EVALUATION OF ANTIBACTERIAL ACTIVITY OF SOME INVASIVE ALIEN SPECIES (IAS) EXTRACTS AGAINST TOMATO BACTERIAL WILT CAUSED BY *Ralstonia solanacearum* (Smith)

M.Sc. THESIS

DERIB ALEMU ABEBE

DECEMBER 2012

JIMMA UNIVERSITY

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M.Sc. Thesis

Submitted to the School of Graduate Studies
Jimma University College of Agriculture and Veterinary Medicine

In Partial Fulfillment of the Requirements for the Degree of
Master of Science in Plant Pathology

DERIB ALEMU ABEBE

December 2012
Jimma University

APPROVAL SHEET

School of Graduate Studies

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As thesis research advisor, We hereby certify that We have read and evaluated this thesis prepared, under our guidance, by Derib Alemu, entitled "**Evaluation of Antibacterial Activity of Some Invasive Alien Species (IAS) Extracts Against Tomato Bacterial Wilt Caused by *Ralstonia solanacearum* (Smith)"**. We recommend that it can be submitted as fulfilling thesis requirement.

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DEDICATION

This work is dedicated to

"**My Parents**"

STATEMENT OF THE AUTHOUR

First, I declare that this M.Sc. thesis is my genuine work and that all sources of materials used for this thesis have been duely acknowledged. This thesis has been submitted in partial fulfillment of the requirements for a M.Sc. Degree in Plant Pathology at Jimma University College of Agriculture and Veterinary Medicine, Ethiopia. I solemnly declare that this thesis has not been submitted to any other institution anywhere for award of any academic degree, diploma, or certificate.

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ACKNOWLEDGEMENTS

The author would like to express his heartfelt appreciation and special gratitude to all persons who, in one way or the other contributed to the accomplishment of this study. Special appreciation and deepest thanks go to the thesis research advisors Dr. Fikre Lemessa, Mulatu Wakjira (Assistant Professor) and Gezahegn Berecha (Assistant Professor) for their continued guidance, inspiration, encouragement, constructive comments and support throughout the study period which made the completion of this study smooth and successful. I would also gratefully acknowledge Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) and my advisors for their financial and logistic support for the project of my study.

I would like to take this opportunity to convey my deep grief to know about the death of my Co-advisor Mr. Mulatu Wakjira. I was really shocked when I got the news as Mulatu was young professional and has a lot to contribute. I would like to express my sorrow about this unfortunate incidence. He was very professional and organized person and I have learned so many things from him. Life and death is a process made by God and it was not in our hands to change the destiny. Nothing can bring him back and he will always be in my thoughts and prayers. May God rest his departed soul in peace.

I extend my thanks to Dr. Girma Adugna for his constructive comments at the early stage of the work. He has been a role model in teaching learning process as well as his highly rated attitude for his students which had been encouraging. Once again, I am grateful to thank all my teachers starting from Grade 1 to M.Sc. Study. On the other hand, all staff of the Jimma University College of Agriculture and Veterinary Medicine are highly acknowledged. My thanks is also extended to Melkassa Agricultural Research Center, that kindly provided tomato seeds for the study.

The University of Debre Berhan, with all the communities and members of our staff are highly acknowledged as they gave me the chance to enjoy the exciting science of Plant Pathology. I have got the key to put our academic energy forward and I will bring my talents to a maximum for our excellence.

I owe special thanks to all my colleagues and friends for their encouragement, ideal support, good atmosphere and relaxation they created during the entire study period. My particular regards are to Melkamu Tiru; deserve greatest thanks and appreciation as he was always with me and ready whenever I called for any help throughout my stay at Jimma.

I am also pleased to express my heartfelt thanks to the members of Plant Pathology, Entomology and Veterinary microbiology laboratory, specially, Mrs. Mulu Uta and Mrs. Demekech Defar, assistant technician in the lab for their patience and continuous encouragement, providing me laboratory facilities and technical assistance.

Last, but not least, the author remains sincere, grateful, and indebted to his family members whose words of encouragement, affection and prayer served me as a source of strength, inspiration and impetus throughout the study. If I have missed names of creditable personalities and institutions in the list, it is not intentional but failure to notice putting pen to paper.

Above all, I thank the almighty God, who made me comfortable all my way and do everything to me previously through and in the future throughout my life.

BIOGRAPHICAL SKETCH

The Author, Mr. Derib Alemu Abebe, was born in Debre Berhan, North Shewa Zone of Amhara Regional State, Northern Ethiopia on 17th May, 1989. He attended his elementary education (grade 1-4) from September 1995 - June 1998 in Baso Primary School at Debre Berhan and also completed his junior elementary school (grade 5-8) in Haramba Elementary School from September 1998 - June 2002. After successfully passing the national exam of the regional state, he joined Haile Mariam Mamo Comprehensive Secondary School in September, 2002 and completed high school as well as preparatory education in natural science in June 2006. Starting from December 2006, he joined Haramaya University and graduated with B. Sc. in Crop Production and Protection on 11th July, 2009. After graduation, He was assigned by Ministry of Education to Debre Berhan University in August, 2009 and worked as Graduate assistant for a semester. In March, 2010 he joined Jimma University College of Agriculture and Veterinary Medicine to pursue his graduate study in Plant Pathology under the sponsor of his current employer, Debre Berhan University.

LIST OF ACRONYMS

AGDW	Above Ground Dry Weight
AGFW	Above Ground Fresh Weight
ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
AVRDC	Asian Vegetable Research and Development Center
BCA	Biocontrol Agent(s)
CFU	Colony Forming Unit(s)
CIP	International Potato Center
CPG	Casmino Peptone Glucose
DIZ	Diameter of Inhibition Zone
DNA	Deoxyribonucleic Acid
EARO	Ethiopian Agricultural Research Organization
FAO	Food and Agriculture Organization
IAS	Invasive Alien Species
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
JUCAVM	Jimma University, College of Agriculture and Veterinary Medicine
MARC	Melkassa Agricultural Research Center
Mg	megagram
MIC	Minimum Inhibitory Concentration
MoARD	Ministry of Agriculture and Rural Development
OD	Optical Density
rpm	revolution per minute
SAS	Statistical Analysis System
TZC	Tetrazolium Chloride/2,3,5-Triphenyl-tetrazolium Chloride
VAM	Vesicular Arbuscular Mycorrhizae

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ABSTRACT

*Tomato (*Lycopersicon esculentum *Mill.) is one of the most widely grown vegetables in the world including Ethiopia. But, its production is constrained by different biotic and abiotic factors. Among biotic, bacterial wilt caused by* Ralstonia solanacearum *is one of the most important pathogens, threatening the production of tomato and potato in Ethiopia. So far there is no single means that would totally manage the disease and provide an absolute cure or fully protect host plants against the pathogen. This study was therefore, initiated with the objectives of evaluating the antibacterial activities of aqueous and two organic solvents (acetone and methanol) extracts of five invasive alien species (*Eichhorina crassipes, Mimosa diplotricha, Lantana camara and Prosopis juliflora) *under* in vitro *and* in vivo *against* R. solanacearum*. Completely randomized and randomized complete block designs with three replications were used for* in vitro *and* in vivo *experiments, respectively.* In vitro *antibacterial test was carried out in disc diffusion sensitivity test. After* in vitro *screening, four botanicals (aqueous extracts of* E. crassipes, M. diplotricha, L. camara *and methanolic extract of* P. juliflora*) with inhibition diameter >10mm were selected and Minimum Inhibitory Concentration was assessed under* in vitro*, and further evaluated under* in vivo *on tomato plants against* R. solanacearum *by applying the botanicals at three time of application (simultaneously, before 2-days and after2-days pathogen inoculation). It is evident from the result that most of the plant extracts exhibited significant inhibition of the bacterial growth compared with the control at 5 % level of significance. All the tested plant species bioactive compounds were relatively more extracted by water than organic solvents. Aqueous extract of* E. crassipes *provided the highest inhibition zone (26 mm), followed by* M. diplotricha *(14 mm). Results from* in vivo *test revealed that most of the treatment combinations significantly reduced disease incidence, disease severity index,* R. solanacearum *population density in the rhizosphere soil and increased biomass of tomato plants. The application of plant extracts at the same time as inoculation resulted in the highest reduction of disease development on tomato plants. More than 91 and 75 % reduction in disease severity index and wilt incidence, respectively, of bacterial wilt was observed in tomato plants treated with leaf extract of* E. crassipes *under greenhouse experiment when it was applied simultaneously with pathogen inoculation. The highest percent reduction of incidence Area Under Disease Progress Curve and severity Area Under Disease Progress Curve were exhibited from aqueous extract of* E. crassipes *with 67.52 and 71.35 %, respectively, followed by aqueous extract of* M. diplotricha *64.08 and 68.69 %, and then* L. camara *aqueous extract with 59.48 and 66.19%. Aqueous extract of* E. crassipes *was found to be the most effective botanical in disease suppression and increase above ground biomass in terms of fresh and dry weight; 52.72 and 11.96 g, respectively, compared to inoculated control (8.79 and 2.79), respectively. The study revealed that aqueous extracts of* E. crassipes *and* M. diplotricha *and* L. camara *are promising botanicals whose effectiveness under field conditions and their mode of action should be explored.*

Key words: antibacterial activity, botanicals, crude extract, *Eichhorina crassipes*, inhibition zone, *Lantana camara*, *Mimosa diplotricha*, *Prosopis juliflora*,

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetables in the world. It is grown on more than five million ha with a production of nearly 129 million Mg (Srinivasan, 2010). China is the world's top tomato grower, accounting for more than one-quarter of the world's tomato production area (FAO, 2010). Egypt and India together account for more than one-fifth of the world total. Turkey and Nigeria are the other major tomato producing countries. Asia and Africa account for about 76% of the global tomato area, with about 65% of world output (FAO, 2010).

In Ethiopia, tomato is an important cash crop and widely cultivated both under irrigation and rain fed throughout the year (Dandena *et al*., 2010). Altitudes between 700 and 2,000 meters characterized by warm, dry days and cooler nights favour optimum growth and development of tomatoes (Lemma, 2002). Tomato has a significant role in human nutrition because of its rich source of lycopene, minerals and vitamins such as ascorbic acid and beta-carotene which are anti-oxidants and promote good health (Dandena *et al*., 2010). The general dietary deficiencies of vitamins in Ethiopian population could be alleviated by a liberal consumption of many vegetables including tomato (Fekadu *et al*., 2004). Considering the significant value of the crop emphasis has been given by the national vegetable crops research program in the country to improve yield and quality of fresh market and processing tomato in order to satisfy the demands of both local and export markets (Lemma, 2002).

In Ethiopia the production and quality is low which is constrained by different biotic and abiotic factors. A number of improved varieties and other agronomic packages have been recommended to the users to overcome the low productivity and quality of tomato in the country. However, the average national yield still remains very low which is around 9 Mg/ha (FAO, 2010) and far less than that of the current world average yield of about 33.6 Mg/ha (FAO, 2010). Diseases caused by different fungal and bacterial pathogens (Getachew *et al*., 2011), increasing temperature, viral diseases and salinity (Dandena *et al*., 2010) are among the major limiting factors in sustaining and increasing tomato production.

Bacterial wilt caused by *Ralstonia solanacearum* (Smith), (synonym- *Pseudomonas solanacearum*) is a soil borne bacterial species and one of the most destructive plant diseases, which is predominantly distributed and damaging in humid climates and at low and medium elevations in the tropical, subtropical and warm temperate regions of the world (Henok *et al*., 2007; Mwangi *et al*., 2008). The host range of the pathogen is exceptionally wide, and many economically important crops as well as many weed hosts have been recognized (Fikre and Zeller, 2007_b). The pathogen, *R. solanacearum,* is a diverse species that differs in host range, geographical distribution, pathogenicity and biochemical properties (Hayward, 1991). It affects as many as 200 plant species representing more than 50 families of particularly members of solanaeceous plants such as tomato, potato, eggplant, pepper and tobacco.

In Ethiopia, bacterial wilt was first recorded in 1956 on potato and eggplant in the Keffa region around Jimma in the western part of the country (Stewart, 1956) and it is one of the most important pathogens (Fikre and Zeller, 2007_b), threatening the production of tomato and potato in different parts of the country. 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 (Fikre and Zeller, 2007_b; Naser *et al*., 2008). Intensity of the disease is increasing from time to time and percentage incidence as high as 45% (Yaynu and Korobko, 1986) and 63% (Bekele, 1996) in potato and 55% (EARO, 2002) in tomato was recorded in major potato producing parts of Ethiopia. Other workers also recorded the disease on potato and tomato from Ziway, Ambo, Bako and Guder areas at higher incidences (Mesfin *et al*., 2009).

So far different control strategies have been employed and suggested such as use of resistant variety, crop sanitation, crop rotation, selection of disease free planting material, disinfection of plant materials (Guo *et al*., 2004), microbial antagonists (Fikre and Zeller, 2007_b) and biological soil disinfestation, soil amendments (Gezahegn *et al*., 2010; Getachew *et al*., 2011) and other cultural practices as single or integrated disease management. However, control through the use of resistant varieties alone has showed little success. 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 (Henok *et al*., 2007). Successful control of the pathogen through crop rotation is also not

always effective since rotation practices recommended for one area may not perform well at other locations in addition to differences in the strains involved (Henok *et al*., 2007). So far, no effective chemical product is available for *Ralstonia* wilt (Xue *et al*., 2009). As a result, the disease is still a major problem causing losses to tomato and potato production due to its wide host range and excellent survival in soil and water (Gezahegn *et al*., 2010). Therefore, alternative management options are strongly considered necessary.

Plants produce antimicrobial agents by secondary metabolism to protect themselves from pathogen attack, and therefore many plant species possess substantial antimicrobial activity (Macdonald, 2008). Plant secondary metabolites, such as essential oils and plant extracts are known to possess insecticidal, antifungal, acaricidal, antibacterial and cytotoxic activities (Rhouma *et al*., 2009). Therefore, they have been intensively screened and applied in pharmacology, pharmaceutical botany, medical and clinical microbiology, plant pathology and food preservation (Rhouma *et al*., 2009). Additionally, several plant extracts have shown antimicrobial activity against bacteria and fungi pathogens *in vitro* and *in vivo* (Hassan *et al*., 2009). Thus, the use of plant extracts with inhibitory activity against plant pathogens could lead to the development of environmentally acceptable chemicals based on the availability of natural products (Macdonald, 2008).

Many workers have reported antimicrobial activities of plant extract and gaining due attention because of their proven attributes such as specificity, biodegradability and low toxicity (Macdonald, 2008; Rhouma *et al*., 2009; Amsalu *et al*., 2011). Therefore, plant extracts or plant secondary metabolites which are not toxic and specific in their action could be considered as an alternative to synthetic bactericides based on the availability of material, hence our exploration is on more available weedy and invasive alien species.

Invasive Alien Species (IAS) as commonly described as exotic plants that are weedy in nature, is species that is new to a region and has a negative impact on the new environment, either, ecologically, economically or socially. IAS are of a great concern in Ethiopia, posing particular problems on biodiversity of the country, agricultural lands, range lands, national parks, water ways, lakes, rivers, power dams, roadsides and urban green spaces with great economic and ecological consequences. Foremost among these are Parthenium weed

(*Parthenium hysterophorus*), Prosopis *(Prosopis juliflora*), Water hyacinth (*Eichhornia crassipes*), Mimosa (*Mimosa diplotricha*) and Lantana (*Lantana camara*). They have been identified by the Environmental Policy and the National Biodiversity Strategy and Action Plan as major threats to biodiversity of the country and economic well being of its people. However, little attempt has been made in terms of research and management of IAS (Taye, 2009).

The reason to choose and work with IAS is (1) IAS may be such successful competitors due to resistance towards different pathogens; these plant species may therefore contain active principles to resist plant pathogen attack, (2) extracts of plants has excellent antimicrobial activity, but the cost of cultivating the plant have encouraged the investigation of readily available material such as IAS, and (3) the IAS are used as raw material for plant derived chemicals then there are large quantities of material readily available for use. Additionally collection of these IAS may protect indigenous plants and at the same time may create economic uses and jobs based on these unwanted species.

Objectives

General Objective:

- ☞ To evaluate the antibacterial activity of some Invasive Alien Species extracts against tomato bacterial wilt caused by *R. solanacearum* under *in vitro* and *in vivo*.

Specific Objectives:

- ✓ To evaluate the antibacterial activity of five selected Invasive Alien Species extracts against *R. solanacearum* under laboratory, *in vitro*, conditions.

- ✓ To determine the minimum inhibitory concentrations of most promising Invasive Alien Species extracts against the target pathogen under laboratory condition.

- ✓ To evaluate *in vivo* inhibitory effects of selected Invasive Alien Species extracts and their time of application on bacterial wilt disease development on tomato seedlings.

2. LITERATURE REVIEW

2.1. Origin and Status of Tomato Production in the World

All related wild species of tomato are native to the Andean region that includes parts of Chile, Colombia, Ecuador, Bolivia and Peru (Costa and Heuvelink, 2005). The most likely ancestor is the wild *L. esculentum* var. cerasiforme (cherry tomato), which is indigenous throughout tropical and subtropical America (Costa and Heuvelink, 2005). As indicated by the same Authors, although the ancestral forms of tomato grew in the Peru–Ecuador area, the first extensive domestication seems to have occurred in Mexico (Costa and Heuvelink, 2005).

The Spanish introduced the tomato into Europe in the early 16th century (Costa and Heuvelink, 2005). European acceptance of the tomato as a cultivated crop and its inclusion in the cuisine were relatively slow. Tomatoes were initially grown only as ornamental plants as the fruits were considered to be poisonous, because of the closely related deadly nightshade (*Solanum dulcamara*). Since the mid-16th century tomatoes have been cultivated and consumed in southern Europe, though they only became widespread in northwestern Europe by the end of the 18th century (Costa and Heuvelink, 2005). According to the same authors, production and consumption of tomatoes expanded rapidly in the USA in the 19th century, and by the end of that century processed products such as soups, sauces and ketchup were regularly consumed.

Tomato is one of the most widely grown vegetables in the world. It is grown on more than 5 million ha with a production of nearly 129 million Mg (Srinivasan, 2010). China is the world's top tomato grower, accounting for more than one-quarter of the world's tomato production area (FAO, 2010). Egypt and India together account for more than one-fifth of the world total whereas Turkey and Nigeria are the other major tomatoes producing countries. Asia and Africa account for about 76% of the global tomato area, with about 65% of world output (FAO, 2010). Although tomato requires a relatively cool, dry climate for high yield and better quality (Srinivasan, 2010), it is adapted to a wide range of climatic conditions from temperate to hot and humid tropics (Srinivasan, 2010).

2.2. History and Trends of Tomato Production in Ethiopia

There is no definite time record regarding the introduction of cultivated tomato in Ethiopia. However, cherry type has been growing for long around big cities and in small gardens (Lemma, 2002). Tomato is a popular and widely grown vegetable crop in Ethiopia, ranking 8th in terms of annual national production (Gemechis *et al*., 2012). It is an important cash crop and widely cultivated both under irrigation and rain fed throughout the year in the country (Dandena *et al*., 2010). Recently, the crop has expanded to commercial production for home use, export and processing industries. While, the bulk of fresh market tomatoes are produced by small scale farmers, processing types are mainly produced by small scale horticultural farms. Farmers are interested in tomato production more than any other vegetables for its multiple harvests, which results in high profit per unit area (Lemma, 2002). Now a day like in many other countries, it is also becoming important in Ethiopia in a variety of dishes. The fresh produce is sliced and used as salad. It is also cooked for making local sauce (*Wot*). The processed products such as tomato paste, tomato juice, tomato ketchup and whole peel tomato are produced for local market and export. It is an important cash generating crop to small scale farmers and provides employment in the production and processing industries. Moreover, it is important source of vitamin A and C as well as minerals. Such diverse use makes the tomato an important vegetable in irrigated agriculture in the country (Lemma, 2002; MoARD, 2009).

Environments characterized as warm, dry day and cooler night, are favorable for optimum growth and development of tomatoes. A temperature range between 21 to 27 ^{0}C day and 10 to 20 ^{0}C night is favorable for plant development, and fruit set in the country (Lemma, 2002; MoARD, 2009). It grows better at a constant day and night temperature and can be grown in many types of soils (MoARD, 2009). However, well-drained friable sandy loam soil with pH of 6.7 is preferable for early and high fruit yield. Tomato is widely produced under irrigation. Production in the rainy season is also possible, but need intensive pest management. Although the bulk of tomato production is concentrated in the Central Rift Valleys, there are favorable growing pockets in the different parts of the country.

Small cherry type tomato fruits have been produced for a long time in Ethiopia in home gardens for household consumption and to some extent as source of income. Lately different fruit types appeared in the big markets such as Addis Abeba, Nazret, and Dire Dawa. Both fresh and processing types of tomatoes are popular. Tomatoes are widely produced by small scale farmers and commercial growers. The bulk of fresh market tomatoes are produced by small scale farmers, along riverbanks and lake areas mainly in central, eastern and northern parts of Ethiopia. Tomato production is increasing in areas where irrigation water is available. It has been found to be the most profitable crop to small scale farmers. For example, at Melkassa Research Center a net income of about 11, 000 - 14, 000 birr/ha was estimated from experimental plot yields which were much higher than any other vegetable crops (MARC, 2000).

2.3. Production Constraints of Tomato in Ethiopia

A number of constraints have contributed to the low yield of tomatoes under farmer's condition. Among these, shortage of improved varieties and recommended production packages, high post harvest losses, lack of disease resistant and high yielding varieties, diseases caused by different fungal and bacterial pathogens (Getachew et al., 2011), increasing temperature, viral diseases and salinity (Dandena *et al*., 2010) are among the prominent ones to mention. Moreover, weather conditions (drought and cold), insect pests, weeds and lack of resources such as, irrigation water, nutrients and high quality seeds are listed as factors that severely limit tomato production in the country (Gemechis *et al*., 2012). The major pests include fruit borer, common armyworm, beet army worm, whitefly, leaf miner and spider mites. The diseases are whitefly-transmitted geminivirus, bacterial spot, bacterial wilt, damping-off, early blight, late blight, fusarium wilt, southern blight, and black leaf mold.

Among diseases, bacterial wilt caused by *Ralstonia solanacearum,* is one of the most important diseases that limit tomato production in Ethiopia. It is the most important soil-borne disease of many solanaeceous crops (potato, tobacco, pepper and eggplant) in Ethiopia (Fikre and Zeller, 2007_a) and its effect is more pronounced during the dry season (Fikre and Zeller, 2007_a; Getachew *et al*., 2011). Incidences of bacterial wilt of tomato as high as 55% were

reported in the major tomato producing regions of Ethiopia (EARO, 2002), where effective control is lacking.

2.3.1. Bacterial Wilt Worldwide

Bacterial wilt caused by *R. solanacearum* is a serious disease in the production of tomatoes and many other crops in tropical, subtropical and warm temperature regions of the world (Abo-Elyousr and Asran, 2009). *R. solanacearum* is an important soil borne bacterial phyto-pathogen with a worldwide distribution and a large host range of more than 200 species in 50 families (Guo *et al*, 2004). Some of its economically important hosts include tomato, pepper, potato, tobacco, banana, cowpea, peanut, cashew, papaya, and olive. The bacterial wilt pathogen *R. solanacearum* is present in all continents throughout wet tropics, sub-tropics and warm temperate regions of the world. As reported by Hayward (2005), this disease is of international concern and is being addressed by collaborative research between national researchers and by International Agricultural Research Centers such as CIP working on potato, ICRISAT working on peanut and AVRDC is working on tomato, pepper and eggplant.

The host range of the pathogen is exceptionally wide, and many economically important crops as well as many weed hosts have been recognized (Fikre and Zeller, 2007_a). The pathogen *R. solanacearum* is a diverse species that differs in host range, geographical distribution, pathogenicity and biochemical properties (Hayward, 1991; Fikre and Zeller, 2007_a). Accordingly, it is classified in to five races and five biovars (Hayward, 1964). Race identification is performed based on host crop while biovar is determined through the oxidation test on disaccharides and hexose alcohols (Hayward, 1964). Race 1 is found on diverse crops, and the other Races (2, 3, 4 and 5) are specific on a few crops (Thera, 2007).

More research work has been done on Race 3 biovar 2 since that is the Race present in both tropical highland climates and in warm temperate climates, characteristic of most developed countries. Still more attention should be paid to Race 1 that occurs exclusively in tropical or sub tropical regions where soil never freezes. Race 2 biovar 3 is a novel variant of the pathogen adapted to cooler environments where soils freeze (Thera, 2007). According to the same author bacterial wilt caused by *R. solanacearum* Race 1 has been reported in China on

pepper, sweet potato, peanut, ginger, eggplant and more over; on eucalyptus in Brazil and South Africa.

Table 1**.** Geographical distribution of host range and biovars of *R. solanacearum* (Smith) Worldwide

Race	Host range	Geographical distribution	Biovar
1	Wide	Asia, Australia, America, Africa	3, 4, 1
2	Banana, Other Musa species	Caribian, Brazil, Phillipens	1
3	Potato, some other Solanaeceous, Geranium	Worldwide except Canada and US	2
4	Ginger	Asia	3, 4
5	Mulberry	China	5

Source: Thera (2007)

2.3.2. Bacterial Wilt in Ethiopia

In Ethiopia, *R. solanacearum* was first recorded in 1956 on potato and eggplant in the Keffa region around Jimma in the western part of the country (Stewart, 1956). Bacterial wilt of tomato is an important disease, threatening the production of potato and tomato in different parts of the country and its importance is increasing from time to time (Fikre and Zeller, 2007_b). It is the most destructive in the mid altitude areas around Shashamane, in the Rift Valley, Bako, Jimma, and many irrigated fields throughout Ethiopia (Fikre and Zeller, 2007_a; Naser *et al*., 2008). Other workers also recorded the disease on potato and tomato from Ziway, Ambo, Bako and Guder areas at higher incidences (Mesfin *et al*, 2009) and intensity of the disease is increasing from time to time. As reported by Yaynu and Korobko (1986) and Bekele (1996) percent incidence as high as 45% and 63% in potato were recorded. Moreover, EARO (2002) indicated that percent incidence up to 55% was recorded on tomato plants in major tomato and potato producing parts of the country. Fikre *et al*. (2010), assessed the genetic diversity of *R. solanacearum* strains from Ethiopia and confirmed as majority of the strains are belonging to race 3 and biovar 2 and few were identified as race 1 for the first time in the country.

2.4. Economic Importance of the Disease

The economic impact of *R. solanacearum* worldwide is not well defined but the disease is known to cause important losses on a large range of economic crops. Although yield losses vary according to host, cultivars, climate, soil type, cropping practices and pathogen's strains, the disease can destroy entire harvest when conditions that favor it are met (Elphinstone, 2005).

Bacterial wilt of potato has been estimated to affect 1.52 million ha in 80 countries. Annual gl obal damage estimates exceed $950 million (Thera, 2007; Dunkle, 2008). For example, it is responsible for yield loss of potato up to 70% in India (Sinha, 1986) and to the extent of 50 to 100% in Kenya, Burundi and Uganda (Mwangi *et al*., 2008). In the southeastern USA where the disease is reported on tobacco, important economic losses in North and South Carolina were estimated at $40 million while average yield losses of the same crop was less than 5% in Zimbabwe and ranged from 10 to 30% in Australia (Elphinstone, 2005). According to the same author, severe losses on ginger were reported in Thailand and India. Disease incidence of up to 55% have been reported in fresh market tomato in Taiwan causing over US$12 million losses per year (Thera, 2007).

2.5. Morphological Characteristics of the Bacteria Wilt of Tomato

R. solanacearum is a strictly aerobic, gram negative, non spore forming, non capsulated, and nitrate-reducing, ammonia-forming, rod-shaped bacterium (Stevenson *et al*., 2001). The bacterium neither hydrolyzes starch nor readily degrade gelatin. It is also sensitive to desiccation and is inhibited by low concentration of sodium chloride in broth culture. Optimum growth generally is between 28 to 32 ^{0}C except certain Race 3 strains pathogenic to potato that are able to grow at lower temperature (Stevenson *et al*., 2001). On tetrazolium chloride (TZC) media the bacteria is fluidal, irregular shape and white with pink centered colonies and avirulent colonies are uniformly round, smaller, and dark red (Kelman, 1954; Tahat and Sijam, 2010). In sterile water the pathogen can be stored for years at room temperature while long storage in liquid media and the lack of oxygen can induce the loss of virulence characterized by a morphological change of the colonies (Kelman, 1954; Tahat and Sijam, 2010).

2.6. Factors Affecting Host Pathogen Interactions

2.6.1. Temperature and light intensity

Temperature is the most important factor affecting the host pathogen interaction as well as the survival of the pathogen in the soil. In general, increase in ambient temperature to between 30 to 35 ^{0}C increases the incidence and rate of onset of bacterial wilt on hosts such as tomato, for many but not all strains of the pathogen (Hayward, 1991). Plants that are resistant at moderate temperature may become susceptible at a higher temperature as resistance is temperature sensitive and strain-specific (Hayward, 1991). As reported by Kongkiattikajorn and Thepa (2006), viability of *Ralstonia* were destroyed by either a 4-day constant temperature of 40 ^{0}C or a 2-h temperature cycle for 2 days at 50 ^{0}C or 3 days at 45 ^{0}C. Furthermore, temperatures of 32 ^{0}C in controlled environment chambers significantly increased severity of bacterial wilt in two tomato lines resistant to bacterial wilt as discussed by Krausz and Thurston (1975).

Few studies have investigated the effect of light intensity and photoperiod on host resistance to bacterial wilt. Reduced light intensity did not reduce resistance aginst *R. solanacearum* isolate in resistant tomato cultivar at 26.6 ^{0}C but significantly decreased resistance at 29.4 ^{0}C (Krausz and Thurston, 1975). They also reported reduced photoperiod significantly decreased resistance of tomato cultivar independent of temperature.

2.6.2. Soil moisture

High soil moisture accumulations resulting from either a high water table or heavy rainfall usually favor development of bacterial wilt (Kelman, 1953). Survival of the pathogen is greatest in wet but well drained soils, whereas survival is affected adversely by soil desiccation and by flooding (Hayward, 1991). Soil moisture significantly affected reproduction and survival of *R. solanacearum* in unsterile soils, regardless of soil type. Colony counts increased within 7 to10 days of introduction into the soil at the highest soil moistures (from flooded to -1 bar), but did not increase in drier soils (from -5 to -15 bars) (Hayward, 1991). Although numbers of *R. solanacearum* declined most in the driest soil treatments, as the soil adjusted to the required pressure potential at the beginning of the experiment, at the end, the driest soil contained generally higher number of viable pathogens than did the wetter treatments. Thus, provided the pressure potential remains constant, some

strains of *R. solanacearum* in certain soils are not sensitive to dry soil conditions. Under natural conditions soil microorganisms are subjected to the stress of cyclical wetting and drying. Dry conditions are a factor contributing to reduction in numbers of *R. solanacearum* in fallow land (Hayward, 1991).

2.6.3. Soil type

Soil is the primary source of the disease. The bacterium can survive in soil for extended periods without a host plant. Some soils are conducive to bacterial wilt and others suppressive. Bacterial wilt is a greater problem in heavy soils and in low-lying areas that can retain soil moisture for long periods.

Some soils in otherwise wilt endemic areas have never supported the disease and in some areas where the disease has been introduced there are contrasting reports on persistence in the soil (Kelman, 1953). More systematic investigation is required of the soils in which bacterial wilt does not occur, or in which the disease does not persist once introduced. A detailed description of these soils would assist in the analysis of the complex of physical, chemical, and biological factors involved in disease suppression.

In Surinam bacterial wilt of tomato and related hosts was absent on the sea shell ridges of the coastal plain in contrast to sandy ridge and clay soils where disease outbreaks occurred frequently (Hayward, 1991). The resistance of the disease to sea shell containing soil was attributed to a complex of processes in the host, of which calcium nutrition was supposed to play a key role. Bacterial growth, maceration of tissues, and symptom development in the early stages of bacterial wilt of tomato was primarily in plant tops of low calcium content, rather than in vessels of older tissue high in calcium content. Soil moisture and antagonistic microorganisms were the most important factors. Soil type, which influences soil moisture, determine the size of antagonistic populations, which in turn affect the survival of *R. solanacearum* in the soil (Hayward, 1991).

2.6.4. Nematode populations

According to Deberdta *et al*. (1999) *R. solanacearum* commonly coexists with poly specific nematode populations in tropical and subtropical areas. The synergistic interaction between root-knot nematode (*Meloidogyne* spp.) and *R. solanacearum* on a variety of hosts is widely recognized (Kelman, 1953). Root infection by nematodes as expressed by root galling index generally correlates with bacterial wilt symptoms as expressed by percentage of plants wilted. This is interpreted as primarily the wounding of roots by nematodes is usually invoked to explain the correlation between nematode infection and bacterial wilt, since this wounding increases the number of sites for bacterial entry. However, the nematode may also act as a modifier of plant tissue in such a way that the tissue becomes more suitable for bacterial colonization.

The effect of cross infection of sedentary plant parasitic nematodes together with *R. solanacearum* in bacterial wilt development on tomato was investigated in a controlled environment on the susceptible tomato cultivar and the polygenically wilt resistant cultivar by Deberdt *et al*. (1999). The result of the study indicated that, infection of tomato roots by root-knot nematodes greatly increased wilt severity in both cultivars and reduced genetic resistance of the resistant cultivar to bacterial wilt.

2.7. Disease Symptoms and Diagnostics on Tomato

Typical symptoms of the disease first appear as drooping of a few young leaves. A sudden complete wilt soon follows. Most of the time, leaves are still green when the plants wilt (Fig.1a), this is a distinct symptom, compared to other vascular diseases like Fusarium wilt, which develop yellowing of leaves (Srinivasan, 2010). Adventitious roots and leaf epinasty are more obvious on tomato when the disease develops slowly under unfavorable climatic conditions or on resistant varieties (Fig.1b). After wilting a vascular system of infected stems appears brown and becomes darker as the disease progresses (Fig.1c). Wilting in tomato can be caused by fungal pathogens, root-knot nematode, or excess soil moisture. To distinguish bacterial wilt from other vascular diseases, a clean stem section from wilted plants can be suspended in water; a milky stream of bacterial cells would flow from xylem elements, 3 to 5

minutes later (Fig. 1d) (Srinivasan, 2010) and a whitish bacterial mass can be seen when pressing an exposed stem slice (Fig.1e).

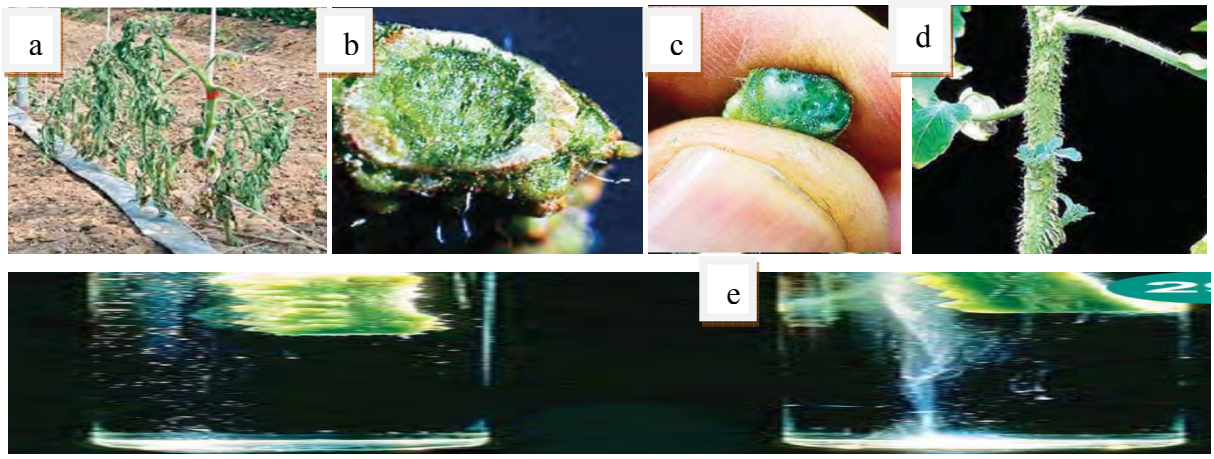

Figure 1. Infected tomato plant displays by *R. solanacearum*. (a) Wilting but not yellowing of leaves. (b) Vascular browning. (c) Whitish bacterial mass, observed when pressing the cut site. (d) Adventitious roots and leaf epinasty. (e) Bacteria streams from an infected stem (right), but not from a healthy stem (left).
Source: Wang and Lin (2005)

2.8. Disease Cycle and Epidemiology

The pathogen, *R. solanacearum,* has 200 plus host ranges (cultivated species and weeds). The pathogen can colonize many weed species without showing symptoms. This may enhance the long-term survival of the pathogen in the soil. It can survive in the soil for a long period depending on the environment. Poorly-drained soils, moderate to high soil temperature and a low to moderate pH are conducive to its survival. The bacterium enters the host through natural wounds in the roots, wounds made during transplanting, or those caused by insects, nematodes *etc*. Infection and disease development are favoured by high temperature (30 - 35 ^{0}C) and high soil moisture (Srinivasan, 2010). The bacterium returns to soil with decaying diseased plant parts and is disseminated by free water, soil movement, or the movement of infected transplant.

2.9. Management of Bacterial Wilt of Tomato

It is difficult to control bacterial wilt disease in the soil (Tahat and Sijam, 2010). Various control strategies have been developed for the control of bacterial wilt, but many are limited in general application, being often crop or site specific, or severely limited in application by socioeconomic conditions. Similarly, the strategy for control of bacterial wilt in cool temperate regions where *R. solanacearum* biovar 2 alone occurs will be very different from control of bacterial wilt in the lowland tropics where strains of wide host range are endemic (Hayward, 1991). No universal solutions exist, but only principles that can be applied and adapted in particular situations.

2.9.1. Host plant resistance

Tahat and Sijam, (2010), indicated that the best strategy to control bacterial wilt caused by *R. solanacearum* is breeding for resistant cultivar. The important strategy of selection or development of resistant cultivars for the control of bacterial wilt has had some success in the case of tobacco and peanut (Kelman, 1953). In potato, no immunity has been identified, and tolerance has been shown to be unsafe in cultivars that harbor latent infections (Hayward, 1991). Resistance genes from other diploid potato species have been identified and used in breeding (Schmiediche, 1988). Furthermore, Tung *et al.* (1990) have made the important finding that genes for adaptation are involved in conferring resistance to bacterial wilt. A heat-tolerant parent gave a higher frequency of resistant offspring in combination with an ascertained source of resistance. Combining ability of the parents was an apparent feature of resistance to bacterial wilt. Widening of the genetic base for both resistance and adaptation to the environment, particularly heat tolerance, is very important in breeding for resistance to bacterial wilt disease.

Although many cultivars in tomato have been developed with useful levels of resistance for certain environments (Kelman, 1953) it has proved difficult to obtain cultivars with stable resistance under conditions of high temperature and humidity in the lowland tropics. Recently emphasis has been given to the importance of improved screening methods to evaluate resistant germplasm. In tomato and many other hosts, young plants are more susceptible than older plants, but natural infection in the field is usually observed at the

flowering stage and onward. Although limited in application, losses from bacterial wilt on tomato in the lowland tropics can be greatly reduced by grafting on to a resistant eggplant rootstock (Hayward, 1991). In addition, Abdalla and Abdulla (1998), found that the degree of susceptibility to bacterial wilt is significantly different among six tomato cultivars which were tested and this indicated that the additives genes were important than non additive genes. Thus, in breeding programs, selection for disease resistant plants after each generation is recommended.

2.9.2. Cultural practices

Crop rotation, intercropping or incorporation, green manure and planting a susceptible crop such as mungbean before the cultivation have been practiced (Hartman *et al.,* 1993). Crop rotation with a non susceptible crop provides some control, but this measure is difficult to use because of the wide host range of the pathogen (Kelman, 1953). Adhikari and Basyat (1998), studied the importance of crop rotation and resistance cultivars for the management of bacterial wilt. They reported that the appearance of bacterial wilt symptoms were delayed by 1 - 3 weeks and the wilt severity was reduced by 20 - 26% when a susceptible tomato was grown after corn (*Zea mays*), lady's finger (*Abelomoschus esculentum*), cowpea (*Vigna unguiculata*), or resistant tomato. Finally it is found that crop rotation with corn, lady's finger, cowpea and resistance cultivars appeared to be useful management strategies to control tomato bacterial wilt. Intercropping has been used as a means of reducing soil populations of the pathogen and root-to-root transmission (Hayward, 1991).

2.9.3. Soil amendment

Amending agricultural soils and soilless growing media with organic matter supplies plant nutrients, increases natural suppressiveness of the soil against soil borne pathogens (Janvier *et al*., 2007). The suppressive effects of organic amendments on the survival of *Ralstonia* vary with soil types. Soil texture, temperature, organic matter content, pH, microbial communities, and moisture content (Van Elsas *et al*., 2005) are among those factors affecting the survival of *R. solanacearum* race 3 biovar 2. Efficient soil management generally improves the composition and activity of soil microbiota thereby enhancing the biological control capacity of the soil (Van Elsas *et al*., 2005). In Taiwan, a soil amendment has been developed that has

a broad spectrum of activity in suppression of a range of soil borne diseases, including bacterial wilt (Hayward, 1991).

Incorporation of household compost, cow dung manure and pig slurry have been found to reduce bacterial wilt incidence and severity. For instance, soil amended with organic materials, inorganic materials (NPK fertilizers) or different combinations of these amendments considerably affected bacterial wilt incidence on potato and increased yield (Lemaga *et al*., 2005). Silicon fertilizer significantly reduced the bacterial population, mean wilt incidence, percent severity index, and corresponding areas of disease incidence, and severity progress curves in the moderately resistant tomato cultivar (Getachew *et al*., 2011). Similarly, sugarcane bagasse resulted in a significant reduction of mean wilt and percent severity index, the corresponding areas under disease incidence and severity progress curves and the bacterial population at 5 days post inoculation, compared to the control (Getachew *et al*., 2011). Furthermore, Gezahegn *et al*. (2010) reported that amending topsoil with different types and rates of amendment can suppress bacterial wilt severity and pathogen survival in the soil.

2.9.4. Disease avoidance

Since high soil temperatures and soil moisture are conducive to bacterial wilt development (Kelman, 1953), losses can be minimized by manipulating the date of planting to accommodate seasons that are less favorable for disease development. For example, in the lowland tropics potato production has been most successful where soils have been selected in which bacterial wilt either does not occur or is not maintained (Hayward, 1991).

2.9.5. Biological control

Biological control is still in its infant phase with few studies reported for bacterial wilt (Nguyen and Ranamukhaarachchi, 2010). Biological control not only increases crop yield and suppresses disease but also avoids environmental pollution. It is important to develop methods for evaluating antagonistic microorganisms and incorporating them into successful disease management. The agents used to control bacterial wilt caused by *R. solanacearum* biologically include avirulent mutants of *R. solanacearum* (Hayward, 1991), genetically

engineered antagonistic bacteria, plant growth promoting rhizobacteria (Guo *et al*, 2004) and antagonistic rhizobacteria such as *Bacillus spp*. and *Pseudomonas spp*. (Fikre and Zeller, 2007_b). As reported by Hayward (1991), the mechanisms involved may include induced resistance or depend upon active colonization of the rhizosphere with antagonistic soil bacteria or bacteriocin and bacteriophage producing strains of *R. solanacearum* or protection by competitive exclusion.

Research on microbial antagonists, such as *Candida ethanolica* has shown promise for bacterial wilt control (Nguyen and Ranamukhaarachchi, 2010). Lwin and Ranamukhaarachchi (2006), reported a satisfactory suppression of the bacterial wilt pathogen by the application of a commercially available mixture of effective microorganisms (EM). Further studies have identified many microorganisms with the potential of suppressing bacterial wilt, although they have not yet been evaluated for effectiveness (Nguyen and Ranamukhaarachchi, 2010). Fikre and Zeller (2007_b), indicated that fluorescent pseudomonads and *Bacillus subtilis* strains significantly reduced the development of bacterial wilt, promote plant growth and increase plant dry weight of tomato plants, compared to untreated control. In addition, Nguyen and Ranamukhaarachchi (2010), reported that three antagonists isolated from soil (*Bacillus megaterium*, *Enterobacter cloacae*, *Pichia guillermondii*) and *Candida ethanolica* showed high potential for disease suppression and also increased fruit weight, biomass and plant height.

Several living microbial products have been commercialized as biological control agents (BCA). Some of these products are a wettable powder of *Bacillus subtilis* (Cohn) Y1336, a water suspension of *Pseudomonas fluorescens* (Migula), and a mixture of wettable powder and granule of *Paenibacillus polymyxa* (Ash, Priest and Col-ins) (Xue *et al*., 2009). However, living microbial BCA have not been widely accepted as an alternative to antibiotics by farmers since they are often regarded as not quite effective, and there is no application method suitable for various BCA and crop cultivation systems.

There has been little study of the potential of vesicular-arbuscular mycorrhizae (VAM) for protection of plants from bacterial wilt. In the Philippines, VAM increased growth and yield of tomatoes and reduced infection by *R. solanacearum*. This was attributed to competition or the mechanical barrier in the form of VAM vesicles and hyphae that inhibit the bacterial pathogen from deeper penetration into host tissues (Hayward, 1991).

2.9.6. Chemical control

Applying chemical pesticides is generally considered as the most effective and fastest strategy for plant disease management. Disease control using chemicals has been difficult because of the localization of the pathogen inside the xylem and its ability for survival at depth in the soil. Some scientists reported that there are no effective bactericides available for chemical control for the *Ralstonia* wilt (Xue *et al*., 2009), while others, reported that it is difficult to control *Ralstonia* wilt with chemicals (Tahat and Sijam, 2010). Soil fumigation with chloropicrin has been reported to achieve limited success if combined with other control methods. Thymol, a plant derived volatile chemical, was shown to reduce disease incidence and increase yield in field experiments (not commercially available) (Momol, 2009).

2.9.7. Use of plant extract

Plants produce antimicrobial agents by secondary metabolism to protect themselves from pathogen attack, and therefore many plant species possess substantial antimicrobial activity (Macdonald, 2008). The antimicrobial activities of plant extracts have been reported by different researchers and gaining due attention because of their proven attributes such as specificity, biodegradability and low toxicity (Macdonald, 2008; Rhouma *et al*., 2009; Amsalu *et al*., 2011). Therefore, they have been intensively screened and applied in pharmacology, pharmaceutical botany, medical and clinical microbiology, plant pathology and food preservation (Rhouma *et al*., 2009). Thus, the use of plant extracts with inhibitory activity against plant pathogens could lead to the development of environmentally acceptable chemicals based on the availability of natural products (Macdonald, 2008).

Even though, very little work has been done to investigate the use of natural plant products to control bacterial wilt; crude medicinal plant extracts of Datura (*Datura stramonium* L), Garlic (*Allium sativum* L.) and Nerium (*Nerium oleander* L.) exhibit antibacterial activity against bacterial wilt of tomato in an experiment conducted both *in vitro* and *in vivo* (Abo-Elyousr and Asran, 2009). Furthermore, Wagura *et al*. (2011), reported that extracts of *Ocimum gratissimum*, *Brassica oleracea* var. *botrytis* and *Ipomoea batatas* were effective to varying degrees in controlling the growth of *R. solanacearum* colonies *in vitro.*

2.9.8. Integrated control

An integrated approach to bacterial wilt control has been advocated, particularly for potato, by combining resistance with proper agronomy. French (1988) has stressed the different weighting to be given to control measures under upland conditions where the pathogen is *R. solanacearum* biovar 2 (race 3) and under lowland conditions where other strains of wider host range and greater longevity in soil may be involved. Use of pathogen free seedbeds to produce clean transplants which can be assisted by fumigating seedbeds and pasteurizing the potting mixture, rotate with non host crops, use of resistant tomato varieties as rootstocks to increase the resistance level of scion varieties (Wang and Lin, 2005) but their reaction may be different from location to location.

3. MATERIALS AND METHODS

3.1. Study Area

The research was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), Jimma, Ethiopia, in Plant Pathology laboratory and greenhouse from July 2011 to May 2012. JUCAVM is located at $7^0$33' N latitude and $36^0$57' E longitude and at altitude of 1710 m a. s. l. The minimum and maximum temperatures in the greenhouse during the study period were 20 and 34 ^{0}C, respectively. However, maximum and minimum temperatures of the area were 26.8 and 11.8 ^{0}C, respectively, with relative humidity of 91% and mean rainfall of 1500 mm per annum (Amsalu *et al*, 2011).

3.2. Collection and Preparation of Plant Materials

Fresh leaf samples from the five major Invasive Alien Species (IAS) viz. *Parthenium hysterophorus* (Family, Asteraceae); *Lantana camara* (Family, Verbenaceae); *Eichhornia crassipes* (Family, Pontederiaceae); *Mimosa diplotricha,* and *Prosopis juliflora*, belonging to the same family, Fabaceae, were collected in July 2011. *P. hysterophorus*, *L. camara* and *M. diplotricha* were collected from natural habitats around Jimma, Ethiopia in August 2011, while *E. crassipes* and *P. juliflora* were collected from natural habitats around Ziway and Afar, Ethiopia, respectively, in July 2011.

The collected samples of each IAS were washed under tap water and surface sterilized with 5% sodium hypochlorite solution for two minutes followed by rinsing thoroughly with sterile water. Then, the plant samples were cut in to smaller size of about 2 - 3 cm long; air dried at room temperature, grinded with the help of sterile pestle and mortar in to fine powder and kept in refrigerator until use.

3.3. Extraction of Plant Materials

3.3.1. Preparation of aqueous extract

Crude plant leaf extract was obtained following standard procedures described by Nduagu *et al*. (2008). A fifty gram of each plant material was separately infused in 250 ml sterilized water to give 20% (w/v) in a 1000 ml conical flask, kept on shaker for 24 hr at 121 rpm. The infusion was filtered afterwards through double layer cheesecloth followed by Whatman No. 1 filter paper and filtrates were centrifuged for 15 min at 6000 rpm. Supernatant of the extract was preserved in air tight bottle until further use in refrigerator.

3.3.2. Preparation of organic solvent extracts

Fifty gram of air dried powder of each plant material was placed in 250 ml acetone (70%) and methanol (70%), in 1000 ml conical flask and kept in rotary shaker at 121 rpm for 24 hr. After 24 hr, they were filtered with double layer cheesecloth and then Whatman No. 1 filter paper and the organic solvents were evaporated in hot air oven at 40 ^{0}C. After solvent evaporation, the remaining crude extract was diluted with sterilized water and kept in air tight bottle and was put in refrigerator until use.

3.4. Isolation and Identification of the Pathogen

The pathogen was originally isolated from symptomatic potato tubers collected from local markets and infected tomato plants which were collected from JUCAVM research field, Jimma, Ethiopia following standard procedures described by Abo-Elyousr and Asran (2009). Samples were surface sterilized by soaking in 2% sodium hypochloride solution for 1 - 2 minutes, rinsed twice in sterile water, cut in to small pieces and placed in a glass of sterile water. After 3 - 5 minutes milky exudates were oozed out from infected samples and a loopful of the resulting suspension was streaked over the surface of Casmino acid-Peptone-Glucose (CPG) agar media in Petri dishes. Plates were incubated at 28 ^{0}C for 48 hr and examined for colony development.

The single colony technique was adopted to obtain pure cultures and identified as *R. solanacearum* based on their cultural characteristics on CPG agar medium as stated in Klement *et al*. (1990), pathogenicity bioassay and some of the biochemical characteristics (catalase, KOH solubility test and utilization of carbohydrates and oxidation of sugar alcohols).

Pathogenecity bioassay was conducted by inoculating three weeks old tomato seedlings (cv. Chali) with suspensions of the pathogen. The seedlings were punctured on the stem with sterilized needle and inoculated with 1ml of pathogen suspension of *R. solanacearum* isolates having a concentration of approximately 10^9 $CFUml^{-1}$. Development of typical wilting symptom was recorded weekly. After development of wilting symptoms re-isolation of the bacteria was made on CPG agar medium to confirm the bacteria (Koch's rule).

The isolated bacterial cultures (strain RsJLMp and RsJUCAVMt) proven to be pathogenic and causing wilt symptoms to tomato and potato plants. On CPG agar medium, colonies of both strains of *R. solanacearum* were visible after 24 - 48 hr of incubation at 28 ^{0}C and had typical cultural characteristics; whitish creamy color, irregularly round, larger in size, fluidal and opaque. The pure culture was maintained for further bioassays in sterilized water at room temperature as indicated by Kelman and Person (1961) to reduce mutation.

3.5. Biochemical Tests of the Pathogen for Identification

3.5.1. Catalase test

Catalase test was performed according to methods described by He *et al*. (1983). One ml of a 3% solution of hydrogen peroxide (H_2S) was added to a Petri dish and a loop full of fresh culture grown on CPG agar medium was added into the solution. Release of bubble from the culture was recorded as catalase positive (Sands, 1990). According to this test the isolated strains of *R. solanacearum* were catalase positive; release gas upon addition of H_2S.

3.5.2. KOH solubility test

It was performed according to Fahy and Hayward (1983) using 24 - 48 hr culture. Two to three drops of 3% KOH were put onto glass slide and the colony of test strain was stirred into the solution with clean loop for 5 - 10 s. When the solution was viscous enough to stick to the loop causing a thin strand of slime, then the test is recorded as KOH soluble (positive). Based on this test both strains of *R. solanacearum* were KOH positive, forming a thin strand of slime and were lifted up with the loop after stirring into the solution.

3.5.3. Carbohydrate utilization and oxidation of alcohols

To test utilization of sugars and sugar alcohols the standard procedures described by Hayward (1964) was followed. The basal medium constituted: $NH_4H_2PO_4$ (1g), KCl (0.2g), $MgSO_4.7H_2O$ (0.2g), bromothymol blue (0.03g), agar (3g) and distilled water 1 L. Before adding the agar the final pH of the medium was adjusted to 7 (an olivaceous green color) by drop wise addition of 40% w/v NAOH solution.

Since sucrose, maltose and lactose solutions are heat liable they were not autoclaved, while glucose, soribitol and mannitol were autoclaved for 20 min as 10% (w/v) solutions (Hayward, 1964). Ten ml of each sugar and sugar alcohol solutions were added to 90 ml of molten cooled Hayward's basal medium and 20 ml volumes of the resulting amended medium were dispensed into previously sterilized test tubes. About 4 ml of inoculum suspension of *R. solanacearum* grown on CPG for 48 hr at 30 ^{0}C was made in distilled water (OD= 0.1 at 600 nm) and with a sterile Pasteur pipette 0.1 ml of the prepared inoculum suspension was inoculated to each tube (Hayward, 1976). Three replicates were used and basal medium with no carbohydrate but inoculated with the pathogen was used as a control. Then it was incubated at 30 ^{0}C and examined at 3, 7 and 14 days for change to acid pH (yellow color) from the top downward and yellow color formation was considered as positive reaction(Hayward, 1964 and 1976). The result of this test revealed that, both isolated strains of *R. solanacearum* (strain RsJLMp and RsJUCAVMt) were utilized carbohydrate sugars (sucrose, maltose, glucose and lactose), but failed to oxidize sugar alcohols (soribitol and mannitol). This indicated that both strains are *R. solanacearum* belong to Biovar 2 Race 3 (Hayward, 1964; EPPO, 2004).

3.6. Media Preparation and Culturing of the Bacteria

The culture medium used was Casmino Peptone Glucose (CPG) agar consisting of 1.0, 10.0, 5.0 and 17.0 gL^{-1} of casmino acid, peptone, glucose and agar, respectively, and then the mixture was boiled while stirring with a magnetic stirrer for 15 minutes to completely dissolve the powdered agar. The solution pH was then adjusted to 6.5 to 7.0 by using dilute solution of NaOH and was autoclaved at 121 ^{0}C for 15 minutes under Autoclave. After autoclaving, the medium was allowed to cool down and poured in to sterilized 9 cm Petri dishes and allowed to solidify. Then after, the bacterial suspension was streaked over the already prepared media and the plates were incubated at 28 ^{0}C for 24 - 48 hr.

3.7. Preparation of Antibacterial Discs

Antimicrobial sensitivity testing was carried out using the disc diffusion sensitivity test which is adopted from Opara and Wokocha (2008). Preparation of antibacterial discs was achieved through the following procedure. Discs (5 mm in diameter) were punctured out from a Whatman No.1 filter paper. Thereafter, the discs were placed in Petri dish diameter allowing about 4 mm in between discs and then sterilized in hot air oven at 160 ^{0}C for 1 hr. After allowing the disc to cool, filter paper discs were pipetted with 5 µl (Abo-Elyousr and Asran, 2009) of each aqueous and organic solvent plant extracts (20%). Sterilized water was used as control.

3.8. *In vitro* Antibacterial Assay

Antibacterial activity of each plant material extracts were determined following the method described by Abo-Elyousr and Asran (2009). Sterile Petri dishes of 9 cm diameter was filled to a depth around 4 mm with CPG agar medium. Thereafter, 100 µl of the bacterial suspension from 24 hr old culture was evenly spread over the medium using sterilized cotton swab as described by Wagura *et al*. (2011). The inoculated plates were allowed to dry for approximately 5 min and four antimicrobial discs were placed per plate with a needle. The plates were then incubated at 28 ^{0}C for 48 hr. After incubation, diameters of inhibition zone around each disc were measured to the nearest millimeter (mm) with a ruler.

3.9. Determination of Minimum Inhibitory Concentration (MIC)

Based on the initial results obtained under *in vitro,* four plant extracts that show the most suppressive effect (maximum inhibition zone) were further tested against *R. solanacearum* at different concentrations to determine the Minimum Inhibitory Concentrations (MIC). Accordingly, most successful IAS extracts that show higher diameter of inhibition zones (> 10 mm) were tested at four concentrations of extracts; 200 mg/ml, 150 mg/ml, 100 mg/ml and 50 mg/ml, by dilution methods of the extract (w/v), by following the same procedures described above. The least concentration of each plant extract showing a clear zone of inhibition was taken as the MIC (Dikbas *et al*., 2009), and was further evaluated under greenhouse, *in vivo*.

3.10. Greenhouse Experiment

3.10.1. Tomato seeds

Healthy seeds of tomato plants (*Lycopersicon esculentum* Mill.) (cv. Chali) which is susceptible to *R. solanacearum* was kindly provided by Melkassa Agricultural Research Center (MARC), Melkassa, Ethiopia.

3.10.2. *In vivo* antibacterial test

The greenhouse experiment was conducted at Jimma University College of Agriculture and Veterinary Medicine, JUCAVM, in Jimma. Based on the efficiency of *in vitro* antibacterial result, four plant extracts (aqueous extracts of *E. crassipes*, *M. diplotericha*, *L. camara* and methanol extract of *P. juliflora*), with inhibition zone of >10 mm in vitro, were chosen for this experiment. Tomato seeds were surface sterilized with 2% sodium hypochlorite for 2 min (Guo *et al*., 2004), and was washed thoroughly with sterilized distilled water and planted in plastic tray filled with sterilized potting medium.

3.10.3. Soil sterilization and transplanting

The soil was sterilized in oven at 82 ^{0}C for 30 minutes by following standard procedures described in (Anonymous, 2012). Sterilized soil (clay and sand mixture at 1:2 v/v) were filled into the plastic pots having a diameter of 20 cm, which were surface sterilized with alcohol into which four tomato seedlings were planted. Then, four weeks old tomato seedlings raised in plastic tray were transplanted in to those pots.

After a week the potted plants were arranged in three groups in a greenhouse. The plants in the first group were drench inoculated around the root areas of seedlings with the pathogens 10^9 CFU ml^{-1} (30 ml/ pot or 7.5 ml/ seedling) (Abo-Elyousr and Asran, 2009; Sangoyomi *et al*, 2011) two days before application with plant extract (40 ml of each extract/ pot or 10 ml/ seedling) (Abo-Elyousr and Asran, 2009). Plants in the second group were inoculated with the pathogen two days after application of plant extracts. Plants in the third group were inoculated with the pathogen at the same time of application as the plant extracts. Inoculated and non-inoculated control pots and plants were treated with an equal volume of distilled water. Watering was done using a hose with fine nozzles and pots were watered independently. A distance of 15 cm between pots was maintained to avoid cross-contamination during watering (Gezahegn *et al*., 2010).

3.10.4. Inoculum preparation and inoculation

For inoculation, the bacterium was grown on CPG agar (Smith *et al*., 1995) at 28 ^{0}C for 48 hr and wild-type bacterial colonies (based on colony morphology) were harvested and suspended in CPG liquid culture, placed on orbital shaker at 120 rpm and grown for three days at room temperature. Cultures grown in CPG liquid medium were collected by centrifugation at 6,000 rpm for 20 min, washed twice in sterilized water, serial dilutions were made and bacterial populations were adjusted approximately to 10^9 CFUml^{-1} (Yao and Allen, 2006).

3.10.5. Quantification of *R. solanacearum* population in rhizosphere soil

To assess the effect of selected IAS extracts on the population density of *R. solanacearum* in rhizosphere soil, the procedures described by Fikre and Zeller (2007_b), were followed. Ten g of pathogen-plant extract infested soil samples were taken from each pot of treatments two months post pathogen inoculation giving 30 g of soil per treatment. The culturable population of the pathogen was determined by the dilution plate method. The soil was mixed thoroughly, and then 10 g was added to sterile water (1 : 9, w/v) and shaken for 30 min on a rotary shaker, serial dilutions were made, and 100 µl (0.1 ml) aliquots were spread on the surface of a semi-selective SMSA medium (Englerbrecht, 1994). Polymyxin B sulphate (1%), 1% crystal violet, 1% tetrazolium salt, 1% bacitracin, 0.1% penicillin (Sigma), 1% chloramphenol, and 1% cycloheximide were used as antibiotics in SMSA medium. After incubating the palates at 28^0C for three days, colonies of *R. solanacearum* were counted and CFU were calculated.

3.11. Data Collected

In the laboratory (*in vitro*) study, diameter of inhibition zones (mm) were measured by taking the average diameter of inhibition zones recorded in the three Petri dishes for each extract. In the greenhouse (*in vivo*) study; wilt incidence (%), disease severity (%), AUDPC (%-days), plant height (cm), fresh and dry weight (g) and *R. solanacearum* population in the rhizosphere soil (CFU/ g dry soil) were recorded and calculated. Disease severity and incidence were assessed at weekly intervals for development of bacterial wilt symptoms. Severity were evaluated based on a six point rating scale (0 to 5) adopted from (Winstead and Kelman, 1952), where: 0 = no wilt symptoms, 1 = one leaf wilted, 2 = two or more leaves wilted, 3 = all leaves except the tip wilted, 4 = whole plant wilted and 5 = death (collapse) of the whole plant.

Disease incidence were assessed and calculated as percentage of wilted plants within each treatment according to the formula:

$$WI = \frac{NPSWS}{NPPT} \times 100$$

Where: WI = wilt incidence, NPSWS = number of plants showing wilt symptoms and NPPT = Number of plants per treatment.

Disease severity indexes (DSI) were calculated by the following equation adopted from Cooke (2006):

$$DSI = \sum[\frac{d \text{ x } n}{N \text{ x } m}] \text{ x } 100$$

Where: d = the disease rating on each plant, n = number of plants in each score, N = the total number of plants examined in each replicate and m = the maximum disease rating possible.

Additionally, the area under disease progress curve (AUDPC) was calculated from percentage of disease incidence and severity according to the midpoint rule (Garret and Mundt, 2000) as:

$$AUDPC = \sum_{i=0}^{n-1} [0.5(x_i + x_{i+1})][t_{i+1} - t_i]$$

Where, x_i = Incidence at the i^{th} assessment or percentage disease severity, t_i= the time of the i^{th} assessment in days from the first assessment date and n= is the total number of days that disease were assessed. Since incidence (x) was expressed in percent and time (t) in days, AUDPC was expressed in %-days (Campbell and Madden, 1990).

Moreover, reduction of wilt incidence, disease severity and AUDPC was calculated using 'PR = [(PC - PT)/PC]*100'; where, PR is percent reduction, PC is percentage value of the control, and PT is percentage value of the treatment group. Plant height was recorded by measuring the above ground part of the plant after two months of pathogen inoculation. Fresh weight was measured by weighing the above ground cuts of the plant at 2 months post inoculation.

Dry matter of plants was measured by weighing the Oven-dried (at 60 ^{0}C for 72 h) above ground cuts of the plants. Finally biomass increase was calculated by using the following formula adopted from (Seleim *et al*., 2011).

$$\text{Biomass increase} = \frac{(\text{average weight of plants with botanicals} - \text{average weights of control plants})}{\text{average weights of control plants}} \text{ x } 100$$

Finally, population densities of *R. solanacearum* in the rhizosphere soil were counted and CFU were calculated per gram (dry weight) of soil by using the following formula (Anonymous, 2012):

$$\text{CFU/ gram of soil} = \frac{\text{CFU counted in a plate}}{dilutin\ facter\ x\ amount\ plated}$$

3.12. Experimental Design and Statistical Analysis

Completely randomized design (CRD) and randomized complete block design (RCBD) with three replications were employed for *in vitro* and *in vivo* experiments, respectively, and *in vitro* experiment was repeated. The experimental data were analyzed using analysis of variance (ANOVA). Statistical analysis were done using SAS software version 9.2 (SAS Institute Inc. 2008). Single and interaction effect of factors were determined using the GLM procedure of SAS. Whenever significant interactions were observed between factors, the level of one factor was compared at each level of other factors and comparison of means at 5% level were made by Tukey's test. Angular transformation for incidence and severity data and Log transformation (Log_{10} (CFU+1) for populations of bacteria in the rhizosphere soil was performed before analysis to normalize variance.

4. RESULTS

4.1. *In vitro* Antibacterial Assay

In vitro studies of antibacterial activities of aqueous, acetone and methanol leaf extracts of five IAS against the growth of *R. solanacearum* were conducted and found to give significantly different results (Table 2). Preliminary screening result confirmed that most of the tested IAS extracts showed antibacterial activity, created inhibition zone around antibacterial discs, against *R. solanacearum*.

The *in vitro* inhibitory activity of methanol extract against the target pathogen (*R. solanacearum*) ranged from inhibition diameter of 0 mm to 11.1 mm. Methanol extract of *P. juliflora* provided the highest diameters of inhibition zone (11.1mm) followed by *L. camara* methanol extract (7 mm). However, the two treatments were statistically different from each other and from methanol extracts of *E. crassipes*, *P. hysterophorous* and *M. diplotricha*, which did not show antibacterial activity (0 mm of inhibition zone) against *R. solanacearum* and statistically at par with control, treated with sterile water.

Similarly with acetone extract, the highest diameter of inhibition zone was displayed by *P. juliflora* (8.6 mm) that was significantly different over the other selected IAS extracts and control. Diameters of inhibition zone of 7.98 and 7.81 mm were offered by acetone extract of *E. crassipes* and *M. diplotricha* respectively, which were not statistically different from each other. Acetone extracts of *P. hysterophorous* also scored 6.12 mm of diameter of inhibition zone. While *L. camara* acetone extract had no inhibitory activity against the target pathogen and that was not statistically different from the control.

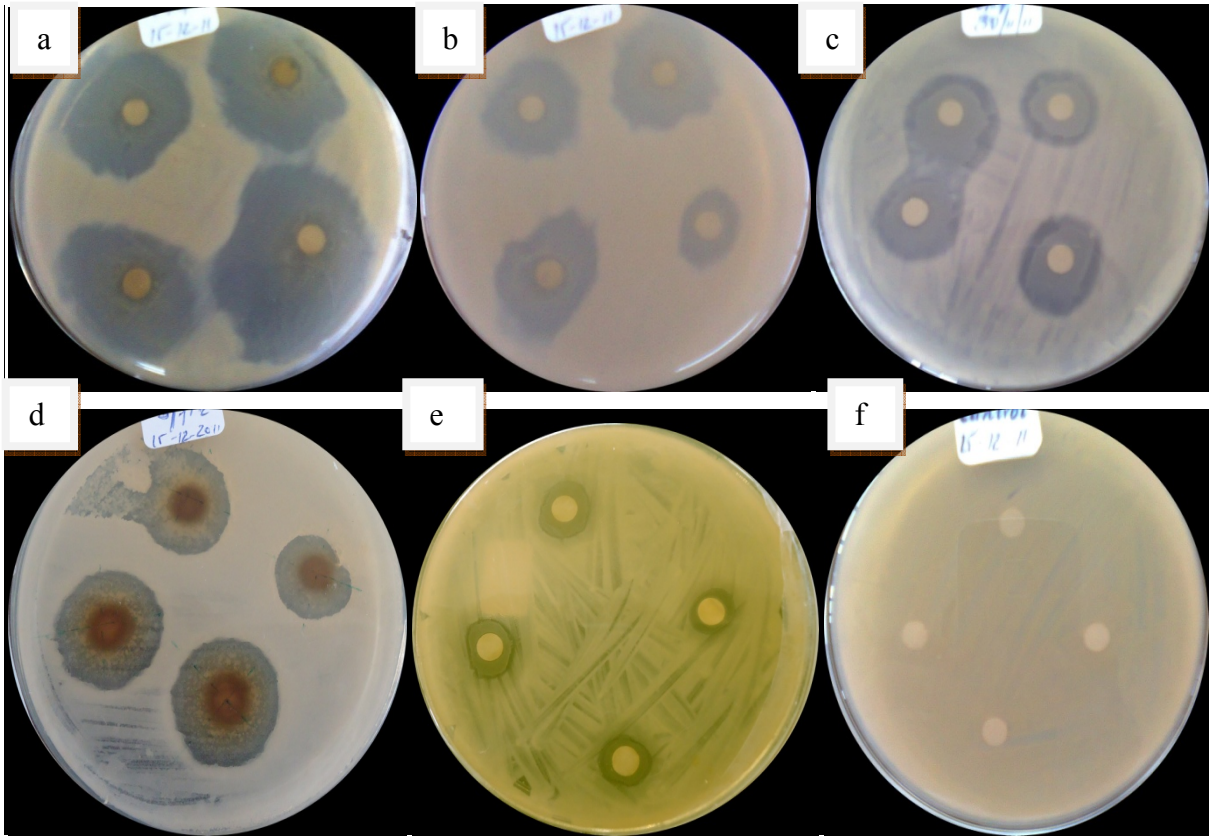

Figure 2. *In vivo* antibacterial activity of the most effective invasive alien species extracts and control treatment against *Ralstonia solanacearum.* (a) *Eichhorina crassipes* aqueous extract. (b) *Mimosa diplotricha* aqueous extract. (c) *Lantana camara* aqueous extract. (e) *Parthenium hysterophorous* aqueous extract. (d) *Prosopis juliflora* 70 % methanol extract. (f) control, untreated plate.

All aqueous extracts had significantly inhibited the growth of *R. solanacearum in vitro* compared with control treated with sterile water. The antibacterial activity of aqueous extract of five IAS extracts was ranged from 8.56 to 26 mm. The highest diameter of inhibition zone was recorded from *E. crassipes* (26 mm) that was statistically different from aqueous extracts of *M. diplotricha, L. camara, P. hysterophorous, P. juliflora* with inhibition zone of 14, 10.87, 8.85 and 8.56 mm respectively, and control.

Table 2. Interaction effect of Invasive Alien Species (*E. crassipes*, *L. camara*, *P. hysterophorous*, *M. diplotricha* and *P. juliflora*) extracts and three extractant on antibacterial activity against *Ralstonia solanacearum in vitro*

Plant species	Mean diameter of inhibition zone (mm)*		
	Solvents used for extraction		
	Methanol	Acetone	Water
E. crassipes	0.00^{i}	7.98^{ef}	26.00^{a}
L. camara	7.00^{g}	0.00^{i}	10.87^{c}
P.hysterophorous	0.00^{i}	6.12^{h}	8.85^{d}
M. diplotricha	0.00^{i}	7.81^{f}	14.00^{b}
P. juliflora	11.10^{c}	8.60^{de}	8.56^{de}
Control	0.00^{i}	0.00^{i}	0.00^{i}

* Data are means of three replicates in two separate experiments. Values followed by similar letter(s) are not significantly different at ($\alpha = 0.05$) according to Tukey test.

4.2. Determination of Minimum Inhibitory Concentration (MIC)

Based on the initial results obtained in *vitro,* four plant extracts that show the most promising effect with maximum diameter of inhibition zones (>10 mm) were further tested against *R. solanacearum* at different concentrations to determine the Minimum Inhibitory Concentrations (MIC). Results in the prescreening study revealed that aqueous extract of *E. crassipes*, *M. diploterícha*, *L. camara* and methanol extract of *P. juliflora* showed higher diameter of inhibition zone compared to all the tested IAS by forming inhibition zone of 26, 14, 10.87 and 11.1 mm, respectively. Accordingly, the antibacterial activities of those extracts were tested at different concentrations (5, 10, 15 and 20%) against *R. solanacearum* to determine the MIC of each extract.

Analysis of variance showed that there was an interaction effect between selected IAS extracts and four concentrations indicating differences in their antibacterial effect against growth of *R. solanacearum* (Fig. 3).

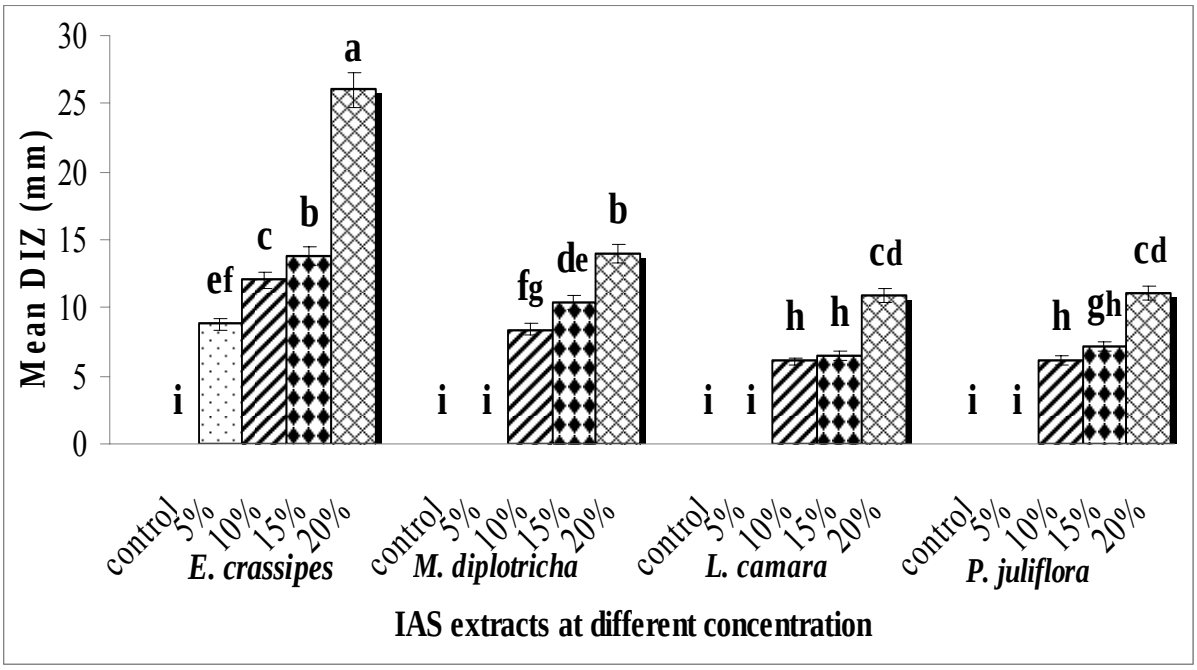

Figure 3. Antibacterial activities of *Eichhorina crassipes, Lantana camara,* and *Mimosa diplotericha* aqueous extracts and methanol extract of *Prosopis juliflora* against *Ralstonia solanacearum* at different concentrations. Values followed by similar letter(s) are not significantly different at ($\alpha = 0.05$) according to Tukey test.

The results of the present study revealed that aqueous extracts of *E. crassipes* at 5, 10, 15, and 20% significantly reduced the growth of *R. solanacearum* compared to the control, treated with sterile water. However, statistically significant differences were not evident between 5% concentration extracts of *M. diplotricha*, *L. camara* and methanol extracts of *P. juliflora* and the control in diameters of inhibition zone. Thus, the Minimum Inhibitory Concentration for inhibition zones against *R. solanacearum* under *in vitro* conditions were 5% for *E. crassipes* aqueous extract and 10% for aqueous extract of *M. diplotricha* and *L. camara* and methanol extract of *P. juliflora*.

4.3. *In vivo* Antibacterial Assay

4.3.1. Effect of botanicals on disease incidence and percent severity index

According to the present study, botanicals (IAS extracts used), time of application and the interaction of the two had significantly ($P<0.05$) affected bacterial disease development in terms of disease incidence and percent severity index. Mean wilt incidence and percent severity index were significantly lower for botanical treated plants than the untreated plants.

Though the applications of all the four plant extracts resulted in reductions of wilt incidence and percent severity index, *E. crassipes* leaf extract was found better in restricting the symptom development of the disease than the rest three plant extracts under greenhouse condition, *in vivo.* Simultaneous application of aqueous extract of *E. crassipes* with pathogen inoculation resulted in reduction of incidence and severity by 75 and 91.7%, respectively, compared to infected control (Table. 3).

Other treatments also resulted in a significant reduction of mean wilt incidence and percent severity index in tomato plants compared to non-treated, infected control. The lower mean wilt incidence reduction was recorded from methanol extract of *P. juliflora* at two days after application (0.00%), followed by two days before application of the same botanical (9.4%) which were not statistically different from the inoculated control. In the case of percent severity index, again the lowest percent reduction were scored by methanol extract of *P. juliflora* when it was applied two days after and before application of the pathogen with 56.2 and 62.5% respectively. But, they were statistically different from the inoculated control.

Table 3. Interaction effects of selected Invasive Alien Species extracts and their application times (2-days pre-inoculation, simultaneously and 2-days post-inoculation) on bacterial wilt disease development in tomato caused by *R. solanacearum*, under greenhouse condition, pot experiment

Plant species (botanicals)	Mean wilt incidence and percent severity index[y] (%)											
	Application times											
	Before 2- days				Simultaneously				After 2- days			
	DI	PR	PSI	PR	DI	PR	PSI	PR	DI	PR	PSI	PR
E. crassipes	(58.3)50.0^{bc}	41.7	(20.0)26.56^{cde}	75.0	(25.0)30.0^{c}	75.0	(6.6)14.76^{f}	91.7	(58.3)50.0^{bc}	41.7	(16.6)24.04^{de}	79.2
L. camara	(66.6)55.0^{b}	33.4	(20.0)26.56^{cde}	75.0	(50.0)45.0^{bc}	50.0	(15.0)22.79^{de}	81.2	(58.3)50.0^{bc}	41.7	(16.6)24.04^{de}	79.2
M. diplotricha	(66.6)55.0^{b}	33.4	(20.0)26.56^{cde}	75.0	(33.3)35.0^{bc}	66.7	(11.6)19.89^{ef}	85.5	(58.3)50.0^{bc}	41.7	(20.0)26.56^{cde}	75.0
P. juliflora	(91.6)79.2^{a}	9.4	(30.0)33.03^{bc}	62.5	(66.6)55.0^{b}	33.4	(23.3)28.85^{cd}	70.8	(100.0)88.8^{a}	0.0	(35.0)36.27^{b}	56.2
Control[z]	(100)88.8^{a}	-	(80)63.44^{a}	-	(100)88.8^{a}	-	(80)63.44^{a}	-	(100)88.8^{a}	-	(80)63.44^{a}	-

PR was calculated based on 'PR = [(DIC-DIT)/DIC] x 100'; where, PR= Percent reduction, DIT is disease incidence by the treatment group, and DIC is disease incidence by the control and PSI is percent severity index. Percent reductions were calculated from the actual data.

[y]-Values in the same column followed by the same letter(s) are not significantly different at ($\alpha = 0.05$) according to Tukey test and values in brackets are actual data before transformation.

[z]-Pathogen inoculated and sterile water treated instead of botanicals.

Note: On uninoculated control, healthy plants, no natural infection occurred and wilt symptom development were not recorded, therefore, zero values have been omitted.

4.3.2. Effect of botanicals on bacterial wilt disease progress

A significant interaction between type of botanicals and time of application in terms of incidence Area Under Disease Progress Curve (iAUDPC), and severity Area Under Disease Progress Curves (sAUDPC) were not evident P> 0.05 (*P= 0.2467* and *P= 0.3053*), respectively. While, main effects of botanicals and time of application had significantly reduced iAUDPC (*P<0.0001* and *P= 0.0007*), and sAUDPC (*P<0.0001* and *P= 0.0007*), respectively.

Disease severity and wilt incidence expressed as iAUDPC and sAUDPC were significantly reduced by botanical treated soils compared to sterile water treated, infected control. The highest percent reduction of iAUDPC and sAUDPC were exhibited from aqueous extract of *E. crassipes* with 67.52 and 71.35%, respectively, followed by aqueous extract of *M. diplotricha* 64.08 and 68.69%, and then *L. camara* aqueous extract with 59.48 and 66.19% (Table 4). Similarly, the same parameters were significantly reduced with methanol extract of *P. juliflora* compared to non treated, control, by 35.48 and 50.48% (Table 4).

Table 4. Effect of selected IAS extracts on incidence area under disease progress curve (iAUDPC) and severity area under disease progress curve (sAUDPC) under greenhouse condition.

Botanicals	Mean iAUDPC[y] (%-days)	PR (%)	Mean sAUDPC[y] (%-days)	PR(%)
E. crassipes	1318.3[c]	67.52	669.37[c]	71.35
L. camara	1645.0[c]	59.48	790.23[c]	66.19
M. diplotricha	1458.3[c]	64.08	731.84[c]	68.69
P. juliflora	2619.2[b]	35.48	1157.41[b]	50.48
Control[z]	4060.0[a]	-	2337.58[a]	-

Percent reduction (PR) was calculated based on 'PR = [(AUDPCC-AUDPCT)/AUDPCC] x 100'.

[y-]Values in the same column followed by the same letter(s) are not significantly different at (α = 0.05) according to Tukey test.

[z-]Pathogen inoculated and sterile water treated instead of botanicals.

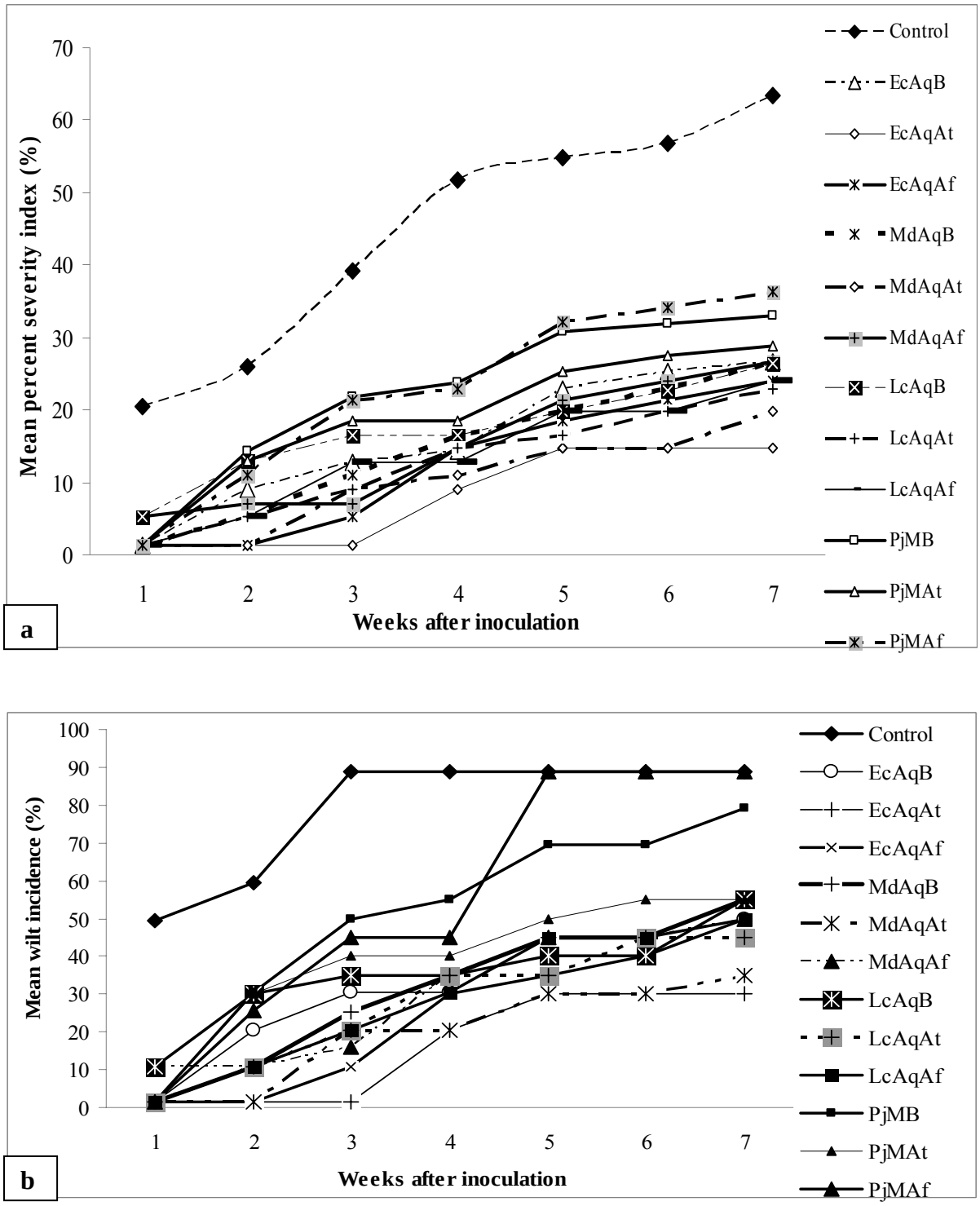

Figure 4. Bacterial wilt development as mean percent severity index (a) and wilt incidence (b) on tomato plants treated with selected plant extracts at different times of application under greenhouse conditions.

Table 5. Effects of application time of selected IAS extracts on incidence area under disease progress curve (iAUDPC) and severity area under disease progress curve (sAUDPC) under greenhouse condition.

Application time	Mean iAUDPC[z] (%-days)	Mean sAUDPC[z] (%-days)
Before 2-days	2422.0[a]	1228.96[a]
Simultaneous	1921.5[b]	1017.48[b]
After 2-days	2317.0[a]	1165.42[a]

[z-]Values in the same column followed by the same letter(s) are not significantly different at (α = 0.05) according to Tukey test.

4.3.3. Effect of botanicals on plant height

According to analysis of variance, main effects (plant species and time of application) alone and the interaction of the two had significantly increased plant height compared to untreated control. Among the leaf extracts tested, best growth was supported by aqueous extract of *E. crassipes* that scored 53.34, 51.84 and 51.17 cm when it was applied simultaneously, two days after and two days before pathogen inoculation, respectively. The result also indicated that these treatements were statistically similar with the negative control, healthy plants, which scored a plant height of 56 cm (Fig. 5).

Simultaneous applications of *L. camara* and *M. diplotricha* aqueous extracts also provided statistically similar results as that of negative controls, for which the scores were 50.67 and 53 cm, respectively. The least was recorded from methanol extract of *P. juliflora* leaf extract which scored 42, 42.67 and 42.83 cm when it was applied two days after, two days before and simultaneously with pathogen inoculation though it was statistically different from infected control, treated with sterile water.

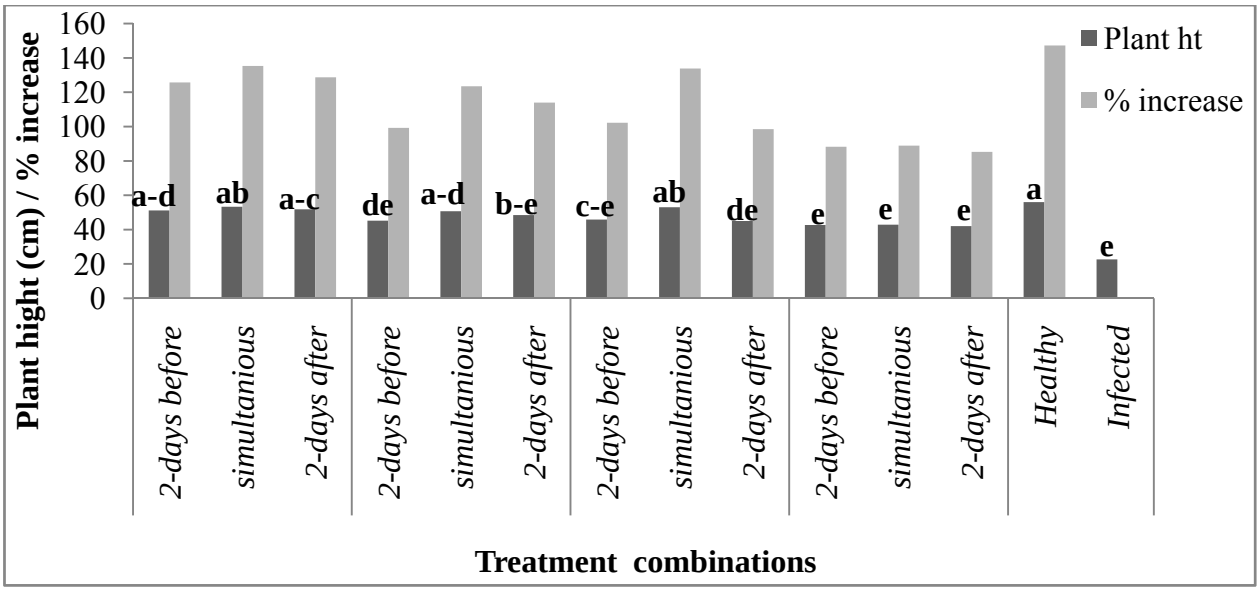

Figure 5. Interaction effect of *Eichhornia crassipes*, *Lantana camara* and *Mimosa diplotricha* aqueous extract and methanol extract of *Prosopis juliflora* and their time of application on plant height of tomato plants infected with *R. solanacearum* under *in vivo*. Values followed by similar letter(s) are not significantly different at (α = 0.05) according to Tukey test.

4.3.4. Effect of botanical type and time of application on shoot biomass

The effects of type of botanicals and their time of application on above ground biomass (fresh and dry weight) per plant are presented in Table 6. Accordingly, the two-way interaction between botanicals and time of application for above ground fresh weight (AGFW) and above ground dry weight (AGDW) were significant at α=0.05.

Aqueous extract of *E. crassipes* applied simultaneously with the pathogen to topsoil gave the highest AGFW with 52.72 g and 499.7% biomass increase over the inoculated control next to non inoculated control, healthy plants, and that was statistically different from other botanicals at α=0.05 (Table 6). Simultaneous application of *E. crassipes* also significantly increased AGDW per plant (11.96 g), followed by two days after pathogen inoculation of application of the *E. crassipes* (9.94 g). Statistically similar results were found from simultaneous application of *L. camara* and *M. diplotricha* for which the score were 9.35 and 9.23 g, respectively. Tomato plants treated with all botanicals at all different time of applications were less in their shoot fresh and dry weights than healthy control plants (Table 6).

Table 6. Interaction effect of some IAS plant extracts and their time of application on above ground fresh and dry weight (g) and biomass increase on tomato plants under greenhouse conditions

Plant species (botanicals)	Above Ground Biomass[x] (g)											
	Application times											
	Before 2- days				Simultaneously				After 2- days			
	AGFW	BI	AGDW	BI	AGFW	BI	AGDW	BI	AGFW	BI	AGDW	BI
E. crassipes	33.1±1.1^{e}	276	8.9±0.3^{d}	219	52.7±1.4^{b}	499	11.9±0.1^{b}	328	40.0±1.8^{d}	355	9.9±0.2^{c}	256
L. camara	42.8±1.3^{d}	387	8.6±0.3^{de}	207	48.3±0.8^{c}	449	9.3±0.5^{cd}	235	31.3±0.9^{e}	256	9.0±0.5^{d}	222
M. diplotricha	31.3±0.3^{e}	256	7.9±0.2^{e}	184	48.7±1.5^{c}	454	9.2±0.4^{cd}	231	30.5±0.9^{e}	247	8.6±0.6^{de}	208
P. juliflora	16.9±0.4^{g}	92	3.7±0.1^{g}	31	33.1±0.9^{e}	277	6.7±0.2^{f}	139	25.6±1.1^{f}	191	6.2±0.1^{f}	121
Control												
Infected[y]	8.8±0.2^{h}	-	2.8±0.1^{g}	-	8.8±0.2^{h}	-	2.8±0.1^{g}	-	8.8±0.2^{h}	-	2.8±0.1^{g}	-
Healthy[z]	113.9±1^{a}	1195	15.5±0.3^{a}	454	113.9±1^{a}	1195	15.5±0.3^{a}	454	113.9±1^{a}	1195	15.5±0.3^{a}	454

PI was calculated based on 'PI = [(AGFWC-AGFWT)/AGFWC] x 100'; where, BI= Biomass increase, AGFWT is above ground fresh weight by the treatment group, and AGFWC is above ground fresh weight by the control and AGDW is above ground dry weight. [x]-Values in the same column followed by the same letter(s) are not significantly different at ($\alpha = 0.05$) according to Tukey test.

[y]-Pathogen inoculated and sterile water treated instead of botanicals.

[z]-Neither pathogen inoculated nor treated with botanicals

4.3.5. Effect of botanicals on population of *R. solanacearum* in the rhizosphere soil

Two months after inoculation the availability of bacteria in plant tissue (stem base) was checked by plating bacterial suspensions extracted from 1 cm stem base of symptomless plants on SMSA media. Latent infection was evident in all treatment combinations except in non infected controls, healthy plants. The population densities of *R. solanacearum* in the rhizosphere soil were assessed two months post-inoculation. A significant (*P <0.0001*) interaction between botanicals used and their time of application were observed in terms of *R. solanacearum* population density in the rhizosphere soil two months after inoculation. The number of culturable bacteria recovered from rhizosphere soil two months after inoculation was significantly lower (2.1 x 10^3) in the rhizosphere soil drenched by *E. crassipes* simultaneously treated with pathogen inoculation pot compared to control treatment (3.22 x 10^7)/g. dry soil. Crude aqueous extract of *E. crassipes* gave the highest percent reduction (50%) in *R. solanacearum* population, followed by *L. camara* (47.72%) and then *M. diplotricha* (44.69%) when applied simultaneously with pathogen inoculation. The lowest was recorded from methanol extract of *P. juliflora* when it was applied two days after and two days before pathogen inoculation for which the score was 9.69 and 9.84%, respectively.

Table 7. Interaction effect of selected IAS extracts and their time of application on *R. solanacearum* population in rhizosphere soil and percent reduction on tomato plants under greenhouse conditions

Plant species (botanicals)	$Log_{10}(CFU+1)$/g.dry soil[y]					
	Application times					
	Before 2- days		Simultaneously		After 2- days	
	CFU	PR	CFU	PR	CFU	PR
E. crassipes	4.50±0.10[e]	31.82	3.30±0.10[g]	50.00	4.65±0.05[de]	29.54
L. camara	4.74±0.05[cd]	28.18	3.45±0.05[fg]	47.72	4.86±0.04[cd]	26.36
M. diplotricha	4.87±0.04[cd]	26.21	3.65±0.05[f]	44.69	4.75±0.05[cd]	28.03
P. juliflora	5.95±0.05[b]	9.84	4.87±0.06[c]	26.21	5.96±0.05[b]	9.69
Control[z]	6.60±0.10[a]	-	6.60±0.10[a]	-	6.60±0.10[a]	-

PR was calculated based on 'PR = [(CFUC-CFUT)/CFUC] x 100'; where, PR= percent reduction, CFUT is colony forming units by the treatment group, and CFUC is colony forming unit by the control. [y-]Values in the same column followed by the same letter (s) are not significantly different at (α = 0.05) according to Tukey test. [z-]Pathogen inoculated and sterile water treated instead of botanicals.

5. DISCUSSIONS

Synthetic pesticides are nowadays widely used for the control of plant diseases throughout the world because of their higher effectiveness in controlling disease causing organisms. However, excessive and unsystematic application of these chemicals has created several environmental and health hazards and also some phytopathogens have developed resistance (Rhouma *et al*., 2009). Green plants have shown to represents a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Dorman and Deans, 2000). Plants produce antimicrobial agents through secondary metabolism to protect themselves from pathogen attack, and therefore many plant species possess substantial antimicrobial activity (Macdonald, 2008).

Preliminary screening result of the present study revealed that except methanolic extract of *E. crassipes*, *M. diplotericha*, *P. hysterophorous* and acetonic extract of *L. camara*, the tested IAS extracts were effective for inhibition of bacterial growth verified by disc diffusion sensitivity test. The present study is in accordance with the works of Barsagade and Wagh (2010), who reported that acetone and methanol leaf extracts of common plants and weeds exhibited antibacterial and antifungal activities against one fungal and two bacterial species. Abo-Elyousr and Asran (2009), also reported that extracts of *Allium Sativum* L., *Datura stramonium* L., and *Nerium oleander* L., showed antibacterial activity and significantly inhibited the growth of *R. solanacearum* isolates *in vitro* compared with untreated control. Moreover, the antibacterial effects of crude medicinal plant extract of *Ocimum gratisimum*, *Brassica oleracae* and *Ipomoea batatas* against *Ralstonia solanacearum* were reported by Wagura *et al.* (2011).

The leaf extracts of plants and weeds have great potential as antimicrobial compounds against different microorganisms. It has been suggested that the antimicrobial activity of plant secondary metabolite is mainly due to the presence of essential oils, flavonoids, triterpenoids and other natural polyphenolic compounds or free hydroxyl groups (Rojas *et al*., 1992). This is supported by Barsagade and Wagh (2010), who indicated that antimicrobial activities of plant and weed extracts may exist in a variety of different components, including aldehyde

and phenolic compounds. According to the result of the present study all the tested botanicals have bioactive compounds; hence all showed antibacterial activity against *R. solanacearum*.

Variation in the effectiveness of the extract against a target microorganism depends upon the chemical compositions of the extracts and membrane permeability of the pathogen for the chemicals and its metabolism. Naturally occurring combination of these compounds can be synergistic and often results in crude extracts having greater antimicrobial activity than the purified, individual constituents. The antimicrobial effect of the extracts could be explained by disturbance of the permeability barrier of the living membrane structure. Cowan (1999), came to similar conclusion. He also indicated substrate deprivation, membrane disruption, bind to adhesines, complex with cell wall, enzyme inhibition and inactivation, metal ion complexation and intercalate into cell wall and/or DNA are the possible mechanisms of plant secondary metabolites against different microorganisms.

Result from the *in vitro* study revealed that the antibacterial activity of all the selected IAS extracts were significantly ($P < 0.0001$) affected by solvents used for extraction against *R. solanacearum* (Table 2). Water (aqueous extract) was relatively more effective than organic solvents (acetone and methanol) and all aqueous IAS extracts showed antibacterial activity against the target pathogen. Thus, result of present study revealed that most of the active ingredients; secondary metabolites having antibacterial activity against *R. solanacearum*, present in leaves of all tested IAS are soluble in water. Whereas, methanol extracts of *E. crassipes*, *M. diplotricha*, *P. hysterophorous* and acetone extract of *L. camara* had no inhibitory activity against the pathogen and statistically similar with the control. Therefore, it appears that secondary metabolites (bioactive compounds), which have antibacterial activity against the target pathogen present in *M. diplotricha, P. hysterophorous* and *E. crassipes* leaves might not solubilize in methanol. Acetone also failed to solubilize active ingredients present in *L. camara* leaves against *R. solanacearum.*

According to the present study antibacterial activity results might have been influenced by the solubility of the active compound(s) in extracting solvents, with high solubility of compounds in water than in organic solvents used. The greater effectiveness of aqueous extract compared with organic solvent (methanol and acetone) extract may be due to differences in constituent and amount of extraction phytochemicals, which are toxic to *R. solanacearum*, present in leaf parts of tested IAS. The result of current study is comparable with Cowan (1999), who reported plant secondary metabolites; starches, polypeptides and lectins which have antimicrobial activity are soluble only in water while, xanthoxyllines, totarol, quassinoids, lactones and phenones are only soluble in methanol. This is in accordance with reports of Sukanya *et al.* (2009), who indicated that antimicrobial activity of various solvent extracts of medicinal plants showed varied level of inhibition against both human and plant pathogenic bacteria. Amsalu *et al.* (2011), also reported that the antifungal activity of eight medicinal plants were influenced by both type of plant species and solvents used for extraction. In addition the potential of each extract differs from one another, suggesting that the toxicity of different extracts may be due to their solubility in extracting solvents. Qasam and Abu-Blan (1996) came to the same conclusion.

Analysis of variance showed that there was an interaction effect between selected IAS extracts and concentrations used ($P<0.005$) indicating differences on their antibacterial effect against growth of *R. solanacearum* (Fig. 3). The effectiveness of those botanicals were increased as their concentration becomes higher and higher. This is most likely due to high concentration of phytochemicals present at higher concentration than the lowest concentration, which in-turn affects the antibacterial activities of those selected botanicals. The result is in accordance with Balestra *et al.* (2009), who reported that *in vitro* test of vegetal extracts from cloves of *Allium sativum* and fruits of *Ficus carica* against bacterial pathogens of tomato including *R. solanacearum* showed antibacterial effect and higher antibacterial activity were provided when they were applied at higher concentrations. Amsalu *et al.* (2011), also found a significant interaction effect between type of plant materials and different concentrations used indicating that the antimicrobial effect of plant extracts depends on not only solvents used for extraction but also concentration levels.

The result of the present study indicated that among IAS crude extracts tested aqueous extract of *E. crassipes* was found to be the best both in inhibiting the bacterial growth *in vitro* test and consistently restricting the disease development on tomato plants under greenhouse conditions. Accordingly, 75 and 91.7% reduction in wilt incidence and disease severity index, respectively, of bacterial wilt was recorded on tomato plants treated with leaf extract of *E. crassipes* in the greenhouse experiment when it was applied simultaneously with pathogen inoculation (Table 3).

The antibacterial activity of *E. crassipes* may be attributed to various chemicals detectable in its extracts. This is supported by many researchers, who reported phytochemical compositions present in crude extracts from *E. crassipes* are tannins, phlobatannin, steroid, terpenoid, alkaloid, flavonoid, phenolic contents, quinone, anthraqninone and cardiac glycosides (Dubey and Lata, 2010; Aboul-Enein *et al*., 2011). Thus, the crude extract contained different antibacterial substances with variable efficiencies and mode of actions may act synergistically in inhibition of bacterial growth. This result is in agreement with those Shanab *et al.* (2010) and Aboul-Enein *et al.* (2011), who reported that the crude extract of *E. crassipes* leaf exhibited antibacterial activities against both the Gram positive and the Gram negative bacteria. Bikash and Bijaya (2011), also verified antibacterial activity of aqueous and methanol hot extraction of *E.crassipes* against different clinical bacteria. Furthermore, the aqueous and different solvent extracts and fractionates of fresh *E. crassipes* showed a significant and remarkable activity against two bacteria and two fungal species compared to the standard (Lalitha and Jayanthi, 2012).

In vitro antibacterial assay of the crude aqueous and methanol extracts of *L.camara* showed significant inhibitory activity against *R. solanacearum,* compared with the control. But, acetone extract of the same plant had no antibacterial activity against the test pathogen, suggesting that acetone failed to solubilize bioactive compounds present in leaves of *L. camara* while they were better extracted in water and methanol. The antibacterial activity of crude aqueous leaf extracts of *L.camara* was also evaluated on tomato plants and resulted in reduction of wilt incidence, disease severity, AUDPC and *R. solanacearum* population in the rhizosphere soil, over the infected control.

This suggested that the presence of active principles in leaves of *L. camara* could be attributed to its antibacterial activity. According to Mary (2011), phytochemical analysis of the methanol and distilled water extracts revealed that leaves of *L. camara* contained; saponin, tannin, steroids and flavonoid; all are reported to possess antibacterial activity. In addition, the leaves are rich in essential oils and phenolic compounds like aesulin, quercetin, isorhamnetine, fisetine, gossypetine, tricine and aesculetine and triterpenoids (Sousa *et al.,* 2010). Moreover, phytochemical analysis of secondary metabolites in leaves of *L. camara* indicated the presence of flavanoids, triterpenoids and alkaloids (Lisa *et al,* 2010). The antibacterial potential of *L. camara* extracts found in this study is supported by different workers, who demonstrated against human pathogenic and phytopathogenic microorganisms (Sonibare and Effiong, 2008; Sharma *et al.*, 2009; Sobia *et al*., 2012).

The *in vitro* antibacterial activity assay of crude extracts of *M. diplotricha* revealed aqueous extract provided the highest antibacterial activity and created inhibition zone of (14 mm), followed by acetone extract of the plant which was produced inhibition zone of (7.81 mm), confirming that the bioactive compounds are better extracted with water than acetone. Antibacterial compounds present in leaves of *M. diplotricha* failed to solubilize in 70% methanol for which no inhibition zone was recorded. It also consistently reduced mean wilt incidence and disease severity index in tomato plants in all three time of applications where simultaneous application performed relatively better (Table 3). Similarly, Wiersum (1983) also reported that use of *M. diplotricha* as a cover crop, in tobacco plantations in Sumatra, reduced the incidence of slime disease, caused by *R. solanacearum*, to very low levels.

Among the five plant species evaluated for their antibacterial activity, *P. juliflora* was the only plant that showed antibacterial activity in all solvents used for extraction. The result of the greenhouse study revealed that methanol extract of *P. juliflora* was inconsistent in its antibacterial efficacy. Hence, the least reduction of mean wilt incidence, disease severity index and AUDPC were recorded in tomato plants drenched with methanol extract of *P. juliflora,* compared with other treatments in all application time. However, higher biomass increase in terms of plant height, AGFW and AGDW were exhibited by crude methanol extract of *P. juliflora* with varied results at different application time, compared to infected control.

The antimicrobial potential of extracts from *P. juliflora* has been evaluated by many researchers against different microorganisms and supported the finding of this study. *P. juliflora* leaf extracts contained alkaloids; juliflorine, juliflocine and benzene insoluble alkaloidal fraction, and all these alkaloids were found to possess significant antibacterial activity as reported by Ahmad (1991). Raghavendra *et al*. (2009), indicated that methanol extract of *P. juliflora* recorded highly significant antibacterial activity among different solvent extracts tested which supports our finding; phytochemicals present in leaf of *P. juliflora* is relatively more soluble in methanol than acetone and water. They also reported that aqueous, methanol and ethanol extracts of *P. juliflora* leaves showed significant antibacterial activity against all tested phytopathogenic bacteria.

The greenhouse experiment of the current study revealed that application of crude aqueous extracts of and methanol extract of selected IAS at different time of application significantly reduced bacterial wilt development and increased biomass on tomato plants, compared to infected control. However, different results were found based on plant species used and their application time. This variation in restricting disease development between IAS and their application time might be due to difference in efficacy as indicated *in vitro* assay and durability of extracts in the soil. This was supported by the work of Abo-Elyousr and Asran (2009), who reported that application of certain plant extracts to soil at a time of inoculation, two days before inoculation and two days after inoculation of the pathogen, significantly reduced disease severity of bacterial wilt on tomato, compared with untreated control. In addition, Hassan *et al.* (2009), also reported soil drenching of some aqueous plant extracts significantly reduced the disease severity of bacterial wilt, caused by *R. solanacearum*, on potato plants compared with inoculated control under both greenhouse and field conditions. Furthermore, Pradhanang *et al.* (2003), reported that thymol, palmarosa and lemongrass oil as soil fumigants suppressed bacterial wilt incidence of tomato caused by *R. solanacearum* in greenhouse pot experiments. Investigations on the mechanisms of disease suppression by plant products have suggested that the active principles present in plant extracts may either act on the pathogen directly or induce systemic resistance in host plants resulting in a reduction of disease development (Hassan *et al*., 2009).

The result of the study also revealed that some of tested botanicals increased plant height and gave statistically similar result as uninoculated, healthy plants, scored plant height of 56 cm. However, all botanical treated plants were scored lesser AGFW and AGDW compared to uninoculated control, healthy plants. This might imply that botanicals by themselves does not increase biomass (promote plant growth) rather they decrease the negative impact of the disease, stunting nature of the disease, which also affects plant height, fresh weight and dry weight of plants. However, significantly higher biomass (plant height, AGFW and AGDW) were recorded from plants treated with botanicals compared with untreated plants, infected control. This is in accordance with the study of Opara and Wokocha (2008), who reported that some aqueous plant extracts increased plant growth parameters including plant height at varying degrees over the infected control against *Xanthomonas campesrtis* Pv. *Vesicatoria* under field experiment. The results of the present study are in agreement with those reported by Abo-Elyousr and Asran (2009), which stated that treated tomato plant with different plant extracts at different application time exhibited lesser shoot fresh and dry weight than healthy plants, uninoculated control. On the other hand did not agree with those reported by El-Arigi *et al*. (2005), who reported that treated potato plant with different plant extracts provided higher shoot and root fresh weight, compared with uninoculated control.

As shown by results from greenhouse test, the number of culturable bacteria recovered from rhizosphere soil two months post inoculation were significantly lower in the rhizosphere soil drenched with botanicals. However, varied percent reduction of *R. solanacearum* population in the rhizosphere soil were recorded from soil drenched with aqueous extract of *E. crassipes, L. camara*, *M. diplotricha* and methanolic extract of *P. juliflora* after two months of pathogen inoculation (Table 7). The variation might be due to difference of the tested botanicals in their bacteriostatic and bactericidal effects on the cells of *R. solanacearum* which in-turn affects disease development on tomato plants. This is in line with Fikre *et al*. (2007_b), who reported that disease development in plants was closely related to population dynamics of *R. solanacearum* in the soil. Similar results were found by Pradhanang *et al.* (2003), and Paret *et al.* (2010), who reported that soil drenching with thymol, palmarosa and lemongrass oil suppressed *R. solanacearum* populations in soil and reduced bacterial wilt incidence at varying degrees in greenhouse pot experiments.

6. SUMMARY AND CONCLUSIONS

The use of plant products instead of synthetic pesticides in plant disease control is an acceptable option in sustainable agriculture. This is due to very high cost of synthetic pesticides as well as their toxic effect on the environment and human-beings, lack of knowledge about appropriate use, difficulty in understanding application doses and resistance development by phytopathogens. The efficacy of plant extracts in controlling plant pathogens has been widely reported but, only few attempts have been demonstrated against bacterial wilt of tomato. Hence, the present study was conducted to demonstrate the possible application of IAS extracts for the management of bacterial wilt of tomato, caused by *R. solanacearum*.

The results of present study showed that aqueous, methanol and acetone extracts of all the five plants (*E. crassipes, M. diplotricha, L.camara, P. juliflora* and *P. hysterophorous*) have varied antibacterial activities against the test pathogen, *R. solanacearum*. However, the level of inhibition was found to be reliant on plant species used, solvents used for extraction, concentration under *in vitro* test and their time of application under greenhouse pot experiment. Results support the fact that the antimicrobial activity of leaf extracts of plants and weeds depends on the phytochemicals present in it.

Consequently, among the plant extracts *E. crassipes M. diplotricha* and *L.camara* were proven to be effective in inhibiting growth of symptoms of bacterial wilt on tomato plants under *in vivo* test. With solvents used for extraction, except *P. juliflora* which was more effective in methanol than in water and acetone, aqueous crude extracts of all the tested plants showed antibacterial activity and inhibit *R. solanacearum* than respective methanol and acetone extracts. The potency of each extract differs from one another, suggesting that the toxicity of different extracts may be due to their solubility in extracting solvents and difference in constituent and amount of antimicrobial principle. The antibacterial effect of the extracts against *R. solanacearum* could be explained by disturbance of the permeability barrier of the living membrane structure.

All the selected plant extracts effectively inhibited the growth of the bacteria at 10, 15 and 20% concentration compared to control. However, except aqueous extract of *E. crassipes*, other tested botanicals failed to inhibit the growth of *R. solanacearum* at 5% concentration and the effectiveness of those botanicals increased as their concentration increased. Results from *in vivo* test also revealed that the inhibitory activities of those selected botanicals vary with application time. Among three times of application evaluated, all selected botanicals performed better when they were applied simultaneously with pathogen inoculation.

Overall, the results indicated that drenching topsoil with aqueous crude extracts of *E. crassipes*, *M. diplotricha*, *L. camara* and methanolic extract of *P. juliflora* at different application time have the potential to suppress the bacterial wilt incidence, severity, and *R. solanacearum* population density in soil. Similarly, drenching of botanicals in the soil provided biomass (fresh weight, dry weight and plant height), increase over the non treated control, with simultaneous application time being the most effective. It is interesting to notice that crude extracts of the tested IAS have potential and could be useful in managing bacterial wilt of tomato, caused by *R. solanacearum*. The aqueous crude extracts of *E. crassipes*, *M. diplotricha* and *L. camara* seems to be promising as they are effective, environmentally safe, economically feasible and gives new opportunities to improve control strategies based on locally available natural products against tomato bacterial wilt in organic and sustainable agriculture. *E.crassipes* was the most effective as an antibacterial agent than other tested botanicals.

Even though the present study of *in vitro* and *in vivo* antibacterial evaluation of IAS plants forms a primary platform for further phytochemical analysis, a better understanding of their mechanisms of action could contribute to an optimal utilization alone or in development of integrated disease management approach against bacterial wilt of tomato. Further research is necessary to determine the identity of the antibacterial compounds present in these IAS extracts. The effectiveness and their practical application of these IAS extracts also need to be studied further under open field conditions.

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8. APPENDICES

8.1. Appendix A. Analysis of Variance (ANOVA) Tables

Appendix Table 1. Summary of ANOVA results for interaction effects of five Invasive Alien Species extracts and three solvents used for extraction on their antibacterial activity against *Ralstonia solanacearum in vitro*

Source of variation	Degree of freedom	Sum square	Mean square	F- value	Pr > F
Treatment	4	179.226	44.806	1239.69	<0.0001
Method	3	1503.110	501.036	13862.50	<0.0001
Tret * Method	12	918.392	76.533	2117.48	<0.0001
Error	40	1.446	0.036		
Total	59	2602.175			
R-Square	MSD	CV (%)	Root MSE	DIZ mean (mm)	
0.999	0.588	3.25	0.19	5.85	

Appendix Table 2. Summary of ANOVA results for antibacterial activity of selected plant extracts against *Ralstonia solanacearum* at different concentrations

Source of variation	Degree of freedom	Sum square	Mean square	F- value	Pr > F
Treatment	3	549.244	183.081	854.02	<0.0001
Concentration	4	1814.431	453.607	2115.95	<0.0001
Tret * Conc	12	259.582	21.632	100.91	<0.0001
Error	40	8.575	0.214		
Total	59	2631.832			
R-Square	MSD	CV (%)	Root MSE	DIZ mean (mm)	
0.99	1.45	6.56	0.46	7.05	

Appendix Table 3. Summary of ANOVA results for the effects of selected plant extracts and their application time on the development (disease incidence) of bacterial wilt caused by *R. solanacearum* on tomato plants in greenhouse pot experiments, *in vivo* test

Source of variation	Degree of freedom	Sum square	Mean square	F- value	Pr > F
Treatment	4	14334.965	3583.741	57.17	<0.0001
Time	2	2193.404	1096.702	17.50	<0.0001
Rep	2	0.057	0.028	0.00	0.9995
Tret * Time	8	1233.217	154.152	2.46	0.0372
Error	28	1755.208	62.686		
Total	44	19516.854			
R-Square	MSD	CV (%)	Root MSE	DI mean (m)	
0.91	23.96	13.05	7.92	60.65	

Appendix Table 4. Summary of ANOVA results for selected plant extracts and their time of application on disease severity (percent severity index) of bacterial wilt of tomato in greenhouse pot experiments, *in vivo* test

Source of variation	Degree of freedom	Sum square	Mean square	F- value	Pr > F
Treatment	4	10808.58	2702.145	468.88	<0.0001
Time	2	259.108	129.554	22.48	<0.0001
Rep	2	13.281	6.640	1.15	0.3305
Tret * Time	8	163.931	20.491	3.56	0.0058
Error	28	161.363	5.763		
Total	44	11406.264			
R-Square	MSD	CV (%)	Root MSE	PSI mean (m)	
0.9858	7.26	7.20	2.40	33.34	

Appendix Table 5. Summary of ANOVA results for selected plant extracts and their time of application on incidence area under disease progress curve (iAUDPC) of bacterial wilt of tomato in greenhouse pot experiments, *in vivo* test

Source of variation	Degree of freedom	Sum square	Mean square	F- value	Pr > F
Treatment	4	47418280.0	11854570.0	108.19	<0.0001
Time	2	2089727.5	1044863.7	9.54	0.0007
Rep	2	265090.0	132545.0	1.21	0.3134
Tret * Time	8	1212260.0	151532.5	1.38	0.2467
Error	28	3068135.0	109576.25		
Total	44	54053492.5			
R-Square		CV (%)	Root MSE	iAUDPC mean (%-days)	
0.9432		14.91	331.023	2220.16	

Appendix Table 6. Summary of ANOVA results for selected plant extracts and their time of application on severity area under disease progress curve (sAUDPC) of bacterial wilt of tomato in greenhouse pot experiments, *in vivo* test

Source of variation	Degree of freedom	Sum square	Mean square	F- value	Pr > F
Treatment	4	17504014.52	4376003.63	235.12	<0.0001
Time	2	353236.96	176618.48	9.49	0.0007
Rep	2	74211.88	37105.94	1.99	0.1551
Tret * Time	8	186967.03	23370.88	1.26	0.3053
Error	28	521126.41	18611.66		
Total	44	18639556.81			
R-Square		CV (%)	Root MSE	sAUDPC mean (%-days)	
0.97		11.99	136.42	1137.28	

Appendix Table 7. Summary of ANOVA results for selected plant extracts and their time of application on plant height of tomato plants infected with *R. solanacearum* in greenhouse pot experiments, *in vivo* test

Source of variation	Degree of freedom	Sum square	Mean square	F- value	Pr > F
Treatment	5	6253.83	1250.76	267.23	<0.0001
Time	2	64.58	32.29	6.90	0.0031
Rep	2	24.19	12.09	2.58	0.0902
Tret * Time	10	106.08	10.61	2.27	0.0370
Error	34	159.14	4.68		
Total	53	6607.83			
R-Square	MSD	CV (%)	Root MSE	Plant height mean (cm)	
0.9759	6.65	4.82	2.16	44.88	

Appendix Table 8. Summary of ANOVA results for selected plant extracts and their time of application on above ground fresh weight (AGFW) of tomato plants infected with *R. solanacearum* in greenhouse pot experiments, *in vivo* test

Source of variation	Degree of freedom	Sum square	Mean square	F- value	Pr > F
Treatment	5	58895.07	11779.01	12746.60	<0.0001
Time	2	1083.86	541.93	586.45	<0.0001
Rep	2	4.88	2.44	2.64.00	0.0856
Tret * Time	10	987.75	98.77	106.89	<0.0001
Error	34	31.42	0.92		
Total	53	61003.00			
R-Square	MSD	CV (%)	Root MSE	Fresh weight mean (g)	
0.999	2.95	2.15	0.96	44.59	

Appendix Table 9. Summary of ANOVA results for selected plant extracts and their time of application on above ground dry weight (AGDW) of tomato plants infected with *R. solanacearum* in greenhouse pot experiments, *in vivo* test

Source of variation	Degree of freedom	Sum square	Mean square	F- value	Pr > F
Treatment	5	842.95	168.59	1819.64	<0.0001
Time	2	16.65	8.32	89.84	<0.0001
Rep	2	0.29	0.15	1.58	0.2210
Tret * Time	10	16.88	1.68	18.22	<0.0001
Error	34	3.15	0.09		
Total	53	879.93			
R-Square	MSD	CV (%)	Root MSE	Dry weight mean (g)	
0.99	0.94	3.53	0.30	8.63	

Appendix Table 10. Summary of ANOVA results for selected plant extracts and their time of application on *R. solanacearum* populations in rhizosphere soil of tomato plants infected with *R. solanacearum* in greenhouse pot experiments, *in vivo* test

Source of variation	Degree of freedom	Sum square	Mean square	F- value	Pr > F
Treatment	4	39.60	9.900	1968.28	<0.0001
Time	2	9.47	4.735	941.43	<0.0001
Rep	2	0.13	0.006	1.26	0.2986
Tret * Time	8	2.88	0.313	62.29	<0.0001
Error	28	0.14	0.005		
Total	44	51.73			
R-Square	MSD	CV (%)	Root MSE	Log 10(CFU+1)/g dry soil mean	
0.99	0.21	1.42	0.07	5.02	

8.2. Appendix B. *In vivo* Antibacterial Experiment Result Figures

E. crassipes (2- days after)	*E. crassipes* (at a time)	*E. crassipes* (2-days before)	Positive control (Infected)	Negative control (Healthy)

Appendix fig. 1. Effects of aqueous crude leaf extract of *E. crassipes* and its time of application on disease development of bacterial wilt, caused by *R. solanacearum,* on tomato plants in greenhouse pot experiments, *in vivo* test.

M. diplotricha (After 2-days)	*M. diplotricha* (at a time)	*M. diplotricha* (Before 2-days)	Positive control (Infected)	Negative control (Healthy)

Appendix fig. 2. Effects of aqueous crude leaf extract of *M. diplotricha* and its time of application on disease development of bacterial wilt, caused by *R. solanacearum*, on tomato plants in greenhouse pot experiments, *in vivo* test.

L. camara (After 2-days)	*L. camara* (At a time)	*L. camara* (Before 2-days)	Positive control (Infected)	Negative control (Healthy)

Appendix fig. 3. Effects of aqueous crude leaf extract of *L. camara* and its time of application on disease development of bacterial wilt, caused by *R. solanacearum,* on tomato plants in greenhouse pot experiments, *in vivo* test.

P. juliflora (After 2-days)	*P. juliflora* (At a time)	*P. juliflora* Before 2-days)	Positive control (Infected)	Negative control (Healthy)

Appendix fig. 4. Effects of methanol crude leaf extract of *P. juliflora* and its time of application on disease development of bacterial wilt, caused by *R. solanacearum,* on tomato plants in greenhouse pot experiments, *in vivo* test.