tubers per plot which led to a highest average tuber number per plant (ATN) of 13.9 and 13.2 tubers for Cruza and Sula respectively. Clone CIP-392661.18, Jalene and Shangai gave moderately high tuber numbers per plot in the same order of their names. The Ethiopian varieties Gudene and Belete, and CIP clones 397006.18 and 399062.118

produced lower tuber numbers per plot (97, 95, 91 and 90 tubers per plot of $4.5m^{2,}$ respectively).

The agronomic traits like stem number per plant (StNo) and plant height (PlHt) also exhibited a significant difference ($P<0.05$) between the genotypes under screening for bacterial wilt resistance.

Five genotypes; CIP- 381381.13, Jalene, CIP-393077.159, Shangai and CIP-391797.22 found to have 3.13- 3.73 stems per plant following Cruza with the highest number of stems (4.3) per plant. CIP-694474.16 had the smallest average stem number (2.1) per plant while the rest are in between. Mean plant height ranged from 36 cm for CIP-391919.3 to the highest 60.92 cm for local and 60.59 cm for Cruza followed by Jalene variety with a plant height of 60.48 cm long. Variety Cruza and the local variety Sula had significantly higher ATN than the other genotypes tested (Table 8). The lowest ATN (4.69 and 5.09 tubers /plant) were recorded from CIP-399062.118 and variety Gudane respectively. Four genotypes CIP-392661.18, Shangai, Cruza and Sula (Chencha local) gave more than ten tubers per plant as 10.42, 12.12, 13.86 and 13.20, respectively (Table 8).

Table 8. Means of 11 traits of 14 potato genotypes tested for bacterial wilt tolerance at Chencha Combined over two cropping seasons (2015 and 16).

Genotypes	ToTNo	DisTNo	UnMrkTNo	UnMrkTW	TotYlth	disYlth	Pdis	ATN	ATW	StNo	PHt
CIP-393077.159	163.50d	30.50 b	54.88 cde	4.16 c	18.89 b	2.42 d	16.31 d	8.34 def	53.14 ab	3.28 bcd	53.60 abc
CIP-694474.16	172.50cd	10.25 e	56.13 cde	1.33 fg	10.99 de	0.81 fg	5.40 ed	8.72 cde	29.24 de	2.10 f	41.69 de
CIP-381381.13	136.88e	17.25 d	50.63 efg	2.11ef	9.55 efg	1.44 ef	16.11 d	6.88 fg	31.79 de	3.73 b	51.82 abcd
CIP-392661.18	204.38 b	7.88 ef	44.88 fgh	1.75 ef	16.53 bc	0.88 fg	4.68 ef	10.42 bc	33.59 d	2.68 cdef	49.79 bcd
CIP-391797.22	135.13 e	32.38 b	61.00 cd	5.17 b	18.19 b	3.99 b	31.53 b	7.42 ef	53.14 ab	3.13 bcde	45.04 cde
CIP-391919.3	110.25 f	2.00 f	38.75 hi	0.63 g	6.28fg	0.05 h	0.78 f	7.28 ef	21.21 f	2.53 cdef	35.96 e
Shangai	192.13bc	8.00 ef	52.50 def	1.90 ef	16.87 bc	0.67 gh	14.97 d	12.12 ab	25.37 ef	3.25 bcd	51.77 abcd
Cruza	267 00 a	11.50 de	63.50 bc	1.71ef	20.13 b	0.70 gh	4.12 ef	13.86 a	29.36 de	4.28 a	60.59 a
CIP-399062.118	90.13 f	4.00 f	34.25 i	0.75 g	5.92 g	0.33 gh	7.08 e	4.69 h	27.54 def	2.78 bcdef	47.20 cd
CIP-397006.18	91.13 f	29.63 bc	52.88 def	4.22 c	9.34 efg	3.72 bc	44.41 a	7.60 ef	26.45 def	2.38 def	34.65 e
Belete	95.13 f	34.00 b	48.38 efgh	4.34 c	13.67 cd	3.20 c	26.79 bc	5.35 gh	56.78 a	2.15 ef	51.41 abcd
Gudenie	97.25 f	24.25 c	40.88 ghi	2.19 e	10.23 def	1.68 e	25.11 c	5.09 h	42.87 c	2.70 cdef	52.01 abcd
Jalenei	196.50bc	91.50 a	125.63 a	9.24 a	18.95 b	8.84 a	48.51 a	9.60 cd	48.22 bc	3.43 abc	60.48 ab
Sula (local)	264.00 a	23.88 c	71.00 b	3.11d	24.32 a	2.03 de	8.17 e	13.20 a	43.27 c	2.85 bcdef	60.92 a
C.V (%)	9.06	15.44	10.11	15.42	16.47	18.60	19.92	11.80	11.45	19.46	12.47

ATN = Average tuber number per plant, ATW = Average tuber weight (g), StNo = Average stem number per plant and PlHt = Average plant hight. **Note:** Means with different letters within the column are significantly different at ($P < 0.05$) based on Tukey's Studentized Range (HSD) Test.

Variety Jalene produced significantly higher tuber numbers (Table 8) with bacterial wilt symptom (DisTNo) and total unmarketable tubers (UnMrkTNo) where out of 197 mean tuber number produced in a plot, 126 tubers (63.96%) were unmarketable and out of which 91 tubers (72.2%) found to have disease symptoms. Likewise, it is clearly seen that the local variety Sula gave the highest tuber yield (TotYlth) of 24.3 ton ha^{-1} followed by Cruza (20.1 ton ha^{-1}). In general, five genotypes; Jalene, CIP-393077.159, CIP-391797.22, Shangai, and CIP-392661.18 gave a relatively better yield ranging from 16.5-18.9 tons ha^{-1} although the proportion of unmarketable and disease infected tubers were higher for Jalene and CIP-391797.22. The lowest unmarketable and disease infected tubers combined with higher tuber yield were obtained from Cruza, Shangai, CIP-392661.18, Sula (Local) and CIP-393077.159.

Cluster analysis

The cluster analysis has grouped the 14 potato genotypes into 4 distinct clusters (CLS) based on 11 traits with 45.8% similarity (Fig. 8a). Each cluster comprises different number of genotypes where CL4 has only one variety Jalene with no similarity with the rest of the clusters where as CL2 comprised 7 genotypes having 63.02% with each other. And the other two clusters CL1 and CL3 contain 2 and 4 genotypes respectively.

Each cluster has peculiar characteristics, for example, Jalenie (CL4) was characterized by high mean for, tuber yield, Tuber number and high disease percentage at the same time. It also showed higher mean for plant height and intermediate mean for stem number, average tuber weight and total tuber number.

Genotypes in CL3 were characterized by lower means for disease incidence and AUDPC and lower means for total tuber number, intermediate plant height and higher

percentages of diseased tuber weight. While genotypes Cruza and Sula (the local variety) grouped in cluster 1, are found to be more tolerant to bacterial wilt which is expressed by lower disease incidence, AUDPC and lower percentage of diseased tuber weight. In addition they are characterized by higher stem numbers, total tuber number and plant height and higher total tuber yield ton ha^{-1}. Whereas genotypes in second cluster (CL2) exhibited an intermediate values of means for most of the traits studied.

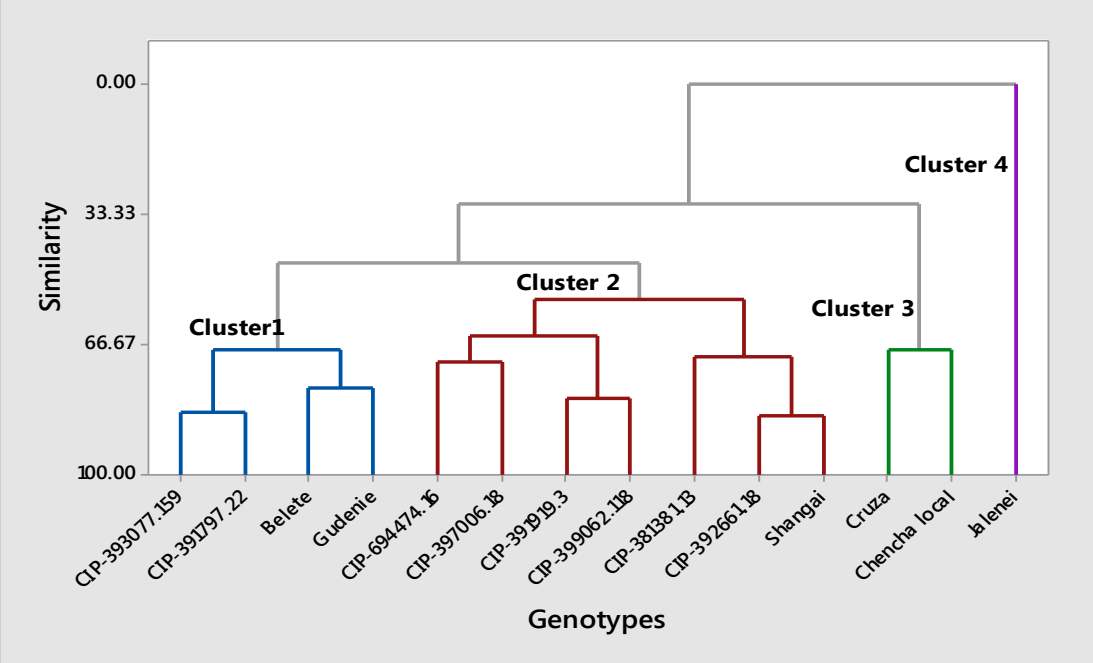


Figure 8a. Dendrogram of 14 potato genotypes tested under natural bacterial wilt infestation conditions combined over two cropping seasons (2015 and 2016).

Principal component analysis-Bi-plot

The clustering of the 14 genotypes was also confirmed with a very similar results of the PCA-biplot (Fig. 8b) that groups the 14 genotypes in to four distinct groups and the genotypes which are in the same group are similar with that of the dendrogram clustering except 2 genotypes:- CIP- 397006.18 and CIP- 393077.159 exchanged their groups. This can indicate the stability of the clustering.

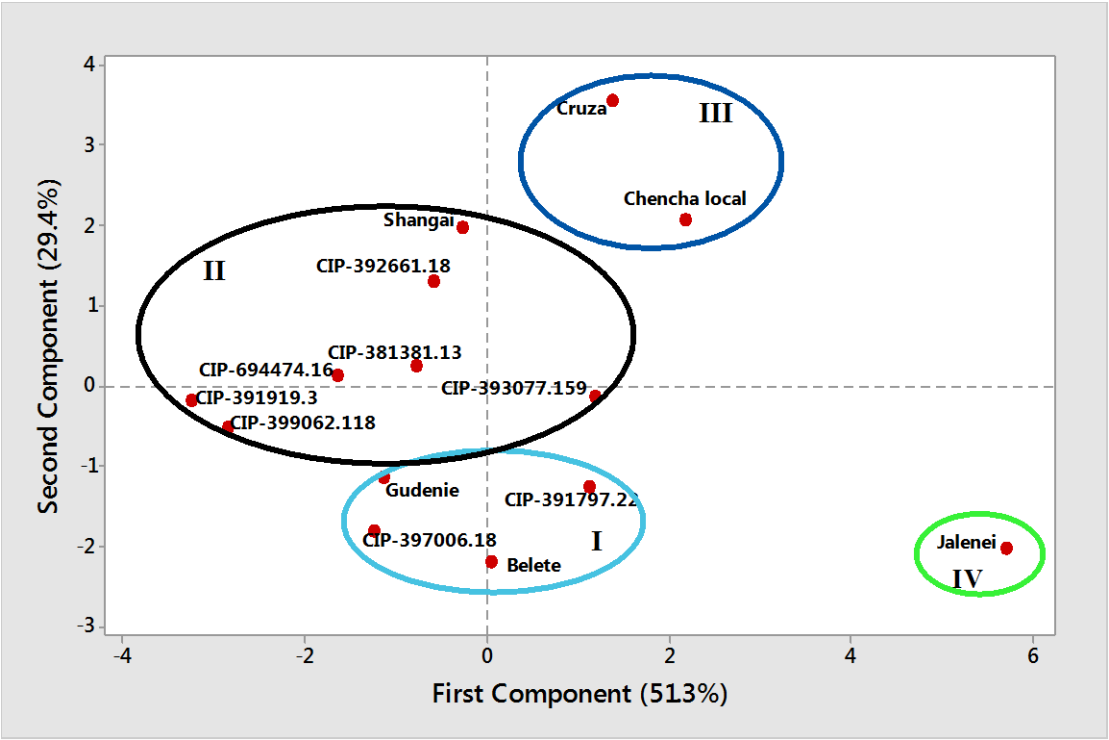


Figure 8b. PCA-bi plot view of 14 potato genotypes tested under natural infestation condition of bacterial wilt for two cropping seasons of 2015 and 2016

Principal component (PC) analysis of the 10 traits of yield and disease (Table 9) showed that the first two PCs accounted for about 80.7% of the total variation with PC1, and PC2, contributing 51.3 and 29.4%, respectively. The first PC that accounted for the highest total variation in the tested genotype had high and positive load for unmarketable tuber number (0.401), unmarketable tuber weight (0.366), number of diseased tuber (0.364), Total tuber yield (0.355), weight of diseased tuber (0.345) and average plant height (0.322) (Table 9).

The second PC had high and positive loading for average tuber number per plant and total tuber number (0.466 and 0.454, respectively) but a negative load for weight of diseased tuber per hectare (-0.341), number of diseased tuber (-0.312) and unmarketable tuber weight (-0.309). Thus the variables with eigenvector of large absolute magnitude (close to unity) reflect a strong influence while those of small

magnitude (near zero) reflect little influence for a particular variable provided that the first few principal components accounts for a substantial portion of the variation.

Table 9. Principal component analysis of ten traits of 14 genotypes, combined over two years (2015 and 2016) under natural disease infestation on farmer's field at Chencha.

PC	Prin1	Prin2
Eigenvalue	5.1331	2.9367
Proportion	0.513	0.294
Cumulative	0.513	0.807
Eigenvectors		
Traits	Prin1	Prin2
ToTNo	0.254	0.454
DisTNo	0.364	-0.312
UnMrkTNo	0.401	-0.07
UnMrkTW	0.366	-0.309
ToTYlth	0.355	0.249
DisTW	0.345	-0.341
ATN	0.210	0.466
ATW	0.266	-0.248
StNo	0.211	0.293
PlHt	0.322	0.231

In this regard, the traits like UnMrkTNo, UnMrkTW, DisTNo, TotYltha, DisTW and PlHt, which had high loading in PC1s were considered to have high contribution for the phenotypic variation in the tested genotypes (Table 9). Traits like ToTNo and ATN have moderate contribution for the variation in the genotypes as the have large load for PC2.

Similarly, the distribution of yield, agronomic and disease traits were plotted on bi-plot view of the principal component (PC) with PC_1 (51.3%) and PC_2 (29.4%) which accounted for 80.7% of the total variations (Fig. 9). Based on this figure, two major groups of traits were formed in which the first group consisted of tuber number, plant height and stem number traits positively correlated with total tuber yield. The traits in

the second group included diseased tuber number, weight and number of unmarketable tubers which are mostly related to disease incidence that affected the yield negatively.

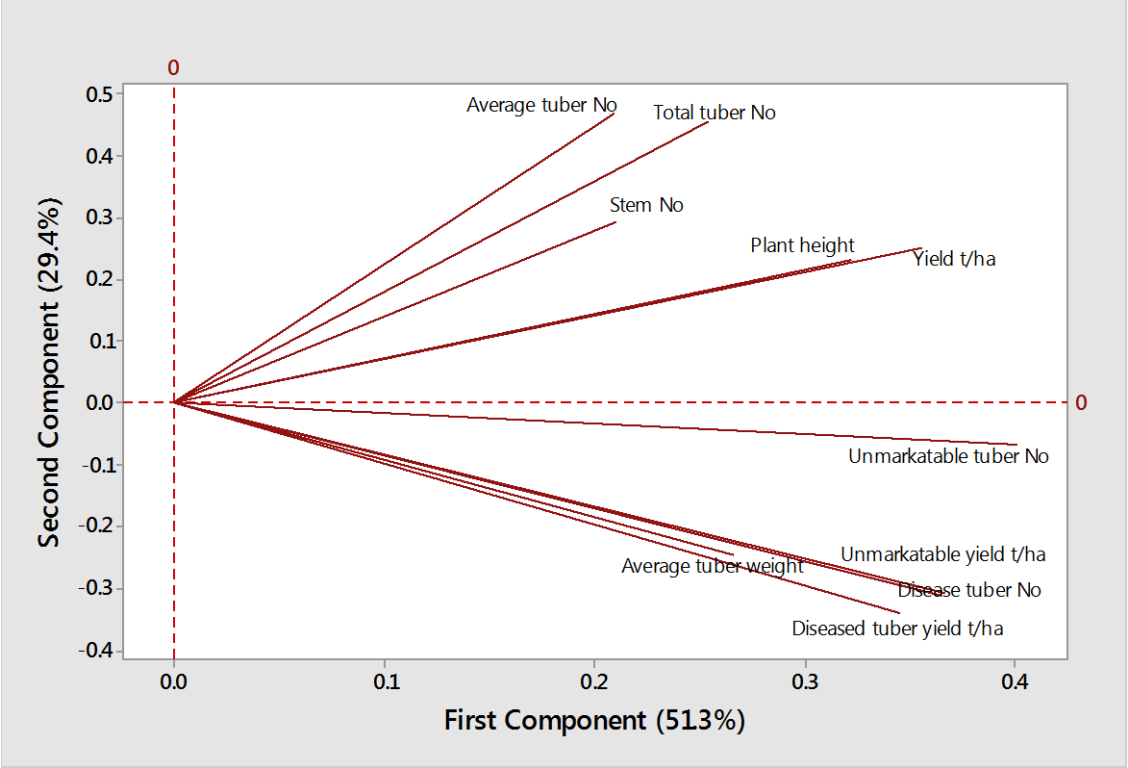


Figure 9. Bi plot view of the principal component analysis (PCA) of ten yield, disease and yield component traits of 14 potato genotypes tested for bacterial wilt tolerance

Phenotypic and genetic variation and heritability

There was a genetic variation among the potato genotypes tested in terms of disease severity, yield and agronomic traits. The estimate of phenotypic (PCV) and genotypic (GCV) variances and broad-sense heritability (H2b) combined over two years (2015 and 2016) are shown in Tables 10. The magnitude of genotypic variance ranged from 0.00 to 2115.9 while the phenotypic variances ranged from 0.49 to 3702.04. High genetic and phenotypic variances were recorded for Total tuber number per plot (ToTN) which is 2115.9 and 3702.04 respectively while the lowest genotypic (0.00)

and phenotypic (0.49) variances were recorded from average stem number per plant (StNo). Accordingly, higher values for the genetic (GCV) and phenotypic (PCV) coefficient of variations were recorded from traits that measured disease severity. Higher values such as 99.9 and 94.04% for diseased tuber weight (DisTW**),** 96.27 and 83.45% for diseased tuber number per plot (DisTN), 83.68 and 66.06% for proportion of diseased tuber (Pdis) and 75.02 and 70.24% for unmarketable tuber weight (ToUnMrkTW) respectively. These high GCVs and PCVs indicated the presence of high genetic variations among the tested genotypes for the traits studied. In contrast traits which express yield and yield components found to have lower values for PCV and GCV for example 39.52 and 16.65% for total yield ton per ha (ToYlth), 38.44 and 29.06% for total tuber number per plot (ToTNo) and 16.72 and 9.35% for average plant height respectively.

The magnitude of H^2b was generally high at each year for all of the characters ranging from 79.88 to 99.56 for year 1 and 93.28 to 99.58 for year 2 (Appendix 10). However, when combined over years (Table 10) most of the characters had lower broad sense heritability indicating the environmental effect of the two years. Therefore it is clearly seen from table 10 that combined broad sense heritability (H^2b) ranged from 17.8% to 87.7% for the traits studied. Relatively highest broad sense heritability was recorded for unmarketable tuber yield (UnmrkTW) (87.7%) followed by diseased tuber weight (DisTW) (81.9%), average tuber weight (ATW) (78.2%) and diseased tuber number (DisTNo) (75.2%). The minimum heritability score was recorded for total tuber yield (TotYlth) (17.8%). This could be due to high environmental effect and low phenotypic and genotypic coefficients of variations of these traits under study.

Table10. Genetic (VG), environmental (VE), and phenotypic (VP) variance, phenotypic (PCV) and genotypic (GCV) coefficients of variation and broad sense heritability (H^2b) of 12 agro morphological traits of 14 potato genotypes tested for bacterial wilt tolerance combined over two years (2015 and 2016).

Triats	Sample mean	VG	VGxL	VE	VP	PCV	GCV	H^2b
ToTNo	158.28	2115.90	3120.90	205.50	3702.04	38.44	29.06	57.16
ToYlth	14.27	5.65	50.98	5.37	31.81	39.52	16.65	17.76
DisTNo	23.38	380.69	248.51	13.01	506.57	96.27	83.45	75.15
DisTW	2.20	4.28	1.85	0.16	5.23	99.90	94.04	81.91
Pdis	18.14	143.59	170.39	13.06	230.42	83.68	66.06	62.32
UnMrkTNo	56.80	309.03	354.97	32.99	490.64	39.00	30.95	62.99
UnMrkTW	3.04	4.56	1.23	0.21	5.20	75.02	70.24	87.67
PrUnMrk	24.52	129.77	138.03	33.86	203.02	58.11	46.46	63.92
ATN	8.60	3.36	10.08	1.02	8.53	33.96	21.31	39.40
ATW	37.28	111.53	57.70	17.79	142.60	32.03	28.33	78.21
StNo	2.94	0.00	0.90	0.33	0.49	23.84	0.00	0.00
PlHt	49.78	21.67	85.75	37.66	69.25	16.72	9.35	31.29

ToTNo= total tuber number number per plot, DisTNo = diseased tuber number, UnMrkTNo = unmarketable tuber number. ToYlth = Total yield t ha^{-1}, DisTW = diseased tuber weight t ha^{-1}, UnMrkTW = unmarketable tuber weight t ha^{-1}, PrUnMrk = proportion of unmarketable tuber yield (%), ATN = average tuber number, StNo = stem number, PlHt = plant height and ATW = Average tuber weight (g).

Phenotypic and genetic correlations among traits

The phenotypic (rP) correlations among traits combined over two years (Table 11) revealed that the correlation coefficients among traits ranged from -0.38 to 0.96. Total tuber yield had strong positive phenotypic correlations ($P < 0.01$) with total tuber number (ToTNo) with the values (0.64), ATW (0.70) and ATN (0.70), respectively.

Traits related to disease incidence including number of diseased tuber (DisTNo), weight of diseased tuber (DisTW) and proportion of diseased tuber from the total yield (Pdis) showed (Table 11) either a weak positive, weak negative ($P < 0.05$) or a non significant ($P > 0.05$) phenotypic correlations with tuber yield.

Other traits that had strong positive phenotypic correlation ($P < 0.01$) between each other were traits related to disease incidence such as DisTNo and DisTW (0.89); DisTNo and Prdis (0.74); DisTNo and unmarketable tuber weight (UnMrkTW) (0.94); unmarketable tuber number (UnMrkTNo) and DisTNo (0.81) and DisTNo and proportion of total unmarketable tuber weight (PrUnMrk) (0.68).

Similarily, the genotypic (rG) correlations among traits combined over two years (Table 12) showed that the genotypic correlations coefficients between the 11 traits ranged from -0.41 to 0.98. Total tuber yield had strong positive genetic correlations ($P < 0.01$) with total tuber number (ToTNo) having (rG = 0.81), ATW (0.56), ATN (0.75), and plant height (0.80).

Total tuber yield showed either a weak positive or negative ($P < 0.05$) or a non significant ($P > 0.05$) genotypic correlations with traits related to disease incidence including number of diseased tuber (DisTNo), weight of diseased tuber (DisTW) and proportion of diseased tuber from the total yield (Pdis) (Table 12).

Traits that are related to disease incidence showed strong positive phenotypic (Table 11) and genetic (Table 12) correlations ($P < 0.01$) between each other such as DisTNo and DisTW (rG = 0.98; rP = 0.89), DisTNo and Prdis (rG = 0.82; rP = 0.74), DisTNo and unmarketable tuber weight (UnMrkTW) (rG = 0.87; rP = 0.94), unmarketable tuber number (UnMrkTNo) and DisTNo (rG = 0.87; rP = 0.81) and DisTNo and proportion of total unmarketable tuber weight (PrUnMrk) (rG = 0.79; rP = 0.68) respectively.

Some traits also showed mixed correlations having strong or medium; positive or negative correlation in one while a non-significant correlation in the other as in the case of total tuber yield (ToYlth) with average stem number per plant (StNo) and

unmarketable tuber weight (UnMrkTW) having a positive phenotypic correlation ($P < 0.01$) ($rp = 0.65$ and 0.40 respectively) (Table 11) where as a non-significant genotypic correlation (Table 16). As it is clearly shown in tabels 11 and 12, average tuber number (ATN) with proportion of total unmarketable tuber weight (PrUnMrk) and ToTNo with PrUnMrk and Pdis had medium ($P < 0.05$) negative phenotypic correlations ($rP = -0.39$; -0.38 and -0.28 respectively) but the genetic correlations for all were not significant.

Table 11. Combined phenotypic correlation coefficients for ten yield, disease and agronomic traits of 14 potato genotypes tested for bacterial wilt tolerance for two years under natural infestation condition at Chencha

Variables	ToTNo	ToYlth	DisTNo	DisTW	Pdis	UnMrkTNo	UnMrkTW	PrUnMrk	ATN	ATW	StNo
ToYlth	0.636**										
DisTNo	0.144 ns	0.076 ns									
DisTW	0.026 ns	0.172 ns	0.891 **								
Pdis	-0.284 **	-0.229 *	0.742 **	0.802 **							
UnMrkTNo	0.513 **	0.101 ns	0.812 **	0.614 **	0.430 **						
UnMrkTW	0.094 ns	0.394 **	0.807 **	0.949 **	0.680 **	0.532 **					
PerunMrk	-0.376 **	-0.282 **	0.681**	0.747 **	0.958 **	0.364 **	0.661 **				
ATN	0.914 **	0.696 **	0.043 ns	-0.009 ns	-0.317 **	0.395 **	0.103 ns	-0.391 **			
ATW	0.016 ns	0.699 **	0.110 ns	0.300 **	0.003 ns	-0.206 *	0.497 **	-0.016 ns	0.014 ns		
StNo	0.319 **	0.654 **	-0.116 ns	0.028 ns	-0.209 *	-0.167 ns	0.189 *	-0.224 *	0.388 **	0.506 **	
PlHt	0.526 **	0.398 **	0.268 **	0.199 *	0.066 ns	0.361 **	0.246 **	0.002 ns	0.382 **	0.215 *	0.372 **

* and ** indicate significance at ($P < 0.05$ and 0.01, respectively); ns = Non significant; ToTNo= total tuber number, DisTNo = diseased tuber number, UnMrkTNo = unmarketable tuber number. ToYlth = Total yield t ha^{-1}, DisTW = diseased tuber weight t ha^{-1}, UnMrkTW = unmarketable tuber weight t ha^{-1}, PrUnMrk = proportion of unmarketable tuber yield (%), ATN = average tuber number, StNo = stem number, PlHt = plant height and ATW = Average tuber weight (g).

Table 12. Combined genotypic correlation coefficients for eleven yield, disease and agronomic traits of 14 potato genotypes tested for bacterial wilt tolerance for two years under natural infestation condition at Chencha.

Variables	ToYlth	DisTNo	DisTW	Pdis	UnMrkTNo	UnMrkTW	PrUnMrk	ATN	ATW	StNo	PlHt
ToTNo	0.813**	0.062 ns	0.006 ns	-0.312 ns	0.477 ns	0.064 ns	-0.405 ns	0.944 **	-0.008 ns	0.527 ns	0.671 *
ToYlth		0.418 ns	0.337 ns	0.028 ns	0.612 *	0.455 ns	0.005 ns	0.753 **	0.558 *	0.453 ns	0.802 **
DisTNo			0.981 **	0.816**	0.869 **	0.970 **	0.792 **	-0.021 ns	0.617 *	0.149 ns	0.379 ns
DisTW				0.792 **	0.873**	0.977 **	0.848 **	-0.043 ns	0.637 *	0.159 ns	0.362 ns
Pdis					0.546 *	0.846 **	0.988**	-0.262 ns	0.365 ns	0.356 ns	0.518 ns
UnMrkTNo						0.831 **	0.485 ns	0.401 ns	0.712 **	0.208 ns	0.443 ns
UnMrkTW							0.828 **	0.017 ns	0.668 **	0.142 ns	0.317ns
PerunMrk								-0.354 ns	0.467 ns	-0.054 ns	-0.060 ns
ATN									-0.174 ns	0.479 ns	0.495 ns
ATW										-0.002 ns	0.441 ns
StNo											0.619 **

* and ** indicate significance at ($P < 0.05$ and 0.01, respectively); ns = Non significant; ToTNo= total tuber number, DisTNo = diseased tuber number, UnMrkTNo = unmarketable tuber number. ToYlth = Total yield t ha^{-1}, DisTW = diseased tuber weight t ha^{-1}, UnMrkTW = unmarketable tuber weight t ha^{-1}, PrUnMrk = proportion of unmarketable tuber yield (%), ATN = average tuber number, StNo = stem number, PlHt = plant height and ATW = Average tuber weight (g).

4.2. Effects of soil amendments on the development of bacterial wilt disease and potato tuber yield under field condition

4.2.1 Disease Incidence

The separate analysis of variance (ANOVA) for year 2015 and 2016 indicated that soil amendments differ significant ($P<0.01$) on their effects on disease incidence (Appendix 11). The effect of the soil amendments on disease incidence was presented as disease severity index (DSI) and area under disease progress curve (AUDPC). The DSI of the two years showed a different trend of disease incidence in the first and second years (Fig. 10).

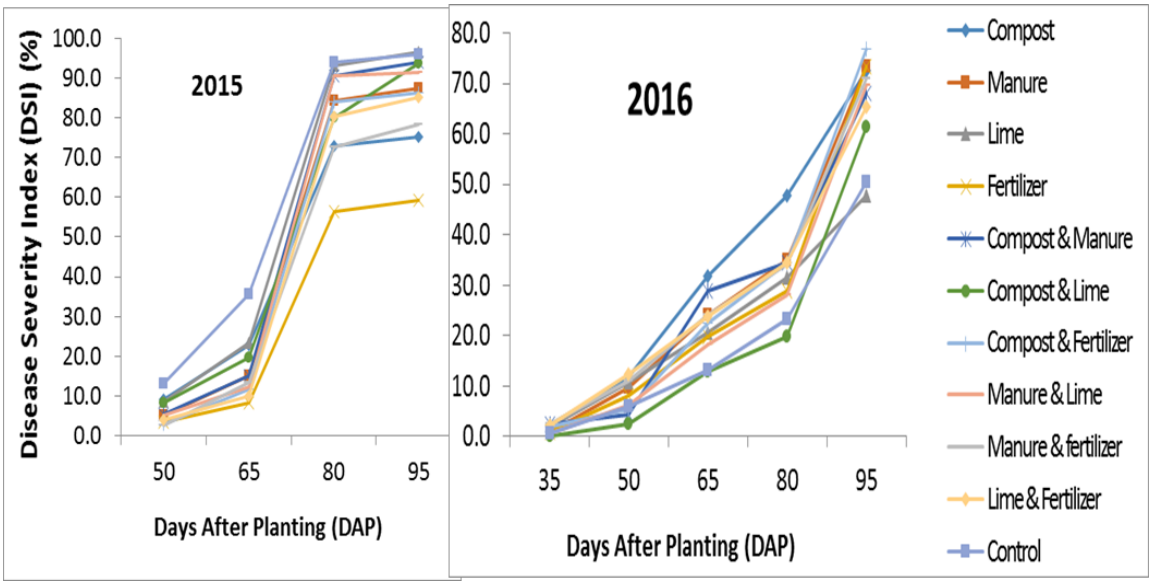
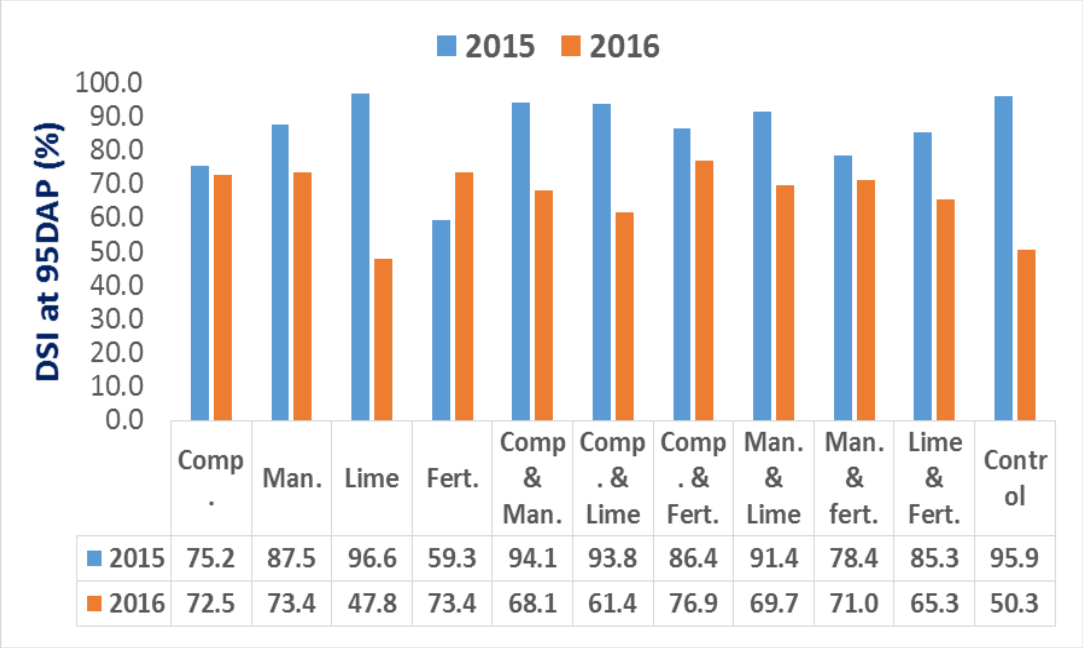


Figure 10. Disease severity index (DSI) of soil amendments tested for bacterial wilt management under natural infestation at Chencha in 2015 and 2016.

The wilt symptom development started early (35 DAP) in the second year. In the first year (2015), disease symptom started lately at around 50 days after planting and percent wilting increased rapidly from 65 days after planting and levelled off after 80 days. While

in the second year (2016), the disease symptom development started as early as 35 days after planting but the percent wilting moves slowly thorough out the five assessment periods having the highest final DSI (77%) from the compost and fertilizer combination treatment at 95 DAP (Fig. 10).

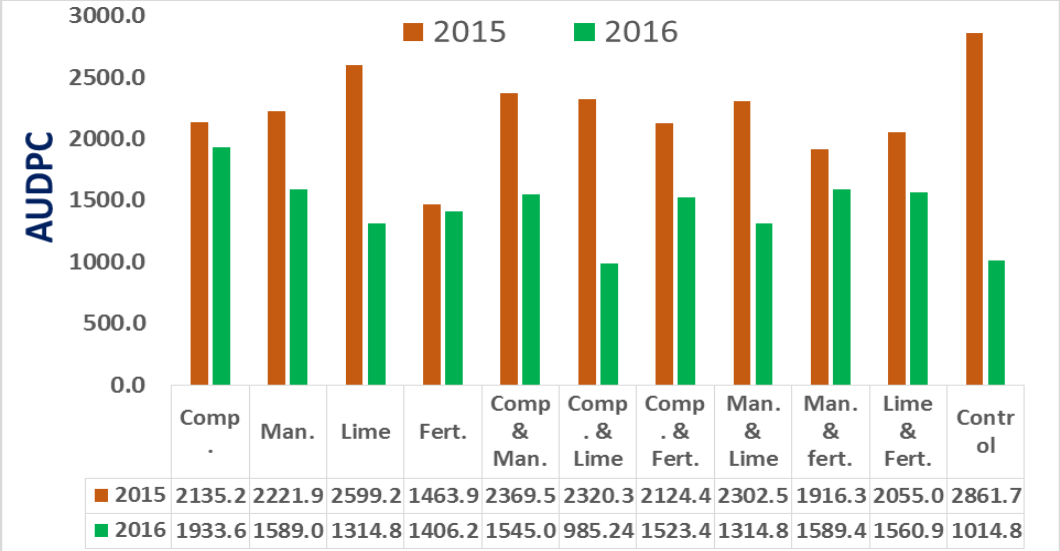
Figure 10 also indicated that the highest final DSI in the first year (95.9 %) was recorded from control plot with no soil amendment at 95 DAP. The lowest DSI 59% and 48% were recorded from fertilizer only and lime only treatments in the first and second year respectively. However, most of the soil amendments showed similar progressive disease development trend throughout the growing period with a final DSI of 60 to 96 % in the first year and 48 to 77% in the second year.



	Comp.	Man.	Lime	Fert.	Comp & Man.	Comp. & Lime	Comp. & Fert.	Man. & Lime	Man. & fert.	Lime & Fert.	Control
2015	75.2	87.5	96.6	59.3	94.1	93.8	86.4	91.4	78.4	85.3	95.9
2016	72.5	73.4	47.8	73.4	68.1	61.4	76.9	69.7	71.0	65.3	50.3

Figure 11. Final Disease Severity index of two years (2015 & 2016) of the soil amendments recorded at 95 days after planting

The final disease incidence, expressed as disease severity index (DSI), of the two years presented separately in Figure 11, depicted that the second year was mostly characterized by lower disease development than the first year for most of the soil amendments except the fertilizer treatment with 73% DSI in 2015 and 59% in 2016. On the other hand, the compost treatment had a very close wilting percentage value in both years (75.2% in year 1 and 72.5% in year 2) (Fig.11). AUDPC also had similar trend (Figure 12) for both years as it is derived from disease severity index. The highest AUDPC value 2861.7 was recorded from control plot in the first year followed by lime only treatment 2599.2 while the lowest AUDPC was recorded from fertilizer only treatment. In the second year, the highest AUDPC (1933.6) was from plots treated with compost and the lowest (985.24) from compost and lime combination. The control plot also showed the second lowest AUDPC (1014.8) in the second year (Fig.12).



	Comp.	Man.	Lime	Fert.	Comp & Man.	Comp. & Lime	Comp. & Fert.	Man. & Lime	Man. & fert.	Lime & Fert.	Control
2015	2135.2	2221.9	2599.2	1463.9	2369.5	2320.3	2124.4	2302.5	1916.3	2055.0	2861.7
2016	1933.6	1589.0	1314.8	1406.2	1545.0	985.24	1523.4	1314.8	1589.4	1560.9	1014.8

Figure 12. Mean AUDPC different soil amendments tested for bacterial wilt management at Chencha for two years (2015 & 2016)

The combined ANOVA showed a significant difference ($P<0.01$) among the treatments, years and the treatment by year interactions in terms of DSI. The combined means of analysis of variance of two years (Appendix 13.) for disease assessment was done excluding the first disease assessment since there was no data for year one. The results of the analysis in Table 13 show that the soil amendments differ significantly in their effect on development of disease symptoms under natural infestation level. The differences between soil amendments were larger in the earlier assessments while narrowing towards maturity of the plants where the values are close to each other during the final recording.

At 50 days after planting the highest DSI (10.5%) was recorded from the plot treated with compost at the rate of 20 ton ha^{-1} followed by lime treatment and control plot while the lowest disease severity (4.1 %) was recorded from the plot treated with a combination of compost and fertilizer. Compost application continued to reach the highest DSI value (27.3%) in the second disease scoring (65 DAP) followed by control and lime only treatments.

Finally, all the treatments showed narrow differences of DSI at 95 days after planting ranging from 72.2 to 81.6% percent wilting. Likewise, the highest AUDPC value (2034.4) was recorded from compost treated plot followed by compost and manure combination, lime only, control and manure with AUDPC values of 1957.3, 1957.0, 1938.3 and 1905.5 respectively (Table 13).

Table 13. Means of disease severity index (DSI) and AUDPC of soil amendments for BW management combined over two years (2015 & 2016) at Chencha

Treatment	50 DAP	65 DAP	80 DAP	95 DAP	AUDPC
Compost	10.47 a	27.34 a	60.34 ab	73.84 abcd	2034.38 a
Manure	7.34 cd	19.53 cd	59.69 ab	80.47 ab	1905.47 abc
Lime	9.53 ab	22.03 bc	62.34 a	72.19 cd	1957.03 ab
Fertilizer	5.78 def	13.91 e	42.57 d	66.35 d	1435.10 e
Compost & Manure	4.84 ef	21.97 bc	62.50 a	81.09 ab	1957.27 ab
Compost & Lime	5.31def	16.25 de	49.84 c	77.56 abc	1652.78 d
Compost & Fertilizer	4.06 f	17.03 de	59.22 ab	81.63 a	1823.91 bcd
Manure & Lime	5.63 def	15.16 e	59.38 ab	80.53 ab	1808.68 bcd
Manure & fertilizer	6.77 cde	18.75 cd	53.36 bc	74.70 abc	1752.87 cd
Lime &Fertilizer	8.13 bc	16.88 de	57.34 ab	75.28 abc	1807.97 bcd
Control	9.53 ab	24.38 ab	58.59 ab	73.13 bcd	1938.28 abc

DSI = disease severity index, AUDPC = area under disease progress curve, DAP=Days after planting. Note: Means with similar letters within a column do not differ significantly at ($P < 0.05$) based on Tukey's Studentized Range (HSD) Test.

In general, the AUDPC was found to be higher for all the treatments used as soil amendments where the smallest AUDPC value (1435.1) was obtained from the plot treated with only the recommended inorganic fertilizer. This may be due to the presence of high infestation of the disease and the shortage of time to see effect of soil amendments

4.2.2. Yield and yield components

The ANOVA for yield and yield component traits showed significant differences ($P < 0.01$ and $P < 0.05$) between the soil amendments in both years (appendix 14). The combined ANOVA for the two years also showed significant differences ($P < 0.01$) among the soil amendments and years for all the traits tested. The interaction between

soil amendments and the year also showed a significant difference ($P< 0.01$) except for the total tuber yield (ToYltha) which did not show a significant difference (Appendix 15).

Soil amendments used in this trial exhibited significant differences ($P < 0.05$) in both the years for most of the traits tested (Appendix 15). Total tuber number (ToTNo), total tuber yield tons ha^{-1} (ToYlth), weight of diseased tubers (DisTW) and the proportion of diseased tuber weight to the total tuber yield (Pdis) were presented separately for each year **(**Table 14). From this table it is clear that all the soil amendments and their combinations found to improve the growth and tuber yield of the potato except the plots treated only with lime where its results mostly showed very close to the control plots with no amendment applied.

The first-year results in Table 14 revealed that the highest total tuber number per plot (ToTNo) was obtained from the combination of manure and lime which is 240 tubers per plot followed by manure, compost and compost and manure giving 239, 226.5, and 226.3, respectively. The lowest number of tuber numbers in year 1 was recorded from control (113.8) and lime treated (129.3) plots. The highest yield of 38.5 tons ha^{-1} in the first year was obtained from treatment combination of manure and inorganic fertilizer followed by compost (35.7), manure (32.7), compost manure combination (32.3) and compost with fertilizer (31.8) tones ha^{-1}. Whereas the minimum tuber yields were harvested from a control and lime only treated plots with 7.1 and 9.4 tons ha^{-1}.

With regard to disease symptom development, weight of diseased tubers (DisWT) and proportion of diseased tubers from the total tuber yield expressed in percentage (Pdis %) (Table 14) were used to measure the effect of the treatments in managing the bacterial

wilt at field infestation level. Thus, the results of the first year (2015) showed that Pdis was higher for all soil amendments except for treatment with only inorganic fertilizer which was significantly lower (63.3%) than the other treatments that reached 81.3% for compost to 95% for the control and the rest lied with in this range.

The results of the second year (2016) also showed that a treatment of manure and fertilizer combination found to give the highest total tuber number about 262 tubers per plot (Table 14) followed by compost and manure treatments with 253 and 245 tubers per

.

Table 14. Means of some yield, disease and yield related traits of soil amendments tested for bacterial wilt management at Chencha for two seasons (2015 and 2016).

Treatments	Year 1 (2015)				Year 2 (2016)			
	ToTNo	ToYlth	DisTW	Pdis (%)	ToTNo	ToYlth	DisTW	Pdis (%)
Compost	226.5 ab	35.7 ab	28.9 ab	81.3 a	253.3ab	41.0 a	4.8 a	11.7 ab
Manure	239.3 a	32.7 abc	27.8 ab	86.1 a	245 ab	34.7 abc	4.9 a	15.5 a
Lime	129.3 e	9.4 d	8.3 d	88.5 a	91.3e	13.4 d	2.0 cd	15.2 a
Fertilizer	159 d	28.0 c	17.5 c	63.3 b	201.8 d	35.0 abc	4.4 ab	13.1 ab
Compost & Manure	226.3 ab	32.3 abc	27.9 ab	86.4 a	251.3 ab	37.8 abc	1.7 d	4.5 c
Compost & Lime	214.5 bc	28.5 c	24.0 bc	83.9 a	231 bc	31.6 bc	1.2 d	4.0 c
Compost & Fertilizer	201.8 c	31.8 bc	29.0 ab	91.2 a	246.8 ab	37.4 abc	1.9 cd	5.1 c
Manure & Lime	240 a	29.5 bc	25.5 ab	86.5 a	229.3 bc	34.5 abc	1.7 d	5.0c
Manure & fertilizer	196.5 c	38.5 a	31.4 a	81.8 a	262.3 a	39.8 ab	3.3 bc	8.3 bc
Lime &Fertilizer	174.8 d	28.8 c	23.8 bc	82.3 a	213.5 cd	29.7 c	4.4 ab	14.8 a
Control	113.5 e	7.1 d	6.8 d	95.0 a	92 e	12.6 d	0.9 d	7.6 bc
C.V (%)	4.34	9.92	11.7	8.43	5.22	11.4	20.74	27.22

ToTNo= total tuber number, ToYlth = Total yield ton per hectare DisTW = diseased tuber weight ton per hectare and Pdis = percent diseased tuber weight (%). Note: Means with similar letters within the column are not statistically different based on Tukey's Studentized Range (HSD) Test.

As in the case of year one, lower number of tubers were obtained from lime only and control plots in the second year. All soil amendments and their combinations gave a higher tuber yield in the second year (Table 14) ranging from 29.7 t ha^{-1} for lime and fertilizer combination to the highest 41 t ha^{-1} harvested from compost treatment. A significantly lower tuber yield, 13.4 and 12.6 t ha^{-1} was obtained still from plots treated with lime only and control plots respectively.

The means of the two years data on 11 traits of yield, yield components and agronomic characteristics (Table 15 and Figure 13) showed that most of the soil amendments and their combinations gave higher total tuber number ranging from 229 to 242 tubers per plot.

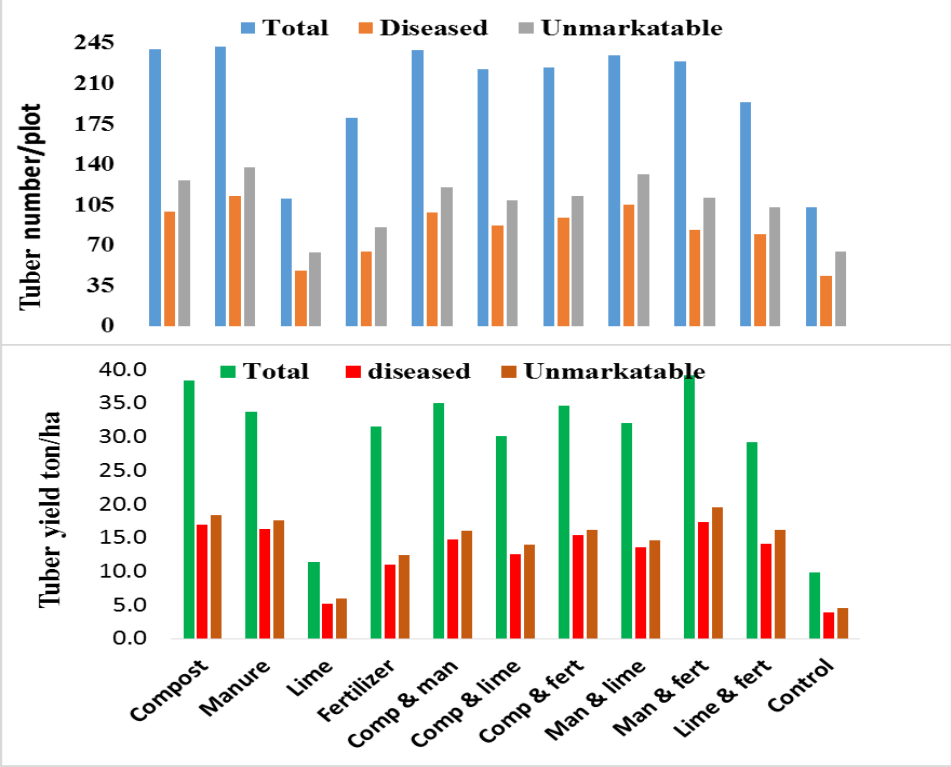


Figure 13. Combined means for total, diseased and unmarketable tuber numbers and weights as affected by soil amendments at Chencha in 2015 & 2016 a= for tuber numbers and b= for tuber yield.

Compost and all its combination with manure, lime and fertilizer help the plant to produce higher number of 239, 223 and 235 tubers per plot, respectively (Fig. 13a). With the same token, manure and its combination with lime and fertilizer also gave 242, 235 and 229 tubers per plot. The combination of lime with fertilizer and fertilizer alone produced medium number of tubers per plot 194 and 180, respectively (Table 15). Treating the soil with lime only didn't show any effect in this experiment as the results of most of the trait means compared were similar to that of the control plots which may be due to the less effectiveness of lime during the season of application.

It is also clearly seen that compost and manure and their combinations with lime and fertilizer and also their mix gave a higher tuber yield more than 30 ton ha^{-1} (Fig. 13b). The highest tuber yield (39.14 t ha^{-1}) was obtained from the treatment combination of Manure and fertilizer followed by compost (38.36 t ha^{-1}) and compost with manure (35.03 t ha^{-1}). The recommended fertilizer alone and when mixed with lime produced a tuber yield of 31.51 and 29.23 tons/ha respectively (Table 15). Whereas, plots treated with lime only gave significantly lower yield (11. 43 tons/ha) than all soil amendments exceeding only the control plot with a tuber yield of 9.85 tons/ha.

The proportion of diseased tuber weight (Pdis) for each treatment was higher for all the soil amendments ranging from 44% for compost and lime combination to 51.8% for plots treated with lime only (Table 15). Manure and control treatments also had higher Pdis of 50.8 and 51.3 %, diseased tuber from the total harvested tuber yield, respectively.

Table 15. Means of tuber number and yield, disease and yield related traits for soil amendment trial combined over 2 years (2015 & 2016) at Chencha

Treatment	ToTNo	DisTNo	UnMrktNo	Unmrktha	ToYltha	DisTW	Pdis	ATN	ATW	StNo	PlHt
Compost	239.86 ab	99.38 ab	126 ab	18.31 ab	38.36 a	16.87 a	46.54 ab	11.99 ab	72.02 abc	2.48 bc	72.73 ab
Manure	242.13 a	112.38 a	137.63 a	17.57 abc	33.68 abc	16.35 abc	50.78 a	12.11ab	62.65 bc	2.4 bc	72.47 ab
Lime	110.25 e	47.75 d	64.00 d	5.93 f	11.43 d	5.17 f	51.82 a	5.51 d	49.61 de	1.95 c	44.40 c
Fertilizer	180.38 d	64.75 cd	85.63 cd	12.43 e	31.51 bc	10.94 e	38.21 b	9.02 c	78.97 a	2.53 bc	72.45 ab
Comp & manure	238.75 abc	98.00 ab	120.50 ab	15.98 bcd	35.03 ab	14.79 abcd	45.43 ab	12.28 a	64.32 bc	2.85 ab	69.65 ab
Comp & lime	222.75 c	87.00 abc	108.63 abc	13.91 de	30.07 bc	12.59 de	43.94 ab	11.14 b	60.96 cd	2.35 bc	65.02 b
Comp & fertilizer	224.25 bc	93.50 ab	112.63 abc	16.09 bcd	34.59 abc	15.41 abcd	48.15 a	11.21 b	69.54 abc	2.68 ab	70.62 ab
Manure & lime	234.63 abc	105.38 ab	131.38 ab	14.56 cde	32.03 bc	13.61 cde	45.74 ab	11.73 ab	61.52 cd	3.36 a	70.55 ab
Manure & fertilizer	229.38 abc	83.00 bc	111.13 abc	19.50 a	39.14 a	17.32 a	45.01 ab	11.93 ab	74.81 ab	3.25 a	79.38 a
Lime & fertilizer	194.13 d	79.50 bc	102.88 bc	16.12 bcd	29.23 c	14.05 bcde	48.91 a	9.71 c	68.27 abc	2.43 bc	74.26 ab
Control	102.75 e	43.50 d	64.13 d	4.57 f	9.85 d	3.84 f	51.27 a	5.26 d	44.31 e	2.5 bc	43.87 c
C.V (%)	4.96	18.74	19.41	14.29	11.3	14.74	11.71	6.07	11.51	15.68	8.78

ToTNo= total tuber number, DisTW = diseased tuber weight ton per hectare; UnMrktNo= Unmarkatable tuber number; Unmrktha = Unmarkatable tuber weight ton per hectare; ToYlth = Total yield ton per hectare; and Pdis = percent diseased tuber weight (%). Note: Means with similar letters within the column are not statistically different based on Tukey's Studentized Range (HSD) Test.

Table 15 also revealed that all the agronomic traits including average tuber number per plant (ATN), average tuber weight (ATW) (gm), stem number per plant (StNo) and plant height (PlHt) (cm) followed similar trends where compost, manure and their combinations with lime and fertilizer resulted in an improved growth. Seven treatments including compost & manure, manure, compost, Manure & fertilizer, manure & lime, compost & fertilizer and compost & lime had higher average tuber numbers (ATN) ranging from 11.4 to 12.28 tubers per plant whereas lime treated and control plots had lower ATN, ATW and PlHt.

4.2.3. Effects of the soil amendments on soil properties

The soil analysis data from this experiment were analyzed separately because different fields were used for the experiments in both years (2015 and 2016). Therefore, a separate analysis of variance (ANOVA) for each year (Appendix 17) indicated that, the soil pH, exchangeable acidity (EA) and manganese (Mn) content were significantly ($P < 0.01$) affected by the soil amendments in the first year while available phosphorus (Av. P), organic carbon (OC), magnesium (Mg) and Iron (Fe) showed differences at $P < 0.05$ significant level. The rest of the soil nutrients including total nitrogen (TN), cation exchange capacity (CEC), potassium (K), calcium (Ca), copper (Cu) and zinc (Zn) didn't significantly differ with the application of soil amendments in the year 2015 (Appendix 18). Similarly, during the second year (2016), pH, OC, K, Ca and Mg showed a significant difference ($P < 0.01$) and EA, Fe and Mn at $p < 0.05$. Total nitrogen (TN), available P, CEC, Cu and Zn were not affected by the application of soil amendments in the second year (Appendix 19).

The mean separation was also done separately for each year and for nutrients which showed a significant difference in the analysis of variance using Tukey's Studentized Range (HSD) Test at $P < 0.05$ (Tables 16 and 17). In addition, the mean results were compared with the soil analysis taken before planting and showed general increase in the pH, available P and manganese content, while lower in all the treatments including the control found to have less iron (Fe) after harvesting of the potato crop in the first year (Table 16). It is clearly observed from table 16 that application of lime and soil amendments combined with lime showed significantly lower manganese (Mn) content of the soil and even lower from the pre-planting content (76.89 ppm).

Mean separation of the second year results (Table 17) depicted that there was an increase in the soil pH in all the treatments where a mixture of compost and lime being the highest soil pH of 5.93 followed by application of lime only with 5.80 soil pH. The minimum pH was observed from the control plot (5.33) but still a bit higher from pre-planting soil pH (5.15) (Table 17). In contrast to the first year, Fe and Mn showed an increase in all the treatments in the second year (table 17). The highest Fe (205.9 ppm) was obtained from compost and lime combination and the lowest (144.9 ppm) from a mixture of compost and lime where as Mn content ranged from 90.18 to 135.78 ppm for compost and lime and Manure and fertilizer combinations respectively, where all are higher than the pre-planting (Table 17).

Table 16. Mean soil chemical properties as influenced by organic amendments for potato bacterial wilt management and tuber yield after harvesting in year 1 (2015).

Treatment	pH	Av. P	Ex. Acidity (Meq/100g)	OC (%)	Mg (meq/100g)	Fe (PPm)	Mn (PPm)
Compost	4.67 d	22.75 ab	0.32 ab	1.26 c	3.81 ab	181.3 bc	81.14 abc
Manure	4.87 bcd	23.83 ab	0.30 ab	1.57 ab	3.61 bc	237.2 a	104.6 a
Lime	5.43 a	8.48 e	0.18 cd	1.30 c	3.21 bc	182.03 abc	55.07 c
Fertilizer	4.67 d	11.39 de	0.40 a	1.32 c	2.71 c	211.54 ab	96.61 a
Compost & Manure	4.83 cd	20.72 abc	0.19 cd	1.69 a	3.40 ab	229.68 ab	103.58 a
Compost & Lime	5.43 a	17.05 abcde	0.12 d	1.30 c	3.87 ab	136.46 c	62.61 c
Compost & Fertilizer	5.00 bcd	14.83 bcde	0.30 ab	1.36 bc	3.84 ab	219.85 ab	107.15 a
Manure &Lime	5.27 ab	25.94 a	0.15 cd	1.47 abc	4.74 a	229.97 ab	64.06 bc
Manure & Fertilizer	5.10 abc	12.31 cde	0.24 cd	1.42 bc	3.33 bc	203.75 ab	97.31 a
Lime & Fertilizer	5.20 abc	19.98 abcd	0.12 d	1.31 c	3.37 bc	194.73 ab	61.68 c
Control	4.83 cd	15.44 bcde	0.32 ab	1.31 c	3.25 bc	231.64 ab	89.82 ab
Before planting	4.53	13.1	0.34	1.34	3.83	254.16	76.89

Av.P = Available phosphorus, EA = Exchangable acidity, OC = Organic carbon, Mg = Maginisium, Fe = Iron and Mn = Manganase.

Table 17. Mean soil chemical properties as influenced by organic amendments of potato for bacterial wilt management and tuber yield after harvesting in year 2 (2016).

Treatment	pH	Ex. Acidity (Meq/100g)	OC (%)	K (meq/100g)	Ca (meq/100g)	Mg (meq/100g)	Fe (PPm)	Mn (PPm)
Compost	5.60 bcd	0.23 abc	1.39 ab	0.60 bc	5.62 d	5.88 a	156.9 cde	121.39 abcd
Manure	5.57 bcd	0.23 abc	1.45 a	0.88 a	6.34 cd	4.83 b	184.07 ab	124.7 abcd
Lime	5.80 ab	0.28 ab	1.34 abc	0.46 cd	8.35 bc	4.57 bc	175.4 bcd	101.18 cde
Fertilizer	5.30 de	0.31 a	1.20 bc	0.24 e	6.73 cd	4.25 bcd	180.02 abc	129.03 ab
Compost & Manure	5.53 bcde	0.23 abc	1.26 abc	0.49 cd	7.79 bcd	4.02 bcde	205.18 a	127.3 abc
Compost & Lime	5.93 a	0.13 c	1.26 abc	0.59 bc	14.43 a	3.98 bcde	144.9 e	90.18 e
Compost & Fertilizer	5.23 e	0.31 a	1.31 abc	0.55 bc	7.96 bc	3.97 bcde	177.97 bcd	114.56 abcde
Manure &Lime	5.70 abc	0.19 bc	1.18 cd	0.73 ab	7.14 bcd	3.84 bcde	169.63 bcde	103.28 bcde
Manure & Fertilizer	5.40 cde	0.24 abc	1.29 abc	0.52 cd	8.92 b	3.82 cde	189.75 ab	135.78 a
Lime & Fertilizer	5.73 ab	0.33 a	0.99 d	0.33 de	8.21 bc	3.39 de	153.25 de	98.81 de
Control	5.33 de	0.29 ab	1.21 bc	0.33 de	6.44 cd	3.24 e	174.7 bcd	124.3 abcd
Before planting	5.15	0.3	1.17	0.86	9,61	4.61	142.6	42.44

EA = Exchangable acidity, OC = Organic carbon, K = Potassium, Ca = Calcium, Mg = Maginisium, Fe = Iron, Zn = Zinc and Mn = Manganase.

4.3. Evaluation of bio-control agents to control bacterial wilt of potato under field condition in Ethiopia

4.3.1 Disease Incidence

The ANOVA of year one has showed in (Appendix 20) that the disease symptom started to develop at initiation of flowering (around 50 DAP) although significance difference ($P < 0.01$) between treatments in disease incidences and severity became clear after 65 DAP.

During the first year (2015) cropping season, planting was done late because the onset of the rain was delayed for about 20 days. This resulted in late planting and there was a smaller rain at the beginning of the season (Fig. 2). Thus the occurrence of disease symptoms was also delayed probably due to unconducive environment to the disease.

In the first year, disease incidence at 50 DAP was very low for all the biocontrol treatments tested ranging from no disease symptom for neem gold at the rate of 0.25 and 1 tons per ha to 1.88% for AMF treated plots (Table 18).

Table 18. Means of disease severity index and AUDPC for biocontrol agents year 1 (2015) at Chencha

Treatment	50 DAP	65 DAP	80 DAP	95 DAP	AUDPC
AMF	1.88 a	5.69 b	32.19 b	42.81ab	917.35 abc
Microbial consortia	1.25 ab	5.31 b	33.13 ab	40.00 ab	895.31 bc
Clean start	0.63 ab	5.94 a	37.19 ab	43.13 ab	979.69 ab
Agriphos-600	1.25 ab	5.00 bc	39.06 a	42.19 ab	996.1 ab
Neem Gold @ 0.1 t/ha	1.25 ab	8.13 a	37.81 ab	45.00 ab	1045.31 a
Neem Gold @ 0.25t/ha	0.00 b	0.31 d	19.06 c	30.63 c	520.32 d
Neem Gold @ 1t/ha	0.00 b	2.81 c	31.56 b	39.38 b	810.94 c
Control	1.25 ab	5.94 ab	35.31 ab	46.88 a	989.07 ab
LSD (alpha=0.05)	1.70	2.42	6.26	7.14	142.49

DSI = disease severity index, AUDPC = area under disease progress curve, DAP=Days after planting, Note: Means with similar letterletters within a column are not significantly different based on Tukey's Studentized Range (HSD) Test.

Application of neem gold at the rate of 0.25 and 1 ton per ha continued to give the lowest DSI and AUDPC throughout the disease assessments in the growing season where as low as nil (0.00) DSI for both rates of Neem gold at 50 DAP and 30.63 and 39.38 % at the final assessment (95 DAP) (Table 18). In addition, the lowest AUDPC (520.32) was recorded from Neem gold at the rate of 0.25 ton per hectare. In contrast, it has been clearly indicated in the same table that the application of Neem gold at the rate of 0.1 t/ha gave the highest (1045.31) AUDPC.

The results of the second year also showed that the disease symptom development started at 35 days after planting. However, it stayed low up to 50 days after planting and then started to increase after 65 days after planting (Table 19). A similar trend has been observed in the disease severity and AUDPC results where plots treated with Neem gold at the rate of 0.25 t ha^{-1} had the least final DSI (40.31%) and AUDPC (956.3) followed by Agrifose-600 with final DSI 42.3%.

Table 19. Means of disease severity index and AUDPC for Biocontrol agents year 2 (2016) at Chencha

Treatment	35 DAP	50 DAP	65 DAP	80 DAP	95 DAP	AUDPC
AMF	6.25 a	20.00 ab	38.44 a	48.44 a	69.69 a	2172.7 a
Microbial consortium	0.94 e	9.69 de	19.69 b	22.50 c	46.56 cd	1134.4 cd
Clean start	1.25 de	9.06 e	22.44 b	31.52 b	56.96 bc	1381.8 bc
Agriphos-600	3.13 bcd	15.63 bc	24.52 b	27.39 bc	42.34 d	1353.9 bc
Neem Gold @ 0.1 t/ha	3.44 bc	20.63 a	39.06 a	44.38 a	66.56 ab	2085.9 a
Neem Gold @ 0.25t/ha	2.19 cde	6.25 e	15.94 b	20.31 c	40.31 d	956.3 d
Neem Gold @ 1t/ha	1.56 cde	14.13 cd	23.26 b	33.92 b	61.20 ab	1540.2 b
Control	4.80 ab	15.51 bc	36.56 a	43.44 a	72.34 a	2011.2 a
LSD (alpha=0.05)	2.08	4.98	9.24	7.76	11.72	358.51

DSI = disease severity index, AUDPC = area under disease progress curve, DAP=Days after planting, Note: Means with similar letters within a column are not significantly different.

In general, it was observed that the biocontrol agents mostly improved the performance of the potato crop and delayed the onset of the disease symptom as compared to other two experimental fields conducted in the same area during the growing seasons.

In terms of AUDPC, the microbial consortia had the second lower value which is 1134.4. This may be due the higher DSI for Agriphose-600 in the first 4 assessments (Table 19) which exhibited a higher disease progress than the microbial consortia. The highest final DSI (72.3%) was from the control treatment followed by AMF with (69.7%) disease severity index while AUDPC was high for AMF (2172.7) followed by the control plot with AUDPC value of 2011.2 (Table 19).

The combined analysis of variance over two years (Appendix 21) revealed significant differences ($p < 0.01$) except in DSI at 80 DAP. The DSI at 35 DAP in the first year was excluded in the combined analysis as all the values were zero because no symptom had developed.

The final disease severity index and AUDPC at 95 days after planting showed that AMF, control and application of neem at the rate of 0.1 t ha^{-1} had significantly higher disease severity than the treatment applied with Neem gold at the rate of 0.25 t ha^{-1} which gave the lowest DSI (35.47) and AUDPC (738.29) (Table 20). Potato plants in this experimental field showed a general better performance with improved tuber yield in both years and combined results (Appendix 20 & 21 and Table 21) which may be due to the application of different biocontrol agents and their effects on the rhizosphere of the soil.

Table 20. Means of disease severity index (DSI) and AUDPC of biocontrol agents tested for BW management combined over two years (2015 & 2016) at Chencha.

Treatment	50 DAP	65 DAP	80 DAP	95 DAP	AUDPC
AMF	10.94 a	22.06 a	40.31 a	56.25 ab	1545.00 a
Microbial consortium	5.47 cd	12.50 bc	27.81 d	43.28 cd	1014.85 b
Clean start	4.84 cd	14.19 b	34.35 bc	50.04 bc	1180.75 b
Agriphos-600	8.44 ab	14.76 b	33.22 cd	42.26 de	1175.02 b
Neem Gold @ 0.1 t/ha	10.94 a	23.59 a	41.09 a	55.78 ab	1565.63 a
Neem Gold @ 0.25t/ha	3.13 d	8.13 c	19.69 e	35.47 e	738.29 c
Neem Gold @ 1t/ha	7.06 bc	13.04 b	32.74 cd	50.29 bc	1175.58 b
Control	8.38 b	21.25 a	39.38 ab	59.61 a	1500.12 a
Mean	7.40	16.19	33.57	49.12	1236.90
C.V (%)	21.58	17.49	10.25	9.21	9.51
LSD (alpha=0.05)	2.54	4.50	5.47	7.19	186.81

DSI = disease severity index, AUDPC = area under disease progress curve, DAP=Days after planting, MS = mean square, Year* Treatment = interaction between year and treatments, R^2 =measure of effect; CV=coefficient of variation (%). **Note:** Means with similar letters are not significantly different.

The disease development through time when combined over two years started to show significant difference at 50 DAP and continued with a sharp increase up to the final disease assessment at 95 days after planting (Fig. 14). Disease severity percentages ranging from 3.13% for Neem gold at the rate of 0.25 t ha^{-1} to 10.94% for both AMF and Neem gold at the rate of 0.1 ton /ha was observed at 50 DAP (Table 20). The highest disease percentage at the final assessment (95 DAP) was 59.61% recorded from control plot followed by AMF (56.25%) and Neem gold @ 0.1 t ha^{-1} (55.78%) (Table 20 and Figure 14).

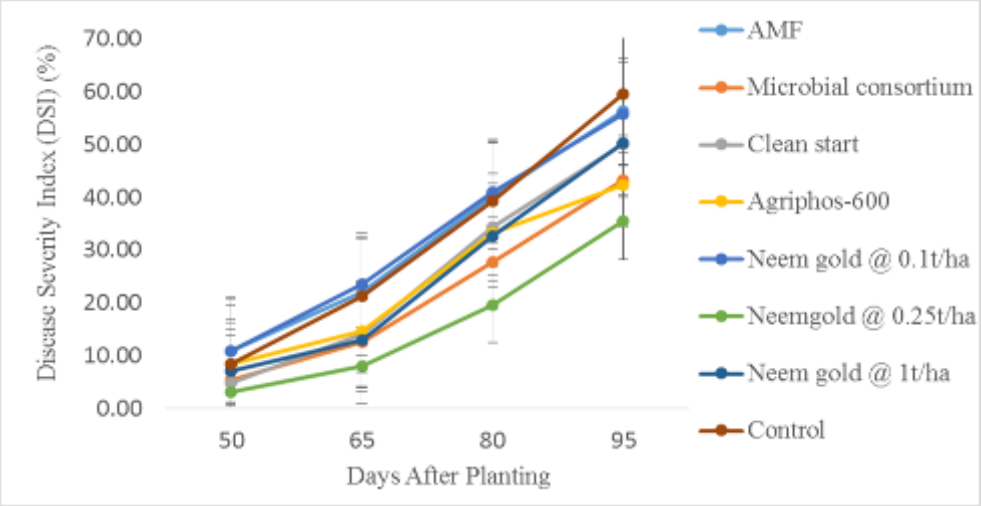


Figure 14. Disease severity index (DSI) of biocontrol agents tested for bacterial wilt management under natural infestation at Chencha combined over two years (2015and 2016). The vertical lines error bars.

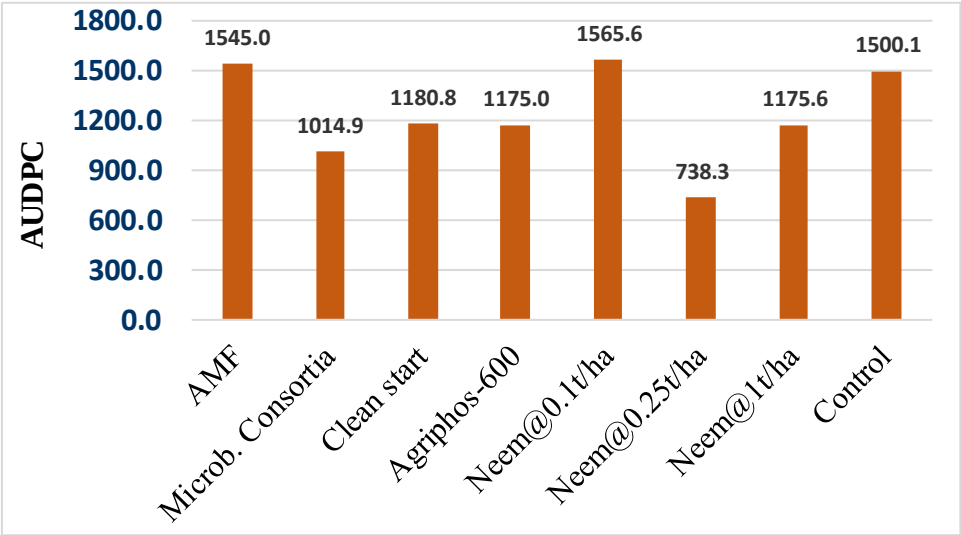


Figure 15. Mean AUDPC of biocontrol treatments under test for their effect on bacterial wilt of potato under natural infestation condition for two years in Chencha.

Similarily, the combined mean of AUDPC showed that the same trend where Neem gold at the rate of 0.1 ton/ ha, AMF and control had the highest AUDPC values 1565.63 1545.00 and 1500.12, respectively while others are intermediate except Neem gold at the rate of 0.25 t ha-1 with a lower AUDPC value of 738.29 (Table 20 and Figure 15). The application of 3 different rates of Neem gold showed a clear difference where the lower rate results are mostly similar to the control plot and the

highest rate of 1 t ha-1 was not found promising. How ever the Neem gold application at the rate of 0.25 t ha-1 found to have lower DSI and AUDPC in both the experimental years and also in the combined over years.

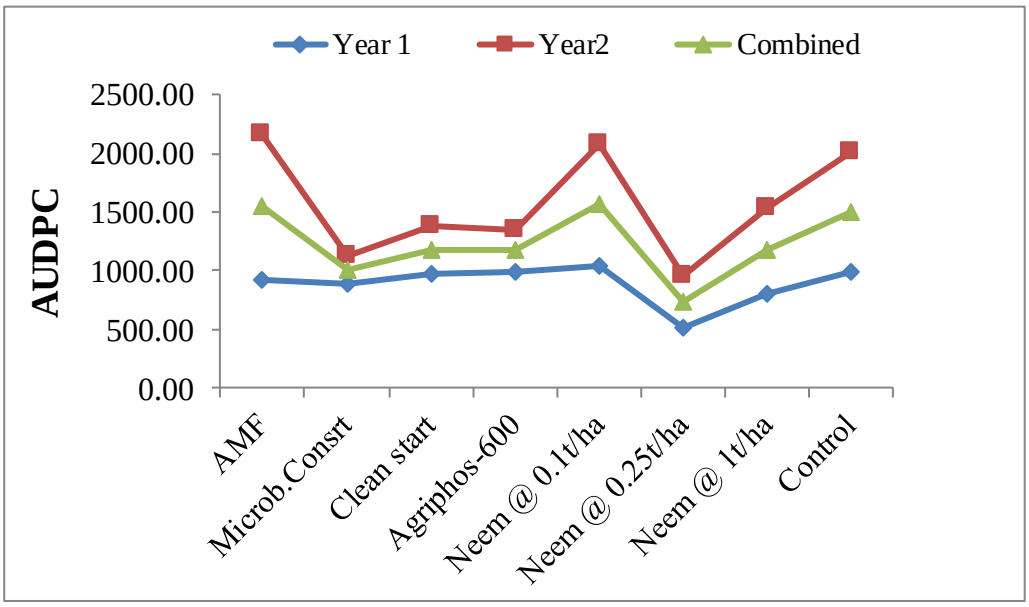


Figure 16. Mean AUDPC of year 1, year 2 and a combination of two years of biocontrol agents tested for bacterial wilt management at Chencha, for two years (2015 & 2016)

It has been clearily indicated in figure 16 that a distinct difference between treatments were observed as the results found to be similar in both the years which help to easily identify the best biocontrol agent to use in that locality. In addition, it can be clearly seen from Figure 16, that treatments which showed lower DSI and AUDPC seems to be more stable during the two years of experimentation while the treatments with higher DSI and AUDPC values such as AMF, control and Neem at 0.1 ton /ha exhibited a good deal of difference as it can be observed from the graph lines of figure 16.

4.3.2. Yield and yield components

The separate analysis indicated that a significant difference ($P<0.01$ and $P< 0.05$) between the biocontrol agents studied for their effect on bacterial wilt disease and

yield except plant height (PlHt) and stem number per plant (StNo) that did not show a significant difference (Apendix 24).

The combined analysis of variance (ANOVA) of the two years also indicated a significant difference ($P<0.01$ and $P< 0.05$) among the treatments in this experiment and years for the traits tested (Appendix 25) except for PlHt where there is no significant difference between the treatments.

The interaction between biocontrol agents and the year also showed a significant difference ($P< 0.01$ and $P<0.05$ only for ToYlth) except for the ATW and PlHt which did not show significant difference (Appendix 25). The biocontrol agents significantly differed ($P \leq 0.05$) in both the years for most of the traits tested (Appendices 21 and 22).

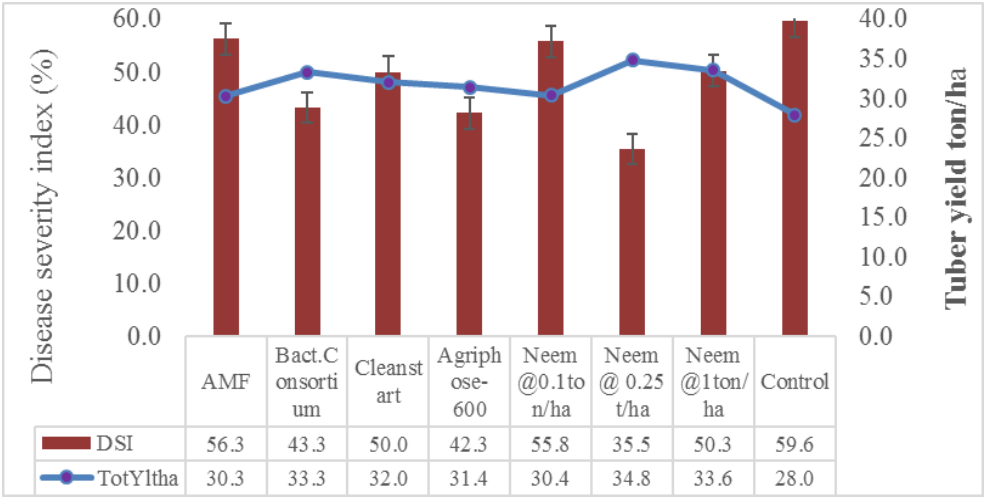


Figure 17. Combined means for total tuber yield and final disease severity index (95DAP) of biocontrol agents tested for bacterial wilt management for two years in Chencha.

The yield was higher in all the treatments including the control (Table 21), ranging from 27.99 t ha^{-1} for control to 34.83 ton per hectare for Neem gold applied at the rate of 0.25 t ha-1. This may show that the application of biocontrol agents can improve

the growth condition of the plant by altering the physico-chemical and the biota condition of the soil.

The total tuber yield and the final severity index (DSI) taken at crop maturity (95 DAP) (Figure 17) indicated that AMF, Neem gold at the rate of 0.1 and 1 t ha-1 and Clean start gave a higher tuber yield more than 30 t ha^{-1} although the higher disease severity percentages greater than 50% were recorded from the same treatments. The control treatment gave the highest final DSI (59.6%) and relatively lowest tuber yield (27.99 tons ha^{-1}).

All the results from this experiment revealed that the application of Neem gold at the rate of 0.25 t ha-1 found to give the highest total tuber yield from all the treatments in both the years and in the interaction that are 35.8, 33.9 and 34.8 ton ha^{-1} for year 1, 2 and the combination of both years, respectively (Appendices 21 and 22, Table 21 and Figure 17). The lowest final DSI (35.5%) and AUDPC (738.3) were also recorded from the same treatment Neem gold at the rate of 0.25 ton ha^{-1} (Fig 14 and 16).

Table 21. Means of 11 traits of potato tuber yield, yield component and disease incidence of biocontrol agents tested for bacterial wilt management Combined over two years (2015 and 2016) at Chencha.

BCA	ToTN	DisTN	UnMrktN	UnmrkTW	ToYlth	disTW	Pdis	ATN	ATW	StNo	PlHt
AMF	182.50 ab	47.88a	71.75 a	8.03bc	30.32 bc	5.84 bc	19.5bc	9.20 ab	74.95b	2.23 b	52.68
Microbial consortia	177.38 bc	29.50c	45.63d	6.42 d	33.31 ab	4.33 e	12.86e	8.87 bc	85.28 a	2.63ab	52.47
Cleanstart	180.88abc	40.13b	53.25 cd	7.6bcd	32.04 ab	5.80 cd	17.9cd	9.04abc	79.8 ab	2.63ab	53.23
Agriphose-600	169.50 bc	39.75b	59.25bc	8.55bc	31.44abc	6.03 bc	19.04c	8.63 bc	82.24ab	2.73ab	53.15
Neem@ 0.1t ha-1	168.13 c	47.38a	64.0 ab	10.18a	30.44 bc	7.52 a	23.86ab	8.41 c	81.2 ab	2.73ab	51.46
Neem gold @ 0.25 t/ha	180.38abc	28.13c	49.63d	7.35cd	34.83 a	4.66 de	13.2de	9.07abc	86.42 a	2.33 b	51.63
Neem@1t ha-1	191.88 a	42.63ab	59.38 bc	8.5 bc	33.58 ab	6.72abc	19.74bc	9.65 a	78.3 ab	3.00 a	54.96
Control	170.75 bc	43.88ab	64.88ab	8.84ab	27.99 c	7.00 ab	25.27a	8.64 bc	72.9 b	2.33 b	49.37
LSD (alpha=0.05)	13.51	6.41	8.33	1.47	3.82	1.17	4.75	0.69	10.23	0.61	8.11

ToTN= total tuber number, DisTN= diseased tuber number, UnMrkTN= unmarketable tuber number, UnMrkTW= unmarketable tuber weight t ha-1, ToYlth= Total yield t ha-1, DisTW = diseased tuber weight t ha-1, Pdis= percent diseased yield (%), ATN= average tuber number, StNo= stem number per plant, PlHt= plant height and ATW= Average tuber weight (g). **Note:** means with different letters are significantly different.

4.3.3. Effects of biocontrol agents on the soil properties

The pre-planting laboratory analysis of the soil samples collected from the experimental field showed that the soil is strongly acidic soil with the pH of 4.78 and 4.66 in year 1 and year 2 respectively. It also contained low levels of available P, total N and Ca, with high level of Mn very low soil organic carbon which showed that the soil is acidic and low in fertility.

The separate analysis for 2015 and 2016 revealed that most of the soil variables did not significantly differ with treatments in both years (Appendix.25). In the first year, available phosphorus (Av. P) and manganese (Mn) showed a significant difference at $P < 0.01$ and zinc (Zn) highly significant at $P < 0.001$. Similarly, the soil properties analyzed did not significantly differ with the biocontrol treatments during the second year except for zinc (Zn). Though most of the treatments did not show significant differences in soil chemical properties, the independent mean separation of the two years indicated that there were differences between the pre planting tests and the results obtained after harvesting of the potato as given in Table 22 and 23. In addition, it was observed that there was a general improvement of soil pH in both years when compared from the pH of the soil during the preplanting tests. The application of biocontrol agents found to affect manganese (Mn) and zinc (Zn) content of the soil significantly.

Table 22. Means of soil chemical properties as influenced by application of biocontrol agents for management of bacterial wilt and tuber yield in year 1 (2015)

Biocontrol Agents	pH	TN (%)	Available P (PPm)	Exchangable Acidity (Meq/100 g)	CEC (Meq/100g)	OC (%)	K (meq/100g)	Ca (meq/100g)	Mg (meq/100g)	Cu (PPm)	Fe (PPm)	Zn (PPm)	Mn (PPm)
AMF	5.04	0.16	8.93 ab	0.31 ab	31.67 ab	1.15	0.38 bc	3.35 ab	7.14	0.74 abc	101.37 abc	1.38 b	134.9 abc
Microbial Consortium	5.07	0.16	6.12 c	0.29 ab	25.77 ab	0.91	0.44 abc	2.89 abc	7.17	0.83 ab	107.31 ab	1.49 b	153.46 ab
Cleanstart	5.18	0.15	6.03 c	0.22 b	32.27 a	1.22	0.54 a	2.47 bc	7.55	0.61 bc	84.88 bc	1.03 c	114.34 bc
Agrifose-600	5.06	0.15	7.35 bc	0.34 ab	24.08 ab	1.21	0.38 bc	2.89 abc	6.77	0.65 bc	87.70 abc	1.43 b	111.41 c
Neem gold (0.1 t /ha)	4.99	0.15	9.62 a	0.38 a	30.56 ab	1.31	0.35 c	5.76a	7.43	0.60 c	79.65 c	0.99 c	122.91 bc
Neem gold (0.25t/ha)	5.09	0.15	6.96 bc	0.37 a	29.99 ab	1.34	0.49 ab	3.15 abc	6.73	0.87 a	110.44 a	1.90 a	174.77 a
Neem gold (1 t/ha)	5.34	0.14	7.03 bc	0.26 ab	22.98 b	1.09	0.48 ab	3.20 abc	7.25	0.57 c	84.88 bc	1.02 c	104.99 c
Control	5.06	0.14	7.50 bc	0.35 a	28.45 ab	1.31	0.48 abc	2.06 c	7.01	0.69 abc	94.65 abc	1.22 bc	123.64 bc
C.V (%)	3.95	7.88	15.27	23.08	18.77	20.53	17.44	22.07	11.20	18.14	14.01	11.82	17.94
LSD (0.05)	Ns	Ns	*	Ns	Ns	Ns	Ns	Ns	Ns	Ns	ns	**	*
Mean (BP)	4.78	0.13	4.72	0.47	31.77	0.96	0.22	2.82	8.66	0.67	139.02	1.18	45.53

Table 23. Means of soil chemical properties as influenced by application of biocontrol agents potato bacterial wilt management and tuber yield in year 2 (2016)

Biocontrol Agents	pH	TN (%)	Available P (PPm)	Exchangable Acidity (Meq/100g)	CEC (Meq/100g)	OC (%)	K (meq/100g)	Ca (meq/100g)	Mg (meq/100g)	Cu (PPm)	Fe (PPm)	Zn (PPm)	Mn (PPm)
AMF	5.28	0.18	11.20	0.32	14.21	1.48	0.54	4.34	2.56	1.96	212.44	0.82 d	196.69
Microbial Consortium	5.22	0.17	10.53	0.31	13.33	1.56	0.47	3.80	2.48	1.71	171.38	2.79 ab	191.66
Cleanstart	5.27	0.16	10.98	0.36	13.57	1.52	0.65	3.75	2.32	1.73	197.63	2.63 c	205.79
Agrifose-600	5.15	0.14	10.17	0.30	13.41	1.52	0.46	3.83	2.46	1.74	168.66	2.65 bc	193.63
Neem gold (0.1 t /ha)	5.17	0.17	11.64	0.32	13.29	1.47	0.48	4.16	2.61	1.67	181.10	2.65 bc	164.99
Neem gold (0.25t/ha)	5.51	0.15	8.59	0.34	13.23	1.52	0.69	4.07	2.60	1.82	169.24	2.78 ab	191.25
Neem gold (1 t/ha)	5.00	0.15	13.03	0.33	14.63	1.47	0.49	3.81	2.43	1.69	191.11	2.65 bc	159.90
Control	5.27	0.18	11.14	0.26	15.27	1.48	0.51	4.58	2.79	1.81	206.83	2.88 a	176.92
C.V (%)	3.22	13.18	18.96	23.00	10.37	8.37	21.76	19.18	13.80	7.43	20.23	3.32	17.04
LSD (0.05)	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	ns	***	ns
Mean (BP)	4.66	0.15	9.42	0.19	15.19	1.32	0.58	4.39	2.62	1.49	212.59	1.79	111.45

CHAPTER V

5. Discussion

5.1. Varietal resitance

Bacterial wilt has become an emerging threat to potato production, reaching epidemic proportions in the Chencha district, with a prevalence of up to 97% (Abdurahman et al., 2017).

The results of the present experiment revealed that the potato genotypes differ in their reaction to bacterial wilt disease when tested under natural infestation condition in both the years. This could be due to differences in weather among the seasons and the resistance to *R. solanacearum* available in potato, which is originated mainly from the cultivated diploid, *Solanum phureja* (Martin and French 1985) which is unstable due to strong host-pathogen-environment interaction (French and Lindo 1982; Tung et al. 1990).

For most genotypes, percent wilting increased rapidly from 60 days after planting and levelled off at 90–100 days after planting. This may be due to the blockage of the xylem tissue by the bacterial mass during the flowering time where the water demand is high. Muthoni et al., (2014) also found the same trend where percent wilting increased rapidly around flowering time. In addition, due to high transpiration rates during the active growth, the plants take up a lot of water together with bacteria in the soil water and hence wilt rapidly.

Varieties Cruza, Shangai and CIP clones showed a general lower tuber number, yield and average tuber weight and also with lower disease incidence in the first year when compared to the Ethiopian varieties including the Chencha local variety (Sula) due to

the small size of tubers. Thus the highest tuber number and tuber yield was harvested from the local variety Sula followed by Jalene in the first year however, in the second year, the highest tuber number and tuber yield were recorded from Cruza followed by Shangai, CIP-392661.18 and then the local variety Sula.

On average, the second year gave the higher tuber yields (ToYlth) and average tuber weight (ATW) and lower proportion of diseased tuber (Pdis) than the first year (Table 7) may be due to the early start of the rain in the second year which helps in early planting and the heavy rains and cool conditions favored crop growth because potato is a cool season crop.

French *et al*., (1998) also stated that no high level of resistance exists in potato cultivars, but some are less susceptible to bacterial wilt than others and can give high yields in the presence of the disease. Some cultivars such as Cruza 148 and Molinera, do not express wilt in cool conditions, but found to be capable of disseminating the disease through progeny tubers with a high rate of latent infection (French *et al*., 1998) when there is conducive environment for disease development.

Despite all these complications, the use of moderately resistance varieties can make an impact on ware potato production in areas where soils are highly infested if bacterial wilt-free seed can be provided (French, 1994; Priou *et al*., 1999b).

As Muthoni *et al*. (2014) reported, resistant potato clones have recently been identified by CIP scientists, and this resistance needs to be incorporated in the variety screening schemes so that those varieties can reach the small holder farmers by replacing the existing susceptible varieties in order to increase potato productivity in sub-Saharan countries.

The higher final disease incidence expressed in DSI (Fig 3) in the second year may be due to higher rainfall in the second season (Fig.2) that may increase infection rate by the pathogen due to high soil moisture that promote survival, reproduction, infectivity, and spread of the bacterium, and hence disease development (Harris 1976; Martin and French 1985).

The variety Cruza was the most tolerant variety followed by Shangai, CIP-392661.18 and the local variety Sula were third and fourth with lower AUDPC Respectively. Cruza was rated as resistant to bacterial wilt in Kenya (Muthoni et al., 2013). Felix et al., 2010 also reported two varieties Kenya Sifa and Kenya Karibu found to be the most resistant to bacterial wilt while Dutch Robjyn and Tigoni as most susceptible. Although such works on potato were not done in Ethiopia so far, Getachew *et al*. (2009) evaluated tomato against the highly aggressive *R. solanacearum* strain originated from Ethiopia and found that six resistant, eleven moderately resistant whereas most genotypes were found highly susceptible.

The cluster analysis grouped the genotypes tested in to four distinct clusters and this confirmed with the biplot from the principal component analysis. This shows the robustness of the clustering of the genotypes. The only excepttions were the two CIP clones CIP-391797.22 and CIP-393077.159. Jalene stood alone in one cluster at zero similarity. However, Cruza and the local variety (Sula) were grouped together in the clustering and PCA biplot. In addition, these varieties showed similar reaction to *R. solanacearum* with lower disease severity and AUDPC and higher yield which calls for further study in the future to know weather it is one of the earlier clons developed by CIP or it is a close relative of variety Cruza..

The broad sense heritabilty analysis revealed higher heritability percentages when combined over two years. This is probably because the heritability of disease resistance and yield are highly determined by the environmental factors.

The combined genotypic and phenotypic correlations among traits over two years were positive, negative and non-significant with high, intermediate and low extent ranged from -0.41 to 0.98 and -0.38 to 0.96 for genotypic and phenotypic, respectively. In general, traits that have positive correlation move in the same direction during selection, i.e., when one is improved, the other also improves positively (Musa, 2016). In this regard, traits that have strong positive correlation with grain yield are useful in indirect selection for yield especially when they have also high heritability. Gemechu, (2012) also suggested that for negatively associated traits, there should be a compromise between selections for both traits or the breeder should set a minimum standard for one trait while selecting for the other, or separate breeding for such traits should be an alternative and for traits with weak association, there may be an independent genetic control between the two traits and improvement in any one of the two would have little effect on the other.Therefore, breeding strategy should be planned in such a way that to select for traits which are positively associated in one go to reduce the time required and also to be cost efficient.

Breeding for resistance to bacterial wilt produces only modest gains and often lacking stability and/or durability (Hayward, 1991; Boucher *et.al*., 1992) and is seldom expressed as immunity since it is overcome by increasing the level of factors favorable for disease development, including temperature, soil moisture, damage to root system, etc. (Martin and French, 1985). As argued by Martin and French (1985), resistance may mean that fewer plants become infected where the expression of

resistance is pathovar- and environment-specific that makes local screening an essential step in the development of resistance varieties.

5.2. Effect of soil amendments

The results of this study revealed that soil amendments differ significantly in terms of their effects on disease incidence in both the years. Most of the organic amendments and their combination showed a general improvement of the plant condition. As stated by Bailey *et al*., (2003), organic amendments to soil have a direct positive impact on plant health and crop productivity through improving the physical, chemical, and biological properties of soil.

A delay in the development of wilt symptom in the first year due to late onset of rain can be because of unconducive condition for the *R. solanacearum* which perform well with the presence of sufficient moisture as stated by Van Elsas et al., (2000) that the survival of *R. solanacearum* was found to be affected by soil type (texture and organic matter), temperature and moisture content.

In general, plots treated with lime alone, was not different from the control plot in most traits tested. This is probably the effect of the lime will not show in the same season of application. Suppression of various pathogens has been reported in organically managed soils van Bruggen and Termorshuizen (2003). *R. solanacearum* was reported to survive least in loamy soil with relatively high organic matter content (4%) whilst survival was highest in soil with lower organic matter content of 2.0-2.5% (van Elsas *et al*., 2000). Efficient soil management may be related to the improvement of composition and/or activity of the soil microbiota (van Elsas *et al*., 2005) which enhances the natural biological control capacity of soil microorganisms.

Schönfeld *et al*., (2003) reported that the addition of household compost was found to increase disease suppression although the mechanism of suppression of *R. solanacearum* was unknown but hypothesized that the effect is related to a shift in the soil microbial community with enhanced antagonism against *R. solanacearum.*

All the organic amendments and their combination gave a better yield than the lime treated and the control plot. Mesiha, (2006) reported the reduction of soil inoculum and bacterial wilt development due to the application of soil amendments which resulted in lower yield losses. This is especially important for smallholder farmers in tropical countries, where the disease is endemic. Similarly, Berga *et al*., (2001) also reported soil amendment with organic materials (*Sesbania sesbana* and *Leucaena diversifolia*) either singly or combined with inorganic fertilizer reduced wilt incidence and increased potato tuber yield in Uganda.

According to Bailey *et al*., (2003), the decomposition of organic matters in soil will affect the viability and survival of a pathogen by releasing natural chemical substances with varying inhibitory properties that may restrict the availability of nutrients. This may increases soil microbial activity having competition effects in the soil and also stimulates the activities of microorganisms (Akathar and Malik, 2000) that are antagonistic to pathogens.

Getachew *et al*. (2011) reported that application of silicon and sugarcane bagasse (an alternative silicon source) significantly reduced the bacterial population and wilt incidence and increased tomato fruit yield. Elsewhere silicon amendment has been reported to significantly reduce bacterial wilt incidence and confer induced host resistance in tomato (Dannon and Wydra, 2004; Diogo and Wydra, 2007). Yadessa *et al*. (2010) also reported that soil amendments with coco peat, farmyard manure

(FYM), compost and green manure reduced bacterial wilt incidence by 81% and enhanced tomato yield compared to un-amended soil. This could be mainly due to improvement in soil physicochemical characteristics and microbial activity of the amended soil to the advantage of crop growth.

The effect of soil amendements on the chemical properties of the soil in this experiment indicated that there were no or little statistical differences in soil response between the different organic amendments, but differences were observed between the organic amendments as a group and the lime treatment alone and the control. In addition, organic amendments improved some of the soil characteristics over the pre-plant soil conditions particularily the pH of the soil. Himathongkham and Riemann, (1999) reported that soil amendment with chicken manure resulted in an increase of the bacterial population during the first two days followed by a decline of the pathogen population due to the accumulation of ammonia or the increase in pH.

Soil amendment with organic materials or NPK fertilizers or with different combination of these amendments is known to significantly affect bacterial wilt incidence and increased potato yields (Berga *et al*., 2005). The suppressive effect of soil amendment on the survival of *R. solanacearum* largely depend on soil type (Michel and Mew, 1998), environmental conditions and the material used for amending the soil which requires specific study for different localities according to the soil type, availablility of the materials and economical feasiblility.

However, as suggested by Kakuhenrize et al 2017, the mechanism of soil amending organic matters in suppressing potato bacterial wilt in the tropical highlands has not been well understood if the yield gain is due to improvement of soil fertility, soil health or both.

5.3. Effect of biocontrol agents

The use of biocontrol agents including the rhizosphere resident microbial antagonists is considered as a promising approach (Henok and Getachew, 2016) in the control of plant diseases. According to Bias (2004) the rhizosphere is a habitat where several important biological processes and interactions takes place primarily due to the influx of mineral nutrients from accumulation of plant roots exudates using mass flow and diffusion. Henok and Getachew (2016) stated that various species of antagonist rhizobacteria such as *Bacillus ceres, pseudomonas putadea* used to control bacterial wilt.

In this study the different biocontrol agents significantly differed in their effect on bacterial wilt disease. Disease symptom gradually increasing towards the end. Application of neem gold seemed to control the disease although there was a unifrom growth of the plants treated with all the treatments in the beginning, the crop become finally infected with the disease towards the maturity of the crop. The use of AMF was not found to effect the development of the disease in this study in contrast with Aguk, (2013) who reported that Microbial inoculates of AMF and rhizobacteria suppressed the bacterial wilt disease compared to the control. This may be due to the problem of establishment the in the area and adaptability problem in the rhizosphere.

The yield, in this experiment, was higher in all the treatments including the control ranging, from 27.99 t ha^{-1} for control to 34.83 ton per hectare for Neem gold applied at the rate of 0.25 t ha-1. This may show that the application of biocontrol agents can improve the growth condition of the plant by altering the physico-chemical property of the soil and the biota condition of the root zone.

The effects of biocontrol agents on the chemical properties of the soil were not found significant except for Zinc and Manganase although there were changes from the pre planting soil test results an improvement in the soil pH. Manganase increased in all the treatments including the control plot particularily in the second year this may be due to the effect of the treatments which balance the availability of manganese to avoid the toxic intake by he plants. In relation to this, Nogueira, et al., (2007) reported the importance of fungi in buffering of toxic heavy metal polluted areas and mentioned the case of excess manganese (Mn) in soil toxic to crops where arbuscular Mycorrhiza fungi may alleviate the toxic effects by producing compounds that affect the balance between Mn-reducing and Mn-oxidizing microorganisms in the mycorrhizosphere and thus affect the level of extractable Mn in the soil. However to know the effect in a better way, plant tissue analysis should be done.

CHAPTER VI

4. General Conclusion and Recommendations

Bacterial wilt, caused by *Ralstonia solanacearum*, is emerging as a major threat to potato production in Ethiopia, (Dereje et al., 2013). In countries like Ethiopia where there is no a formal seed certification scheme and lack of regional quarantine measures (Bekele & Abebe, 2013; Gorfu et al**.,** 2013) bacterial wilt will continue to be a mojor constraint in potato industry hindering the great potential of potato to achieve food security for an ever-growing human population in the country (Abdurahman et al., 2017). And hence, there is concesus on the need for integrated disease management strategy against the devastation caused by bacterial wilt disease in Ethiopia.

- From the first experiment it was concluded that varieties differ in their response to bacterial wilt disease and five out of 14 genotypes tested were tolerant to the disease. The variety Cruza had the lowest disease incidence, and can be considered for verification in other potato growing areas.

- Varieties also differ in yield and yield components significantly and from the tested genotypes Cruza, Shangai and CIP-392661.18 showed better adaptability in the study area with lower disease incidence and reasonable tuber yield.
- The Ethiopian variety Jalene was the most susceptible with highest AUDPC value.

- In general five genotypes (Cruza, Shangai, CIP-392661.18, Sula (Local) and CIP-393077.159 showed better tolerance/ performance under natural field infestation at Chencha.

- The local variety Sula was found to tolerate disease and gave a better tuber yield. The mechanisms conferring disease tolerance and better yields in this genotype need to be study further at molecular level so as to guide future breeding work in the search for bacterial wilt resistance
- Potato varieties and clones found to tolerate bacterial wilt can be used in the integrated management of the disease provided that the disease-free seeds are available.
- Ammending the soil with organic amendments and their combination can reduce the effect of the disease by improving the yield.
- Different alternative organic amendments can be used according to the availability and the cost of preparation.
- Soil amendments can also improve the physicochemical properties and by doing that the microbiological activity of the soil can be altered.

- The use of biocontrol agents can improve the plant growth and delayed the onset of disease symptom which helps the potato plant to form tubers of a marketable size.

- Arbuscular micorhize (AMF) in this study was not found effective it may be due to the problem of establishment contents the rhizobia around the root may not be conducive as its effect was highly determined by such

- The use of Neem kernel was found to be more effective in reducing bacterial wilt infection as well as improving yield in both years although the results found to be determined by the rate of application.

- However , it is very difficult to produce pathogen-free seed potatoes in places where bacterial wilt of potato is widespread, i.e. where the pathogen is already established

- Plant tissue analysis could have been done to see the effects of soil amendment and biocontrol agents on the chemical properties of the soil and the nutrient use efficiency which is very important in improving yield and plant defense system.

The following Recommendations can be given to minimize the effect of bacterial wilt disease:

- From the results of this study an integrated disease management can be applied using tolerant varieties like cruza, organic amendment and application of neem @ 0.25t/ha to reduce the effect of BW in Chencha area.
- Use of an integrated disease management (IDM) approaches should be promoted using various methods which are applicable and economical to each localities to minimize the effect of the disease and produce reseanoble yield.

- However, emphasis should be given to create a clean seed flow from disease free areas to the infected localities so that the inoculum can be contained for future eradication
- A well-established quality control system should be developed to avoid the transmission of the disease through latently infected potato seed tubers to disease free areas.

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Appendices

Appendix 1. ANOVA table for disease severity index (DSI) and AUDPC of 14 potato genotypes for the variety screening trail year1 (2015) and year 2 (2016) at Chencha

	DSI at 50 DAP	DSI at 65 DAP	DSI at 80 DA)	DSI at 95 DAP	AUDPC
Year 1 (2015)					
MS Block	12.78 **	148.40 **	121.66 **	157.95 **	207022 **
MS TRT	162.10 **	1479.01 **	2074.62 **	1967.17 **	2620403 **
C.V (%)	35.39	17.47	11.32	8.27	9.79
R^2	0.9523	0.97	0.974	0.9515	0.9763
Mean	4.70	24.24	38.21	70.51	1500.83
Year 2 (2016)					
MS Block	5.26 ns	7.51 ns	5.62 ns	3.35 ns	7145.21 ns
MS TRT	135.87 **	440.18 **	980.64 **	1510.98 **	1285536 **
C.V (%)	37.35	25.11	24.83	16.41	20.51
R2	0.9306	0.9363	0.9077	0.9481	0.9389
Mean	4.94	12.6100	23.24	32.00	814.73

Level of significance = $P < 0.01$, DSI = disease severity index, AUDPC = area under disease progress curve, DAP = Days after planting, MS = mean square, R^2 = measure of effect; CV=coefficient of variation (%)

Appendix 2. Means of Disease severity index (DSI) and AUDPC for 14 potato genotypes in the variety screening trail year 1 (2015) at Chencha

Variety	DSI at 50 DAP	DSI at 65 DAP	DSI at 80 DA)	DSI at 95 DAP	AUDPC
CIP-393077.159	8.47 b	24.38 d	42.91 cd	72.67 cd	1617.90 de
CIP-694474.16	0.00 d	5.00 g	10.21 e	43.30 fg	552.80 f
CIP-381381.13	3.35 cd	35.94 c	52.50 c	84.77 abc	1987.40 cd
CIP-392661.18	0.00 d	3.75 g	15.31 e	54.95 ef	698.00 f
CIP-391797.22	6.32 bc	41.56 bc	65.94 b	90.94 ab	2341.90 bc
CIP-391919.3	1.09 d	9.02 fg	14.17 e	34.79 g	616.90 f
Shangai	0.39 d	8.75 fg	19.13 e	60.83 ed	877.40 f
Cruza	0.64 d	11.88 efg	18.01 e	43.44 fg	778.90 f
CIP-399062.118	1.88 d	16.56 def	37.81 d	82.33 bc	1447.10 e
CIP-397006.18	1.68 d	21.25 de	47.47 cd	91.56 ab	1730.10 de
Belete	19.14 a	49.38 b	65.61 b	83.20 bc	2492.20 b
Gudenie	18.13 a	66.56 a	78.13 a	95.94 ab	3025.80 a
Jalenei	3.44 cd	37.81 c	51.51 c	99.38 a	2111.00 c
Sula (Chencha local)	1.31 d	7.5 fg	16.25 e	49.08 efg	734.10 f
Mean	4.70	24.24	38.21	70.51	1500.83
C.V (%)	35.39	17.47	11.32	8.27	9.79
LSD (alpha=0.05)	4.20	10.70	10.93	14.74	371.19

Appendix 3. Means of disease severity index (DSI) and AUDPC for 14 potato genotypes in the variety screening trail year 2 (2016) at Chencha

Variety	DSI at 50 DAP	DSI at 65 DAP	DSI at 80 DA)	DSI at 95 DAP	AUDPC
CIP-393077.159	4.38 cde	11.25 cd	22.19 bc	32.19 cd	775.80 bc
CIP-694474.16	5.00 cd	17.19 c	27.55 b	47.80 b	1066.90 b
CIP-381381.13	12.19 b	28.75 b	46.38 a	70.31 a	1745.70 a
CIP-392661.18	0.63 de	1.88 e	8.14 cd	30.43 cd	383.10 cd
CIP-391797.22	4.38 cde	9.38 cde	22.79 b	30.79 cd	746.20 bc
CIP-391919.3	1.04 de	3.13 e	4.88 d	10.07 ef	203.30 d
Shangai	1.25 de	2.78 e	3.27 d	4.62 f	134.70 d
Cruza	0.00 e	3.13 e	7.04 d	7.79 ef	210.80 d
CIP-399062.118	8.75 bc	15.31 cd	30.58 b	42.45 bc	1072.40 b
CIP-397006.18	3.75 de	8.13 de	25.75 b	29.94 cd	760.80 bc
Belete	0.94 de	8.21 de	16.48 bcd	20.81 de	533.40 cd
Gudenie	5.00 cd	16.45 c	29.32 b	32.43 cd	967.30 b
Jalenei	21.25 a	38.75 a	58.75 a	64.64 a	2106.70 a
Sula (Chencha local)	0.63 de	12.23 cd	22.19 bc	23.75 d	699.10 bc
C.V (%)	37.35	25.11	24.83	16.41	20.51
LSD ($P < 0.05$)	4.66	8.00	14.58	13.27	421.99

Appendix 4. Mean squares (MS), R2, and CV for combined ANOVA of DSI and AUDPC of 14 potato genotypes tested for bacterial wilt tolerance for two years at Chencha.

Traits	Mean Squares			R^2	CV
	Year	Genotype	Year * Genotype		
DSI at 50 DAP	1.60 ns	145.46 **	153.41 **	0.93	39.29
DSI at 65 DAP	3785.8 **	1293.12 **	626.07 **	0.95	22.75
DSI at 80 DAP	6279.02 **	2223.51 **	831.76 **	0.95	17.31
DSI at 95 DAP	41527.30 **	2627.88 **	850.28 **	0.97	11.10
AUDPC	1.30 **	2784657 **	1121282 **	0.96	14.75

** = significant at P < 0.01, DSI = disease severity index, AUDPC = area under disease progress curve, DAP=Days after planting, MS = mean square, Year* Genotype = interaction between year and genotype, R^2 =measure of effect; CV=coefficient of variation (%).

Appendix 5. Means of Disease severity index (DSI) and AUDPC for 14 potato genotypes in the variety screening trail Combined over 2 years (2015 &2015) at Chencha

Variety	DSI at 50 DAP	DSI at 65 DAP	DSI at 80 DA)	DSI at 95 DAP	AUDPC
CIP-393077.159	6.42 c	17.81 d	32.55 d	52.43 cd	1196.84 d
CIP-694474.16	2.50 de	11.09 defg	18.88 e	45.55 de	809.89 e
CIP-381381.13	7.77 bc	32.34 bc	49.44 ab	77.54 a	1866.58 a
CIP-392661.18	0.31 e	2.81 h	11.73 ef	42.67 de	540.58 efg
CIP-391797.22	5.35 cd	25.47 c	44.36 bc	60.87 bc	1544.05 b
CIP-391919.3	1.07 e	6.07 gh	9.52 f	22.43 h	410.10 g
Shangai	0.82 e	5.76 gh	11.20 ef	32.73 fg	506.05 fg
Cruza	0.32 e	7.50 fgh	12.52 ef	25.61 gh	494.84 fg
CIP-399062.118	5.31 cd	15.94 de	34.20 d	62.39 b	1259.78 bcd
CIP-397006.18	2.72 de	14.69 def	36.61 cd	60.75 bc	1245.43 cd
Belete	10.04 ab	28.79 c	41.04 bcd	52.00 cd	1512.80 bc
Gudenie	11.56 a	41.51 a	53.72 a	64.19 b	1996.53 a
Jalenei	12.34 a	38.28 ab	55.13 a	82.01 a	2108.82 a
Sula (Chencha local)	0.97 e	9.87 efgh	19.22 e	36.42 ef	716.62 ef
Mean	4.82	18.42	30.72	51.26	1157.78
C.V (%)	39.29	22.75	17.31	11.10	14.75
LSD (alpha=0.05)	3.27	7.25	9.20	9.84	295.30

Appendix 6 Analysis of variance for traits of tuber yield and yield component of 14 potato genotypes tested for bacterial wilt tolerance at Chencha for two seasons (2015 and 16).

Traits	Year 1 (2015)			Year 2 (2016)		
	MS	R^2	CV	MS	R^2	CV
ToTN	18999.96 **	0.96	10.52	23995.90 **	0.99	7.08
DisTN	4245.3 **	0.99	13.21	814.32 **	0.98	18.78
UnMrkTNo	4244.36 **	0.97	8.85	1133.63 **	0.96	10.45
UnMrkTW	30.04 **	0.98	17.83	16.71 **	0.97	12.45
TotYlth	190.18 **	0.95	20.02	273.55 **	0.92	14.28
DisTW	30.35 **	0.98	20.34	19.03 **	0.99	14.92
Pdis	1604.89 **	0.96	18.62	933.05 **	0.98	19.66
ATN	45.93 **	0.94	11.32	66.36 **	0.95	11.90
ATW	761.02 **	0.96	12.61	628.44 **	0.89	10.24
StNo	0.86 **	0.77	14.35	6.99 **	0.84	17.88
PHt	169.04 *	0.65	11.39	777.14 **	0.86	13.66

* and ** indicate significance (P<0.05 and 0.01, respectively); ToTNo= total tuber number, DisTNo= diseased tuber number, UnMrkTNo= unmarketable tuber number, UnMrkTW= unmarketable yield ton ha^{-1}, ToYlth= Total yield ton ha^{-1}, DisTW = diseased yield ton ha^{-1}, Pdis= percent diseased yield (%), ATN= average tuber number, StNo= stem numberper plant, PlHt= plant height and ATW= Average tuber weight (g).

Appendix 7. Means of 11 traites of 14 potato genotypes tested for bacterial wilt tolerance at Chencha, year 1 (2015)

Genotype	ToTNo	DisTNo	UnMrkTNo	UnMrkTW	TotYlth	disYlth	Pdis	ATN	ATW	StNo	PHt
CIP-393077.159	171.75 cde	41.50 cd	77.75 bcd	3.88 cd	10.93 bc	2.53 d	23.73 de	8.80 cde	29.98 c	2.15 bcd	48.90 abc
CIP-694474.16	184.5 cd	3.00 g	72.00 cde	0.52 g	5.63 de	0.08 e	1.32 h	9.23 cd	13.68 def	1.40 d	50.67 abc
CIP-381381.13	143.50 def	20.50 e	72.50 bcde	1.42 fg	5.54 de	0.99 e	18.12 def	7.18 def	17.39 de	1.55 d	42.55 c
CIP-392661.18	179.00 cde	6.50 fg	51.25 g	0.53 g	6.30 d	0.23 e	3.62 gh	9.21 cd	15.40 de	2.10 bcd	52.83 abc
CIP-391797.22	119.50 fg	57.75 b	81.50 bc	6.73 b	11.46 b	6.52 b	57.08 a	6.06 fg	42.67 b	2.05 bcd	50.53 abc
CIP-391919.3	187.75 c	4.00 fg	68.50 cdef	0.40 g	6.68 cd	0.10 e	1.56 h	9.81 c	15.34 ed	2.45 abc	47.12 bc
Shangai	139.75 ef	14.00 ef	51.50 g	1.01 g	3.06 de	0.87 e	28.42 d	6.99 def	9.90 ef	1.40 d	44.83 c
Cruza	207.50 c	12.50 efg	62.75 defg	0.56 g	6.38 cd	0.30 e	4.98 gh	10.52 c	14.36 def	1.85 cd	50.67 abc
CIP-399062.118	90.25 g	3.75 fg	47.25 g	0.35 g	1.43 e	0.13 e	8.77 fgh	4.52 g	7.12 f	2.15 bcd	47.24 bc
CIP-397006.18	110.25 fg	40.50 d	70.50 cdef	3.15 de	5.40 de	2.94 d	53.83 ab	6.45 efg	18.88 d	1.80 cd	43.05 c
Belete	90.00 g	51.50 bc	60.25 efg	4.82 c	11.51 b	4.76 c	42.43 bc	4.88 fg	52.86 a	2.00 bcd	54.61 abc
Gudenie	84.50 g	39.75 d	54.75 fg	2.58 ef	5.61 de	2.46 d	44.05 bc	4.23 g	29.96 c	2.40 abc	60.96 ab
Jalenei	265.75 b	124.50 a	179.00 a	9.42 a	22.57 a	9.16 a	41.17 c	13.29 b	38.10 b	2.75 ab	59.82 ab
Sula(Chencha local)	322.50 a	40.75 cd	89.00 b	3.63 cde	25.07 a	3.32 d	13.18 efg	16.13 a	35.00 bc	2.95 a	63.25 a
Mean	164.04	32.89	74.18	2.78	9.11	2.46	24.44	8.38	24.33	2.07	51.22
C.V (%)	10.52	13.21	8.85	17.83	20.02	20.34	18.62	11.32	12.61	14.35	11.39
LSD (alpha=0.05)	43.57	10.98	16.58	1.25	4.61	1.26	11.50	2.39	7.75	0.75	14.73

Appendix 8. Means of 11 traites of 14 potato genotypes tested for bacterial wilt tolerance at Chencha, year2 (2016)

Genotype	ToTNo	DisTNo	UnMrkTNo	UnMrkTW	TotYlth	disYlth	Pdis	ATN	ATW	StNo	PHt
CIP-393077.159	155.25 de	19.50 b	32.00 cde	4.44 bc	26.86 bc	2.32 c	8.90 cde	7.87 cde	76.31 a	4.40 bcd	58.30 abc
CIP-694474.16	160.50 d	17.50 b	40.25 c	2.14 fgh	16.34 d	1.55 def	9.48 cd	8.22 cde	44.80 efg	2.80 def	32.70 de
CIP-381381.13	130.25 efg	14.00 bcd	28.75 def	2.80 efg	13.56 d	1.89 cd	14.10 c	6.59 def	46.20 defg	5.90 ab	61.10 ab
CIP-392661.18	229.75 bc	9.25 de	38.50 cd	2.98 def	26.76 bc	1.54 def	5.75 defg	11.63 b	51.78 bcdef	3.25 def	46.75 bcd
CIP-391797.22	150.75 def	7.00 ef	40.50 c	3.62 cde	24.92 bc	1.46 defg	5.98 def	8.78 cd	63.62 ab	4.20 bcde	39.55 de
CIP-391919.3	32.75 k	0.00 g	9.00 g	0.86 i	5.88 e	0.00 i	0.00 g	4.74 f	27.07 h	2.60 ef	24.80 e
Shangai	244.50 b	2.00 fg	53.50 b	2.80 efg	30.67 ab	0.47 hi	1.52 fg	17.26 a	40.84 fg	5.10 abc	58.70 abc
Cruza	326.50 a	10.50 cde	64.25 a	2.86 def	33.88 a	1.10 efgh	3.27efg	17.21 a	44.37 efg	6.70 a	70.50 a
CIP-399062.118	90.00 ij	4.25 efg	21.25 f	1.15 hi	10.41 de	0.54 hi	5.39 defg	4.87 f	47.96 cdef	3.40 cdef	47.15 bcd
CIP-397006.18	72.00 j	18.75 b	35.25 cde	5.30 b	13.28 d	4.53 b	34.99 b	8.75 cd	34.01 gh	2.95 def	26.25 e
Belete	100.25 hi	16.50 bc	36.50 cde	3.86 cd	15.82 d	1.65 cde	11.16 cd	5.82 ef	60.69 bc	2.30 f	48.20 bcd
Gudenie	110.00 hij	8.75 de	27.00 ef	1.81 ghi	14.85 d	0.90 fgh	6.17 def	5.96 ef	55.78 bcde	3.00 def	43.05 cd
Jalenei	127.25 fgh	58.50 a	72.25 a	9.06 a	15.34 d	8.52 a	55.84 a	5.91 ef	58.35 bcd	4.10 cde	61.15 ab
Sula (Chencha local)	205.50 c	7.00 ef	53.00 b	2.60 efg	23.57 c	0.73gh	3.16 efg	10.28 cb	51.54 bcdef	2.75 def	58.60 abc
C.V (%)	7.08	18.78	10.45	12.45	14.28	14.92	19.66	11.90	10.24	17.88	13.66
LSD (alpha=0.05)	27.27	6.56	10.41	1.04	7.01	0.73	5.88	2.66	12.99	1.72	16.68

Appendix 9. Mean squares (MS), R^2, and coefficient of variation (CV) for combined ANOVA of 11 traits of 14 genotypes tested at Chencha in 2015 and 2016.

Traits	Mean Squares			R^2	CV
	Year	Genotype	Year * Genotype		
ToTNo	3714.51 **	29616.33 **	13379.53 **	0.971	9.06
DisTNo	10184.14 **	4052.59 **	1007.07 **	0.986	15.44
UnMrkTNo	33811.75 **	3925.13 **	1452.87 **	0.975	10.11
UnMrkTW	7.62 **	41.61 **	5.13 **	0.972	15.42
ToYltha	2985.58 **	254.46 **	209.27 **	0.953	16.47
DisTW	7.40 **	41.80 **	7.59 **	0.980	18.60
Pdis (%)	4451.08 **	1843.32 **	694.62 **	0.973	19.92
ATN	6.26 *	68.24 **	44.05 **	0.946	11.80
ATW	18793.56 **	1140.86 **	248.59 **	0.963	11.45
StNo	85.40 **	2.99 **	4.86 **	0.879	19.46
PlHt	231.21 *	554.04 **	392.14 **	0.801	12.47

* and ** indicate significance (P<0.05 and 0.01, respectively); ToTNo= total tuber number, DisTNo= diseased tuber number, UnMrkTNo= unmarketable tuber number, UnMrkylth= unmarketable yield ton ha^{-1}, ToYlth= Total yield ton ha^{-1}, DisYlth= diseased yield ton ha^{-1}, Pdis= percent diseased yield (%), ATN= average tuber number, StNo= stem numberper plant, PlHt= plant height and ATW= Average tuber weight (g).

Appendix 10. Broad sense heritability (H^2_b) of year 1 (2015) and 2 (2016) of the traits of 14 potato genotypes tested for bacterial wilt tolerance

Triats	Year 1			Year 2			Combined over years		
	S^2_g	S^2_e	H^2_b (%)	S^2_g	S^2_e	H^2_b (%)	H^2b (%)	$\sqrt{\sigma^2}$g	$\sqrt{\sigma^2}$ph
ToTNo	4675.60	297.52	98.43	5971.10	111.41	99.54	57.16	46.00	60.84
ToYlth	46.74	3.22	98.31	66.51	7.52	97.25	17.76	2.38	5.64
DisTNo	1056.60	18.89	99.56	201.90	6.74	99.17	75.15	19.51	22.51
DisTW	7.53	0.25	99.18	4.74	0.08	99.58	81.91	2.07	2.29
Pdis	396.05	20.71	98.71	231.97	5.18	99.44	62.32	11.98	15.18
UnMrkTNo	1050.30	43.10	98.98	279.17	16.97	98.50	62.99	17.58	22.15
UnMrkTW	7.45	0.25	99.17	4.14	0.17	98.98	87.67	2.14	2.28
PrUnMrk	333.53	27.92	97.95	202.25	39.02	95.40	63.92	11.39	14.25
ATN	11.26	0.90	98.04	16.31	1.10	98.34	39.40	1.83	2.92
ATW	187.90	9.42	98.76	150.64	25.89	95.88	78.21	10.56	11.94
StNo	0.19	0.08	90.48	1.63	0.47	93.28	0.00	0.00	0.70
PlHt	33.76	34.01	79.88	184.11	40.70	94.76	31.29	4.66	8.32

Appendix 11. Mean squares (MS), R^2, and CV for separate ANOVA of disease severity index (DSI) and AUDPC of soil amendments tested for two years at Chencha.

Disease severity index (DSI) (%)	Year 1 (2015)			Year 2 (2016)		
	MS	R^2	CV	MS	R^2	CV
35DAP	-	-	-	3.05 **	0.77	37.95
50 DAP	42.0 **	0.89	22.14	43.54 **	0.91	15.17
65 DAP	252.2 **	0.95	12.72	136.94 **	0.94	8.57
80 DAP	505.2 **	0.86	6.48	214 81 **	0.88	10.43
95 DAP	502.4 **	0.86	6.07	364.59 **	0.86	7.07
AUDPC	520816.7 **	0.90	6.50	296189.8 **	0.93	6.59

** = significant at $P < 0.01$, AUDPC = area under disease progress curve, DAP=Days after planting, MS = mean square, R^2 = measure of effect; CV = coefficient of variation (%)

Appendix 12. Mean disease severity index at 15 days interval Soil Amendment trial year 1 (2015) and year 2 (2016) at Chencha

Treatment	Year 1 (2015)					Year 2 (2016)					
	50 DAP	65 DAP	80 DAP	95 DAP	**AUDPC**	35 DAP	50 DAP	65 DAP	80 DAP	95 DAP	**AUDPC**
Compost	9.06 b	22.81 b	72.88 d	75.19 b	2135.2 cd	2.19 ab	11.88 a	31.88 a	47.81 a	72.5 abc	1933.6 a
Manure	5.00 de	15.00 cd	84.38 abcd	87.50 ab	2221.9cd	0.94bcd	9.69 ab	24.06 b	35.00 b	73.44 ab	1589.07b
Lime	8.44 bc	23.44 b	93.13 ab	96.56 a	2599.2ab	1.88 abc	10.63 ab	20.63 bc	31.56 b	47.81 e	1314.85c
Fertilizer	3.44 e	8.13 e	56.40 e	59.27 c	1463.9 e	0.94 bcd	8.13 bc	19.69bc	28.75bc	73.44 ab	1406.25bc
Comp. and Man.	5.31 cde	15.00cd	90.63abc	94.06 a	2369.5bc	2.50 a	4.38 de	28.94 a	34.38 b	68.13abc	1545.0bc
Comp.and Lime	8.13 bcd	19.69bc	80.00 cd	93.75 a	2320.3bc	0.00 d	2.50 e	12.81 d	19.69 d	61.37 cd	985.24 d
Comp.and Ferti.	2.81 e	11.56de	84.06abcd	86.38ab	2124.4cd	1.88 abc	5.31cde	22.5 bc	34.38 b	76.88abc	1523.44bc
Manure and Lime	5.00 de	12.19de	90.63 abc	91.38 a	2302.5 bc	0.63 cd	6.25 cd	18.13 c	28.13bc	69.69abc	1314.85 c
Manure and ferti.	2.60 e	13.44de	72.5 d	78.44 b	1916.3 d	2.50 a	10.94 ab	24.06 b	34.23 b	70.97abc	1589.44 b
Lime and Ferti	4.06 e	10.0 de	80.31 bcd	85.25 ab	2055.0 cd	2.19 ab	12.19 a	23.75 b	34.38 b	65.31 bc	1560.94 b
Control	13.13 a	35.63 a	94.06 a	95.94 a	2861.7a	0.63cd	5.94 cd	13.13 d	23.13 cd	50.31 de	1014.85d
Mean	6.09	16.99	81.72	85.79	2215.45	1.48	7.9800	21.78	31.95	66.35	1434.32
C.V (%)	22.14	12.72	6.48	6.07	6.50	37.95	15.17	8.57	10.43	7.07	6.59
LSD (alpha=0.05)	3.31	5.31	13.29	12.80	354.07	1.39	2.98	4.59	8.19	11.54	231.28

Appendix 13. Mean squares (MS), R2, and CV for combined ANOVA of DSI and AUDPC of soil amendments tested for bacterial wilt management for two years at Chencha.

Days after planting	Mean Squares			R2	CV
	Year	Treatment	Year by Treatment		
50	79.0 **	36.6 **	48.94 **	0.91	17.9
65	504.7 **	137.5 **	251.62 **	0.94	10.9
80	54510.1**	290.2 **	429.84 **	0.98	7.65
95	8316.6**	178.5 **	68 .19 **	0.92	6.45
AUDPC	13423641**	228416**	588590 **	0.96	6.63

** = significant at P < 0.01, DSI = disease severity index, AUDPC = area under disease progress curve, DAP=Days after planting, MS = mean square, Year* Treatment = interaction between year and treatments, R^2 =measure of effect; CV=coefficient of variation (%).

Table 14. Analysis of variance for some traits of tuber yield and yield component of soil amendments tested for bacterial wilt management at Chencha for two years (2015&16).

Traits	Year 1 (2015)			Year 2 (2016)		
	MS	R^2	CV	MS	R^2	CV
ToTN	7600.04 **	0.97	4.34	15116.16**	0.98	5.22
DisTN	7306.06 **	0.83	15.23	507.52 **	0.95	14.87
UnMrkTNo	7489.17 **	0.75	17.25	1089.25 **	0.89	15.07
UnMrkTW	282.79 **	0.93	11.55	20.99 **	0.84	22.08
TotYlth	402.19 **	0.95	9.92	381.00 **	0.91	11.4
DisTW	282.08 **	0.93	11.67	9.37**	0.9	20.74
Pdis	259.87 **	0.68	8.43	85.91**	0.83	27.22
ATN	18.76 **	0.96	5.23	39.15 **	0.97	6.45
ATW	1258.33 **	0.9	11.32	101.43 *	0.56	10.28
StNo	0.82 **	0.74	14.13	0.68 *	0.49	16.57
PHt	395.42 **	0.77	8.71	816.48 **	0.91	8.81

* and ** indicate significance (P<0.05 and 0.01, respectively); ToTNo= total tuber number, DisTNo= diseased tuber number, UnMrkTNo= unmarketable tuber number, UnMrkTW= unmarketable yield ton ha^{-1}, ToYlth= Total yield ton ha^{-1}, DisTW = diseased yield ton ha^{-1}, Pdis= percent diseased yield (%), ATN= average tuber number, StNo= stem numberper plant, PlHt= plant height and ATW= Average tuber weight (g).

Appendi15. Mean squares (MS), R^2, and coefficient of variation (CV) for combined ANOVA of 11 traits of soil amendment trials tested at Chencha in 2015 and 2016.

Traits	Mean Squares			R2	CV
	Year	Treatment	Year by Treatment		
ToTNo	6984.7**	20745.7**	1970.5**	0.97	4.96
DisTNo	343625**	4104.8**	3708.8**	0.97	18.74
UnMrkTNo	331363.6**	5018.8**	3559.6 **	0.94	19.41
UnMrkTW	6972.**	184.8**	118.9**	0.98	14.29
ToYltha	369.6**	775.1**	8.13 ns	0.92	11.3
DisTW	8751.1**	163.6**	127.8 **	0.98	14.74
Pdis (%)	122867.3**	124.4**	221.4**	0.99	11.71
ATN	16.1**	53.2**	4.7**	0.96	6.07
ATW	574.3*	852.3**	507.5**	0.82	11.51
StNo	13.1**	1.3**	0.18 ns	0.73	15.68
PlHt	3226**	1104.5**	107.4*	0.88	8.78

* and ** indicate significance ($P < 0.05$ and $P < 0.01$, respectively); ToTNo= total tuber number, DisTNo= diseased tuber number, UnMrkTNo= unmarketable tuber number, UnMrkylth= unmarketable yield ton ha^{-1}, ToYlth= Total yield ton ha^{-1}, DisYlth= diseased yield ton ha^{-1}, Pdis= percent diseased yield (%), ATN= average tuber number, StNo= stem numberper plant, PlHt= plant height and ATW= Average tuber weight (g).

Appendix 16. Mean squares (MS), R^2, and CV for separate ANOVA on the response of soil chemical properties due to the application of soil amendments tested for two years at Chencha.

	Year 1 (2015)				Year 2(2016)			
	MS	C.V	R^2	Mean	MS	C.V	R^2	Mean
pH	0.24 **	4.92	0.66	5.03	4.18 **	3.41	0.73	5.56
TN	0.00 ns	7.33	0.48	0.16	0.00 ns	6.98	0.56	0.16
Av. P	93.77 *	30.77	0.65	17.52	2.98 ns	20.86	0.38	7.77
EA	0.03**	26.31	0.78	0.24	0.01 *	25.22	0.57	0.25
CEC	2.28 ns	6.15	0.81	16.94	5.13 ns	12.95	0.19	26.26
OC	0.05 *	10.2	0.57	1.39	0.04 **	9.49	0.62	1.26
K	0.05 ns	22.67	0.53	0.72	0.10 **	23.24	0.82	0.52
Ca	6.98 ns	27.38	0.49	7.08	16.67 **	16.06	0.88	7.99
Mg	0.85 *	15.79	0.61	3.61	1.61 **	13.98	0.91	4.16
Cu	0.07 ns	13.72	0.53	1.76	0.01 ns	11.77	0.3	0.97
Fe	2740.9 *	15.83	0.71	205.29	8893.1 *	8.82	0.71	173.8
Zn	1.04 ns	27.14	0.45	2.98	0.31	18.22	0.54	2
Mn	1173.7**	18.88	0.71	83.96	661.55*	14.1	0.58	115.5

*and ** = significant at $P < 0.05$ and $P < 0.01$, ns= not significant, TN= Total Nitrogen, Av.P = Available phosphorus, EA = Exchangable acidity, CEC = cathion exchange capacity, OC = Organic carbon, K = Potassium, Ca = Calcium, Mg = Maginisium, Cu = Copper, Fe = Iron, Zn = Zinc and Mn = Manganase.

Appendix 17 Means of on the response of soil analysis results of soil chemical properties due to the application of soil amendments tested at Chencha year 1 (2015).

Treatments	pH	TN	Av. P	EA	CEC	OC	K	Ca	Mg	Cu	Fe	Zn	Mn
Compost	4.67 d	0.16	22.75 ab	0.32 ab	17.48	1.26 c	0.64	6.22	3.81 ab	1.79	181.3 bc	3.74	81.14 abc
Manure	4.87 bcd	0.14	23.83 ab	0.30 ab	17.07	1.57 ab	0.74	5.08	3.61 bc	2.00	237.2 a	3.88	104.6 a
Lime	5.43 a	0.16	8.48 e	0.18 cd	15.92	1.30 c	0.65	9.94	3.21 bc	1.57	182.03 abc	2.82	55.07 c
Fertilizer	4.67 d	0.16	11.39 de	0.40 a	15.11	1.32 c	0.72	5.32	2.71 c	1.79	211.54 ab	2.68	96.61 a
Comp & Man	4.83 cd	0.17	20.72 abc	0.19 cd	17.65	1.69 a	0.85	5.39	3.40 ab	1.52	229.68 ab	3.62	103.58 a
Comp & Lime	5.43 a	0.17	17.05 abcde	0.12 d	18.11	1.30 c	0.75	8.30	3.87 ab	1.67	136.46 c	3.00	62.61 c
Comp & Fert	5.00 bcd	0.16	14.83 bcde	0.30 ab	17.33	1.36 bc	0.71	6.72	3.84 ab	1.91	219.85 ab	2.72	107.15 a
Man &Lime	5.27 ab	0.16	25.94 a	0.15 cd	17.67	1.47 abc	1.03	8.54	4.74 a	1.81	229.97 ab	3.24	64.06 bc
Man & Fert	5.10 abc	0.16	12.31 cde	0.24 cd	16.95	1.42 bc	0.75	7.36	3.33 bc	1.88	203.75 ab	2.20	97.31 a
Lime & Fert	5.20 abc	0.17	19.98 abcd	0.12 d	16.53	1.31 c	0.54	7.29	3.37 bc	1.59	194.73 ab	2.10	61.68 c
Control	4.83 cd	0.16	15.44 bcde	0.32 ab	16.53	1.31 c	0.60	7.79	3.25 bc	1.78	231.64 ab	2.81	89.82 ab
C.V (%)	4.92	7.33	30.77	26.31	6.15	10.2	22.7	27.4	15.79	13.7	15.83	27.14	18.88
LSD (0.05)	0.42	Ns	9.18	0.11	Ns	0.24	Ns	Ns	0.97	ns	55.31	ns	27

Appendix 18. Means of on the response of soil analysis results of soil chemical properties due to the application of soil amendments tested at Chencha year 2 (2016).

Treatments	pH	TN	Av. P	EA	CEC	OC	K	Ca	Mg	Cu	Fe	Zn	Mn
Compost	5.60	0.17	8.00	0.23	24.41	1.39	0.60	5.62	5.88	0.84	156.9	2.45	121.39
Manure	5.57	0.14	7.85	0.23	26.87	1.45	0.88	6.34	4.83	1.00	184.07	1.89	124.7
Lime	5.80	0.16	7.37	0.28	25.69	1.34	0.46	8.35	4.57	0.99	175.4	1.85	101.18
Fertilizer	5.30	0.15	7.64	0.31	26.94	1.20	0.24	6.73	4.25	0.94	180.02	2.02	129.03
Comp & Man	5.53	0.16	9.51	0.23	27.18	1.26	0.49	7.79	4.02	1.08	205.18	1.96	127.3
Comp & Lime	5.93	0.16	7.42	0.13	24.90	1.26	0.59	14.43	3.98	0.98	144.9	2.30	90.18
Comp & Fert	5.23	0.16	9.48	0.31	27.97	1.31	0.55	7.96	3.97	0.99	177.97	2.04	114.56
Man &Lime	5.70	0.15	7.86	0.19	25.88	1.18	0.73	7.14	3.84	0.91	169.63	1.19	103.28
Man & Fert	5.40	0.17	7.44	0.24	27.45	1.29	0.52	8.92	3.82	1.00	189.75	2.22	135.78
Lime & Fert	5.73	0.16	6.42	0.33	24.22	0.99	0.33	8.21	3.39	0.98	153.25	2.06	98.81
Control	5.33	0.17	6.46	0.29	27.33	1.21	0.33	6.44	3.24	0.97	174.7	2.05	124.3
C.V (%)	3.41	6.98	20.86	25.22	12.95	9.49	23.24	16.06	13.98	11.77	8.82	18.22	14.1
LSD (0.05)	0.32	Ns	Ns	0.11	ns	0.2	0.21	2.19	0.99	ns	26.01	ns	27.74

Appendix 19. Mean squares (MS), R^2, and CV for separate ANOVA of disease severity index (DSI) and AUDPC of biocontrol agents tested for two years at Chencha.

Disease severity index (DSI) (%)	Year 1 (2015)			Year 2 (2016)		
	MS	R^2	CV	MS	R^2	CV
35DAP	-	-	-	13.74 **	0.86	29.72
50 DAP	1.79 *	0.58	76.37	107.26 **	0.89	15.14
65 DAP	22.15 **	0.88	20.9	332.97 **	0.88	14.17
80 DAP	159.79 **	0.91	7.96	442.66 **	0.93	9.63
95 DAP	97.43 **	0.87	7.29	632.97 **	0.9	8.67
AUDPC	112448.6 **	0.94	6.71	841563.98 **	0.93	9.57

*And ** = significant at Alpha=0.01, and 0.05, respectively, DSI = disease severity index, AUDPC = area under disease progress curve, DAP=Days after planting, MS = mean square, Year* Treatment = interaction between year and treatments, R^2 =measure of effect; CV=coefficient of variation (%).

Appendix 20. Means of 11 traits of potato tuber yield and yield component of biocontrol agents tested for bacterial wilt management at Chencha in 2015.

BCA	TotTN	DisTN	UnMrktN	UnmrkTW	ToYlth	DisTW	Pdis	ATN	ATW	StNo	PlHt
AMF	208 ab	48.5 bc	85.0 a	9.9 c	32.9 ab	6.4 d	19.7 c	10.5 a	70.6 b	2.4	41.7
Bacterial consortium	192 ab	52.0 ab	75.3 ab	10.4 bc	34.0 ab	7.5 bcd	22.1bc	9.6 ab	80.0 ab	2.6	42.0
Cleanstart	186 b	61.5 a	82.8 ab	11.9 abc	33.1 ab	8.5 abc	26.0 abc	9.3 b	80.3 ab	2.7	40.5
Agriphose-600	187 b	56.5 ab	87.3 a	12.7 ab	32.7 ab	9.1 ab	28.1ab	9.5 ab	77.7 ab	2.0	43.5
Neem@0.1t ha-1	188 ab	56.8 ab	81.3 ab	14.6 a	34.9 ab	10.6 a	30.2 a	9.4 ab	83.4 ab	2.5	43.1
Neem@0.25t ha-1	188 ab	39.0 c	67.8 b	10.4 bc	35.8 a	6.7 cd	18.8 c	9.4 ab	85.7 a	2.1	41.8
Neem@1t ha-1	211 a	53.3 ab	78.5 ab	11.1 bc	37.0 a	8.5 abcd	23.0 abc	10.5 a	79.1 ab	2.6	43.3
Control	189 ab	47.8 bc	80.8 ab	11.0 bc	30.3 b	8.1bcd	27.3 ab	9.4 ab	71.9 ab	2.2	41.6
Mean	193.44	51.9	79.8	11.5	33.8	8.17	24.4	9.7	78.6	2.4	42.2
C.V (%)	5.2	8.7	7.97	10	6.47	10.99	12.5	5.4	7.49	17.8	6.51
LSD (alpha=0.05)	23.87	10.75	15.09	2.73	5.19	2.13	7.2	1.24	13.97	Ns	Ns

ToTN= total tuber number, DisTN= diseased tuber number, UnMrkTN= unmarketable tuber number, UnMrkTW= unmarketable tuber weight t ha-1, ToYlth= Total yield t ha-1, DisTW = diseased tuber weight t ha-1, Pdis= percent diseased yield (%), ATN= average tuber number, StNo= stem number per plant, PlHt= plant height and ATW= Average tuber weight (g). Note means with different letters are significantly different.

Appendix 21. Means of 11 traits of potato tuber yield and yield component of biocontrol agents tested for bacterial wilt management at Chencha in 2016.

BCA	TotTN	DisTN	UnMrktN	UnmrkTW	ToYlth	DisTW	Pdis	ATN	ATW	StN0	PlHt
AMF	157.0 cd	47.3 a	58.50 a	6.15 a	27.75 bc	5.28 ab	19.35 ab	7.85 cd	79.34 ab	2.10 d	63.70
Microbial consortium	162.5 bc	7.0 e	16.00 e	2.50 c	32.68 ab	1.17 d	3.59 d	8.13 bc	90.60 a	2.65 bcd	62.90
Clean start	176.3 a	18.8 d	23.75 de	3.35 bc	31.02 abc	3.06 c	9.87 c	8.81 a	79.28 ab	2.60 bcd	65.95
Agriphose-600	152.5 cd	23.0 d	31.25 d	4.39 b	30.19 abc	3.00 c	10.02 c	7.82 cd	86.83 ab	3.50 a	62.80
Neem@0.1t ha-1	148.0 d	38.0 bc	46.75 bc	5.77 a	26.03 c	4.48 b	17.52 ab	7.40 d	78.97 ab	3.00 abc	59.80
Neem@0.25t ha-1	173.0 ab	17.3 d	31.50 d	4.27 b	33.90 a	2.59 c	7.62 cd	8.76 ab	87.15 ab	2.55 cd	61.45
Neem@1t ha-1	173.3 ab	32.0 c	40.25 c	5.93 a	30.20 abc	4.95 ab	16.43 b	8.77 ab	77.55 ab	3.40 ab	66.65
Control	152.8 cd	40.0 ab	49.00 b	6.65 a	25.72 c	5.92 a	23.21 a	7.84 cd	73.92 b	2.45 cd	57.15
Mean	161.91	27.91	37.13	4.87	29.69	3.81	13.45	8.17	81.70	2.78	62.55
C.V (%)	3.41	11.32	9.58	10.62	8.52	11.65	18.12	3.34	8.60	12.72	10.99
LSD (alpha=0.05)	13.09	7.49	8.44	1.23	6.00	1.05	5.78	0.65	16.67	0.84	ns

ToTN= total tuber number, DisTN= diseased tuber number, UnMrkTN= unmarketable tuber number, UnMrkTW= unmarketable tuber weight t ha-1, ToYlth= Total yield t ha-1, DisTW = diseased tuber weight t ha-1, Pdis= percent diseased yield (%), ATN= average tuber number, StNo= stem number per plant, PlHt= plant height and ATW= Average tuber weight (g). Note means with different letters are significantly different.

Appendix 22. Mean squares (MS), R^2, and CV for combined ANOVA of DSI and AUDPC of biocontrol agents tested to manage bacterial wilt of potato for two years at Chencha.

	50 DAP	65 DAP	80 DAP	95 DAP	AUDPC
MS Year	2672.2 **	8170.1 **	10.8 ns	3966.0 **	7513903 **
MS TRT	63.78 **	239.5 **	414.9 **	542.7 **	663933 **
MS Year*TRT	45.4 **	115.6 **	187.5 **	187.7 **	290079 **
C.V (%)	21.6	17.5	10.3	9.2	9.51
R^2	0.97	0.97	0.89	0.91	0.96
Mean	7.40	16.19	33.6	49.1	1236.9

*and ** = significant at Alpha=0.01, and 0.05, respectively, DSI = disease severity index, AUDPC = area under disease progress curve, DAP=Days after planting, MS year = mean square for year, MS TRT = mean square for the treatments; MS Year* Treatment = mean square for interaction between year and treatments, R^2 =measure of effect; CV=coefficient of variation (%).

Appendix 23. Mean squares (MS), R^2, and coefficient of variation (CV) of ANOVA for 11 traits of soil amendment trials tested at Chencha for each year.

Traits	Year 1 (2015)			Year 2 (2016)		
	MS	R^2	CV	MS	R^2	CV
ToTN	398.27 *	0.60	5.2	484.4 **	0.86	3.41
DisTN	190.78 **	0.78	8.73	741.5 **	0.96	11.32
UnMrkTN	149.63 *	0.59	7.97	795.2 **	0.95	9.58
UnMrkTW	9.41 **	0.72	10.00	8.7 **	0.92	10.62
TotYlth	17.22 *	0.65	6.47	35.4 **	0.66	8.52
DisTW	7.09 **	0.77	10.99	10.3 **	0.95	11.65
Pdis	67.98 **	0.78	12.47	177.9 **	0.91	18.12
ATN	1.08 *	0.60	5.38	1.2 **	0.85	3.34
ATW	107.65 *	0.59	7.49	132.5 *	0.48	8.60
StNo	0.27 ns	0.36	17.79	0.9 **	0.72	12.72
PHt	4.26 ns	0.25	6.51	38.8 ns	0.22	10.99

* and ** indicate significance (P<0.05 and 0.01, respectively); ToTN= total tuber number, DisTN= diseased tuber number, UnMrkTN= unmarketable tuber number, UnMrkTW= unmarketable tuber weight t ha-1, ToYlth= Total yield t ha-1, DisTW = diseased tuber weight t ha-1, Pdis= percent diseased yield (%), ATN= average tuber number, StNo= stem number per plant, PlHt= plant height and ATW= Average tuber weight (g).

Appendix 24. Mean squares (MS), R^2, and coefficient of variation (CV) for combined ANOVA of 11 traits of soil amendment trials tested at Chencha in 2015 and 2016.

Traits	Mean Squares			R^2	CV
	Year	Treatment	Year by Treatment		
ToTNo	15907.5 **	512.6 **	370.02 **	0.87	4.78
DisTNo	9216 **	445.2 **	487.04 **	0.96	10.11
UnMrkTNo	29155.6 **	594.1 **	350.71 **	0.97	8.96
UnMrkTW	701 65 **	10.0 **	8.12 **	0.96	11.3
ToYltha	271.0 **	38.1 **	14.45 *	0.73	7.57
DisTW	304.5 **	9.7 **	7.72 **	0.95	12.34
Pdis (%)	1919.5 *	155.4 **	90.46 **	0.9	15.8
ATN	37.6 **	1.2 **	1.03 **	0.86	4.85
ATW	155.2 *	175.6 *	64.57 ns	0.52	8.03
StNo	2.8 **	0.55 *	0.65 **	0.63	15
PlHt	6634.9 **	21.2 ns	21.78 ns	0.86	9.75

* and ** indicate significance (P<0.05 and 0.01, respectively); ToTN= total tuber number, DisTN= diseased tuber number, UnMrkTN= unmarketable tuber number, UnMrkTW= unmarketable tuber weight t ha-1, ToYlth= Total yield t ha-1, DisTW = diseased tuber weight t ha-1, Pdis= percent diseased yield (%), ATN= average tuber number, StNo= stem number per plant, PlHt= plant height and ATW= Average tuber weight (g).

Appendix 25. Mean squares (MS), R^2, and CV for separate ANOVA on the response of soil chemical properties due to the application of biocontrol agents tested for two years at Chencha.

	Year 1 (2015)				Year 2(2016)			
	MS	C.V	R^2	Mean	MS	C.V	R^2	Mean
pH	0.036 ns	3.95	0.35	5.1	0.06 ns	3.22	0.55	5.23
TN	0.00 ns	7.88	0.36	0.15	0.00 ns	13.18	0.39	0.16
Av. P	4.77 **	15.27	0.72	7.44	4.78 ns	18.96	0.51	10.89
EA	0.01 ns	23.08	0.64	0.31	0.00 ns	23	0.86	0.32
CEC	37.51 ns	18.77	0.54	28.22	1.71 ns	10.37	0.33	13.87
OC	0.06 ns	20.53	0.51	1.19	0.003 ns	8.37	0.13	1.5
K	0.01 ns	17.44	0.76	0.44	0.23 ns	21.76	0.46	0.54
Ca	0.83 ns	22.07	0.63	2.97	0.273 ns	19.18	0.2	4.04
Mg	0.25 ns	11.2	0.61	7.13	0.06 ns	13.8	0.23	2.53
Cu	0.04 ns	18.14	0.65	0.7	0.027 ns	7.43	0.62	1.77
Fe	395.57ns	14.01	0.62	93.79	900.17 ns	20.23	0.34	187.3
Zn	0.29 ***	11.82	0.9	1.31	1.383 ***	3.32	0.99	2.48
Mn	1665 **	17.94	0.771	130.05	781.0 ns	17.04	0.39	185.1

*, ** and *** = significant at $P \leq 0.05$, $P < 0.01$ and $P < 0.001$, ns= not significant, TN= Total Nitrogen, Av.P = Available phosphorus, EA = Exchangable acidity, CEC = cathion exchange capacity, OC = Organic carbon, K = Potassium, Ca = Calcium, Mg = Maginisium, Cu = Copper, Fe = Iron, Zn = Zinc and Mn = Manganase.

Appendix 26. Field layout of Experimental fields at Chencha:

Experiment 1: Variety screening

1		2		3		4		5		6		7		8		9		10		11		12		13		14	Rep I

12		6		1		11		10		3		14		7		5		4		9		2		8		13	Rep II

11		14		3		12		9		13		4		8		1		10		2		6		5		7	REP III

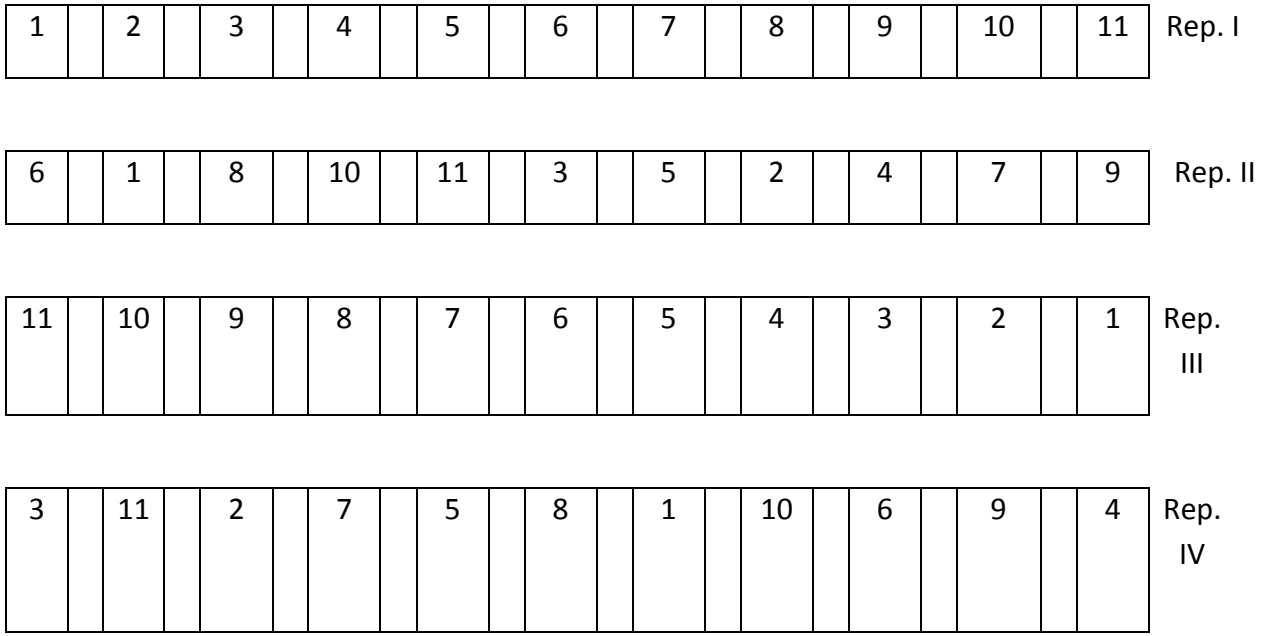
14		13		12		11		10		9		8		7		6		5		4		3		2		1	Rep IV

Potato Genotypes

No.	Clones/Variety	No.	Clones/Variety	No.	Clones/Variety	No.	Clones/Variety	No.	Clones/Variety
1.	CIP-393077.159	4.	CIP-392661.18	7.	Shangai	10.	CIP-397006.18	13	Jalene
2.	CIP-694474.16	5.	CIP-392797.22	8.	Cruza	11.	Belete	14	Sula (Chencha local)
3.	CIP-381381.13	6.	CIP-391919.3	9.	CIP-399062.118	12.	Gudanei		

Appendix 26 continued……

Experiment 2: Soil Amendment



Plot size: 3 m X 3m (9m2) with spacing of 75 cm between rows and 30 cm between plants.

Appendix 26 continued……

Treatments application for soil amendment trials in year 1(2015) at Chencha

N0.	Treatments for soil Amendment	Applied soilAmendment	Fertilizer with Equivalent N	Remarks
1	Compost @ 20 t/ha	18kg compost	176g DAP + 57.3g Urea	
2	Manure @ 20 t/ha	18kg manure	176g DAP + 55g Urea	
3	Lime @1.5 t/ha	1350g Lime (CaCo3)	-	Rate of application differs based on the lime requirement of the particular field
4	Recommended rate fertilizer	-	176g DAP + 148.5g Urea	Recommended rate @ 195kg DAP and 165kg Urea per hectare
5	½ compost + 1/2 manure	9kg Compost + 9kg Manure	176g DAP + 56g Urea	
6	Compost + Lime	18kg Compost + 1350g Lime	176g DAP + 57.3g Urea	
7	½ compost + Fertilizer	9kg Compost + Fertilizer	176g DAP + 103g Urea	
8	Manure + Lime	18kg Manure + 1350g Lime	176g DAP + 55g Urea	
9	½ Manure + Fertilizer	9kg manure + Fertilizer	176g DAP + 101.5g Urea	
10	Lime + Fertilizer	1350g + full dose fertilizer	176g DAP + 148.5g Urea	
11	Control	No amendment	No fertilizer	

Appendix 26 continued……

Treatments application for soil amendment trials in year 2 (2016) at Chencha

N0.	Treatments for soil Amendment	Applied soilAmendment	Fertilizer with Equivalent N	Remarks
1	Compost @ 20 t/ha	18kg compost	176g DAP + 111g Urea	
2	Manure @ 20 t/ha	18kg manure	176g DAP + 53.5g Urea	
3	Lime @1.6 t/ha	1440 g Lime (CaCo3)	-	Rate of application differs based on the lime requirement of the particular field
4	Recommended rate fertilizer	-	176g DAP + 148.5g Urea	Recommended rate @ 195kg DAP and 165kg Urea per hectare
5	½ compost + 1/2 manure	9kg Compost + 9kg Manure	176g DAP + 82.2g Urea	
6	Compost + Lime	18kg Compost + 1350g Lime	176g DAP + 111g Urea	
7	½ compost + Fertilizer	9kg Compost + Fertilizer	176g DAP + 129.7g Urea	
8	Manure + Lime	18kg Manure + 1350g Lime	176g DAP + 53.5g Urea	
9	½ Manure + Fertilizer	9kg manure + Fertilizer	176g DAP + 101g Urea	
10	Lime + Fertilizer	1350g + full dose fertilizer	176g DAP + 148.5g Urea	
11	Control	No amendment	No fertilizer	

Appendix 26 continued…….

Experiment 3: Biocontro agents.

1		2		3		4		5		6		7		8	Rep. I
6		4		2		7		8		5		3		1	Rep. II
8		3		1		5		7		4		2		6	Rep. III
3		7		2		6		5		1		8		4	Rep. IV

Treatments

No.	Biocontrol Agents	No.	Biocontrol Agents
1	Arbuscular michrizal Fungi (AMF)	5	Neem gold (0.1 t /ha)
2	Microbial Consortium	6	Neem gold (0.25t/ha)
3	Clean start	7	Neem gold (1 t/ha)
4	Agriphose-600	8	Control

Appendix 27. Pictures showing some activities during the research in the field and in the laboratories.

Photos taken during Isolation, Identification and inoculation for patogenecity test of the samples collected from Chencha experimental fields. **Clockwise:** Samples from stem and tubers; growing in AGAR media; testing the pathogen using strip test for Ralstonia; Selective media for R.solanacearum; inoculating the soil; inoculated plants in the green house.

Appendix 27. Continued……

Photos taken during different field activities. **Clockwise:** Compost preparation and transportation; field planting; poster presentation at EAPR 2017, France; harvesting of the crop and health breaks with the farmers.