MANAGEMENT OF BACTERIAL WILT (*Ralstonia solanacearum)* OF POTATO (*Solanum tuberosum*) USING PLANT EXTRACTS AND HOST PLANT RESISTANCE

GIDRAF ONDURU OKEYO

A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Doctor of Philosophy Degree in Crop Protection of Egerton University

EGERTON UNIVERSITY

AUGUST, 2024

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this university or any other for the award of a degree.

Signature:…… ……  …….. Date:……02/08/2024.....

Gidraf Onduru Okeyo

KD122/15514/19

Recommendation

This thesis has been submitted with our approval as university supervisors.

Signature:…  … Date:……5th Ausgust 2024……

Dr. Miriam K. Charimbu, PhD

Department of Crops, Horticulture and Soils

Egerton University.

Signature:…  ……..…….. Date: ……3rd August 2024……

Dr. Jane G. Nyaanga, PhD

Department of Crops, Horticulture and Soils

Egerton University.

Signature:……  …… Date:……02.08.2024…….

Dr. Thiago Mendes, PhD

Genetics, Genomics and Crop Improvement Science Division

International Potato Center (CIP).

DEDICATION

I dedicate this PhD research to my lovely wife Mrs. Lilian Akinyi and my children Fidel, Wiladson, and Tabitha for their moral support, spiritual encouragement, and patience during the study period. I also dedicate this work to the entire Orwa family led by Rev. Zakayo Okeyo Orwa and Mrs. Elsa Ouma and all friends for their immense prayers, love, and motivation to ensure I got the merited academic qualification. The inspirational words "*Nyasaye ongeyo*" ("God knows") from Rev. Zakayo Okeyo Orwa acted as a reference phrase whenever I faced difficulties during the research period.

ACKNOWLEDGEMENTS

I want to thank the almighty God for His immense protection and guidance through this bristly path of academic achievement. Special thanks to the Center of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM) and the Department of Crops, Horticulture and Soil Sciences, Egerton University for the PhD scholarship and admission to pursue my studies. Further acknowledgements are addressed to The University of Nairobi (UoN) for funding part of the research through The Kenya Climate Smart Agriculture Project (KCSAP) and International Livestock Research Institute (ILRI) for permission to use their laboratory. I also acknowledge Agricultural Development Corporation (ADC) Molo and International Potato Centre (CIP) for provision of study materials (potato genotypes). My supervisors, Dr. Miriam K. Charimbu, Dr. Jane G. Nyaanga, and Dr. Thiago Mendes remains this work's linchpin. Much appreciation goes to Prof. Ogweno J. Otieno, Prof. George O. Abong, Prof. John N. Huria, Dr. Shadrack O. Nyawade, Dr. Oliver O. Okumu, Dr. Lilian Waiboci and Dr. Joshua O. Owade for their unconditional mentorship. I am also indebted to Mr. Fredrick Nganga, ILRI, Mr. Kiambi Mike, ADC, Mr. Titus N. Mwangangi, UoN, and Mr. Herman Machavure, CIP for their technical guidance.

ABSTRACT

Potato (*Solanum tuberosum* L.) is ranked second after maize among Kenya's most grown food crops. However, its production is constrained by bacterial wilt (*Ralstonia solanacearum*) disease. The objectives of this study were: to evaluate different potato genotypes for resistance to bacterial wilt in the field; to determine *in-vitro* antibacterial activity of different plant extracts against *R. solanacearum* of potatoes; to elucidate the bioactive compounds effective in managing *R. solanacearum* and to determine the synergistic effect of plant extracts and host plant resistance in the management of bacterial wilt (*Ralstonia solanacearum*) of potatoes under greenhouse conditions. Thirty potato genotypes were screened for resistance to *R. solanacearum* in the field at Turi, Nakuru County and Kiandu, Nyeri County using an alpha lattice design with 3 replicates for 2 seasons. *In-vitro* screening of antibacterial activity of plant extracts against *R. solanacearum* was conducted using disk diffusion technique and the experiment was arranged in a Completely Randomized Design (CRD). The bioactive compounds from the most effective plant extracts were identified by gas chromatography-mass spectrometry (GC-MS). Greenhouse experiment was arranged in a CRD of 2*6 factorial arrangements with 3 replicates. Data was collected on disease incidence, disease severity, plant growth and yield parameters, colony counts and growth inhibition zones. Data were subjected to analysis of variance (ANOVA) at $p<0.05$ using R software, version 4.3.1 and the treatment means separated using Tukey's Honestly Significant Difference (HSD). Field screening results ranked genotypes CIP 515004.535 and CIP 515008.561 as highly resistant and Shangi, Asante, Tigoni, Arka and CIP 515008.535 as highly susceptible. *In-vitro* results revealed that *Pelargonium zonale* leaves extract as the most effective extract with growth inhibition zone of 18.73mm followed by *Psisdium guajava* with growth inhibition zone of 14.27mm. Both *P. zonale* and *P. guajava* had 7 similar antibacterial compounds; *Fumaric acid, Pyrogallol, 4-Hydroxybenzoic acid, Shikimic acid, Protocatechuic acid, 3, 4, 5-Trihydroxybenzoic acid ethyl ester* but *P. zonale* had *Lactic acid* as an additional compound. Sherekea exhibited the highest average efficacy from the two plant extracts; 0.14 for disease incidence, 1178.21 for AUDPC, 1.84 for stem height, 2.66 for bacterial wilt colony counts and 0.28 for yield (Kgs) respectively from the greenhouse experiment. These results revealed effective synergistic effect of botanicals and host plant resistance in management of potato bacterial wilt and hence should be promoted as integrated disease management option.

TABLE OF CONTENT

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ADC	Agricultural Development Corporation
AEZ	Agro-Ecological Zones
AMDIS	Automated mass spectral deconvolution and identification system software
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AUDPC	Area under disease progress curve
BCAs	Biological Control Agents
BDB	Blood disease bacterium
BW	Bacterial Wilt
BWI	Bacterial Wilt Incidence
CABI	Center for Agriculture and Biosciences International
CFDA-SE	Carboxy-fluorescein Diacetate Succinimidyl Ester
CFUs	Colony Forming Units
CIP	International potato center
CPG	Casamino acid-Peptone-Glucose
CRD	Complete Randomized Design
CSA	Climate smart-agriculture
DAP	Days after planting
DI	Disease incidence
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FWI	Final wilt incidence
GC-MS	gas chromatography-mass spectrometry
GSH	Glutathione
HSD	Honestly Significance Difference
ILRI	International Livestock Research Institute
KOH	Potassium hydroxide
MIC	Minimum Inhibitory Concentration

MLSA	Multilocus sequence analysis
NAST	Number of asymptomatic tubers
NCM	Nitrocellulose Membrane
OD	Optical density
PE	Plant emergence
PCR	Polymerase Chain Reaction
PRNVBC	Percent reduction in number of bacterial colonies
PST	Proportion of symptomatic tubers
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT	Retention time
SH	Stem height
TZC	Triphenyl Tetrazolium Chloride
USD	United States Dollar

CHAPTER ONE
INTRODUCTION

1.1 Background information

Potato (*Solanum tuberosum L.)* is the second most grown staple food crop in Kenya after maize (Bornventure *et al.,* 2017). The crop plays an important role in food security and poverty alleviation through creation of employment and income generation to all players along its value chain (Mwakidoshi *et al.,* 2021). In Kenya potato is grown by over 800,000 farmers either as a cash or food crop and employs over 2.5 million people who work as market agents, transporters and processors (Muthoni *et al*., 2014). The crop is mainly grown in the high-altitude areas 1,500-3,000masl (meters above sea level) which include: the slopes of Mt. Kenya, such as Meru, Embu and Kirinyaga and parts of Laikipia, Aberdare ranges that covers parts of Nyeri, Muranga, Kiambu and Nyandarua, Mau Escarpment (Mau, Narok and Molo), Tinderet, Nandi Escarpment and Cherangani hills. Other areas such as Kericho, Kisii and Taita hills have also been reported as emerging potato growing areas (Janssens *et al*., 2013).

Potato production in Kenya has steadily increased due to expansion of arable lands but the yield and quality of the harvested tubers has remained low (Wang'ombe & van Dijk, 2013). The average potato yield in Kenya ranges from 8 – 15 tons/ha and this is 2 to 3 times lower than the achievable yields of 40 tons/ha (Mwakidoshi *et al.,* 2021). Contributing factors for low yield include, poor land preparation, planting and fertilizer application, insufficient control of pests and diseases, unfavorable climatic conditions during crop growth and post-harvest losses among others (Muthoni & Nyamongo, 2009).

Bacterial wilt caused by *Ralstonia solanacearum* is a major threat to crop production worldwide (Jiang *et al.,* 2017). Bacterial wilt pathogen consists of a species complex of four phylotypes; *R. solanacearum* (phylotype II), *Ralstonia pseudosolanacearum* sp. nov. (*R. solanacearum* (phylotypes I and III), and *Ralstonia syzygii subsp. indonesiensis* subsp. nov (*R. solanecearum* (phylotype IV) which causes vascular wilts to hundreds of plant species globally (Boschi *et al.,* 2017; Jiang *et al.,* 2017; Rahman *et al.,* 2010; Safni *et al.,* 2014). *Ralstonia pseudosolanacearum* sp. nov. (*R. solanacearum* (phylotypes I and III) are documented as the major *R. solanacearum* phylotypes affecting global potato production (Boschi *et al.,* 2017; Safni *et al.,* 2014).

The pathogen is estimated to affect over 1.7 million hectares of land under potato production globally in about 80 countries with yield losses approximated at USD 950 million per annum (Mwankemwa, 2015; Patil *et al.,* 2012). East African countries such Kenya and Uganda have reported yield losses ranging from 30-100% (Muthoni *et al.,* 2014). The continuous recycling of farm saved seed potato tubers has aggravated the yield losses in potato fields due to seed degeneration arising from seed borne pathogens mainly bacterial wilt caused by *Ralstonia solanacearum* (Thomas-Sharma *et al.,* 2016). Bacterial wilt pathogen lacks proper documented management strategy and causes yield losses ranging between 30-00% in potato fields. Crop rotation with non-host crops has been suggested to suppress spread of this pathogen but this has not been efficient since the pathogen survives in association with other weed hosts which offers an alternative source of nourishment in the absence of potatoes (Patil *et al.,* 2012).

Use of synthetic chemical compounds such as antibiotics (streptomycin, ampicillin, tetracycline and penicillin) and soil fumigants (Metham sodium), cultural management options (field sanitation, use of disease free planting materials, crop rotation among others and biological management options (plant extracts, antagonistic microbes and animal wastes) have been proposed for adoption and use by different farmers for management of bacterial wilt pathogen (Katafiire *et al.,* 2005; Sarkar & Chaudhuri, 2016; Whipps, 2001; Yuliar *et al.,* 2015). Likewise, use of resistant potato varieties as a management option for the control of bacterial wilt has been recommended because it is cheap and practical compared to other management options (Muthoni *et al.,* 2012). However, most of the proposed management options have displayed limited success. Some of the challenges experienced with these management options include high phytosanitary standards associated with chemical control, increased labor requirements associated with cultural management options, absence of commercialized biological control agents and lack of complete immunity to *R. solanacearum* from resistant varieties due to complex nature/genetic variability of *R. solanacearum* species (Patil *et al.,* 2012). Many laboratory experiments worldwide have screened thousands of species of higher plants both for pharmaceuticals and pest control products (Sarmah *et al.,* 2009). In many cases, these substances serve as plant defense mechanisms against attack and damage by microorganisms, insects, and herbivores. The current study sought to screen different potato varieties for resistance as well as determining the efficacy of different bioactive compounds in selected plants for the management of bacterial wilt pathogens on potatoes.

1.2 Statement of the problem

Potatoes are one of the major food and income generating crops cultivated by many small-scale farmers in Kenya. Its production is however faced with several challenges, key among them is bacterial wilt (*Ralstonia solanacearum)* disease. This is a soil borne pathogen that infects the host plant through wounds on the root hairs and below ground stem parts. The pathogen colonizes the vascular bundles and hinders transport of water and nutrients and hence wilting of the infected plant. Bacterial wilt pathogens can cause up to 30-100% yield loss in potato fields if left uncontrolled. It is mainly disseminated from one field to another through infested seeds sought from informal sources. Few management strategies with limited effectiveness have been documented. For instance, cultural methods such as crop rotations are largely impractical because the farms are too small to allow effective implementation. Furthermore, the pathogen has a wide host range, and it persists for a long period of time in the soil. Few chemicals have been registered in Kenya for managing bacterial wilt, but they are often ineffective due to resistance development by bacterial wilt pathogen coupled with high phytosanitary standards associated with their use and high retail costs. Use of resistant varieties and biological control agents have also been proposed by various scientists for its management. However, limited commercialization of biopesticides against *R. solanacearum* and lack of complete immunity to *R. solanacearum* expressed by resistant varieties due to genetic variability have affected their efficacy against *R. solanacearum* pathogen. Thus, there is need for advanced research on eco-friendly management options such as host plant resistance and botanicals for the management of bacterial wilt pathogen on potatoes.

1.3 Objectives

1.3.1 Main objective

To contribute to increased food security using host plant resistance and plant extracts in the management of bacterial wilt (R*alstonia solanacearum)* of potato in Kenya.

1.3.2 Specific objectives

i. To screen for resistance levels of different potato genotypes against R*alstonia solanacearum* in the field.
ii. To determine the antibacterial activity of different plant extracts against *Ralstonia solanacearum* of potatoes in-vitro.
iii. To elucidate the bioactive compounds against *Ralstonia solanacearum* in selected plant extracts.

iv. To determine the synergistic effect of plant extracts and host plant resistance in the management of bacterial wilt (*Ralstonia solanacearum*) of potatoes under greenhouse conditions.

1.4 Hypotheses

i. There are no significant differences in potato genotypes resistance to R*alstonia solanacearum.*

ii. Different plant extracts have no antibacterial activity against *Ralstonia solanacearum* of potatoes in-vitro.

iii. Selected plant extracts against *Ralstonia solanacearum* have dissimilar bioactive compounds.

iv. There is no synergistic effect between plant extracts and host plant resistance in the management of bacterial wilt (*Ralstonia solanacearum*) of potatoes under greenhouse conditions.

1.5 Justification

Potato production in Kenya has experienced tremendous growth over the past one decade with an increase in acreage (Wang'ombe & van Dijk, 2013). This growth is attributed to its importance as source of starch and staple food crop to meet the increased demand of growing Kenyan population (Birch *et al.,* 2012). The high demand for potatoes is further attributed to increased urbanization and fast-food industries. Potatoes provide more calories, vitamins, and nutrients per area of land sown than other staple crops making it the second most important food crop after maize. It is also a valuable cash earner to many smallholders in areas where traditional cash crops like coffee and tea are either scarce or absent. The potato sector therefore offers job opportunities and contributes to food security and poverty alleviation which are key elements towards the achievement of Sustainable Developments Goal (SDGs) 1 and 2.

However, the sector has faced various production constraints such as low rainfall, lack of clean seeds and crop diseases among others. Among the diseases, field infestation by bacterial wilt (*Ralstonia solanacearum)* pathogen has led to low yields and poor-quality tubers. Yield losses from *R. solanacearum* are estimated at 30-100% in the absence of proper management strategies (Abdurahman *et al.,* 2019). There is an urgent need to address the yield loss arising from *R. solanacearum* since it would be a serious threat to potato production and hence affecting food security and livelihoods of different actors in the potato value chain.

Development of resistant cultivars remains the best option for management of bacterial wilt pathogen. Breeding programs to develop resistant genotypes were initiated in many parts of the world, but acceptable cultivars with good resistance to bacterial wilt are yet to be identified in Kenya. This is due to absence of high-level gene-for-gene resistance traits encoded by single dominant genes since the available sources of resistance are usually polygenic hence difficult to transfer all the identified quantitative trait loci (QTL) into desirable cultivars (Muthoni *et al.*, 2020). According to potato variety catalogue 2019, more than 60 potato varieties have been registered and released in Kenya for commercialization but none of them is reported to be resistant to bacterial wilt pathogen. In addition, the resistance has been shown to be very unstable due to its strong host-pathogen-environment interaction (Muthoni *et al.*, 2020). Screening potato genotypes for resistance under local environmental conditions is the first important step for effective resistance breeding and provides a safe and easily adapted management strategy against bacterial wilt pathogen of potatoes leading to increased yields. Recent research has also focused on natural plant products (biopesticides) as alternatives for plant disease management in developing countries such as Kenya. These products are cost-effective, easy to prepare and safe for the environment. There is however a need for identification of bioactive compounds and evaluating the efficacy of candidate plant extracts for management of bacterial wilt pathogen in potato fields. The findings from this study will provide alternative integrated bacterial wilt management options among potato farmers in Kenya as well as around the globe and hence increased potato yields and income generation to stakeholders along its value chain.

1.6 Definition of terms

Bioactive compound: A type of chemical found in small amounts in plants or certain foods which has positive or negative effects on living organisms, tissues or cells.

Biovar: Refers to a variant prokaryotic strain that differs physiologically or biochemically from other strains in a particular species.

Botanical: A natural substance obtained from plants (plant parts or extracts) and is used typically in medicinal or cosmetic products.

Plant disease epidemiology: Refers to the study of the outbreak of disease, its course, intensity, cause and effects and the various factors governing it.

Plant disease incidence: Refers to the number of plant units or proportion of plants that are visibly diseased in relation to total number of plants assessed.

Plant disease severity: Refers to the area of the sampling unit (plant surface) affected by disease expressed as a proportion of the total area.

Host range: Refers to the number of host species (plant species) which can be infected by a specific pathogen.

Virulence: The ability of an organism to infect the host and cause a disease.

CHAPTER TWO
LITERATURE REVIEW

2.1 Taxonomy and origin of potato

Cultivated potato (*Solanum tuberosum* L.) and its wild relatives belong to the *Solanum* sect. *Petota* (Solanaceae), consisting of approximately 110 *Solanum* species (Naeem *et al.,* 2023). Intensive taxonomic work by various taxonomists since potato domestication, grouped potato into 494 epithets for wild taxa and 626 epithets for cultivated taxa respectively. However, recent classifications recognize approximately 100 wild species, and four cultivated species partitioned into four clades (Ovchinnikova *et al.,* 2011; Spooner *et al.,* 2016). The four cultivated species comprise of *S. ajanhuiri*, *S. curtilobum*, *S. juzepczukii* and *S. tuberosum* which is further sub-divided into the Andigenum and Chilotanum cultivar groups (Rodríguez *et al.,* 2010). The taxonomic classifications were guided by different taxonomic philosophies from different taxonomists and species concepts (Spooner *et al.,* 2016). The taxonomists also integrated the multiple evidence and phylogenetic relationships between taxa to provide framework for further investigation of complex groups and rare endemic species (Peralta *et al.,* 2021).

The origin of potato crop and its wild relatives is primarily centred around Western South America (de Haan & Rodriguez, 2016; Soare & Chiurciu, 2021). According to Sukhotu and Hosaka (2006), the crop is assumed to have originated from domestication of indigenous potato cultivars in South America, the highlands of Andes and lowlands of south-central Chile. Its domestication resulted in worldwide spread and the crop was first reported in Europe at around16th century before spreading to all parts of the world (de Haan & Rodriguez, 2016). In Africa potato crop was first domesticated ant around 1830 in in South Africa and later in east Africa at around 1880 by British and German colonialists (Black, 2008).

2.2 Potato production in Kenya

Globally, potato is widely grown because of its tubers which are used as food by various stakeholders along its value chain and as raw material for various industries (Soare & Chiurciu, 2021). The crop is used as vegetable and/or for manufacturing of alcoholic beverages, starch and processed products like French fries in industries (Sandilya *et al.,* 2023). The potato crop is an essential source of energy, protein, and micronutrients like iron and zinc as well as key nutrients to the diet including vitamin C, potassium, and dietary fibre. The crop is also a major source of

vitamins B1, B3, and B6 and minerals such as iron, potassium, phosphorus, and magnesium and contains folate, pantothenic acid, and riboflavin (Devaux *et al.*, 2021; Khalid *et al.*, 2020).

Potato crops are currently grown on approximately 20 million hectares of land worldwide yielding about 366 million tons annually. The highest production zones are concentrated in the temperate region of the northern hemisphere where the crop is mainly grown as a cash crop (Devaux *et al.*, 2021). In the tropical region, the crop is produced both for staple food and as a cash crop in the highlands of the Andes, the African highlands and the Rift valley, and the volcanic mountains of West Africa and Southeast Asia (Muthoni *et al.*, 2010). The crop is also grown as a winter crop in the subtropics of the Mediterranean region, North India, and southern China (Devaux *et al.*, 2021). In terms of consumption, potato is ranked as the third most important food crop after wheat and rice with countries like China, India and Russia as the leading producers (Devaux *et al.*, 2021; Gelaye, 2023; Soare & Chiurciu, 2021).

Potato was introduced in Kenya in the late 19th century by the British East African Trading Company who restricted its production by then to the white settlers in the white highlands (Durr & Lorenzl 1980). Today potato crop is ranked second after maize as the most important staple food crop in Kenya where it is grown by more than 800,000 farmers and this has been aided by its ability to thrive well in high altitude areas (1,500-3,000 m above sea level) compared to maize (Janssens *et al*., 2013; Onditi *et al*., 2012). The crop is grown along the slopes of Mt. Kenya (Meru, Embu, Kiringa and parts of Laikipia, Aberdare ranges; Nyeri, Muranga, Kiamba and Nyandarua), the highlands on Mau Escarpment (Mau, Narok and Molo), Tinderet, Nandi Escarpment, Cherangani hills, Kericho, Kisii and Taita hills (Janssens *et al*., 2013). Its increased production has ranked Kenya as the fifth biggest potato producing country in sub-Saharan Africa (Muthoni & Nyamongo, 2009).

2.3 Production constraints

Despite the steady increase of potato production in Kenya due to expansion of arable lands, the sector has poor yields ranging from 8 – 15 tons/ha which are 2 to 3 times lower than the achievable yields of 40 tons/ha (Mwakidoshi *et al.*, 2021; Wang'ombe & van Dijk, 2013). These low yields are attributed to poor land preparation, lack of certified seeds, poor planting and fertilizer application, insufficient control of pests and diseases mainly bacterial wilt disease, unfavorable climatic conditions during crop growth and substantial harvest and post-harvest losses among others (Kyamanywa *et al*., 2011; Muthoni & Nyamongo, 2009). Among pests and diseases,

bacterial wilt (*Ralstonia solanacearum*) is the key constraint to potato production in Kenya contributing to reduced potato yields (Muthoni *et al.*, 2014).

Crop rotation is a major component for increased potato production since it optimizes energy use efficiency (EUE) through enhancement of optimal fertility and chemical use as well as reduction of greenhouse gas (GHG) emissions (Khakbazan *et al.,* 2019; Mohr *et al.,* 2011). Similarly, crop rotation is essential for the management of soilborne pathogens such as bacterial wilt, Fusarium wilt and potato cyst nematodes among others (Kakuhenzire *et al.,* 2013). However, since most potato farmers in sub-Saharan Africa (SSA) are smallholder farmers with small farm sizes which cannot accommodate crop rotation, they have abandoned crop rotation as a practice and this has led to buildup of soilborne diseases hence low yields in various potato fields (Mohr *et al.,* 2011). Most farmers use farmer saved seeds in potato production which has resulted in spreads of pests and diseases in potato fields. This is attributed to limited certified seed supply and the high costs of available certified seeds. For instance, Kenya has only 30 registered seed merchants who supply less than 5% of the seeds used by potato farmers (Atieno *et al.,* 2023).

Potato crops require high amounts of nitrogen, phosphorus and potassium elements during plant growth (Koch *et al.,* 2020). Nitrogen is essential for protein synthesis and tuber growth, Phosphorus promotes root growth, rapid tuber formation and starch synthesis while potassium is crucial for translocation of sugars to the tubers and starch synthesis for increased tuber growth and filling (Innocent *et al.,* 2021). However, overreliance on inorganic fertilizers can cause adverse effects on physicochemical and biological properties of the soil as well as nutritional imbalance (Gelaye, 2023; Innocent *et al.,* 2021). Thus, integrated nutrient management which combines both organic and inorganic fertilizers is crucial for optimal potato production since organic fertilizers has the capacity to improve soil fertility and structure for the flourishing of soil fauna and flora (Alemayehu *et al.,* 2020; Gelaye, 2023; Sofo *et al.*, 2022).

Climate variability is a major factor affecting global production of major crops such as maize, wheat, cassava, potato, and beans (Waaswa *et al.,* 2022). This is because climate change affects atmospheric temperatures, CO_2 concentration in the atmosphere, rainfall distribution, ultraviolet radiation and pest population (insect pests, disease causing pathogens and weeds) which are the major factors affecting optimal crop production (Hijmans, 2003; Quiroz *et al.,* 2018). However, these effects vary across regions and the crop response to these factors is also region dependent (Quiroz *et al.,* 2018). Globally, potato production has recorded up to 32% yield

reduction under non climate change adaptation and mitigation measures. The yield decrease arises from droughts, floods, high temperatures and irregular rainfall patterns (Haverkort *et al.,* 2013; Hijmans, 2003; Waaswa *et al.,* 2022). Thus, there is need for adoption of global climate smart-agriculture (CSA) practices such as irrigation, deep-ploughing, crop rotation, mixed cropping, terracing, mulching, zero or minimum tillage, cover crops and improved varieties aimed at mitigation and adaptation to climate variability (Waaswa *et al.,* 2022). These CSA practices enhance increased potato productivity through reduction of greenhouse gas emissions and improved resilience (Caitlin, 2014; Khatri-Chhetri *et al.,* 2017; Mereu *et al.,* 2018; Parker *et al.,* 2019).

Fields infestation by various insect species have affected potato production globally resulting in about 16% yield losses (Kroschel *et al.,* 2020). However, under uncontrolled conditions, this has sometimes escalated to approximately 30-70% yield loss (reduced tuber quality and yield) depending on the insect species (Kroschel & Schaub, 2013; Mujica & Kroschel, 2013). About 49 insect species are documented to affect potato production globally (Kroschel *et al.,* 2020). These include Colorado potato beetle [*Leptinotarsa decemlineata* (Say) (Chrysomelidae)], aphid [*Aphis spp.* (Aphididae)] potato tuber moth [*Phthorimaea operculella* (Zeller) (Gelechiidae)]; pea leaf miner [*Liriomyza huidobrensis* (Blanchard) (Agromyzidae)], Andean potato weevils [*Premnotrypes spp.* (Curculionidae)] and potato leafhopper [*Empoasca fabae* (Harris) (Cicadellidae)] among others (Radcliffe & Lagnaoui, 2007). These insect pests can either cause direct damage to infested potato plants through their feeding effect and/or indirectly through transmission of diseases such as potato viruses. Insect pest infestation in potato fields have resulted in excessive pesticide use posing human and environmental health concerns and this has called for development and implementation of integrated pest management (IPM) approaches (Kroschel *et al.,* 2020).

Potato production is also affected by disease pathogens such as fungi (late blight caused by *Phytophthora infestans*, Early blight caused by *Alternaria solani*, potato wart caused by *Synchytrium endobioticum* and tuber powdery scab caused by *Spongospora subterranea*) (Adolf *et al.,* 2020) viruses (*Potato virus Y* (PVY), *Potato virus A* (PVA), *Potato virus V* (PVV), *Potato virus X* (PVX), *Potato virus S* (PVS) and *Potato leaf roll virus* (PLRV) among others) (Kreuze *et al.,* 2020), bacteria (*Clavibacter spp., Ralstonia spp., Pectobacterium spp., Dickeya spp., Streptomyces spp.,* and *Liberibacter spp.*) (Charkowski *et al.,* 2020) and nematodes (Root-lesion nematodes (RLN) caused by *Pratylenchus* spp., potato cyst nematodes (PCN) caused

by *Globodera* spp., and root-knot nematodes (RKN) caused by *Meloidogyne* spp.(Esteves *et al.,* 2015). Among these disease-causing pathogens, late blight (*P. infestans*) and bacterial wilt (*Ralstonia solanacearum*) are the main diseases affecting potato production globally (Boschi *et al.,* 2017; Lal *et al.,* 2016). Late bight is a heterothallic fungal pathogen which undergoes both sexual and asexual reproduction (Demissie, 2019). The pathogen has two mating types (A1 and A2) (Njoroge *et al.,* 2019; Pule *et al.,* 2013; Were *et al.,* 2013). For sexual reproduction to occur both A1 and A2 must come into contact and successful sexual cycle might result in intolerable epidemics (Njoroge *et al.,* 2019). If left uncontrolled, late blight pathogen can cause up to 100% yield losses in potato fields (Demissie, 2019; Muchiri *et al.,* 2009; Nyankanga *et al.,* 2004). Thus, there is need for development and implementation of Integrated Disease Management (IDM) strategy which incorporates cultural management practices, use of resistant cultivars and chemical sprays with the aim of reduced yield losses in potato fields (Demissie, 2019; Namanda *et al.,* 2004; Nyankanga *et al.,* 2004).

2.4 Bacterial wilt

Bacterial wilt of potatoes is caused by *Ralstonia solanacearum* from the non-fluorescent pseudomonads rRNA homology group II (García *et al.,* 2019). *R. solanacearum* is a rod-shaped gram-negative bacterium of approximately 0.5-1.5 µm in length and has a single polar flagellum used in locomotion (CABI, 2020). The pathogen was first described by Erwin F. Smith who named its causal agent *Bacillus solanacearum* (Smith, 1896) and later changed the name to *Pseudomonas solanacearum* (Smith, 1911). With the discovery of the new genus called Burkholderia by Yabuuchi *et al.* (1992), all the pathogens in the non-fluorescent pseudomonads were transferred to the new genus. Further studies by Yabuuchi *et al.* (1995) concluded that *Burkholderia solanacearum* was distinct from other members within the genus Burkholderia and proposed a new genus Ralstonia and hence the name *Ralstonia solanacearum.*

Studies by Wicker *et al.,* (2012) further subdivided the *R. solanacearum species* complex into eight clades based on multilocus sequence analysis (MLSA). Phylotypes I and III were each contained in a single clade (1 and 6, respectively), phylotype II consisted of four separate clades (2, 3, 4 and 5) while Phylotype IV was constituted of two clades (7 and 8). Clade 7 was composed of blood disease bacterium (BDB) and *R. solanacearum* phylotype IV strains while clade 8 was composed of *R. syzygii* strains and *R. solanacearum* phylotype IV strains isolated from clove trees. Phylotype IV was reported to be the most divergent and ancestral phylotype, while phylotypes I,

II and III exhibited ongoing diversification between them (Wicker *et al.,* 2012). Similarly, results from the MLSA study indicated that phylotypes I and III are more closely related compared to phylotypes II and IV, and the dendrograms also documented the close relationship between the phylotype IV strains. It was later discovered that *R. solanacearum* is a heterogeneous species of plant pathogenic bacteria causing vascular wilt to more than 450 plant species from ≥ 50 families globally (CABI, 2020; Milijasevic-Marcic *et al.,* 2015; Safni *et al.,* 2014). Within its species complex, *R. solanacearum* (phylotype II), *R. pseudosolanacearum* sp. nov. (*R. solanacearum* (phylotypes I and III), and *R. syzygii subsp. indonesiensis* subsp. nov (*R. solanecearum* (phylotype IV) is known to affect global production of solanaceous crops mainly in the tropical and temperate regions (Boschi *et al.,* 2017; Rahman *et al.,* 2010; Safni *et al.,* 2014).

2.4.1 Geographic distribution and status of bacterial wilt of potatoes in Kenya

Studies by Safni *et al.* (2014) reclassified *R. solanacearum* into three distinctive species depending on origin; *R. solanacearum* (Phylotype II originating from South America), *R. pseudosolanacearum* (Phylotype I and III originating from Asia and Africa respectively) and R. syzygii (Phylotype IV originating from Indonesia). Geographic distribution of *R. solanacearum* pathogen is affected by several factors, mainly host prevalence and conduciveness of climatic conditions (Karim & Hossain, 2018). Due to its wide host range (more than 450 plant species from ≥ 50 families), the pathogen has a worldwide distribution mainly in the tropical, sub-tropical and warm temperate areas (CABI, 2020; EFSA *et al.,* 2019; EPPO, 2023; Milijasevic-Marcic *et al.,* 2015; Safni *et al.,* 2014) (Plate 2.1).

In Asia, the bacterial wilt pathogen is present in Bhutan, Brunei, China, India, Indonesia, Iran (widespread), Japan, North and South Korea, Lao, Malaysia, Myanmar, Nepal (localized), Pakistan, Philippines, Saudi Arabia, Singapore, Sri Lanka, Taiwan, Thailand, Turkey (few occurrences) and Vietnam (CABI, 2020; EFSA *et al.,* 2019; EPPO, 2023). In Africa, the pathogen is present in Angola, Benin (wide spread), Burkina Faso, Burundi, Cameroon (localized), Congo, Congo Democratic Republic, Cote d'Ivoire, Egypt, Eswatini, Ethiopia (wide spread), Gambia, Ghana, Guinea, Kenya (wide spread), Lesotho, Libya, Madagascar (wide spread), Malawi, Mali, Mauritius, Niger (wide spread), Nigeria, Reunion, Rwanda (wide spread), Senegal, Sierra Leone, Somalia, South Africa, Tanzania, Togo, Uganda (wide spread), Zambia and Zimbabwe (CABI, 2020). In Europe the pathogen is present in Moldova and Serbia; present in few occurrences Belgium, Bulgaria, France, Germany, Hungary, Italy, Poland, Romania, Russia and Spain;

transient under eradication in Czechia (transient under eradication), Greece (transient under eradication), Ukraine (transient under eradication) and United Kingdom (transient under eradication) and localized in Netherlands (CABI, 2020; EFSA *et al.,* 2019; EPPO, 2023).

In North and Central America, the pathogen is present in Belize (widespread), Costa Rica, Cuba, Dominican Republic, El Salvador, Grenada, Guadeloupe, Guatemala, Honduras, Jamaica, Martinique (widespread), Mexico, Nicaragua, Panama, Saint Vincent and the Grenadines, Trinidad and Tobago (widespread) and the USA (widespread) (CABI, 2020; EFSA *et al.,* 2019; EPPO, 2023). In South America, the pathogen is present in Argentina, Bolivia (widespread), Brazil, Chile, Colombia, Ecuador, French Guiana, Guyana (widespread), Paraguay (localized), Peru, Suriname, Uruguay (widespread) and Venezuela (CABI, 2020; EFSA *et al.,* 2019; EPPO, 2023). In Oceania, the pest is present in Australia, Cook Islands, Federated States of Micronesia, Fiji, French Polynesia, Guam, Micronesia, New Caledonia (localized), New Zealand, Papua New Guinea, Samoa, Tonga and Vanuatu (CABI, 2020; EFSA *et al.,* 2019; EPPO, 2023).

Bacterial wilt disease of potatoes was first reported in Kenya at around 1940s in Embu currently known as Embu County (Otipa *et al.,* 2003; Robinson & Ramos, 1964). The pathogen is believed to have been introduced by latently infected seed potato tubers imported from Europe (Todd, 1969). Currently bacterial wilt pathogen has spread to all potato growing areas in Kenya affecting more than 70% of potato farms with yield losses ranging from 50-100% if left uncontrolled (Muthoni *et al.,* 2014). Since the discovery of this pathogen, it has exhibited an exponential rate of spread countrywide due to trade of infected and or latently infected seed potato tubers between potato farmers (Muthoni *et al.,* 2014; Robinson & Ramos, 1964).

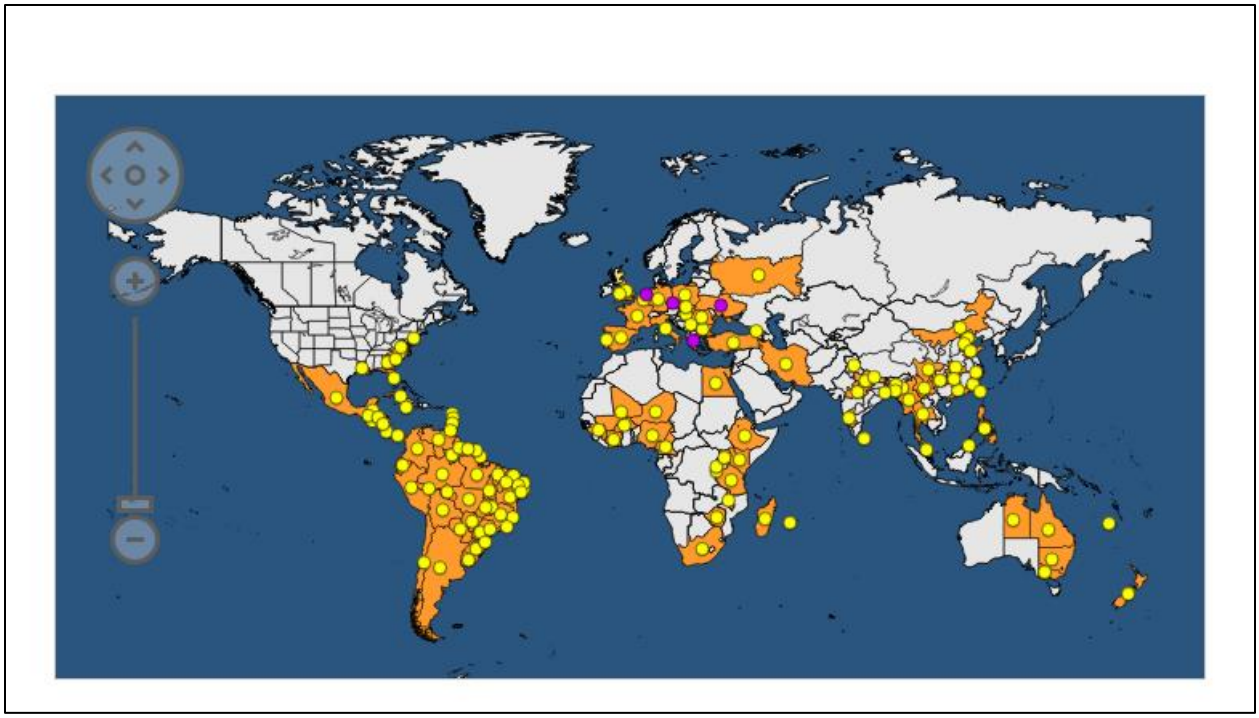


Plate 2. 1: Global distribution map for the *Ralstonia solanacearum* species complex.

Yellow and orange indicate reported presence and purple stands for reported transient under eradication presence.

Source: EPPO (2023).

2.4.2 Host range

Ralstonia solanacearum is known to have a wide host range apart from economically important crops such as potatoes, tomatoes, tobacco and bananas (Zinnat *et al.,* 2018). However, this varies with the pathogenic varieties (races) within *solacearum* species (CABI, 2020). More than 450 plant species from ≥ 50 families both in the tropics and sub-tropics are reported to be susceptible to either one or more races of *R. solanacearum* (CABI, 2020; EFSA *et al.*, 2019; EPPO, 2023; Milijasevic-Marcic *et al.*, 2015; Safni *et al.,* 2014). Based on varied host range, the pathogen was subdivided into five main races: race 1 which affects most of solanaceous plants and weeds, race 2 affects triploid bananas and heliconia, race 3 also known as potato race is restricted to potatoes, race 4 affects ginger and race 5 affects mulberry (He *et al.*, 1983; Sarkar & Chaudhuri, 2016). The most important hosts for *R. solanacearum* are potato (*Solanum tuberosum*), banana and plantain (*Musa* spp.*)*, tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), tobacco (*Nicotiana tabacum*), numerous ornamental plants and some weeds [bittersweet nightshade (*Solanum dulcamara*), black nightshade (*Solanum nigrum*), gallant soldier (*Galinsoga*

parviflora), Shaggy soldier (*Galinsoga ciliata*), pink knotweed (*Polygonum capitata*), Common Purslane (*Portulaca oleracea*), Narrawa burr (*Solanum cinereum*) and Common Nettle (*Urtica dioica*)](CABI, 2020; EPPO, 2023; Uwamahoro *et al.*, 2020). Some of the other reported susceptible crops include onion (Allium *cepa*), custard apple (*Annona reticulata*), straw berry (*Fragaria × ananassa*), radish (*Raphanus sativus*), cassava (*Manihot esculenta)*, and sweet potato (*Ipomoea batatas*), castor bean (*Ricinus communis*), groundnut (*Arachis hypogaea*), rubber plant (*Hevea brasiliensis*) and cotton (*Gossypium* spp.) among others (CABI, 2020; Zinnat *et al.*, 2018). Most of these host plants especially weeds exhibit latent infection symptoms which has made their identification difficult and hence aiding the pathogen survival during cultural control processes such as crop rotation, field sanitation and close season (Pradhanang *et al.*, 2000).

2.4.3 Economic importance of bacterial wilt pathogen

Bacterial wilt caused by *Ralstonia solanacearum* is a serious threat to potato production worldwide mainly in the tropical, subtropical and areas with warm temperate climates (Karim & Hossain, 2018). Economic losses from bacterial wilt pathogen are difficult to estimate since direct yield loss is dependent on myriads of factors such as host susceptibility, cultivar, climate, soil type, cropping practice and pathogen strain. Thus, crop damage extent is usually expressed on a crop-by-crop basis and can range from minimal crop loss to very high economic damage (Jiang *et al.*, 2017). The pathogen is estimated to affect over 1.7 million hectares of land under potato production globally with yield losses approximated at USD 950 million per annum (Mwankemwa, 2015). The most affected countries are Kenya, China, Uganda, Indonesia, Bangladesh, Bolivia, and Peru (Muthoni *et al.*, 2014). In Kenya, bacterial wilt disease has been reported to affect over 70% of potato farms leading to high yield losses ranging from 50 to 100% (Kaguongo *et al.*, 2010). Infection by bacterial wilt pathogen may result in wilting of the entire plant stand in the field, tuber rot in the store and or spread of the pathogen to clean fields through planting latently infected tubers (Mwankemwa, 2015).

2.4.4 Disease symptoms

Different potato varieties and cultivars respond differently to infection by bacterial wilt pathogens due to varied host-pathogen-environment interactions (Aliye *et al.*, 2015; Muthoni *et al.*, 2014). Bacterial wilt symptoms can occur on leaves, stems and tubers (Plate 2.2). Above ground symptoms include wilting, stunting and chlorosis (yellowing of foliage) (Priou *et al.*, 1999). Wilt symptoms first appear as wilted leaves towards the end of the branches, usually one

side of a leaflet or a single branch during the day but recover at night under cool temperatures (Champoiseau *et al.,* 2009). Under favorable environmental conditions which facilitate rapid disease development, the infected plant wilts quickly without yellowing symptoms (Priou *et al.,* 1999). As the disease progresses, the affected leaves develop a bronze tint and streaky brown discoloration may be observed on the stem slightly above the soil. In severe cases, epinasty may be observed on the affected petioles, the affected plant fails to recover and die. The affected vascular bundles exude a white, slimy mass of bacteria when broken or cut (CABI, 2020; Priou *et al.,* 1999).

Below ground symptoms are usually observed on the tubers and they may or may not be visible externally depending on the stage of development and severity of the disease (CABI, 2020; Priou *et al.,* 1999). Affected tubers exude bacterial ooze from the eyes and stem-end attachments. Soil masses adhere to the dry bacterial exudates leading to smutty appearance of the affected tubers (CABI, 2020; Priou *et al.,* 1999). When the affected tubers are cut, a brown discoloration of the vascular ring and the adjacent tissues which may extend to the pith and/or tuber cortex is observed. A white-milky bacterial ooze is usually noticed from the freshly cut section of the infected tubers while creamy fluid exudate usually appears spontaneously on the vascular ring of the cut surface (Champoiseau *et al.,* 2009; Priou *et al.,* 1999).

2.4.5 Pathogen detection and identification

2.4.5.1 Visual assessment and vascular flow test

Visual symptoms are the first diagnostic tool for bacterial wilt disease causing pathogens on potatoes (Mwankemwa, 2015). These include the above ground symptoms such as stunting, yellowing, wilted leaves tend to recover during the night, brown discoloration of stems slightly above soil level and permanent wilting of the infected plants (*Aslam et al.,* 2017; CABI, 2020; Champoiseau *et al.,* 2009; Priou *et al.,* 1999). Below ground symptoms include tubers exuding bacterial ooze from the eyes and stem-end attachments and soil masses adhering to the dry bacterial exudates forming smutty appearance (CABI, 2020; Champoiseau *et al.,* 2009; Priou *et al.,* 1999). The affected vascular bundles exude a string of white, slimy mass of bacteria (bacterial stream) when cut and immersed in a clear glass of water (Karim & Hossain, 2018). However, this diagnostic method is not effective for host plants with latent infection or asymptomatic symptoms and cannot identify the race and/or biovars of the target pathogen.

Plate 2. 2: Visual and vascular flow symptoms.

A= bacterial wilt symptom on the leaf, B = brown discoloration of the vascular systems of transversely cut stem, C = white-milky bacterial ooze from the freshly cut section of the infected tuber and D = streams of bacterial ooze from the cut portion of infected potato stem.

Source: Champoiseau *et al.* (2009) and Priou *et al.* (1999).

2.4.5.2 Colony characteristics on normal and selective media

The suspected infected plant parts are collected and taken to the laboratory for isolation of bacterial pathogen on normal and selective media mainly nutrient agar and Triphenyl Tetrazolium Chloride (Kelma's TZC agar) media (Karim & Hossain, 2018; Kelman, 1954). After 36-48 hours of incubation at 28^{o}C, white and/or creamy colored, irregularly shaped, highly fluidal, and opaque bacterial colonies will be observed on both media however, the virulent colonies will appear as white colonies with pink centers as opposed to non-virulent colonies which will display uniform round and dark red colonies on Kelma's TZC agar (Vanitha *et al.,* 2009). Kelma's TZC agar media is a selective media and can differentiate virulent from non-virulent colonies of *R. solanacearum* pathogen (Karim & Hossain, 2018). If the pathogen is isolated on casamino acid-peptone-glucose

(CPG) medium, virulent strains appear as white to cream colored, irregularly round, fluidal and opaque colonies while nonvirulent colonies are uniformly round, smaller and butyrous (García *et al.,* 2019).

2.4.5.3 Biovar tests

This is a biochemical assay which uses disaccharides and sugar alcohols to group isolated *Ralstonia solanacearum* pathogens into 5 biovars (biovar I, II, III, IV and V) (Hayward & Hartman, 1994). The classification is based on the ability of different biovars to utilize carbon in hexose alcohols (mannitol, sorbitol and dulcitol) and/or to produce acids from 3 disaccharides (lactose, maltose and cellobiose) (Karim & Hossain, 2018; Kinyua *et al.,* 2014) (Table 2.1). The inoculated carbon sources are incubated for up to 7 days and positive results (oxidation of carbon sources) are denoted by color change to yellow (Kinyua *et al.,* 2014). Based on previous biovar tests results, Biovar 2 (race 3) is documented as the potato low temperature race and is endemic in high latitudes, and high altitudes areas ranging from 1500-2500 m above sea level. Race 3/biovar 2A (R3bv2A) causes bacterial wilt of potato in over 90% of cases worldwide but is more endemic in the cool tropical highlands and in the higher latitudes such as southern Sweden and southern Argentina ((Mihovilovich *et al.,* 2017). However, due to latent infection in seed potato tubers harvested from cool climatic areas, R3bv2A has spread, and infected potato plants grown in warmer locations. Race 1/biovars 1, 3 and 4 are documented to be endemic with severe bacterial wilt symptoms in warmer subtropical and tropical lands (Mihovilovich *et al.,* 2017). However, due to overlap of host range and lack of race-cultivar specificity by *R. solanacearum* strains, tests to define races are cumbersome. Thus, molecular characterization focusing on the variation of selected marker genes resulting in the identification of Phylotypes and sequevars should be adopted as a classification scheme since it adds more valuable information about the geographical origin and/or the pathogenicity of *R. solanacearum* strains (Kinyua *et al.,* 2014; Mihovilovich *et al.,* 2017).

Table 2. 1: Identification of biovars of *R. solanacearum* based on the utilization of certain carbohydrates

Carbohydrates		Biovar 1	Biovar 2	Biovar 3	Biovar 4	Biovar 5
Hexose alcohol	Mannitol	-	-	+	+	+
	Sorbital	-	-	+	+	-
	Dulcitol	-	-	+	+	-
Disaccharides	Lactose	-	+	+	-	+
	Maltose	-	+	+	-	+
	Cellobiose	-	+	+	-	+

+ denotes utilization of hexose alcohol and acid production from disaccharides

Source: Hartman and Hayward (1994)

2.4.5.4 Pathogenicity test

Pathogenicity test has been used by various research scientists to detect *R. solanacearum* pathogen (Gutarra *et al.,* 2017). The suspected pathogen is first isolated following standard procedures, and the pathogenicity of the isolates is tested on susceptible cultivars of potatoes, tomatoes, eggplants and pepper among others (Sagar *et al.,* 2013). The inoculum is optimized and then inoculated to the test plants either through stem stab method or through soil inoculation and observation of symptom development mainly wilt incidence (Gutarra *et al.,* 2017; Mahdy *et al.,* 2012; Sagar *et al.,* 2013). Soil inoculation is always done as soil drench or root inoculation of 3-4 weeks old plants. Prior to inoculation, the roots are wounded and 30-50 ml of 10^8 CFU/mL [optical density (OD) at 600 nm wavelength] bacterial suspension is poured close to the crown of the plant (Hong *et al.,* 2012). For stem stab or stem injection method, the seedling stems are injected with sterilized needles and 100 µl of bacterial suspension at 10^8 CFU/mL [optical density (OD) at 600 nm wavelength] is injected is injected into the stabbed stem (García *et al.,* 2019). In both cases the inoculated plants are incubated either in growth rooms or in greenhouses with approximate temperatures of 30˚C and the plants assessed for disease symptoms mainly disease incidence and wilt index (García *et al.,* 2019). Singh *et al.* (2018) developed a rapid pathogenicity technique which is performed under gnotobiotic condition in microfuge tubes by using 6-7 days old tomato seedlings for root inoculation. The inoculated tomato seedlings exhibited wilt symptoms within 48 hours of incubation and the virulent assay can be completed within two weeks.

2.4.5.5 Serological tests

The Enzyme-Linked Immunosorbent Assay (ELISA) is the main serological technique that has been used effectively for detection and identification of *R. solanacearum* because it is fast, cheap, not complicated and can be used under field condition (García *et al.,* 2019; Machmud & Suryadi, 2008). The basic technique behind ELISA technique is the antigen and antibody reaction which is observed as either positive or negative through ELISA reader and/or through color formation. Amendments have been made by various researchers to improve sensitivity and specificity of the detection of *R. solanacearum* on asymptomatic plants, tubers and soil using ELISA technique (Priou *et al.,* 2010). As a result, post-enrichment enzyme-linked immunosorbent assay (ELISA) on nitrocellulose membrane (NCM) with improved sensitivity and specificity in detection of *R. solanacearum* on latently infected tubers was developed by International Potato Centre (CIP) (Priou *et al.,* 1999, 2001). Similarly, another post-enrichment double-antibody sandwich (DAS) ELISA which is highly sensitive and specific to detection and semi-quantification of *R. solanacearum* in soil was optimized at CIP (Priou *et al.,* 2006). The main drawback of this detection method is its inability to detect *R. solanacearum* at very low concentration and *cannot* distinguish between virulent and avirulent strains.

2.4.5.6 PCR detection using specific primers

PCR-based techniques are DNA based techniques which were developed to reduce the drawbacks of other detection methods such as isolation on selective medium, pathogenicity test and serological tests which are lengthy, time consuming and cannot distinguish virulent and avirulent strains (Singh *et al.,* 2014). Most of these techniques have been developed and are being used to detect phyto-pathogenic bacteria. Different conserved genes such as 16S rDNA (16), flic C, cytochrome c1 signal peptide, and hypersensitive response and pathogenicity (hrp) gene have been used to design primers for amplification process (Berg *et al.,* 2005; Kang *et al.,* 2007; Schönfeld *et al.,* 2003). Some of them like a hrp gene has proved useful and has been used to distinguish between virulent and avirulent strains of *R. solanacearum* from different sources (asymptomatic plant, irrigation water and soil) and infected crop samples (Ito *et al.,* 1998; Singh *et al.,* 2014). PCR-based detection is an efficient technique however its drawback lies with its technical complexity and expensiveness. Similarly, presence of numerous compounds such as phenolics or acidic polysaccharides in plants and humic acid in soils have resulted in inconsistent results which hinders the enzymatic PCR procedure (Patel *et al.,* 2022).

2.4.6 Disease cycle and epidemiology

Ralstonia solanacearum is usually soil and water borne pathogen and can survive in the soil inform of pellets and water reservoir over long periods of time (years) acting as primary source of inoculum in subsequent seasons (Champoiseau, 2008; Zinnat *et al.,* 2018). For example, race 3 survived for a period of 2-3 years in Australia under a bare fallow (CABI, 2020). Previous research studies have also reported that *R. solanacearum* can survive in water for 40 years, but the pathogen is more commonly found in the debris of diseased plants, vegetative propagative organs and wild hosts (Anton *et al.,* 2021). However, the pathogen population in an infested soil is dependent on availability of alternate hosts, soil type, soil pH, soil nutrient supply [soil organic carbon (SOC), total nitrogen (TN), available potassium (AK) and available phosphorus (AP)], and cultural practices carried out in that field (Cao *et al.,* 2022; Karim & Hossain, 2018; Li *et al.,* 2017; van *Elsas et al.,* 2000). Studies by Li *et al.,* (2017), reported that *R. solanacearum* population develops more quickly and severely in acidic soil conditions (pH 4.5-5.5) since these pH conditions suppresses the population and antagonistic activities of antagonistic bacteria such as *Pseudomonas fluorescens* and *Bacillus cereus*. Similarly, studies by Cao *et al.* (2022), reported that soils with high soil organic carbon (SOC), total nitrogen (TN), available potassium (AK) and available phosphorus (AP) contents had suppressed *R. solanacearum* population compared to conducive soils. The pathogen can also survive on alternate hosts like susceptible weeds, latently infected tubers, seeds and plant residues of symptomatic plants and acts as a source of inoculum in the subsequent seasons (Choudhary *et al.,* 2018).

The pathogen enters the host plant through wounds on the root hairs and below ground stem parts created by other soil microorganisms such as root-knot nematodes and mechanical injuries during cultural practices and or insect damages (Allen *et al.,* 2005; Champoiseau, 2008). Upon entry, the pathogen moves and colonizes the vascular bundles especially the lumen and mesophyll of xylem vessels from where it multiplies and produce large amounts of extracellular polysaccharides (EPS) causing vessel blockage and finally wilting and death of the infected plant (CABI, 2020; Charkowski *et al.,* 2020; Li *et al.,* 2017). The speed of movement is however dependent on the colonized plant part. *R. solanacearum* pathogen is disseminated from infested to non-infested fields through contaminated irrigation water, latently infected potato tubers either externally on the tuber surface, in the lentils and/or in the vascular tissues (Hossain & Karim, 2018). The pathogen can also be disseminated mechanically through infested equipment's during

farm operations such as pruning, use of cuttings for propagation and harvesting, insect feeding mainly biting and chewing insects such as Colorado potato beetle, xylem feeding bugs and through nematode infestation (Charkowski *et al.,* 2020; Choudhary *et al.,* 2018; Dossoumou *et al.,* 2023; Hossain & Karim, 2018). Long distance trade of asymptomatic infected vegetative propagation materials such as seed potato tubers have resulted in the spread and establishment of *R. solanacearum* pathogen in several ecoregions around the world (Charkowski *et al.,* 2020; Gutarra *et al.,* 2017). Currently, transmission through infected water, infested soil and movement of infected vegetative plant parts are deemed as the main dissemination modes of *R. solanacearum* pathogen for most host plants than transmission via true seed (Charkowski *et al.,* 2020).

2.5 Factors affecting growth and multiplication of *Ralstonia solanacearum* pathogen

Temperature and soil moisture are the main factors influencing *R. solanacearum* biology and development. High temperatures ranging between 29-35˚C enhance rapid pathogen growth and disease development (Champoiseau, 2008). However, the optimal temperature ranges between 24-35˚C and this varies with different races (CABI, 2020). Wilts symptoms are most severe at temperature ranges between 25-35˚C but the susceptible host plants usually express decreased symptom expression at temperatures above 35˚C and below 18˚C respectively (Bhanwar, 2022). Studies by Sahu *et al.* (2017) revealed that the thermal death point of *R. solanacearum* is 37˚C and hence no pathogen growth beyond 37˚C. The pathogen expression is rarely noticed under low temperatures usually below -10˚C but it still survives in latent state (Champoiseau, 2008). Most of the *R. solanacearum* strains are non-pathogenic at temperatures below 20˚C (Bocsanczy *et al.,* 2012). Temperatures also affect the resistance levels of various cultivars. For instance, resistant cultivars to *R. solanacearum* at moderate temperatures become susceptible at high ambient temperatures (Hayward, 1991; Prior *et al.,* 1996; Singh *et al.,* 2014; Yeon *et al.,* 2022).

High soil moisture content and extended rainy seasons are conducive for increased *R. solanacearum* pathogen reproduction and survival in the soil resulting in high disease incidences (Nesmith & Jenkins, 1985). For instance, Jiang *et al.* (2021) reported higher levels of *R. solanacearum* disease incidence from soils with 60% to 70% water holding capacity and this can be attributed to several factors. First, the high moisture levels in the soils might have created optimal conditions for plant growth leading to increased root exudation and improved growth and colonization of the plant by the *R. solanacearum* pathogen (Boyer & Kramer, 1995; Funk & Larson, 2016; Islam & Toyama, 2004; van Elsas *et al.,* 2000). The high moisture levels can also

have a direct effect to the *R. solanacearum* pathogen by providing a medium for increased multiplication, movement and colonization of the host plant (Aung *et al.,* 2018; Beattie, 2011; Velásquez *et al.,* 2018). Additionally, soil moisture affects the availability of oxygen and nutrient availability in the soil which can affect the growth of *R. solanacearum* pathogen as well as the strength and antagonistic activity of antagonistic microbes against *R. solanacearum* in the rhizosphere (Brockett *et al.,* 2012; Cavagnaro, 2016; Chen *et al.,* 2007; Dalsing *et al.,* 2015; Mainiero & Kazda, 2005). In contrast to optimal soil moisture levels, previous research findings have reported that non-optimal soil moisture levels have led to over expression of plant resistance genes in most of the host plants resulting in low disease indices (disease incidences and severities) (Jiang *et al.,* 2018; Mondal *et al.,* 2014; Sinha *et al.,* 2016).

Apart from temperature and soil moisture, other factors such as soil physicochemical properties (microbiome composition, soil type, soil structure, organic matter content in the soil, soil pH and nitrogen availability in the soil, and salt content) may also influence the *R. solanacearum* biology and survival in the soil (Jiang *et al.,* 2021). For instance, van Bniggen and Termorshuizen (2003) reported low survival rates of *R. solanacearum* in loamy soils with high organic matter content vis-à-vis the high survival rates recorded in soils with low organic matter content (van Elsas *et al.,* 2000). This was attributed to enhanced soil microbiota and hence natural biological control capacity of various antagonistic microbes against *R. solanacearum* pathogen (Lee *et al.,* 2021; van Elsas *et al.,* 2005; Wei *et al.,* 2018, 2019; Wen *et al.,* 2020). Previous research findings have also documented increased bacterial wilt disease symptom expression in acidic soils and soils with high nitrogen content (Gu *et al.,* 2020; Li *et al.,* 2017a, Li *et al.,* 2017b; Wang *et al.,* 2017). However, soils with high carbon, nitrogen, phosphorus and potassium availability have been linked with healthy plant rhizosphere (Wang *et al.,* 2017; Wei *et al.,* 2018; Wu *et al.,* 2020).

2.6 Management of bacterial wilt

2.6.1 Planting resistance varieties

Bacterial wilt resistance cultivars have been bred by various scientists and have served as the most economical and environmentally friendly strategy in management of bacterial wilt disease (Yuliar *et al.,* 2015). Wild potato relatives such as *Solanum stenotomum, S. chacoense, S. phureja* and *S. commersonii* Dun have been used as the main source of resistant traits in the production of resistant potato cultivars (Muthoni *et al.,* 2020). *S. phureja* Juz. and Bukasov are the most widely used in production of resistant cultivars against bacterial wilt of potato through hybrid breeding

and protoplast fusion techniques (He *et al.,* 2024). For example, the somatic hybrid (potato genotype BP9) produced from *S. tuberosum* and *S. phureja* reduced bacterial wilt incidence by 90-100% under field condition (Fock *et al.,* 2000). However, the success of breeding for resistance to bacterial wilt pathogen has been affected by; availability and diversity of resistance source, linkage between resistance and other agronomic traits, varied strains of *R. solanacearum*, host-pathogen interaction and adopted selection methodology (Boshou, 2005; Elphinstone, 2005; Genin, 2010; Patil *et al.,* 2012). Other studies also reported latent infection of *R. solanacearum* on resistant cultivars (Prior *et al.,* 1996; Uwamahoro *et al.,* 2020). Bacterial wilt resistant cultivars have displayed negative correlation with yield and tuber quality and hence high rejection rates by farmers (Yuliar *et al.,* 2015). Additionally, some sexual hybrids of potato with resistant genotypes of *S.chacoense, S. sparsipillum, S. raphanifolium, S. microdontum* and *S. multidissectum* have shown moderate resistant levels to bacterial wilt pathogen and wild traits like high glycoalkaloid content which have affected their adoption and commercialization (Muthoni *et al.,* 2020).

2.6.2 Phytosanitation and cultural practices

Phytosanitation (planting disease-free tuber seeds, and quarantine measures) and cultural practices (crop rotation, intercropping, delayed planting, soil amendments, positive selection, and negative selection) are the most widely practiced bacterial wilt management strategy in bacterial wilt endemic areas and/or in areas where the pathogen is present but has not been established (Champoiseau *et al.,* 2010; Muthoni *et al.,* 2012). Proper field sanitation helps to reduce inoculum buildup in the soil (Charkowski *et al.,* 2020). Field sanitation techniques include planting of certified seed potato tubers, quarantine measures, planting on disease free fields, cleaning and disinfection of farm tools between farms and proper disposal of infected plant parts (Gobena, 2020; Kassa, 2016; Muthoni *et al.,* 2012; Muthoni *et al.,* 2014). During crop growth and after harvesting, the field should be regularly monitored and infected plants and plant parts (weeds, volunteer crops, haulms and tubers) should be removed from the farm and buried deep away from irrigation canals or burnt (Choudhary *et al.,* 2018). Field sanitation techniques are usually aimed at avoiding the survival of the pathogen on crop fields and thus limiting its spread to other disease-free areas through different disease dissemination mechanisms (Priou *et al.,* 1999; Tessema *et al.,* 2023). Although certified seed tubers are advocated as a management option against bacterial wilt disease in potato fields, its effectiveness has been hindered by insufficient volumes of certified seed potato tubers produced by the formal seed system especially in the developing countries (Lung'aho *et al.,*

1997). Thus, farmers rely heavily on seed tubers from informal sources which has led to recurrent infections in potato fields (Kinyua *et al.*, 2001; Muthoni *et al.*, 2010). Similarly, the use of quarantine as a management option against bacterial wilt disease has not been effective due to presence of informal seed systems in developing countries and the porous international boundaries that promote uncontrolled movement of both seed and ware potatoes (Muthoni *et al.*, 2010).

Crop rotation by non-host crops such as cereals and grasses has helped reduce *R. solanacearum* buildup in the soil (Charkowski *et al.,* 2020; Uwamahoro *et al.*, 2018). For instance, low bacterial wilt incidences and higher potato yields have been reported in rotational programs involving potatoes, wheat, sweet potato, maize, millet, carrots, cow peas, sorghum, onion, common beans, cabbage and *Crotalaria falcata* compared to pure potato stands (Kakuhenzire *et al.,* 2013; Kassa, 2016; Katafiire *et al.,* 2005; Muthoni *et al.,* 2012). Crop rotation as a bacterial wilt management strategy is based on several principles such as use of non-host crops (host exclusion technique), use of crops with suppressive effect against *R. solanacearum* due to their exudates and secondary metabolites, N fixing plants, plants with high residual matter and their adaptability to the specific environs (Mwaniki *et al.,* 2017). However, the effective rotational period varies with various biotic and abiotic factors affecting pathogen survival ability in the soil (Choudhary *et al.,* 2018). Small farm sizes, especially in developing countries like sub-Saharan Africa, have also hindered effectiveness of crop rotation as a management practice. Most farmers especially in sub-Saharan Africa are smallholder farmers with small pieces of land and thus, have continuously cultivated potatoes on the same pieces of land while a few have practiced very short rotations that are inadequate to reduce the inoculum build up in the bacterial wilt infested fields (Kaguongo *et al.,* 2008; Lemaga, 1997; Riungu, 2011).

2.6.3 Antagonistic microbes/Biological Control Agents (BCAs)

Use of biological control agents (BCAs) has been on the increase due to environmental concerns accompanied by chemical control methods (Whipps, 2001). These agents are self-sustaining, easily established in the infected soils after first application and have a long-term disease suppression effect after application (Quimby *et al.,* 2002; Whipps, 2007). The BCAs are believed to suppress *R. solanacearum* populations in the soil through competition for nutrients, space, antibiosis, parasitism and induced resistance (Agrios, 2005; Cook & Baker, 1983). About 90% of BCAs against *R. solanacearum* are bacteria while fungi only constitute 10% (Yuliar *et al.*, 2015; Wang *et al.,* 2023). Some examples of reported BCAs against *R. solanacearum* from

bacterial origin include *Acinetobacter* spp., *Burkholderia nodosa, Bacillus* spp., *Chryseobacterium daecheongense, Chryseobacterium indologenes,* Bacteriophages, *Enterobacter* spp.(*Enterobacter cloacae*), *Pseudomonas putida*, *Pseudomonas fluorescens, Paenibacillus macerans*, Streptomyces, *Pantoea ananatis*, *Pseudomonas oleovorans* (Huang *et al.,* 2013; Ling *et al.,* 2010; Long *et al.,* 2004; Mamphogoro *et al.,* 2020; Marković *et al.,* 2023; Mohamed *et al.,* 2020; Sun *et al.,* 2023; Upreti &Thomas 2015; Xue *et al.,* 2009; YA & Toyota, 2008; Zhou *et al.,* 2008). Similarly, BCAs against *R. solanacearum* from fungal origin include *Pythium oligandrum, Gigaspora margarita, Glomus mosseae, Scutellospora* spp., *Parmotrema tinctorum* and *Trichoderm*a spp. (*Trichoderma harzianum* and *Trichoderma asperellum* T34*) (*Gomes *et al.,* 2003; Hase *et al.,* 2006; Mohamed *et al.,* 2020; Tahat *et al.,* 2012; Yan *et al.,* 2021*).* Demerits associated with BCAs are poor performance due to inconsistent colonization; restriction to single and/or a few pathogens by some species of antagonistic microbes, high rates of inoculum use which is not economical and difficulties in isolation, storage and application (Yuliar *et al.,* 2015).

2.6.4 Plant extracts/residues

Previous study results have revealed the efficacy of different plant extracts in the management of *R. solanacearum* pathogen (Mutimawurugo *et al.,* 2020; Yuliar *et al.,* 2015). The mode of action against various plant diseases exhibited by plant extracts is dependent on the active compounds within that specific extract (Mulugeta *et al.,* 2020). Similarly, the mode of action is also dependent on the application method for instance; it is assumed that the mode of action of plant extracts against bacterial wilt pathogen is through antimicrobial activity and/or through improved physical, chemical and biological soil properties for those applied as organic amendment (Cardoso *et al.,* 2006). For example, freshly cut aerial parts from pigeon pea (*Cajanus cajan*) and crotalaria (*Crotalaria juncea*) incorporated at concentrations of 20–30% and incubated for 30 days in a bacterial wilt inoculated tomato field, suppressed bacterial wilt disease at 45 days after the inoculation (Cardoso *et al.,* 2006).

Some extracts act as plant strengtheners through stimulation of plant growth, plant metabolism as well as defense and hence protecting the plant from predisposing factors (biotic and a biotic stress) to disease infection (Dubey *et al.,* 2008; Guleria & Tiku, 2009; Yakin *et al.,* 2017). Other plant extracts exhibit bactericidal effect on plant pathogens through creation of unfavorable environmental conditions for growth and reproduction of target pathogens e.g., disruption of the cell membrane leading to out flow of cell content and death of the target pathogen (Gonelimali *et*

al., 2018; Mulugeta *et al.,* 2020). Examples of effective plant extracts against *R. solanacearum* include Rosella (*Hibsicus sabdariffa*), pomegranate (*Punica granatum*) and blue gum (*Eucalyptus globulus*), tobacco (*Nicotiana tabacum* L.), wild marigold (*Tagetes minuta* L.), garlic (*Allium sativum* L.), malabar nut (*Adhatoda vasica*), French marigold (*Tagetes patula*), sodom apple (*Calotropis procera*) and spring onion (*Allium fistulosum*) (Bereika *et al.,* 2020; Deberdt *et al.,* 2012; Din *et al.,* 2016; Mutimawurugo *et al.,* 2023). Antimicrobial compounds such as flavonoids, 5-(3-buten-1-ynyl)-2,2'-bithienyl (BBT) and 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (BBTOAc) from *Tagetes patula* were reported to suppress *R. solanacearum* under *in vitro* experiments (Mutimawurugo *et al.,* 2020; Terblanche & Villiers, 1998).

The plant-based products have been applied/used in various forms for the management of *R. solanacearum* both in the greenhouse and in the field. Some of the adopted application methods include soil amendments (incorporation as green manure and dried powder), extracted compounds applied as soil drench and foliar sprays and by injecting the extracted compounds of the test plants (in the selected plant parts such as stems and/or tubers) using sterile needles (Bereika *et al.,* 2020; Cardoso *et al.,* 2006; Din *et al.,* 2016; Mutimawurugo *et al.,* 2023).

Plants and plant-derived products have been used as a good source of bioactive compounds both in the pharmaceutical and biopesticide industries (Koparde *et al.,* 2019; Okwute, 2012). For biopesticides, five families (Apocynaceae, Flacourtiaceae, Fabaceae, Lamiaceae, and Asteraceae) from the plant kingdom are documented as the main source of bioactive compounds which can be used in the manufacture of biopesticides (Gakuya *et al.,* 2013; Céspedes *et al.,* 2014). Examples of plant-derived bioactive compounds with biopesticide activity include chitosan, salicylic acid, benzoic acid, benzothiadiazole, saponins, alkaloids, flavonoids, terpenes, proteins, peptides, blasticidin, mildiomycin, polyoxins, tannins and phenolic compounds (Din *et al.,* 2016; Mutimawurugo *et al.,* 2020; Singh *et al.,* 2020). Several techniques have been adopted for analysis of bioactive compounds in various plant extracts. These include common phytochemical screening assays, chromatographic techniques [high performance liquid chromatography (HPLC), gas chromatography mass spectrometry (GC-MS), thin layer chromatography (TLC) and liquid chromatography mass spectrometry (LC-MS)] and non-chromatographic techniques [immunoassay and Fourier Transform Infra-Red (FTIR)] (Sasidharan *et al.,* 2011; Shakeel, 2023).

2.6.5 Animal wastes

Numerous studies have indicated the efficacy of animal waste on plant disease management. However, only a few study results have demonstrated its efficacy on bacterial wilt pathogens (Yuliar *et al.,* 2015). Studies by Gorissen *et al.* (2004) and Islam and Toyota (2004) reported suppressed *R. solanacearum* population in the soil due to application of pig slurry and poultry manure. Similarly, studies by Ding *et al.* (2023) also reported suppressed *R. solanacearum* population in the infested tomato field due to application of composted cow manure. It has been proposed that the efficacy of animal waste is achieved through the shift of microbial population leading to higher numbers of natural biological control agents (BCAs) (Gorissen *et al.,* 2004, Islam & Toyota, 2004). It is also suggested that the efficacy of composted animal wastes against *R. solanacearum* is achieved through release of toxic compounds, induced host plant immunity and reassembly of the rhizosphere microbial community (Ding *et al.,* 2023). However, the efficiency of these organic wastes is affected by the type of soils which has also been reported to affect *R. solanacearum* populations. Similarly, the efficacy of animal wastes also depends on the type of feed fed to the animal. This is because if the animal is fed on latent bacterial wilt infected plant products and/or plant parts and the resultant wastes are not allowed to undergo full decomposition, then this type of animal waste can act as source of inoculum and spread of bacterial wilt disease in the field (Kirigo, 2019).

2.6.6 Chemical control

Efficacy of different chemical compounds has been investigated against bacterial wilt pathogens (Sarkar & Chaudhuri, 2016). These include use of antibiotics such as streptomycin, ampicillin, tetracycline and penicillin which started as early as 1952 and soil fumigation (Robison *et al.,* 1954; Farag *et al.,* 1982; Murakoshi & Takahashi, 1984). However, the chemical control strategies have not been effective due to the soil-borne and systemic nature of bacterial wilt pathogen (pathogen's complexity) (Sangoyomi *et al.,* 201; Wang *et al.,* 2023; Yuliar *et al.,* 2015). In addition, the chemical control strategies are not feasible due to environmental considerations such as degradation of ozone layer by methyl bromide which was used as a fumigant and ground water contamination with toxic compounds (Muthoni *et al.,* 2014; Mwankemwa, 2015).

2.7 knowledge Gaps

Host plant resistance and use of botanicals have been advocated by various research scientists as ecofriendly management options against *Ralstonia solanacearum* in the infested

potato fields (Wang *et al.,* 2023). However, the two management options have not been exploited fully due to various setbacks. Factors such as the diversity and availability of resistance sources, the genetic relationships between agronomic and resistance traits and host-pathogen-environment interaction mechanisms have negatively impacted on the efficacy of host plant resistance. For instance, most of the reported highly resistant potato varieties against *R. solanacearum* pathogen have shown negative correlation with various agronomic traits, mainly yield traits and this has negatively affected their adoption and commercialization. Most of the research studies which have been conducted to assess antibacterial potency of various plant extracts (botanicals) against *R. solanacearum* usually stops at the laboratory level (*in-vitro*) and does not proceed to either greenhouse or field levels. Additionally, most of the *in-vitro* studies have failed to identify various antibacterial compounds against *R. solanacearum* pathogen from the assed plant extracts. Furthermore, due to the wide host range and complexity of *R. solanacearum*, the pathogen has proved difficult to manage by a single management approach. Hence, the current research is focused on screening of different potato genotypes for resistance against *R. solanacearum* pathogen, assessment of the antibacterial activity of selected plant extracts against *R. solanacearum* pathogen and determination of antibacterial compounds from the effective plant extracts against *R. solanacearum*. The current study also assesses the synergy between host plant resistance and botanicals in the management of *R. solanacearum* pathogen under greenhouse conditions. Thus, the results from this study can help in the development of integrated disease management (IDM) options against *R. solanacearum* in potato production fields.

CHAPTER THREE

SCREENING FOR RESISTANCE LEVELS OF DIFFERENT POTATO GENOTYPES AGAINST BACTERIAL WILT (*Ralstonia solanacearum*) INFECTION IN THE FIELD

Abstract

Bacterial wilt (*Ralstonia pseudosolanacearum* sp. *nov.)* is a major disease affecting global potato production. Resistant potato cultivars have been proposed as the cheapest and most practical management option. There is, however, a need for frequent screening of different potato germplasms to identify those with resistance traits for adoption and commercialization. A field study was conducted for two successive seasons: season one (October 2021 to January 2022) and season two (April to August 2022) in two different trial sites to assess resistance levels of thirty potato genotypes against bacterial wilt in naturally infected potato fields. The two trial sites were Kiandu in Nyeri County, Kenya located at Agro-ecological Zone II and Turi in Nakuru County, Kenya located at AEZ III. The experiment was laid down in 3 x 7 alpha lattice design with 3 replicates in season one and 3 x 9 alpha lattice design with 3 replicates in season two. Biovar identification test was conducted to identify the specific biovars and phylotypes of isolated bacteria from the two trial sites. Data was collected on bacterial colony counts, identified biovars, plant emergence, disease severity, disease incidence and yield parameters. Results from the two trial sites showed varied responses of all the tested potato genotypes against natural infection by bacterial wilt pathogen. Overall results in the two seasons ranked genotypes CIP 515004.535 and CIP 515008.561 as highly resistant and Shangi, Asante, Tigoni, Arka and CIP 515008.535 as highly susceptible. All the clones ranked as highly resistant (CIP 515004.535 and CIP 515008.561) had very low yields (transformed average tuber weights) of 0.37 t/ha compared to susceptible commercialized varieties (Shangi 1.14 t/ha, Asante 0.97 t/ha, Tigoni 1.02 t/ha, and Arka 0.70 t/ha). Thus, future research should focus on integrating high yielding traits on these genotypes for increased chances of adoption and commercialization. Additionally, almost all the highly resistant, resistant, moderately resistant and moderately susceptible ranked genotypes exhibited internal bacterial wilt symptoms from dissected tubers and hence are only suitable for ware potato production as opposed to seed potato production.

3.1 Introduction

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most devastating bacterial diseases affecting crop production worldwide (Hassan *et al.,* 2009). The pathogen affects more

than 450 plant species in the tropical, subtropical and temperate regions of the world. Plants species belonging to the Solanaceae family such as potato, tomato, pepper, eggplant and tobacco are the most susceptible to this pathogen (Manda *et al.,* 2020; Siri *et al.,* 2011). Yield loss due to potato field infestation by *R. solanacearum* pathogen varies from one region to another and is dependent on host-pathogen-environment interactions. For instance, yield losses ranging from 5-95% have been reported in Uruguay (Siri *et al.,* 2011) while East African countries such Kenya and Uganda have reported yield losses ranging from 30-100% (Muthoni *et al.,* 2014).

Bacterial wilt pathogen is soil borne and is mainly disseminated from infested to non-infested fields through latently infected tubers and/or propagative materials, contaminated irrigation water, plant feeding by insect vectors and nematodes and mechanically through infested farm equipment (Charkowski *et al.,* 2020; Choudhary *et al.,* 2018; Dossoumou *et al.,* 2023; Karim & Hossain, 22018). Because of its soil-borne nature coupled with other survival factors such as ability to persist in the soil over a long period of time, wide host range, wide biological variations and its ability to remain latent in some host plants leading to unintentional spread, bacterial wilt control in potato fields has proved difficult (Gutarra *et al.,* 2017; Sharma *et al.,* 2017; Uwamahoro *et al.,* 2020). Physical, cultural and chemical controls have been adopted as management options to combat bacterial wilt disease but none of these strategies has been able to control the disease completely (Mamphogoro *et al.,* 2020).

Therefore, use of potato cultivars with durable resistance to bacterial wilt pathogen is regarded as the cheapest and most practical management option (Siri *et al.,* 2011; Wang *et al.,* 2023). Different bacterial wilt resistant potato cultivars have been bred by various scientists across the globe; Kenya Karibu, Kenya sifa, Ingabire, Mauritius clone (89016), and Cruza-148 (CIP-720118) in Kenya, Molinera and Lopez' in Mexico, Prisca in Madagascar, Ndinamagara in Burundi, Rwanda and Democratic Republic of Congo, CIP clones (388575.5, 388575.9), clones 390005.11, 388574.2B and 388580.18A in Uganda (Muthoni *et al.,* 2012; Muthoni *et al.,* 2014; Uwamahoro *et al.,* 2020).

Although use of resistance cultivars is perceived as the cheapest alternative in bacterial wilt management, it also has some limitations such as incomplete immunity to bacterial wilt pathogen from resistant cultivars due to genetic variability of the pathogen species (Patil *et al.,* 2012). Some of the resistant potato cultivars exhibit latent infection symptoms and hence acting as a source of pathogen spread in successive seasons (Prior *et al.,* 1996; Yuliar *et al.,* 2015). Additionally, the

effectiveness of resistant cultivars is usually affected by host-pathogen-environment interaction and race diversity (Genin, 2010) i.e., existence of different pathogenic races at different sites and/or existence of more than one race in one site (Muthoni *et al.,* 2014; Ravelomanantsoa *et al.,* 2018). Some cultivars have been reported to exhibit variable reactions in different environments and in different seasons within the same environment (Ateka *et al.,* 2001; French *et al.,* 1998). This therefore calls for frequent screening of different potato germplasms to identify those with resistance traits against bacterial wilt pathogens. These germplasms can act as potential parents in various breeding programs aimed at developing bacterial wilt resistant cultivars. Thus, the present study is aimed at screening different potato genotypes to determine their resistance levels to *R. solanacearum* in naturally infected fields.

3.2 Materials and methods

3.2.1 Experimental sites

Field experiments were conducted at Turi, Nakuru County and Kiandu, Nyeri County in Kenya. The trial site at Turi was located at latitude 0º 17' 11'' S, longitude 35º 46' 16'' E and at an elevation of 2426 m above sea level. The area lies under Agro-ecological Zone III (AEZ III) with an average annual rainfall of 1000-1250 mm and mean annual temperature of <15ºC (Jaetzold & Schmidt, 1983). The main soil type in the Turi area is Nitisols (Maina *et al.,* 2020). The trial site at Kiandu was located at latitude 0º 29' 5'' S, longitude 36º 58' 34'' E and at an elevation of 1779 m above sea level. Kiandu lies under AEZ II with an average annual rainfall of 750-1000 mm/year and mean annual temperature range of 15-17ºC (Jaetzold and Schmidt, 1983). The main soil type in Kiandu area is Humic Nitisol (Andic) (Ichang'I, 1987). The specific farms were selected after a regular scout for natural bacterial wilt incidences during the 2021 long rain season as described by Mihovilovich *et al.* (2017) from which two sites i.e., one site at Turi and the other site at Kiandu with slightly uniform bacterial wilt incidences of about 30% were selected respectively.

The average mean rainfall received in both experimental sites during the two trial seasons were 71.5 mm for Turi and 54.5 mm for Kiandu in the first season (Short rains, 2021) and 109.9 mm for Turi and 65.25 mm for Kiandu in the second season (Long rains, 2022). The average mean temperatures recorded in both experimental sites during the two trial seasons were 17.8ºC for Turi and 19.34ºC for Kiandu in the first season (Short rains, 2021) and 17.75ºC for Turi and 18.8ºC for Kiandu in the second season (Long rains, 2022) (Table 3.1).

Table 3. 1: Rainfall and temperatures in the experimental sites during the experimental period

Parameters	Short rains 2021		Long rains 2022	
	Turi	Kiandu	Turi	Kiandu
Average rainfall (mm)	71.50	54.50	109.90	65.25
Average Temperature (°C)	17.80	19.34	17.75	18.80

3.2.2 Description of potato genotypes

The test genotypes were sourced from international potato center (CIP) and Agricultural Development Corporation (ADC) Molo. These consisted of 15 CIP clones selected for bacterial wilt tolerance based on the previous experimental results from CIP in-house trials and 15 commercial varieties from ADC which were used as checks (Table 3.2). The clones were acquired at the second-generation stage while the commercial varieties were acquired at the basic seed stage. Seed bulking was done for two seasons at the University of Nairobi, Upper Kabete campus. Twenty-one genotypes were planted per site in season one as opposed to season two in which 27 genotypes were planted per site. This is because some clones and one commercial variety (purple gold) were low yielding and did not produce enough tubers hence had to be bulked again. In season one, both the two trial sites had 19 similar genotypes plus two different genotypes; CIP 515008.503 and CIP 515004.535 for Kiandu as well as CIP 515002.528 and CIP 515008.561for Turi respectively. Three genotypes (CIP 515008.503, CIP 515008.535, CIP 515008.561) were lost during the second seed bulking process and hence were not planted in either of the trial sites in season two. Both the two trial sites were planted with 27 similar genotypes in season two.

Table 3. 2: List of genotypes used in the study

Genotypes	Serial No.	Trial site per season				Type		Disease attributes				Yield (t/ha)
		Kiandu S1	Kiandu S2	Turi S1	Turi S2	CV	CIP clone	LB	BW	PCN	Viruses	
Arka	1	√	√	√	√	√	×	UN	UN	UN	UN	UN
Asante	2	√	√	√	√	√	×	MT	S	UN	T-PLRV	35-45
Chulu	3	√	√	√	√	√	×	T	UN	UN	T-PVX	≥40
Dutch Robyjin	4	√	√	√	√	√	×	S	UN	UN	UN	≤30
Kenya Karibu	5	√	√	√	√	√	×	T	T	UN	T-most viruses	35-45
Kenya Mpya	6	√	√	√	√	√	×	R	UN	UN	R-PLRV	≥40
Konjo	7	√	√	√	√	√	×	R	UN	UN	R-PLRV, PVX	≥40
Lenana	8	√	√	√	√	√	×	R	UN	UN	R-PLRV, PVX	≥40
Nyota	9	√	√	√	√	√	×	T	UN	UN	UN	≥40
Purple Gold	10	×	√	×	√	√	×	R	UN	UN	R-PLRV, PVX	≤30
Shangi	11	√	√	√	√	√	×	MS	UN	UN	UN	30-40
Sherekea	12	√	√	√	√	√	×	R	UN	UN	R-PVX	≥40
Tigoni	13	√	√	√	√	√	×	HT	S	UN	ER-PVX	35-45
Unica	14	√	√	√	√	√	×	MR	UN	UN	HR-PLRV, PVX	≥45
Wanjiku	15	√	√	√	√	√	×	T	UN	UN	T-PVX, ET-PVY	≥40
CIP 515009.573	16	√	√	√	√	×	√	UN	UN	UN	UN	UN

CIP 515002.516	17	√	√	√	√	×	√	UN	UN	UN	UN	UN
CIP 515008.530	18	√	√	√	√	×	√	UN	UN	UN	UN	UN
CIP 515008.521	19	√	√	√	√	×	√	UN	UN	UN	UN	UN
CIP 515004.535	20	√	√	×	√	×	√	UN	UN	UN	UN	UN
CIP 515014.567	21	×	√	×	√	×	√	UN	UN	UN	UN	UN
CIP 515011.555	22	×	√	×	√	×	√	UN	UN	UN	UN	UN
CIP 515013.558	23	×	√	×	√	×	√	UN	UN	UN	UN	UN
CIP 515006.507	24	×	√	×	√	×	√	UN	UN	UN	UN	UN
CIP 515008.555	25	×	√	×	√	×	√	UN	UN	UN	UN	UN
CIP 515007.614	26	×	√	×	√	×	√	UN	UN	UN	UN	UN
CIP 515002.528	27	×	√	√	√	×	√	UN	UN	UN	UN	UN

CIP 515008.535	28	√	×	√	×	×	√	UN	UN	UN	UN	UN
CIP 515008.503	29	√	×	×	×	×	√	UN	UN	UN	UN	UN
CIP 515008.561	30	×	×	√	×	×	√	UN	UN	UN	UN	UN

CIP = International Potato Center, CV = commercial variety, S1 = Season 1, S2 = Season 2, √ = Positive/present for the respective column, × = Negative/absent for the respective column, LB = late blight , BW= bacterial wilt, PCN = potato cyst nematode, UN = unknown, MT = moderately tolerant, T = tolerant, S = susceptible, MS = moderately susceptible, R = resistant, HT = highly tolerant, MR = moderately resistant, HR = highly resistant, ER = extremely resistant, PLRV = *Potato leaf roll virus*, PVX = *Potato virus X*, PVY = *Potato virus Y*.

3.2.3 Determination of *Ralstonia solanacearum* population in the selected sites

Soil samples were collected from the two selected sites before commencement of each field experiment per season to determine *Ralstonia solanacearum* population in the experimental plots. A soil auger was used to collect 100 g of soil samples from 15-30 cm depth in each experimental plot per site following a zigzag sampling procedure. The samples were mixed thoroughly to form composite samples from which 100 g of soil samples (three samples per block) were sampled per plot and placed in plastic bags, labeled and sent to the laboratory for *R. solanacearum* population analysis. In the laboratory, 1 g of soil per sample was suspended in 10 mL of sterile distilled water and shaken for 30 minutes. Ten-fold serial dilution was conducted on each sample to attain 10^{-3} CFU/mL from which 1 mL/sample was cultured on Kelma's TZC agar media as described by Kelman, (1954) and Karim and Hossain (2018) using pour plate method. The TZC plates were incubated at 28 ± 1^0C for 48 hours. After 48 hours, the number of bacterial wilt colonies were counted, and their population determined using the following formula;

$$CFU/mL = \frac{Total\ number\ of\ colonies}{Plated\ volume\ (mL)} * dilution\ factor \quad (1)$$

3.2.4 Biovar identification through Carbohydrate fermentation test

The test media was prepared per carbohydrate (hexose alcohol and disaccharide) by mixing 3.10 g of protease peptone with 5 g of Sodium hypochlorite, 7.2 mL of 0.25% phenol red solution and 10 g of each carbohydrate source in separate one-litre bottles. Ten millilitres of each stock solution was dispensed in universal bottles and the test media autoclaved at 121°C for 15 minutes for all the carbohydrates except for lactose, maltose and trehalose which were autoclaved for 3 minutes at the same temperature since the three carbohydrates are subject to breakdown with prolonged autoclaving. Three universal bottles were used per carbohydrate. Upon cooling, two universal bottles per carbohydrate were inoculated with 3 loopful of the test bacterium isolated from the soil samples collected from each of the trial sites while one non-inoculated universal bottle per carbohydrate acted as untreated control. All the universal bottles were incubated at 28 ± 1^0C for 5 days.

3.2.5 Field assessment of potato genotypes for tolerance to *Ralstonia solanacearum*

Field experiments were conducted for two seasons (2021 short rain and 2022 long rain seasons respectively) at two different sites to assess resistance levels of different potato genotypes against *Ralstonia solanacearum*. The experiments were laid down in an alpha lattice design with three blocks each having seven plots (treatments) in season one and three blocks each having 9 plots (treatments) in season two. In both seasons, the experiment was replicated three times, and the treatments were randomized within each replicate. The total number of treatments were seven and nine per block in season one and two respectively. In each block, furrows were prepared at 75 cm spacing and diammonium phosphate fertilizer added on the furrows at the rate of 500 kg/ha and mixed thoroughly with soil. To avoid disease escape, uniform wounds were created on each tuber using sterile knives before planting. At the onset of rains, ten well sprouted seed potato tubers per genotype were planted manually on the furrows in the respective plots with the sprouts facing upwards at 30 cm spacing between the tubers. This translates to a plant population of 44,400 tubers per hectare. One meter guard row was planted around the entire experimental plot in each experimental site per season. After emergence, all the potato agronomic practices except application of bactericides were conducted until plant maturity.

3.2.6 Data collection

Data was collected on the number of bacterial colonies, plant emergence, disease severity, disease incidence and yield parameters [tuber grading (ware, seeds and chats) and tubers wights (kg)]. Plant emergence data was collected at 45 days after planting while disease incidence and severity were collected at 60, 74 and 88 days after planting respectively. Disease incidence was scored by counting the number of symptomatic plants per treatment (genotype). Percent disease incidence was calculated using the following formula (Okeyo *et al.,* 2018);

$$\text{Percent disease incidence (\%)} = \frac{\text{Number of symptomatic plants}}{\text{Total number of plants assessed}} \times 100 \qquad (2)$$

Disease severity was recorded using a scale of 1-3 based on the degree of wilting of the affected plant where by 1 = healthy plant, 2 = ≤50% of the plant foliage wilted and 3 = ≥50% of

plant foliage wilted leading to death (Plate 3.1). Percent disease severity (%) was calculated using the following formula;

$$S = 100(\frac{\sum n}{N * Max.score\ in\ the\ scale})$$

(3)

Whereby S = percent disease severity, Σn = summation of wilt scores, and N = total number of plants evaluated per treatment (Mihovilovich *et al.*, 2017).

Plate 3. 1: Bacterial wilt disease severity scale (1 = healthy plant, 2 = ≤50% of the plant foliage wilted and 3 = ≥50% of plant foliage wilted.

Source: Mihovilovich *et al.* (2017).

The percent disease severity data per treatment were used to calculate area under disease progress curve (AUDPC) values using the following formula;

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

(4)

Whereby yi= percent severity score at the ith observation, ti = time in days at the ith observation and n= total number of observations.

The harvested tubers were graded as symptomatic tubers (for tubers showing either externally visible and/or internal bacterial wilt symptoms) and asymptomatic tubers (for tubers without both external and internal bacterial wilt symptoms). The harvested tubers were graded into

ware, seeds and chats. The graded tubers (ware + seeds) were weighed, and the weights converted to tons per hectare using the following formula.

$$TTYA = \frac{TTWPL * PLD}{1000} \quad (5)$$

Whereby TTYA = Total Tuber Yield Adjusted (Tons/ha), TTWPL = Total Tuber Weight/Plant (kg) and PLD = Plant Population Density (De Haan *et al.*, 2014). In each trial season, the resistance levels of each genotype were ranked according to Mihovilovich *et al.* (2017) (Table 3.3).

Table 3. 3: Bacterial wilt resistance ranking scale for potatoes

Resistance levels	Average wilt severity score	% Visible infected tubers	Rank
Highly resistant	1	0	1
Resistant[1]	1	≤ 15	2
Moderately resistant	1.01 - 1.30	≤ 30	3
Moderately susceptible	1.31 - 1.60	-	4
Susceptible	1.61 - 2.20	-	5
Highly susceptible	≥ 2.20	-	6

3.2.7 Data analysis

The data on *Ralstonia solanacearum* population in the field was analyzed using R software, version 4.2.2 (R Studio Team, 2020). The *R. solanacearum* population data was subjected to Levene tests to test for equal variance (Levene, 1960) while all the other data sets except disease severity, were subjected to normality tests using Shapiro-Wilk tests (Shapiro & Wilk, 1965) at $p<0.05$. Where the data lacked equal variance as well as normal distribution, the data were transformed using the formula;

$$\text{Log}\,(X + 1) \quad (6)$$

The bacterial population data was subjected to T-test analysis while field data; percent emergence, final bacterial wilt incidence and yield parameters [proportion of symptomatic tubers, number of symptomatic tubers and weights (t/ha)] were tested using mixed model analysis of variance (ANOVA) with genotypes, trial sites and seasons considered as fixed factors and various

responses as random factor. Whenever significant interactions between genotype, site and season were detected, each seasonal data was subjected to analysis of variance (ANOVA) in alpha lattice design. Treatment means were separated using Tukey's Honestly Significant Difference (HSD) at $p \leq 0.05$ with the agricolae package. The following statistical model was fitted:

$$Yij(l) = \mu + \pi i + Bi(l) + Tj + Ej + Si + S(k) + GEij + \varepsilon ij(l) \quad (7)$$

Where by $Y_{ij(l)}$ = the response value of the jth treatment in the lth block within ith replication μ= effect of overall mean, π_i = replicate effect, B_i = incomplete blocking effect, T_j = effect due to ith potato genotype, E_i = effects due to jth environment, S_i= seasonal effect, S_k= effects due to k^{th} location GE_{ij} = interaction effects due to jth genotype with ith Environment, and $\varepsilon_{ij(l)}$ = effects due to random error.

3.3 Results

3.3.1 Bacterial colony counts

The mean *Ralstonia solanacearum* colony counts were significantly different at $p \leq 0.05$ between Turi and Kiandu trial sites per season. Turi trial site recording the highest bacterial colony counts of 2.11 x 10^5 CFU/mL in season one and 4.97 x 10^5 CFU/mL in season two compared to Kiandu trial site which recorded 104.83 CFUs/ mL in season one and 235.67 CFU/mL in season two respectively (Table 3.4).

Table 3. 4: *Ralstonia solanacearum* colony counts from soil samples in the two trial sites during season one and two

	Ralstonia solanacearum colony counts (CFU/mL)	
Sites	Season 1	Season2
Turi, Molo	2.11 x 10^5 a	4.97 x 10^5 a
Kiandu, Nyeri	1.05 x 10^5 b	2.36 x 10^5 b
Grand mean	3.16 x 10^5	7.33 x 10^5
p-value ($\alpha = 0.05$)	0.00	0.00

The values are the average *Ralstonia solanacearum* counts. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$.

3.3.2 Biovar identification of bacterial isolates from the two experimental sites

The isolated bacteria from the two trial sites oxidized all the disaccharide sugars and hexose alcohols. The positive result was indicated by color change from red to yellow in inoculated universal bottles compared to the non-inoculated checks (Plate 3.2).

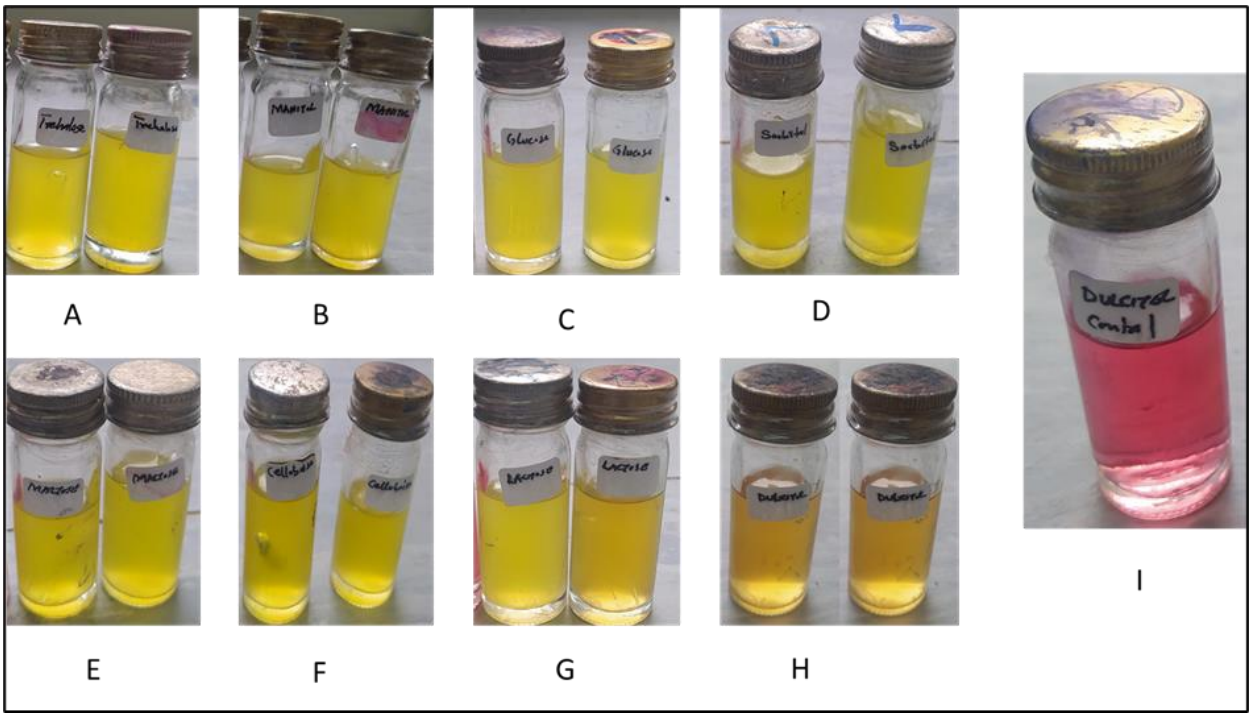


Plate 3. 2: Biovar identification of bacterial isolates from Kiandu and Turi experimental sites based on oxidation of disaccharides sugars and hexose alcohols. A) Trehalose, B) Mannitol, C) Dextrose, D) Sorbitol, E) Maltose, F) Celluboise, G) Lactose, H) Dulcitol I) negative check.

3.3.3 Mixed model analysis of variance (ANOVA) results

The analysis of variance revealed significant interactions between genotypes and site (Genotype x Site), genotype and season (Genotype x Season) and genotype, site and season (Genotype x Site x Season) on percent plant emergence, bacterial wilt incidence at 88 DAP, proportion of symptomatic tubers, number of asymptomatic tubers and weight (t/ha). Similarly, all the parameters showed significant interaction between trial sites and seasons (Site x Season) except percent plant emergence and number of asymptomatic tubers (Appendix 3).

3.3.4 Plant emergence, growth and bacterial wilt incidence

The mean percent plant emergence varied from one genotype to another across the trial sites per seasons. In season one, the mean percent plant emergence was significantly different at $p \leq 0.05$ across all the tested genotypes in Kaindu while those in Turi were not significantly different (Table 3.5). In season two, the mean percent plant emergence was significantly different at $p \leq 0.05$ across all the tested genotypes in both Kaindu and Turi sites. The mean percent plant emergence in season one was 3.90 while that of season two was 3.84. Kiandu trial site recorded the highest mean percent plant emergence compared to Turi in both seasons.

Table 3. 5: Transformed mean percent plant emergence of the tested potato genotypes in season one and two

	Transformed mean percent emergence			
	Season one		season two	
Genotypes	Kiandu	Turi	Kiandu	Turi
Keny karibu	2.00 ± 0.00 a	1.96 ± 0.05 a	2.00 ± 0.00 a	1.92 ± 0.08 ab
Unica	2.00 ± 0.00 a	1.92 ± 0.12 a	1.97 ± 0.03 ab	1.99 ± 0.03 a
Konjo	2.00 ± 0.00 a	1.94 ± 0.08 a	1.91 ± 0.05 ab	1.76 ± 0.04 bc
Sherekea	1.99 ± 0.03 ab	1.99 ± 0.03 a	1.99 ± 0.03 a	1.92 ± 0.06 ab
Dutch Robyjin	1.99 ± 0.03 ab	1.97 ± 0.06 a	1.84 ± 0.12 ab	1.92 ± 0.12 ab
Tigoni	1.99 ± 0.03 ab	1.94 ± 0.08 a	1.94 ± 0.03 ab	1.94 ± 0.08 ab
Arka	1.99 ± 0.03 ab	2.00 ± 0.00 a	1.92 ± 0.12 ab	1.92 ± 0.06 ab
CIP 515009.573	1.99 ± 0.03 ab	1.90 ± 0.01 a	1.93 ± 0.03 ab	1.99 ± 0.03 a
CIP 515008.530	1.99 ± 0.03 ab	2.00 ± 0.00 a	1.97 ± 0.06 ab	1.94 ± 0.08 ab
Kenya Mpya	1.97 ± 0.03 ab	1.97 ± 0.03 a	1.97 ± 0.03 ab	2.00 ± 0.00 a
Nyota	1.97 ± 0.03 ab	1.96 ± 0.05 a	1.80 ± 0.10 b	1.83 ± 0.04 abc
Asante	1.97 ± 0.03 ab	1.94 ± 0.06 a	1.94 ± 0.03 ab	1.96 ±0.05 ab
CIP 515002.516	1.97 ± 0.03 ab	1.84 ±0.10 a	1.96 ± 0.05 ab	1.71 ± 0.00 c
CIP 515008.521	1.97 ± 0.03 ab	2.00 ± 0.00 a	1.91 ± 0.05 ab	1.91 ± 0.00 abc
Wanjiku	1.97 ± 0.06 ab	1.99 ± 0.03 a	1.99 ± 0.03 a	1.96 ± 0.05 ab
Shangi	1.96 ± 0.05 ab	1.91 ± 0.05 a	1.95 ± 0.09 ab	1.82 ± 0.13 abc
CIP 515008.503	1.96 ± 0.06 ab	-	-	-
CIP 515008.535	1.94 ± 0.03 ab	1.88 ± 0.15 a	-	-

Lenana	1.94 ± 0.08 ab	1.83 ± 0.04 a	1.88 ± 0.09 ab	1.97 ± 0.06 a
CIP 515004.535	1.90 ± 0.10 ab	-	1.94 ± 0.06 ab	1.97 ± 0.06 a
Chulu	1.84 ± 0.12 b	1.94 ± 0.06 a	1.96 ± 0.05 ab	1.80 ± 0.10 abc
CIP 515008.561	-	1.83 ± 0.15 a	-	-
CIP 515002.528	-	1.82 ± 0.13 a	1.99 ± 0.03 a	1.95 ± 0.09 ab
CIP 515014.567	-	-	1.99 ± 0.03 a	1.83 ± 0.04 abc
CIP 515011.555	-	-	1.99 ± 0.03 a	1.93 ± 0.13 ab
CIP 515006.507	-	-	1.99 ± 0.03 a	1.89 ± 0.03 abc
CIP 515008.555	-	-	1.97 ± 0.06 ab	1.99 ± 0.03 a
CIP 515013.558	-	-	1.89 ± 0.03 ab	1.97 ± 0.06 a
Purple gold	-	-	1.83 ± 0.04 ab	1.83 ± 0.04 abc
CIP 515007.614	-	-	1.80 ± 0.08 b	1.94 ± 0.03 ab
Mean	1.97	1.93	1.93	1.91
MSE	0.00	0.01	0.00	0.00
MSD	0.15	0.25	0.18	0.21

The values are the average percent plant emergence ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$. MSE = mean square error, MSD = mean square displacement, - = absence of a particular genotype in a specific trial site per season.

Bacterial wilt incidence (BWI) varied across genotypes, trial sites and seasons. In both trial sites, BWI commenced at 60 days after planting (DAP) for both seasons except Kiandu trial site in season one. Most genotypes showed rapid increase of BWI from 60 – 88 DAP in both the two trial sites and seasons. The wilt incidences at 88 DAP were significantly different at $p \leq 0.05$ among potato genotypes used in the study in both the two trial sites and seasons. In season one (short rains), genotypes; Shangi, recorded the highest final BWI at Kiandu trial site while CIP 515008.530, CIP 515008.521 and CIP 515004.535 recorded the least. CIP 515002.528 recorded the highest wilt incidence at 88 DAP at Turi trial site while CIP 515002.516, CIP 515008.530, CIP 515008.521 and CIP 515008.561 recorded the least (Table 3.6).

Table 3. 6: Transformed mean bacterial wilt incidences in Kiandu and Turi trial sites in season one

Genotypes	Transformed % disease incidence				
	Kiandu		Turi		
	74 DAP	88 DAP	60 DAP	74 DAP	88 DAP
CIP 515002.528	-	-	0.00 ± 0.00 a	1.99 ± 0.03 a	1.99 ± 0.03 a
Shangi	0.83 ± 0.73 ab	1.95 ± 0.06 a	0.00 ± 0.00 a	1.80 ± 0.12 a	1.86 ± 0.07 a
Nyota	0.71 ± 0.61 ab	1.87 ± 0.04 a	0.00 ± 0.00 a	1.75 ± 0.05 a	1.85 ± 0.06 a
Tigoni	1.65 ± 0.11 a	1.87 ± 0.04 a	0.00 ± 0.00 a	1.60 ± 0.05 a	1.83 ± 0.04 a
Kenya Mpya	0.95 ± 0.82 ab	1.73 ± 0.07 ab	0.36 ± 0.63 a	1.77 ± 0.02 a	1.84 ± 0.01 a
Asante	0.00 ± 0.00 b	1.70 ± 0.07 ab	0.00 ± 0.00 a	1.82 ± 0.05 a	1.87 ± 0.02 a
CIP 515008.535	0.36 ± 0.63 b	1.66 ± 0.11 abc	0.00 ± 0.00 a	1.16 ± 1.01 ab	1.26 ± 1.09 ab
Arka	0.00 ± 0.00 b	1.65 ± 0.10 abcd	0.00 ± 0.00 a	1.68 ± 0.05 a	1.68 ± 0.05 ab
Chulu	0.00 ± 0.00 b	1.64 ± 0.28 abcd	0.00 ± 0.00 a	0.72 ± 0.63 ab	0.82 ± 0.71 abc
Dutch Robyjin	0.00 ± 0.00 b	1.59 ± 0.25 abcd	0.00 ± 0.00 a	0.91 ± 0.83 ab	1.74 ± 0.14 a
Unica	0.44 ± 0.76 b	1.51 ± 0.19 abcd	0.00 ± 0.00 a	1.59 ± 0.20 a	1.69 ± 0.15 ab
Konjo	0.00 ± 0.00 b	1.50 ± 0.31 abcd	0.00 ± 0.00 a	0.90 ±0.82 ab	1.44 ± 0.23 ab
Kenya karibu	0.00 ± 0.00 b	1.38 ± 0.10 abcd	0.00 ± 0.00 a	0.44 ± 0.77 ab	0.88 ± 0.76 abc
CIP 515009.573	0.00 ± 0.00 b	1.37 ± 0.36 abcd	0.00 ± 0.00 a	1.67 ± 0.08 a	1.87 ± 0.09 a
Lenana	0.00 ± 0.00 b	1.33 ± 0.38 abcd	0.00 ± 0.00 a	1.12 ± 0.97 ab	1.82 ± 0.09 a
Wanjiku	0.00 ± 0.00 b	1.31 ± 0.24 abcd	0.00 ± 0.00 a	1.49 ± 0.15 ab	1.64 ±0.15 ab
CIP 515002.516	0.00 ± 0.00 b	0.80 ± 0.70 bcde	0.00 ± 0.00 a	0.00 ± 0.00 b	0.00 ± 0.00 c
CIP 515008.503	0.00 ± 0.00 b	0.71 ± 0.61cde	-	-	-

Sherekea	0.00 ± 0.00 b	0.69 ± 0.60 de	0.00 ± 0.00 a	0.44 ± 0.77 ab	0.44 ± 0.76 bc
CIP 515008.530	0.00 ± 0.00 b	0.00 ± 0.00 e	0.00 ± 0.00 a	0.00 ± 0.00 b	0.00 ± 0.00 c
CIP 515008.521	0.00 ± 0.00 b	0.00 ± 0.00 e	0.00 ± 0.00 a	0.00 ± 0.00 b	0.00 ± 0.00 c
CIP 515004.535	0.00 ± 0.00 b	0.00 ± 0.00 e	-	-	-
CIP 515008.561	-	-	0.00 ± 0.00 a	0.00 ±0.00 b	0.00 ± 0.00 c
Mean	0.25	1.25	0.02	1.09	1.26
MSE	0.11	0.09	0.02	0.2431	0.16
MSD	1.05	0.96	0.44	1.55	1.25

The values are the average disease incidence ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$. DAP = days after planting, MSE = mean square error, MSD = mean square displacement. Bacterial wilt incidence (BWI) was not reported in Kiandu trial site at 60 DAP.

In season two (long rains), genotypes; Asante recorded the highest BWI at 88 DAP (2.0) at Kiandu trial site while CIP 515004.535 recorded the least (zero BWI). Arka recorded the highest BWI at 88 DAP (1.89) at Turi trial site while Wanjiku, CIP 515009.573, CIP 515013.558, CIP 515002.516, Sherekea, CIP 515014.567, CIP 515008.521 and CIP 515004.535 recorded the least (zero BWI) (Table 3.7). Both Kiandu and Turi trial sites recorded insignificantly different grand mean of BWI score at 88 DAP (1.25 and 1.26 respectively) in season one while Kiandu recorded the highest grand mean of final BWI (1.51) in season two.

Table 3. 7: Transformed mean bacterial wilt incidences in Kiandu and Turi trial sites in season two

Genotypes	Transformed % disease incidence					
	Kiandu			Turi		
	60 DAP	74 DAP	88 DAP	60 DAP	74 DAP	88 DAP
Asante	1.3 ± 0.0 ab	1.95 ± 0.06 a	2.00 ± 0.00 a	0.00 ± 0.00 b	1.52 ± 0.10 abc	1.85 ± 0.05 a
Arka	1.46 ± 0.13 a	1.82 ± 0.16 ab	1.99 ± 0.03 a	0.36 ± 0.63 ab	1.78 ± 0.04 a	1.89 ± 0.05 a
Dutch Robyjin	1.59 ± 0.06 a	1.87 ± 0.12 ab	1.99 ± 0.03 a	0.00 ± 0.00 b	0.44 ± 0.76 bcd	0.99 ± 0.88 abcde
Konjo	1.55 ± 0.13 a	1.74 ± 0.12 ab	1.95 ± 0.05 ab	0.00 ± 0.00 b	0.93 ± 0.82 abcd	1.66 ± 0.05 ab
CIP 515002.528	0.00 ± 0.00 c	1.78 ± 0.07 ab	1.94 ± 0.04 ab	0.00 ± 0.00 b	0.35 ± 0.60 cd	1.35 ± 0.28 abcd
Kenya Mpya	1.67 ± 0.07 a	1.86 ± 0.05 ab	1.90 ± 0.05 ab	0.38 ± 0.65 ab	0.44 ± 0.76 bcd	1.57 ± 0.07 abcd
Shangi	1.63 ± 0.14 a	1.76 ± 0.11 ab	1.89 ± 0.03 ab	0.80 ± 0.70 ab	1.52 ± 0.19 abc	1.76 ± 0.18 ab
Tigoni	0.00 ± 0.00 c	1.58 ± 0.07 abc	1.87 ± 0.03 ab	0.00 ± 0.00 b	1.39 ± 0.24 abc	1.56 ± 0.36 abcd
Unica	1.27 ± 0.13 ab	1.73 ± 0.11 ab	1.84 ± 0.06 ab	0.00 ± 0.00 b	0.35 ± 0.60 cd	1.43 ± 0.32 abcd
CIP 515007.614	0.00 ± 0.00 c	1.65 ± 0.16 abc	1.80 ± 0.05 ab	0.00 ± 0.00 b	1.39 ± 0.18 abc	1.59 ± 0.03 abc
Nyota	0.94 ± 0.82 abc	1.49 ± 0.15 abc	1.77 ± 0.06 ab	0.00 ± 0.00 b	0.00 ± 0.00 d	0.42 ± 0.72 cde
Lenana	0.00 ± 0.00 c	1.15 ± 0.08 abcde	1.75 ± 0.05 ab	0.00 ± 0.00 b	0.00 ± 0.00 d	1.37 ± 0.29 abcd
Purple gold	1.50 ± 0.10 a	1.61 ± 0.03 abc	1.75 ± 0.03 ab	0.38 ±0.65 ab	1.49 ± 0.04 abc	1.70 ± 0.06 ab
CIP 515009.573	1.51 ± 0.09 a	1.55 ± 0.12 abc	1.72 ± 0.03 ab	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 e
Wanjiku	0.35 ± 0.60 bc	1.36 ± 0.24 abcd	1.69 ± 0.09 abc	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 e
CIP 515008.555	0.00 ± 0.00 c	1.52 ± 0.17 abc	1.68 ± 0.05 abc	0.00 ± 0.00 b	0.00 ± 0.00 d	0.36 ± 0.63 de
CIP 515006.507	0.93 ± 0.80 abc	1.37 ± 0.05 abcd	1.66 ± 0.05 abc	0.39 ±0.68 ab	0.39 ± 0.68 bcd	1.04 ± 0.96 abcde
Chulu	0.74 ± 0.65 abc	1.52 ± 0.17 abc	1.61 ± 0.17 abc	1.28 ± 0.17 a	1.69 ± 0.09 ab	1.90 ± 0.06 a

Kenya Karibu	0.00 ± 0.00 c	0.69 ± 0.60 cdef	1.51 ± 0.19 abc	0.00 ± 0.00 b	0.49 ± 0.85 abcd	0.55 ± 0.95 bcde
CIP 515013.558	0.85 ± 0.75 abc	1.26 ± 0.13 abcde	1.38 ± 0.12 abc	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 e
CIP 515002.516	0.00 ± 0.00 c	1.25 ± 0.25 abcde	1.37 ± 0.05 abc	0.00 ± 0.00 b	0.00 ±0.00 d	0.00 ± 0.00 e
Sherekea	0.36 ± 0.63 bc	0.44 ± 0.76 def	1.15 ± 0.15 bcd	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 e
CIP 515011.555	0.00 ± 0.00 c	0.90 ± 0.78 bcdef	0.90 ± 0.78 cd	0.00 ± 0.00 b	1.30 ± 0.29 abcd	1.58 ± 0.22 abc
CIP 515014.567	0.00 ± 0.00 c	0.35 ± 0.60 ef	0.88 ± 0.76 cd	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 e
CIP 515008.521	0.00 ± 0.00 c	0.00 ± 0.00 f	0.39 ± 0.68 de	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 e
CIP 515008.530	0.00 ± 0.00 c	0.35 ± 0.60 ef	0.35 ± 0.60 de	0.00 ± 0.00 b	1.28 ± 0.22 abcd	1.39 ± 0.08 abcd
CIP 515004.535	0.00 ± 0.00 c	0.00 ± 0.00 f	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 e
Mean	0.66	1.28	1.51	0.13	0.62	0.96
MSE	0.11	0.10	0.06	0.09	0.17	1.14
MSD	1.08	1.01	0.81	0.97	1.31	1.22

The values are the average disease incidence ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$. DAP = days after planting, MSE = Mean square error, MSD = mean square displacement.

3.3.5 Area under disease progress curve

Area under disease progress curve (AUDPC) varied among the tested genotypes across seasons and trial sites. In season one (short rains), genotypes; Tigoni recorded the highest AUDPC (3.25) at Kiandu trial site while CIP 515008.530 and CIP 515004.535 recorded the least (3.07). CIP 515002.528 recorded the highest AUDPC (3.31) at Turi trial site while CIP 515002.516, CIP 515008.530, CIP 515008.521 and CIP 515008.561 recorded the least (3.07). In season two (long rains), genotypes; Dutch Robyjin recorded the highest AUDPC (3.39) at Kiandu trial site while CIP 515004.535 recorded the least (3.07). Arka recorded the highest AUDPC (3.29) at Turi trial site while Wanjiku, CIP 515009.573, CIP 515013.558, CIP 515002.516, Sherekea, CIP 515014.567, CIP 515008.521 and CIP 515004.535 recorded the least (3.07) (Table 3.8).

Table 3. 8: Transformed mean area under disease progress curve recorded in the two trial sites in season one and two

Genotypes	Transformed area under disease progress curve			
	Season one		season two	
	Kiandu	Turi	Kiandu	Turi
CIP 515002.528	-	3.31 ± 0.02 a	3.26 ± 0.04 bcde	3.11 ± 0.01 cde
Purple gold	-	-	3.23 ± 0.01 defg	3.16 ± 0.03 bcde
Tigoni	3.25 ± 0.01 a	3.23 ± 0.02 abc	3.26 ± 0.02 bcde	3.19 ± 0.08 abcd
Shangi	3.20 ± 0.04 ab	3.29 ± 0.03 a	3.33 ± 0.04 abc	3.22 ± 0.05 abc
Nyota	3.20 ± 0.02 ab	3.29 ± 0.00 a	3.21 ± 0.01 efgh	3.08 ± 0.02 e
Kenya Mpya	3.18 ± 0.03 abc	3.30 ± 0.03 a	3.34 ± 0.04 ab	3.17 ± 0.07 bcde
CIP 515008.535	3.17 ± 0.04 abcd	3.21 ± 0.13 abcd	-	-
Unica	3.16 ± 0.02 abcde	3.21 ± 0.04 abcd	3.24 ± 0.01 cdef	3.11 ± 0.03 cde
Arka	3.14 ± 0.02 bcdef	3.27 ± 0.02 ab	3.35 ± 0.01 ab	3.29 ± 0.01 a
Asante	3.14 ± 0.02 bcdef	3.28 ± 0.03 a	3.37 ± 0.02 a	3.20 ± 0.01 abcd
Chulu	3.13 ± 0.05 bcdef	3.09 ± 0.03 cde	3.18 ± 0.02 efghij	3.26 ± 0.02 ab
Konjo	3.13 ± 0.05 bcdef	3.14 ± 0.07 bcde	3.32 ± 0.01 abcd	3.15 ± 0.02 bcde
Lenana	3.11 ± 0.04 cdef	3.20 ± 0.05 abcd	3.15 ± 0.01 fghijk	3.09 ± 0.01 de
CIP 515013.558	-	-	3.15 ± 0.04 fghijk	3.07 ± 0.00 e
CIP 515007.614	-	-	3.22 ± 0.01 efgh	3.16 ± 0.03 bcde
CIP 515009.573	3.11 ± 0.03 cdef	3.25 ± 0.01 ab	3.21 ± 0.03 efgh	3.07 ± 0.01 e

Dutch robyjin	3.11 ± 0.06 cdef	3.19 ± 0.04 abcde	3.39 ± 0.03 a	3.11 ± 0.04 cde
CIP 515008.555	-	-	3.19 ± 0.05 efghi	3.08 ± 0.02 e
CIP 515006.507	-	-	3.17 ± 0.04 efghij	3.12 ± 0.04 cde
Wanjiku	3.10 ± 0.02 def	3.20 ± 0.07 abcde	3.17 ± 0.03 efghij	3.07 ± 0.00 e
CIP 515002.516	3.08 ± 0.02 ef	3.07 ± 0.00 e	3.10 ± 0.02 ijk	3.07 ± 0.00 e
Sherekea	3.08 ± 0.01 ef	3.08 ± 0.03 de	3.11 ± 0.06 ijk	3.07 ± 0.00 e
Kenya Karibu	3.08 ± 0.01 ef	3.09 ± 0.02 de	3.14 ± 0.03 ghijk	3.12 ± 0.09 cde
CIP 515011.555	-	-	3.13 ± 0.06 hijk	3.17 ± 0.06 abcde
CIP 515008.503	3.08 ± 0.01 ef	-	-	-
CIP 515008.521	3.08 ± 0.02 f	3.07 ± 0.00 e	3.07 ± 0.01 k	3.07 ± 0.00 e
CIP 515008.530	3.07 ± 0.00 f	3.07 ± 0.00 e	3.09 ± 0.03 jk	3.13 ± 0.01 cde
CIP 515014.567	-	-	3.09 ± 0.03 ijk	3.07 ± 0.00 e
CIP 515004.535	3.07 ± 0.00 f	-	3.07 ± 0.00 k	3.07 ± 0.00 e
CIP 515008.561	-	3.07 ± 0.00 e	-	-
Mean	3.13	3.19	3.21	3.13
MSE	0.00	0.00	0.00	0.00
MSD	0.08	0.14	0.10	0.12

The values are the average area under disease progress curve ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$, MSE = mean square error, MSD = mean square displacement, - = absence of a particular genotype in a specific trial site per season.

3.3.6 Yield parameters and occurrence of bacterial wilt on harvested tubers

The proportion/percentage number of symptomatic tubers were significantly different at $p \leq 0.05$ across all the tested genotypes in both the trial sites and seasons. Dutch Robyjin recorded the highest proportion of symptomatic tubers (2.0) across the two trial sites in the two trial seasons while CIP 515008.521 recorded the least [zero across the trial sites in the two seasons except at Kiandu trial site in season two (069)] (Table 3.9).

Table 3. 9: Transformed mean proportion/percentage number of symptomatic tubers per genotype recorded in Kiandu and Turi trial sites in season one and two

Genotypes	Transformed proportion (%) mean number of symptomatic tubers			
	Season one		Season two	
	Kiandu	Turi	Kiandu	Turi
Unica	2.00 ± 0.00 a	2.00 ± 0.00 a	2.00 ± 0.00 a	1.96 ± 0.05 ab
Dutch Robyjin	2.00 ± 0.00 a	2.00 ± 0.00 a	2.00 ± 0.00 a	2.00 ± 0.00 a
Shangi	1.99 ± 0.03 a	2.00 ± 0.00 a	2.00 ± 0.00 a	2.00 ± 0.00 a
Asante	1.96 ± 0.05 a	2.00 ± 0.00 a	1.96 ± 0.05 a	1.96 ± 0.00 ab
Arka	1.94 ± 0.06 a	1.99 ± 0.03 a	2.00 ± 0.00 a	2.00 ± 0.00 a
Tigoni	1.91 ± 0.05 a	1.88 ± 0.09 a	1.90 ± 0.10 a	1.67 ± 0.03 ab
CIP 515002.516	1.86 ± 0.21 a	1.97 ± 0.03 a	1.76 ± 0.08 a	1.63 ± 0.14 abc
CIP 515009.573	1.86 ± 0.13 a	1.90 ± 0.10 a	1.77 ± 0.24 a	1.52 ± 0.02 abc
Kenya Karibu	1.85 ± 0.06 a	1.91 ± 0.05 a	2.00 ± 0.00 a	1.96 ± 0.05 ab
Konjo	1.84 ± 0.20 a	1.78 ± 0.24 a	1.48 ± 0.15 abc	1.52 ± 0.17 abc
Nyota	1.82 ± 0.20 a	1.79 ± 0.14 a	0.79 ± 0.70 bcd	0.35 ± 0.60 cd
Wanjiku	1.77 ± 0.15 ab	1.97 ± 0.06 a	1.70 ± 0.09 a	1.68 ± 0.02 ab
Sherekea	1.68 ± 0.19 ab	2.00 ± 0.00 a	1.28 ± 0.23abc	1.32 ± 0.00 abc
CIP 515008.503	1.65 ± 0.30 ab	-	-	-
Kenya Mpya	1.19 ± 0.26 abc	1.79 ± 0.17 a	1.73 ± 0.04 a	0.35 ± 0.60 cd
Lenana	1.01 ± 0.90 abcd	1.88 ± 0.11 a	1.48 ± 0.15 abc	0.99 ± 0.86 abcd
Chulu	0.62 ± 1.07 bcd	1.93 ± 0.13 a	1.78 ± 0.07 a	1.38 ± 0.10 abc
CIP 515008.530	0.35 ± 0.60 cd	1.93 ± 0.03 a	0.79 ± 0.70 bcd	1.43 ± 0.01 abc
CIP 515004.535	0.35 ± 0.60 cd	-	0.00 ± 0.00 d	0.00 ± 0.00 d
CIP 515008.521	0.00 ± 0.00 d	0.00 ± 0.00 b	0.69 ± 0.60 cd	0.00 ± 0.00 d
CIP 515008.535	0.00 ± 0.00 d	0.00 ± 0.00 b	-	-
CIP 515002.528	-	0.35 ± 0.60 b	0.00 ± 0.00 d	0.35 ± 0.60 cd
CIP 515008.561	-	0.00 ± 0.00 b	-	-
CIP 515014.567	-	-	1.92 ± 0.08 a	1.63 ± 0.03 abc
CIP 515007.614	-	-	1.87 ± 0.15 a	1.24 ± 0.10 abcd
CIP 515013.558	-	-	1.76 ± 0.04 a	0.94 ± 0.08 abcd

CIP 515006.507	-	-	1.75 ± 0.12 a	1.75 ± 0.12 ab
CIP 515008.555	-	-	1.72 ± 0.12 a	0.00 ± 0.00 d
CIP 515011.555	-	-	1.67 ± 0.10 a	0.69 ± 0.06 bcd
Purple gold	-	-	1.56 ± 0.12 ab	1.14 ± 0.16 abcd
Mean	1.41	1.58	1.53	1.24
MSE	0.13	0.02	0.06	0.16
MSD	1.15	0.49	0.79	1.30

The values are the average percent symptomatic tubers ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$, MSE = mean square error, MSD = mean square displacement, - = absence of a particular genotype in a specific trial site per season.

Number of asymptomatic tubers varied across genotypes, trial sites and seasons and were significantly different at $p \leq 0.05$. Genotypes Shangi and Sherekea recorded the highest number of asymptomatic tubers (1.86 in Kiandu and 1.99 Turi trial) in season one while CIP 515008.535 had the least (0.96 in Kiandu and 1.17 in Turi trial sites respectively). In season two, CIP 515002.528 recorded the highest number of asymptomatic tubers (1.76 in Kiandu and 1.68 in Turi) while CIP 515004.535 recorded the least (1.01 in Kiandu and 0.82 in Turi respectively) (Table 3.10).

Table 3. 10: Transformed mean number of asymptomatic tubers per genotype recorded in Kiandu and Turi trial sites in season one and two

	Mean transformed number of asymptomatic tubers			
	Season one		season two	
Genotypes	Kiandu	Turi	Kiandu	Turi
Shangi	1.86 ± 0.05 a	1.64 ± 0.10 abcd	1.59 ± 0.05 abc	1.78 ± 0.13 a
Dutch Robyjin	1.85 ± 0.16 a	1.94 ± 0.18 a	1.25 ± 0.25 cdefgh	1.56 ± 0.09 ab
Unica	1.84 ± 0.08 a	1.74 ± 0.05 abc	1.58 ± 0.04 abc	1.68 ± 0.08 ab
Kenya Mpya	1.71 ± 0.04 ab	1.81 ± 0.10 ab	1.16 ± 0.12 efgh	1.46 ± 0.15 ab
Wanjiku	1.64 ± 0.03 abc	1.75 ± 0.02 abc	1.52 ± 0.17 abcd	1.60 ± 0.10 ab
Konjo	1.60 ± 0.10 abcd	1.38 ± 0.11 bcd	1.26 ± 0.15 cdefgh	1.20 ± 0.29 bc
CIP 515009.573	1.59 ± 0.10 abcd	1.61 ± 0.10 abcd	1.37 ± 0.04 bcdefg	1.71 ± 0.07 ab
Tigoni	1.57 ± 0.06 abcd	1.51 ± 0.05 abcd	1.56 ± 0.06 abcd	1.50 ± 0.10 ab

Nyota	1.54 ± 0.10 abcd	1.67 ± 0.18 abcd	1.22 ± 0.06 defgh	1.46 ± 0.19 ab
Lenana	1.54 ± 0.03 abcd	1.60 ± 0.04 abcd	1.54 ± 0.07 abcd	1.78 ± 0.04 a
Sherekea	1.52 ± 0.10 abcde	1.99 ± 0.04 a	1.26 ± 0.10 cdefgh	1.56 ± 0.15 ab
Arka	1.49 ± 0.08 abcde	1.68 ± 0.05 abcd	1.58 ± 0.18 abc	1.47 ± 0.25 ab
Kenya Karibu	1.47 ± 0.03 abcde	1.48 ± 0.08 abcd	1.53 ± 0.03 abcd	1.38 ± 0.28 abc
Asante	1.46 ± 0.17 abcde	1.57 ± 0.15 abcd	1.36 ± 0.21 bcdefgh	1.64 ± 0.10 ab
CIP 515002.516	1.41 ± 0.16 abcdef	1.65 ± 0.12 abcd	1.36 ± 0.23 bcdefgh	1.35 ± 0.35 abc
CIP 515008.521	1.26 ± 0.08 bcdef	1.66 ± 0.06 abcd	1.05 ± 0.06 gh	0.85 ± 0.14 c
CIP 515008.530	1.24 ± 0.15 cdef	1.87 ± 0.04 ab	1.63 ± 0.12 ab	1.63 ± 0.13 ab
CIP 515008.503	1.15 ± 0.09 def	-	-	-
Chulu	1.07 ±0.42 ef	1.38 ± 0.25 bcd	1.49 ± 0.15 abcde	1.48 ± 0.12 ab
CIP 515004.535	0.98 ± 0.24 f	-	1.01 ± 0.09 h	0.82 ± 0.41 c
CIP 515008.535	0.96 ± 0.19 f	1.17 ± 0.50 d	-	-
CIP 515002.528	-	1.66 ± 0.13 abcd	1.76 ± 0.08 a	1.68 ± 0.09 ab
CIP 515008.561	-	1.25 ± 0.31 cd	-	-
CIP 515011.555	-	-	1.53 ± 0.08 abcd	1.44 ± 0.16 ab
CIP 515006.507	-	-	1.44 ± 0.01 abcdef	1.50 ± 0.24 ab
CIP 515014.567	-	-	1.40 ± 0.16 bcdef	1.32 ± 0.13 abc
CIP 515008.555	-	-	1.16 ± 0.15 efgh	1.19 ± 0.06 bc
CIP 515013.558	-	-	1.11 ± 0.18 fgh	1.43 ± 0.15 ab
CIP 515007.614	-	-	1.04 ± 0.04 gh	1.38 ± 0.04 abc
Purple gold	-	-	1.04 ± 0.15 gh	1.26 ± 0.10 abc
Mean	1.46	1.62	1.36	1.45
MSE	0.02	0.03	0.01	0.03
MSD	0.45	0.56	0.35	0.58

The values are the average asymptomatic tubers ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$, MSE = mean square error, MSD = mean square displacement, - = absence of a particular genotype in a specific trial site per season.

Transformed mean tuber wights (t/ha) varied across genotypes, sites and seasons and were significantly different at $p \leq 0.05$. Genotypes Unica and Sherekea recorded the highest mean tuber

weights (1.23t/ha and 1.57t/ha) at Kiandu and Turi trial sites respectively in season one while CIP 515008.535 and CIP 515008.561 recorded the least mean tuber weights (0.24t/ha and 0.37t/ha) in Kiandu and Turi trial sites respectively. In season two, CIP 515002.528 and Unica recorded the highest mean tuber weights (1.16t/ha and 1.52t/ha) in Kiandu and Turi respectively while CIP 515008.555 and CIP 515008.521 recorded the least mean tuber weights (0.40t/ha and 0.27t/ha) in Kiandu and Turi trial sites respectively (Table 3.11).

Table 3. 11: Transformed mean tuber wights (t/ha) recorded in Kiandu and Turi trial sites in season one and two

Genotypes	Mean transformed tuber weight (t/ha)			
	Season one		Season two	
	Kiandu	Turi	Kiandu	Turi
Unica	1.23 ± 0.12 a	1.49 ± 0.11 ab	1.01 ± 0.08 abcd	1.52 ± 0.05 a
Kenya Mpya	1.08 ± 0.08 ab	1.36 ± 0.08 abc	0.49 ± 0.12 ghi	1.17 ± 0.23 abc
Dutch Robyjin	1.01 ± 0.19 abc	1.31 ± 0.02 abc	0.48 ± 0.14 ghi	0.99 ± 0.12 abcd
Shangi	1.00 ± 0.08 abc	1.22 ± 0.15 abc	0.92 ± 0.21 abcde	1.42 ± 0.07 ab
Konjo	0.96 ± 0.03 abcd	1.10 ± 0.13 bcde	0.53 ± 0.12 fghi	1.17 ± 0.31 abc
Wanjiku	0.94 ± 0.07 abcd	1.33 ± 0.01 abc	1.07 ± 0.12 ab	1.34 ± 0.03 ab
CIP 515009.573	0.88 ± 0.19 bcde	1.14 ± 0.06 bcde	0.64 ± 0.13 defghi	1.31 ± 0.03 abc
CIP 515002.516	0.88 ± 0.08 bcde	1.27 ± 0.17 abc	0.87 ± 0.33 abcdef	0.90 ± 0.40 bcde
Lenana	0.88 ± 0.03 bcde	1.25 ± 0.09 abc	1.03 ± 0.18 abc	1.46 ± 0.03 ab
Kenya Karibu	0.79 ± 0.11 bcdef	1.20 ± 0.12 abcd	0.83 ± 0.11 abcdefgh	1.10 ± 0.30 abc
Nyota	0.77 ± 0.19 bcdef	1.32 ± 0.11 abc	0.68 ± 0.16 cdefghi	1.05 ± 0.41 abcd
Asante	0.69 ± 0.11 cdefg	1.19 ± 0.16 abcd	0.63 ± 0.32 efghi	1.38 ± 0.05 ab
Sherekea	0.67 ± 0.02 defgh	1.57 ± 0.04 a	0.65 ± 0.23 defghi	0.98 ± 0.09 abcde
Tigoni	0.61 ± 0.05 efghi	1.31 ± 0.07 abc	0.97 ± 0.11 abcde	1.19 ± 0.25 abc
Arka	0.58 ± 0.13 efghi	0.80 ± 0.10 def	0.71 ± 0.27 bcdefghi	0.71 ± 0.06 cdef
CIP 515008.503	0.47 ± 0.17 fghij	-	-	-
CIP 515008.521	0.39 ± 0.04 ghij	1.01 ± 0.8 cdef	0.47 ± 0.12 hi	0.27 ± 0.10 f
Chulu	0.35 ± 0.18 hij	0.77 ± 0.02 efg	1.07 ± 0.02 ab	1.16 ± 0.06 abc

CIP 515008.530	0.33 ± 0.10 ij	1.20 ± 0.05 abcd	1.03 ± 0.08 abc	1.02 ± 0.23 abcd
CIP 515004.535	0.30 ± 0.12 ij	-	0.45 ± 0.13 i	0.36 ± 0.35 ef
CIP 515008.535	0.24 ± 0.07 j	0.62 ± 0.30 fg	-	-
CIP 515002.528	-	1.19 ± 0.05 abcd	1.16 ± 0.14 a	1.37 ± 0.14 ab
CIP 515008.561	-	0.37 ± 0.19 g	-	-
CIP 515006.507	-	-	0.96 ± 0.11 abcde	1.15 ± 0.19 abc
CIP 515011.555	-	-	0.85 ± 0.11 abcdefg	1.09 ± 0.15 abc
CIP 515014.567	-	-	0.73 ± 0.19 bcdefghi	0.98 ± 0.15 abcd
CIP 515013.558	-	-	0.63 ± 0.16 efghi	1.13 ± 0.12 abc
Purple gold	-	-	0.47 ± 0.20 ghi	1.02 ± 0.07 abcd
CIP 515007.614	-	-	0.47 ± 0.06 hi	1.08 ± 0.06 abcd
CIP 515008.555	-	-	0.40 ± 0.03 i	0.47 ± 0.13 def
Mean	0.72	1.14	0.75	1.07
MSE	0.01	0.02	0.01	0.04
MSD	0.33	0.41	0.36	0.61

The values are the average yield (t/ha) ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$, MSE = Mean square error, MSD = mean square displacement, - = absence of a particular genotype in a specific trial site per season.

3.3.7 Correlation between bacterial wilt incidence, area under disease progress curve and proportion of symptomatic and asymptomatic tubers and yield

Correlation coefficients of the tested traits (bottom diagonal) and their p-values (top diagonal) are illustrated in (Table 3.12). In both seasons, final wilt incidence (FWI) and area under disease progress curve (ADP) exhibited significant positive correlation at $p \leq 0.05$. Correlations between yield (t/ha) and FWI were negative and insignificant across seasons and trial sites except Kiandu season one. Similarly, correlation between yield (t/ha) and ADP was negative and insignificant across seasons and trial sites except Kiandu season two and Turi season two. The correlation between proportion of symptomatic tubers (ST) and number of asymptomatic tubers (AST) was inconsistent with other traits [FWI, ADP and yield (t/ha)].

Table 3. 12: Pearson correlation coefficients and p-values of various traits for the 30 genotypes in different sites during the two trial seasons

	Kiandu Season 1						Kiandu season 2				
	FWI	ADP	ST	AST	Yield		FWI	ADP	ST	AST	Yield
FWI	1.00	0.01^{*}	0.42^{ns}	0.01^{*}	0.03^{*}	FWI	1.00	0.00^{*}	0.49^{ns}	0.11^{ns}	0.07^{ns}
ADP	0.96	1.00	0.26^{ns}	0.08^{ns}	0.06^{ns}	ADP	0.98	1.00	0.37^{ns}	0.08^{ns}	0.03^{*}
ST	-0.48	-0.63	1.00	0.63^{ns}	0.79^{ns}	ST	0.41	0.52	1.00	0.12^{ns}	0.10^{ns}
AST	-0.83	-0.84	0.29	1.00	0.03^{*}	AS	-0.79	-0.85	-0.78	1.00	0.01^{*}
Yield	-0.92	-0.87	0.17	0.92	1.00	Yield	-0.85	-0.92	-0.81	0.96	1.00
	Turi season 1						Turi season 2				
	FWI	ADP	ST	AST	yield		FWI	ADP	ST	AST	yield
FWI	1.00	0.00^{*}	0.09^{ns}	0.07^{ns}	0.11^{ns}	FWI	1.00	0.00^{*}	0.63^{ns}	0.08^{ns}	0.08^{ns}
ADP	0.99	1.00	0.10^{ns}	0.06^{ns}	0.09^{ns}	ADP	0.98	1.00	0.73^{ns}	0.05^{*}	0.04^{*}
ST	-0.82	-0.80	1.00	0.51^{ns}	0.45^{ns}	ST	-0.29	-0.21	1.00	0.75^{ns}	0.72^{ns}
AST	-0.85	-0.86	0.39	1.00	0.07^{ns}	AST	-0.83	-0.87	-0.20	1.00	0.03^{*}
Yield	-0.79	-0.82	0.45	0.84	1.00	Yield	-0.83	-0.90	-0.22	0.92	1.00

FWI = Final wilt incidence, ADP = area under disease progress curve, ST = Proportion of symptomatic tubers and AST = Number of asymptomatic tubers. * = significant at $p \leq 0.05$ and ^{ns} = not significant at $p \leq 0.05$.

3.3.8 Ranking of potato genotypes in terms of resistance levels to bacterial wilt pathogen based on disease severity scores

Average ranking of each genotype in terms of their resistance levels to bacterial wilt pathogen per experimental site per season are illustrated in (Table 3.13). Average ranking revealed that genotypes CIP 515004.535 and CIP 515008.561 were highly resistant, CIP 515008.521 was resistant while Sherekea, CIP 515002.516, CIP 515008.530, CIP 515008.503, CIP 515008.555, CIP 515014.567 and CIP 515013.558 were moderately resistant. Shangi, Asante, Tigoni, Arka, CIP: 515008.535 and CIP: 515007.614 were ranked as highly susceptible genotypes.

Table 3. 13: Ranking of potato genotypes in terms of resistance levels to bacterial wilt pathogen based on disease severity scores

Genotypes	Resistance levels of each genotype				Average ranking
	Season one		Season two		
	Kiandu	Turi	Kiandu	Turi	
Chulu	5	3	4	5	4
Sherekea	3	3	3	2	3
Shangi	6	6	6	5	6
Kenya Karibu	3	6	5	3	4
Kenya Mpya	5	3	6	5	5
Nyota	6	6	5	3	5
Unica	5	5	5	4	5
Wanjiku	4	5	5	2	4
Dutch Robyjin	4	5	6	4	5
Asante	5	6	6	5	6
Lenana	4	5	5	3	4
Tigoni	6	5	6	5	6
Konjo	5	4	6	5	5
Arka	5	5	6	6	6
Purple gold	-	-	5	4	5
CIP 515009.573	4	5	5	2	4
CIP 515002.516	3	2	4	2	3
CIP 515008.530	2	2	3	4	3

CIP 515008.521	3	1	3	1	2
CIP 515008.535	5	6	-	-	6
CIP 515008.503	3	-	-	-	3
CIP 515004.535	2	-	1	1	1
CIP 515008.561	-	1	-	-	1
CIP 515002.528	-	6	6	4	5
CIP 515008.555	-	-	5	1	3
CIP 515007.614	-	-	6	5	6
CIP 515014.567	-	-	4	2	3
CIP 515011.555	-	-	4	5	5
CIP 515013.558	-	-	3	2	3
CIP 515006.507	-	-	5	4	5

1 = Highly resistant, 2 = Resistant, 3 = Moderately resistant, 4 = Moderately susceptible, 5 = Susceptible, 6 = Highly susceptible, - = absence of a particular genotype in a specific trial site per season.

3.4 Discussion

Initial bacterial wilt colony counts revealed that Kiandu trial site had almost half the bacterial wilt colony counts recorded in Turi trial site. However, both the two trial sites expressed similar above ground bacterial wilt symptoms (incidence) during the field scouting process. The variation can be attributed to different agricultural activities carried out in these farms in the previous season, source of seed potato tubers planted during the scout season and abiotic factors (edaphic factors, temperatures and soil moisture) which might have affected bacterial wilt disease establishment and spread (Bocsanczy *et al.*, 2012; Bhanwar, 2022; Buddenhagen, 1985; Jaing *et al.*, 2021). Bacterial wilt colony counts in both trial sites increased in the second season compared to season one and this can be attributed to infected potato haulms which were spread evenly and left to decompose in the trial sites after season one harvest. Additionally, it can also be attributed to continuous planting of potatoes in the same plots since the scouting period which could have facilitated inoculum buildup in the two trial sites (Agutaa, 2015; Muthoni *et al.*, 2014).

The isolated bacteria from the two trial sites oxidized all the disaccharide sugars and hexose alcohols and hence can be classified as *R. pseudosolanacearum* sp. nov. biovar III race 1 [*Ralstonia solanacearum* (phylotype I)] (Boschi *et al.*, 2017; Khasabulli *et al.*, 2017; Popoola *et al.*, 2015;

Rahman *et al.,* 2010). The biovar test results from Turi isolates confirmed the findings of Rostand *et al.* (2018) who reported biovar III (phylotype I) as the most prevalent phylotype causing bacterial wilt of potato in Nakuru County. However, the test results from Kiandu isolates contrasted the findings of Sharma *et al.* (2022) who reported phylotype II as the most prevalent strain in Nyeri county and this variation can be attributed to difference in sampling sites.

The mean percent plant emergence varied from one genotype to another across sites and seasons. The variation between genotypes can be attributed to different genetic traits of each genotype coupled with their response to wounds created during planting. Kiandu trial site recorded the highest overall percent plant emergence in both seasons, and this could be due to high temperatures recorded in Kiandu at planting compared to those recorded in Turi trial site (Kooman *et al.*, 1996). Season two recorded an overall low percent plant emergence in both trial sites compared to season one. The low percent plant emergence in season two might have resulted from high rainfall amounts at planting compared to season one. This could have caused excessive soil moisture which favored establishment and spread of pre-emergence damping-off diseases since non-emerged tubers were found to rot during hilling (Lamichhane *et al.,* 2017).

Bacterial wilt incidence (BWI) and Area under disease progress curve (AUDPC) varied across genotypes, trial sites and seasons. In both trial sites, BWI commenced at 60 days after planting (DAP) for both seasons except Kiandu trial site in season one. The late bacterial wilt disease symptom expression at Kiandu trial site in season one can be attributed to low initial bacterial wilt populations according to colony count results. Most genotypes began to express BWIs at flowering and the BWI increased rapidly from 60 – 88 DAP in both the two trial sites and seasons. This is because at this stage of plant growth, the potato crop absorbs a lot of water from the soil to replace the high amount of water lost through the transpiration. High amounts of bacterial wilt inoculum could have also been absorbed together with the soil water leading to xylem blockage and hence increased BWI. The final BWIs and AUDPCs were significantly different among potato genotypes used in the study in both the two sites and seasons. This is due to the different response exhibited by various potato varieties and/or cultivars to bacterial wilt infection owing to varied host-pathogen-environment interactions (Aliye *et al.,* 2015; Muthoni *et al.,* 2014). Turi and Kiandu trial sites recorded insignificantly different mean final BWI in season one while Kiandu recorded the highest mean final BWI in season two. In general, season one recorded the highest final disease indices (BWI and AUDPC) compared to season two and this could be because

season one was a short rain season with low rainfall amounts and high temperatures compared to season two which was a long rain season with high rainfall amounts and low temperatures. The high temperatures could have enhanced rapid bacterial wilt disease development and spread at both trial sites in season one and Kiandu trial site in season two (Champoiseau, 2008).

The proportion/percentage number of symptomatic tubers were significantly different across all the tested genotypes in both the two trial sites and seasons. In both trial seasons, almost all the genotypes failed to exhibit externally visible bacterial wilt symptoms on the harvested tubers but when cut, the infected tubers exhibited brown discoloration of the vascular ring and the adjacent tissues extending to the pith and/or tuber cortex. A white-milky bacterial ooze was observed from the freshly cut section of the infected tubers while creamy fluid exudate was detected on the vascular rings of the cut surface (Champoiseau *et al.,* 2009; Priou *et al.,* 1999). The invisible external symptoms on most of the assessed tubers may be attributed to late disease establishment since disease symptoms appeared at flowering (CABI, 2020, Priou *et al.,* 1999). Similarly, the invisible external symptoms can also be attributed to low potato seed degeneration effect since basic seeds and second-generation seeds were used for commercial varieties and clones respectively (Thomas-Sharma *et al.,* 2016). Some genotypes like CIP 515008.535 exhibited high above ground wilt symptoms (bacterial wilt incidences and AUDPCs) but their tubers failed to show both external and internal bacterial wilt symptoms. This can be attributed to their colored flesh which makes it difficult to observe internal tuber wilt symptoms upon tuber dissection. In general, season two had the highest proportion/percent number of symptomatic tubers compared to season one and this can be attributed to high bacterial wilt colony counts recorded in season two as opposed to season one (Champoiseau, 2008; Muthoni *et al.,* 2014).

The number of asymptomatic tubers and tuber weight (t/ha) were significantly different and varied across genotypes, experimental sites and seasons. This variation can be attributed to the inherent yield potential of each genotype, environmental effects from specific trial sites and interaction between the test genotypes and environment (Placide *et al.,* 2019; Placide *et al.,* 2022). Turi trial site recorded a high number of asymptomatic tubers and tuber weights (t/ha) in both the two trial seasons compared to Kiandu trial site. The high yield parameters in Turi can be attributed to low temperatures especially during tuber bulking and high rainfall amounts received in Turi compared to Kiandu trial site (Dahal *et al.,* 2019; Kumar *et al.,* 2003; Muthoni *et al.,* 2014; Placide

et al., 2022). The yield results confirmed the findings of Placide *et al.* (2022) who reported varied yield results across genotypes, trial sites and seasons.

Bacterial wilt incidence at 88 DAP and area under disease progress curve exhibited significant positive correlation. This is an indication that area under disease progress curve increased with increase in wilt incidence and this was similar to the findings of Soesanto, *et al.*, (2019) who reported positive correlation between area under disease progress curve and bacterial wilt incidence on tomatoes. Yield (t/ha) correlated negatively with bacterial wilt incidence at 88 DAP and area under disease progress curves. Low disease indices (disease incidence and disease severity) reduce potato plant damage resulting in increased crop growth and hence increased yields (Adhikari & Basnyat, 1998; Rivard *et al.,* 2012; Soesanto, *et al.,* 2019). Proportion/percent number of symptomatic tubers and number of asymptomatic tubers exhibited inconsistent correlation with other traits [final wilt incidence, area under disease progress curve and yield (t/ha)]. The inconsistent correlation can be attributed to different sets of genes expressed by bacterial wilt pathogens during both asymptomatic and symptomatic disease expression. It can also be attributed to different resistance levels of various genotypes used against bacterial wilt pathogens (Muthoni *et al.,* 2014; Swanson *et al.,* 2005).

Genotype ranking varied across sites and seasons, and this was consistent with research findings of various scientists who reported varied resistance levels of various potato genotypes to bacterial wilt infection (Ateka *et al.,* 2001; Felix *et al.,* 2010; Muthoni *et al.,* 2014). The average rankings classified genotypes CIP 515004.535 and CIP 515008.561 as highly resistant, CIP 515008.521 as resistant, Sherekea, CIP: 515002.516, CIP: 515008.530, CIP: 515008.503, CIP: 515008.555, CIP 515014.567 and CIP 515013.558 as moderately resistant, chulu, Kenya Karibu, Wanjiku and Lenana as moderately susceptible, Kenya Mpya, Nyota, Unica, Dutch Robyjin, Konjo, Purple gold, CIP 515002.528, CIP 515011.555 and CIP 515006.507 as susceptible while Shangi, Asante, Tigoni, Arka and CIP 515008.535 were highly susceptible. Most of the highly resistant, resistant, moderately resistant and moderately susceptible genotypes especially the CIP clones had very low yields [tuber weights (t/ha)] which affected the seed balking process and may also affect their future adoption and commercialization by farmers. This confirmed the study findings of Yuliar *et al.* (2015) who reported negative correlation of bacterial wilt resistant cultivars with yield and tuber quality and hence high rejection rates by farmers and consumers. Previous studies ranked varieties; Kenya Dhamana (CIP 800228), Kenya Sifa, Kenya Karibu,

Mauritius clone (89016), Cruza-148 (CIP 720118) and Ingabire as resistant to bacterial wilt while varieties; Asante (CIP 381381.20), Tigoni (CIP 381381.13), Nyayo, and Dutch Robyjin were the most susceptible (Ateka *et al.*, 2001; Felix *et al.*, 2010; Muthoni *et al.*, 2014). This study also confirmed the high susceptibility of Asante, Tigoni and Dutch Robyjin against bacterial wilt pathogens.

3.5 Conclusion and Recommendations

The study results ranked genotypes CIP 515004.535 and CIP 515008.561 as highly resistant, CIP 515008.521 as resistant, Sherekea, CIP 515002.516, CIP 515008.530, CIP 515008.503, CIP 515008.555, CIP 515014.567 and CIP 515013.558 as moderately resistant, Chulu, Kenya Karibu, Wanjiku, Lenana and CIP 515009.573 as moderately susceptible, Kenya Mpya, Nyota, Unica, Dutch Robyjin, Konjo, Purple gold, CIP 515002.528, CIP 515011.555 and CIP 515006.507 as susceptible while Shangi, Asante, Tigoni, Arka and CIP 515008.535 were highly susceptible. All the highly resistant and resistant clones had very low yields [tuber weights (t/ha)] compared to commercialized varieties which may affect their future adoption and commercialization by farmers. Thus, future studies should focus on integrating high yielding traits on these genotypes for increased chances of adoption and commercialization by farmers. Additionally, all the highly resistant, resistant, moderately resistant and moderately susceptible genotypes except CIP 515008.561 displayed internal bacterial wilt symptoms from dissected tubers and hence can only be suitable for ware potato production as opposed to seed potato production. Advanced bacterial wilt detection techniques such as serological tests and molecular detection should be done on genotypes without symptomatic tubers to validate absence of latent infection symptoms from such genotypes.

CHAPTER FOUR

ANTIBACTERIAL ACTIVITY OF GUAVA, MORINGA, CAMPHOR BUSH AND PELARGONIUM EXTRACTS ON BACTERIAL WILT (*Ralstonia solanacearum*) OF POTATO

Abstract

In-vitro efficacy of various botanicals (plant extracts) have been reported against Bacterial wilt (*Ralstonia solanacearum*) of potatoes. However, this research area has not been fully explored. Thus, antibacterial activity of ethanol and acetone plant extracts from guava (*Psidium guajava*), drumstick (*Moringa oleifera*), camphor bush (*Tarchonanthus camphoratus*) and pelargonium (*Pelargonium zonale*) against *R. solanacearum* was evaluated *in-vitro* at a concentration of 100 mg/mL of 1% Dimethlysulfoxide (DMSO) using disk diffusion technique. The *R. solanacearum* was isolated from infected haulms collected from potato growing fields at the University of Nairobi. The most effective extracts were subjected to further screening at different concentrations to determine their minimum inhibitory concentrations (MICs). All the four plant extracts showed varied antibacterial efficacy. *P. zonale* leaves extract was the most effective with growth inhibition zones of 18.73mm and 18.60mm for ethanol and acetone solvents respectively. The average growth inhibition zones for each plant extract were not significantly different at $p \leq 0.05$ among extraction solvents. The minimum inhibitory concentration (MIC) results showed that antibacterial activity of *P. zonale* and *P. guajava* leaf started at 6.25 mg/mL with growth inhibition zones of 7.67 and 8.0 mm for ethanol and acetone solvents respectively. *P. zonale* and *P. guajava* leaf extracts exhibited significantly higher antibacterial activity at $p \leq 0.05$ compared to other extracts. Thus, further research should be conducted to assess their antibacterial potency against *R. solanacearum* both *in-vivo* and under field conditions.

4.1 Introduction

Bacterial wilt caused by *Ralstonia solanacearum* is the second most devastating disease after late blight (*Phytophthora infestans*) affecting global potato production (Ahmed *et al.,* 2022; Mutimawurugo *et al.,* 2019). The pathogen is soil-borne and can persist in the soil over a long period of time usually ≥ 3 years and hence its management has proved difficult in infested potato fields (Gutarra *et al.,* 2017; Sharma *et al.,* 2017). Integrated disease management has been proposed for adoption by various farmers for the management of this disease (Uwamahoro *et al.,* 2018). Since the pathogen colonizes the xylem vessels and tubers, it is difficult to control upon

successful disease establishment on the host plant and hence integrated management options should be aimed at preventing initial disease infection (Karim & Hossain 2018; Mutimawurugo *et al.,* 2019). However, effectiveness of integrated disease management against bacterial wilt pathogen is affected by site-specific nature and diversity of bacterial wilt pathogen resulting in extensive use of conventional bactericides (Biswal & Dhal 2018; Sarkar & Chaudhuri 2016).

Due to negative environmental effects associated with conventional bactericides, efforts have been focused on development of botanicals as eco-friendly management options against bacterial wilt pathogens (Karim & Hossain 2018; Oboo *et al.,* 2014). The phytobiocidal effect of various plant extracts against *R. solanacearum* has been demonstrated by several researchers both *in-vitro* and *in-vivo*. For instance, Mutimawurugo *et al.* (2020) reported *in-vitro* antibacterial activity of tobacco, wild marigold and garlic against bacterial wilt pathogen with inhibition zones ranging from 18-20 mm and Minimum Inhibition Concentrations (MICs) ranging from 6.25-12.5 mg/mL respectively. Studies by Biswal (2015) revealed *in-vitro* antibacterial activity of aqueous plant extracts from 20 different plant species: *Moringa oloifera*, *Psidium guajava* and *Bauhinia recimosa* among others against *R. solanacearum* of potatoes in India. Similarly, Oboo *et al.* (2014) also reported *in-vivo* antibacterial activity of *Tarchonanthus camphoratus* against *R. solanacearum* in Kenya.

Despite the documented positive efficacy results of antibacterial activity of various plant extracts against bacterial wilt pathogens, the research area has not been fully exploited (Borges *et al.,* 2018; Mutimawurugo *et al.,* 2020). For instance out of 7 registered commercial products; ENRICH BM WP (Bronopol 27%w/w), METHAM SODIUM 51 LS (Metham sodium 510g/L), METACOP 450 WP and EXTRA-COP 450 WP (Metalaxyl-M 50g/L + Copper Oxychloride 400g/L), CHALIM 150 Powder (Calcium hypochlorite 150 g/Kg), KOBE 1.2 SL (Chrysophanol 12g/L) and KHITO 1.2 SL (Chrysophanol 12g/L) for the management of bacterial wilt pathogen in Kenya, only KOBE 1.2 SL (Chrysophanol 12g/L) and KHITO 1.2 SL (Chrysophanol 12g/L) are derived from plant extracts (https://www.pcpb.go.ke/biopesticides-on-crops/). The under exploitation of botanicals in the management of bacterial wilt has been attributed to rapid degradation, volatilization of their bioactive compounds under field condition and inadequate knowledge on various formulations with slow-release actions (Borges *et al.,* 2018). Both KOBE 1.2 SL (Chrysophanol 12g/l) and ENRICH BM WP (Bronopol 27%w/w) control bacterial wilt disease through induction of host plant resistance (Liu *et al.,* 2016; https://agroduka.com/enrich-

bm). However, *in-vitro* efficacy of bronopol against both gram-negative and gram-positive bacteria have also been reported (Stretton & Manson, 1973).

Despite the documented positive efficacy results of antibacterial activity of various plant extracts against *R. solanacearum* of potatoes, this research area has not been fully explored (Borges *et al.,* 2018; Mutimawurugo *et al.,* 2020). Therefore, the purpose of this work was to test the antibacterial activity of acetone and ethanol extracts of various plant species *in vitro* against *R. solanacearum* isolated from infected potato haulms collected from potato growing fields at the University of Nairobi, Kenya.

4.2 Materials and methods

4.2.1 Sample collection

Infected plants showing typical bacterial wilt symptoms were collected from potato growing fields at the University of Nairobi, Upper Kabete Campus. The station is located at a mean altitude of 1980 m, latitude 1° 15 S and longitude 36° 41' E, in Lower Highland Zone II (LH2) of the Agro-ecological zone (AEZs) of Kenya (Jaetzold *et al.,* 2007). Ten infected plant samples were collected in khaki paper bags, placed in a cool box and taken to the food microbiology laboratory at the University of Nairobi.

4.2.2 Isolation and purification of *Ralstonia solanacearum* pathogen

To confirm their infection, the samples were tested for bacterial ooze production and bacterial wilt pathogen isolated from samples which produced bacterial ooze. The bacterial wilt pathogen was isolated on a selective medium; Triphenyl tetrazolium chloride (Kelman's TZC agar) as described by Kelman (1954) and Karim and Hossain (2018).

4.2.3 Bacterial wilt pathogen identification and confirmatory tests

The isolated bacterium was identified based on morphological, physiological, cultural, biochemical and pathogenicity tests according to She *et al.* (2017). The virulent and non-virulent colonies were differentiated using colony characteristics on Kelman's TZC medium. A loopful of the test bacterium was smeared on a clean glass slide with a drop of sterile water to determine Gram staining. The smeared bacterium was air-dried and then heat-fixed over a Bunsen flame. The smear was then stained with crystal violet, Lugol's iodine and safranin with 3 rinses using water according to Rahman *et al.* (2010). After counterstaining, the slide was blot-dried and examined under a light microscope at x100 magnification with a drop of immersion oil. The Potassium hydroxide (KOH) solubility test was carried out according to Priou *et al.* (1999) but with slight

modification. As opposed to Priou *et al.* (1999) who carried out KOH directly on bacterial ooze from potato tuber, in this study, KOH test was conducted using isolated bacteria on a sterile glass slide. Biovar identification through carbohydrate fermentation test was carried out according to Rahman *et al.* (2010) but with slight modifications as outlined in section 3.3.4.

4.2.4 Pathogenicity test

The pathogenicity test was performed using one week old seedlings from certified seed potato tubers (Shangi variety) as described by Priou *et al.* (1999). Ten certified seed potato tubers were planted in pots (polythene sleeves) containing sterile soil media in a greenhouse and allowed to emerge. From the emerged seedlings, 5 seedlings were inoculated while the remaining 5 non-inoculated seedlings were used as checks. Bacterial cultures of 4.5 x 10^8 CFU/mL were prepared by culturing the isolated bacteria on Casamino Acid-Peptone Glucose (CPG) media without triphenyl tetrazolium chloride (TZC) at 28±1^{0}C for 48 hours. The bacterial cells were harvested by washing the cultures in sterile distilled water. Two days prior to inoculation, the test plants were starved without irrigation and wounds created around the root zone using sterile scalpels. Ten milliliters of 4.5 x 10^8 CFU/mL bacterial suspension was inoculated around the root zone of 5 emerged seedlings using a syringe while the other 5 seedlings were inoculated with 10 ml of sterile distilled water. The plants were maintained and monitored for symptom development for a period of 2-5 weeks. The temperatures inside the glass house ranged from 25-32°C while the relative humidity ranged from 80-90%. Bacterial pathogen was re-isolated from the symptomatic seedlings using TZC media.

4.2.5 Preparation of plant extracts

Four plants namely guava (*Psidium guajava*), drumstick (*Moringa oleifera*), camphor bush (*Tarchonanthus camphoratus*) and pelargonium (*Pelargonium zonale*) were used in this study (Table 4.1). Fresh leaves of pelargonium (*Pelargonium zonale*) and guava (*Psidium guajava*) were collected from Taita Taveta and Mau Narok, Kenya, respectively. Moringa leaves and seeds were sourced from Kajiado County. The identities of the test plants were confirmed by a taxonomist and sample specimens kept at the Department of Crops, Horticulture and Soil Sciences (CHS), Egerton University, Kenya.

The crude extracts were extracted from leaves in all the selected plants except for *Moringa oleifera* in which the extracts were taken both from leaves and seeds as described by Biswal (2015) but with slight modifications. As opposed to Biswal (2015) who used only water (a polar solvent)

as an extraction solvent, in this study two solvents; ethanol (polar solvent) and acetone (non-polar solvent) were used as extractants. Additionally, the extracted compounds were concentrated to pastes through solvent evaporation to estimate extract yields. Healthy plant parts were collected and washed under running tap water followed by shade drying at room temperature for three weeks. After complete drying, the plant materials were ground into fine powders. Before extraction, the pH of each plant extract was determined according to Silas *et al.* (2012) with slight modifications. As opposed to Silas *et al.* (2012) who soaked 100 g of each plant sample in 5 liters of distilled water for a period of 90 days with continuous pH testing on a daily basis, in this study 5 g of each ground plant material was dissolved in 50 mL of distilled water in the ratio of 1:10. The mixtures were shaken on a mechanical shaker (end to end reciprocating shaker) at 600 revolutions per minute for 1 hour and the pH of each solution measured using a pH meter. After pH determination, 20 g of fine powder of each plant material was soaked in 200 mL of each extraction solvent (ethanol and acetone) with regular stirring for 48 hours. After 48 hours, the solutions were filtered through double layers of muslin cloth and the filtrates collected in different sterile bottles. The filtrates were centrifuged at 9000 rpm for 10 minutes and the supernatants filtered through Whatman filter papers to remove the remaining particles. The filtrates were concentrated to pastes at temperatures slightly below the boiling points of each solvent (50^0C for acetone extracts and 60^0C for ethanol extracts) in two different water baths. The boiling point of acetone is 56^0C while that of ethanol is 78^0C. The beakers were stirred regularly to prevent the pastes from sticking on their walls. The pastes were air-dried overnight, weighed and stored at 4^0C. Percent extract yields were calculated using Eq. (1) as outlined by Mostafa *et al.* (2018).

$$Yield\ (\%) = \frac{\text{Weight of extracted plant residue (paste)}}{\text{Weight of raw plant sample}} * 100 \quad (8)$$

Table 4.1: Plant species and plant parts used in the study

Plant species	Family	Local name	Common name	Plant part
Moringa oleifera	Moringaceae	Moringa	Drumstick tree	leaves and seeds
Tarchonanthus camphoratus	Asteraceae	Leleshwa	Camphor bush	Leaves
Psidium guajava	Myrtaceae	Mapera	Common guava	Leaves
Pelargonium zonale	Geraniaceae	Geraniums	Pelargoniums	Leaves

4.2.6 Antibacterial bioassays

The antibacterial activity experiment was laid out in a completely randomized design (CRD) with 5 replicates (5 disks per plate) and 9 treatments (Table 4.2). The antibacterial activity of extracts from the four plants was tested using disk diffusion technique according to Mostafa *et al.* (2018). One hundred milligrams of each plant extract (paste) was reconstituted in 1 mL of 1% Dimethyl sulfoxide (DMSO). Twenty milliliters of molten TZC medium were poured on each sterile petri dish and allowed to cool and solidify. About 100 µL of bacterial suspension at a concentration of 4.2×10^5 CFU/mL was added on the surface of each petri dish and spread gently and uniformly using a sterile L-shaped glass rod. Sterile disks of 6 mm diameter prepared through punching and sterilization of Whatman filter papers were impregnated with 15 µL of each plant extract and allowed to drain for 30 minutes. For negative control, the disks were impregnated with 15 µL of sterile distilled water and 1% DMSO respectively while those of positive control were impregnated with 15 µL of KOBE 1.2 SL (Chrysophanol 12 g/l) (registered plant extract in management of bacterial wilt of potatoes) and ENRICH BM (Bronopol 27%w/w) registered bactericide for management of bacterial wilt of potatoes. The disks were placed on the inoculated TZC media and plates refrigerated at 4°C for 2 hours for optimal diffusion of applied treatments and then incubated at 28±1°C for 48 hours. The presence of growth inhibition zones was observed, the diameters measured using a calibrated ruler and considered as a sign of antibacterial activity.

Table 4.2: Treatment description of antibacterial assay experiment

Treatments	Treatment type
	Plant extracts Conc. 100 mg/mL
Treatment 1	*Pelargonium zonale* leaves
Treatment 2	*Psidium guajava* leaves
Treatment 3	*Tarchonanthus camphoratus* leaves
Treatment 4	*Moringa oleifera* leaves
Treatment 5	*Moringa oleifera* seeds
	Positive controls at commercial rates
Treatment 6	ENRICH BM (Bronopol 27%w/w)
Treatment 7	KOBE 1.2 SL (Chrysophanol 12g/l)
	Negative controls
Treatment 8	Distilled water
Treatment 9	1% DMSO

Positive control comprised registered conventional bactericide [ENRICH BM (Bronopol 27%w/w)] and botanical [KOBE 1.2 SL (Chrysophanol 12g/l)] form management bacterial wilt of potatoes at commercial rates. BM (Bronopol 27%w/w) was sourced from Osho Chemical Industries Limited while KOBE 1.2 SL (Chrysophanol 12g/l) was sourced from Amiran Kenya limited. Negative control comprised distilled water and 1% Dimethyl sulfoxide (DMSO).

4.2.7 Determination of minimum inhibitory concentration (MIC) of effective plant extracts

The minimum inhibitory concentration (MIC) experiment of most effective plant extracts was laid out in a complete randomized design (CRD) with 5 replicates (5 disks per plate) and 5 treatments (different concentrations) (Table 4.3). The most effective extracts (*Pelargonium zonale* and *Psidium guajava*) which exhibited high antimicrobial activity at 100 mg/mL were subjected to further screening to determine the minimum inhibitory concentration (MIC) using disk diffusion method as described by Mostafa *et al.* (2018). Different concentrations of these two plant extracts were prepared by further dilution of 100 mg/mL concentrations to attain 50 mg/mL, 25 mg/mL, 12.50 mg/mL, 6.25 mg/mL and 3.13 mg/mL. Twenty milliliters of molten TZC medium per petri dish was poured on sterile petri dishes and allowed to cool and solidify. One hundred microliters of bacterial suspension at a concentration of 4.2×10^5 CFU/mL were added on the surface of each petri dish and spread gently and uniformly using a sterile glass rod. Different sterile disks (6 mm

diameter) were impregnated with 15 µL of each concentration and allowed to drain for 30 minutes. The disks were then placed on the inoculated TZC media and plates were placed in the refrigerator at 4^0C for 2 h for optimal diffusion of applied treatments and then incubated at 28 ± 1^0C for 48 hours. The presence of growth inhibition zones was observed, and their diameters were measured and recorded.

Table 4. 3: Treatment description of minimum inhibitory concentration (MIC) experiment

Treatments	Treatment type
	Conc. Mg/mL
Treatment 1	50.00
Treatment 2	25.00
Treatment 3	12.50
Treatment 4	6.25
Treatment 5	3.13

Pelargonium zonale and *Psidium guajava* extracts were used for MIC experiment

4.2.8 Data analysis

The antibacterial activity data were subjected to analysis of variance (ANOVA) using R software, version 4.2.2 (R Studio Team, 2020). Treatment means were separated using Tukey's Honestly Significant Difference (HSD) at $p \leq 0.05$ with the agricolae package. The following statistical model was fitted:

$$Yij = \mu + Ti + \varepsilon ij \quad (9)$$

Whereby Means μ= general mean, $T_{i=}$ Treatment effect and ε_{ij} = effects due to random error.

4.3 Results

4.3.1 Confirmatory and biovar identification through carbohydrate fermentation tests

The bacterial isolate colonies had an irregular, spherical, white fluidal appearance with pink cores (Plate 4.1A). A mucoid (elastic and viscous) thread was observed when a wire loop was raised from the bacterial solution a few centimeters from the glass slide used for potassium hydroxide (KOH) test (Plate 4.1B). The microscopic result indicated that the bacterial isolate cells did not preserve the crystal violet color in the Gram stain test, but they did retain the pink color of the counter strain, and their form was rod shaped. This indicates that the bacterial isolate was a Gram-negative bacterium (Plate 4.1C). The isolated bacterial pathogen oxidized all the

disaccharide sugars and hexose alcohols. This was indicated by color change from red to yellow in inoculated universal bottles compared to the checks (non-inoculated bottles) (Plate 4.2).

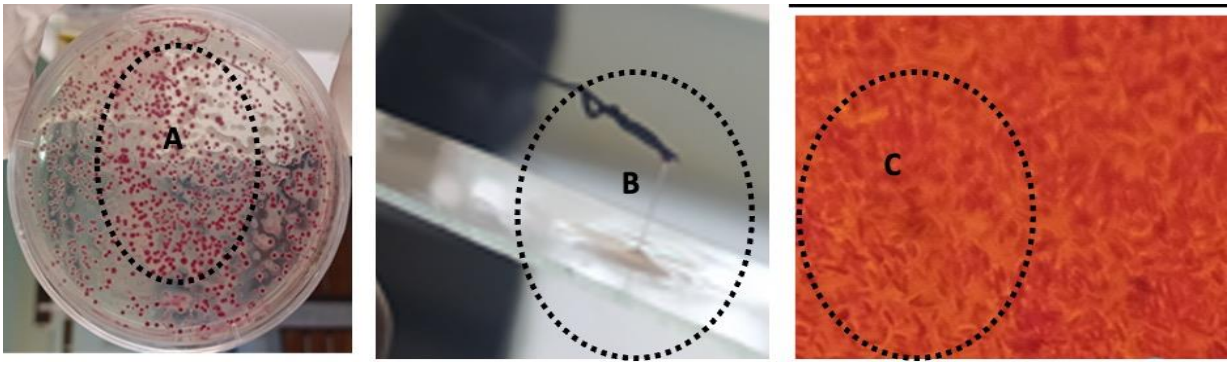


Plate 4. 1: A) Colony characteristics of *R. solanacearum* on TZC medium, B) The formation of *R. solanacearum* mucoid threads because of a KOH solubility test and C) Rod-shaped Gram-Negative *R. solanacearum* bacteria under a light microscope.

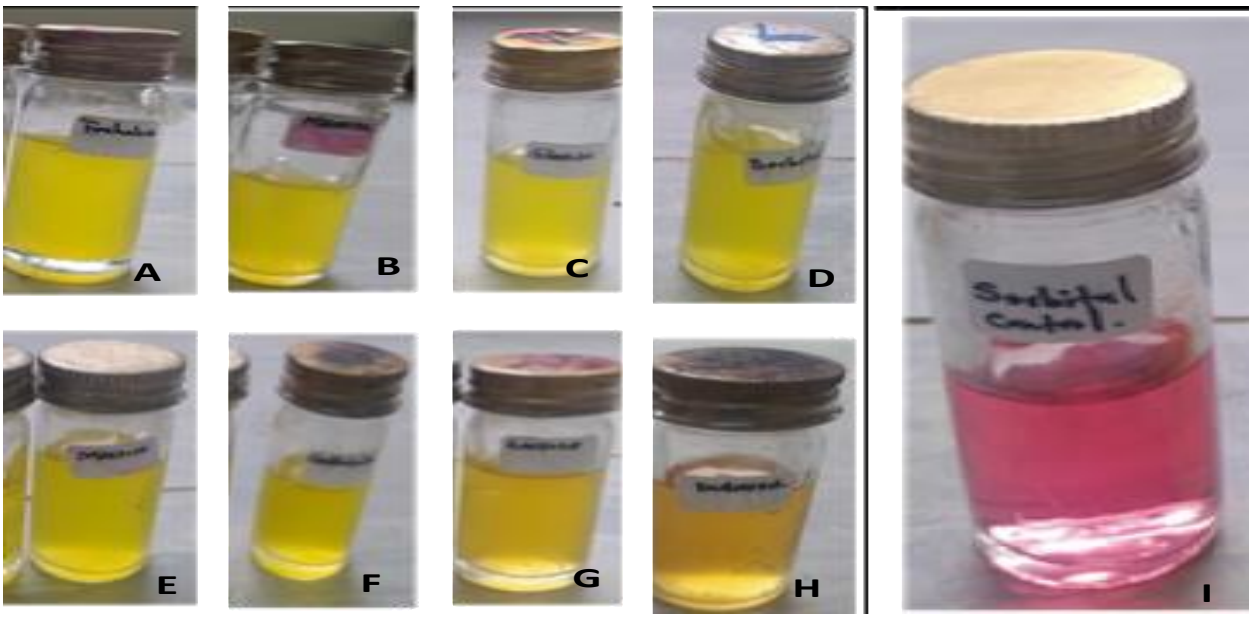


Plate 4. 2: Biovar identification of the isolated bacterium based on oxidation of disaccharides sugars and hexose alcohols. A) Trehalose, B) Mannitol, C) Dextrose, D) Sorbitol, E) Maltose, F) Celluboise, G) Lactose, H) Dulcitol I) negative check.

4.3.2 Pathogenicity test

The inoculated seedlings began to exhibit wilt symptoms 7 days after inoculation. The initial symptoms appeared as wilted apical leaves during the day, but the affected seedlings recovered at night. As the symptoms progressed, the wilted leaves failed to recover and the whole

infected plants withered two weeks later. No symptoms were observed on non-inoculated plants (Plate 4.3). The isolated bacteria from the symptomatic plants displayed the same morphological characteristics as that of the original pathogen on TZC media. The isolated bacterium was identified as *R. pseudosolanacearum* sp. nov. based on morphological and biochemical traits as well as pathogenicity tests.

Plate 4. 3: Symptom expression in test plants used for pathogenicity test

4.3.3 Plant extract yields

The pH and yield percentage of plant extracts extracted by both acetone and ethanol are shown in (Table 4.4). Extracts from 20 g of dried and ground powder of plant material produced varied yields ranging from 0.22–3.02 g for those extracted using acetone solvents and 1.39–4.59 g for those extracted using ethanol solvents. Except for Moringa seed powder, Ethanol solvent recorded high percentage yields compared to acetone.

Table 4. 4: The pH of plant extracts and their extract yields per extraction solvent

		Extract yield (g)		Percent extract yield (%)	
Plant species	pH	Acetone	Ethanol	Acetone	Ethanol
Moringa oleifera leaves	5.75	2.41	4.59	12.05	22.95
Moringa oleifera seeds	5.33	3.02	2.93	15.10	14.65
Tarchonanthus camphoratus	5.51	0.50	1.39	2.50	6.95
Psidium guajava	5.26	1.62	3.40	8.10	17.00
Pelargonium zonale	4.78	0.22	3.81	1.10	19.05

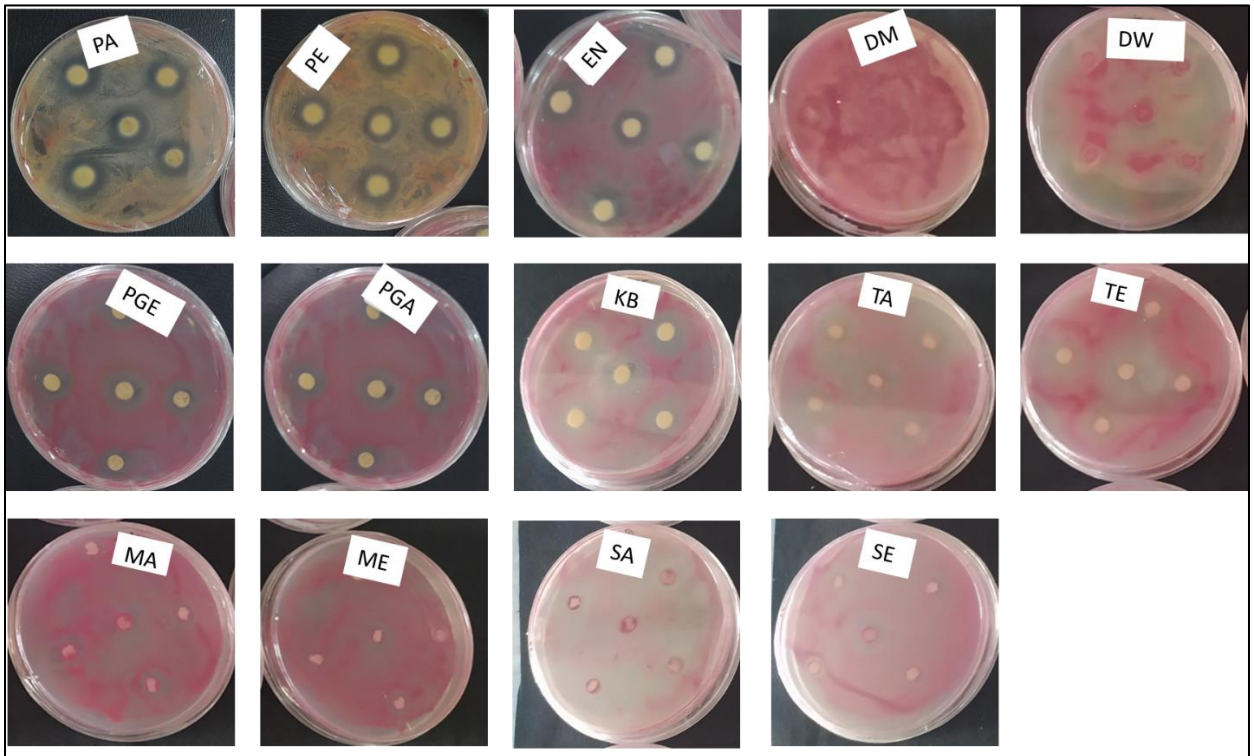
4.3.4 Antibacterial bioassays

Antibacterial activity of plant extracts from four different plant species and the controls are presented in (Table 4.5). All the tested extracts displayed varied antibacterial potency against *R. solanacearum*. The antibacterial activity of the four plant extracts differed significantly at $p \leq 0.05$ compared to negative and positive controls. From both solvents, extracts from *Pelargonium zonale* leaves were the most effective at 100 mg/mL of 1% DMSO based on average growth inhibition zones followed by *Psidium guajava*. The antibacterial activity of *P. zonale* was significantly different at $p \leq 0.05$ to those of negative controls and positive controls except ENRICH BM (Bronopol 27% w/w). The antibacterial activity of *P. guajava* was significantly different at $p \leq 0.05$ to those of negative and positive controls respectively. Antibacterial activity of each plant extract and controls were depicted by clear zones around the impregnated disks (Plate 4.4).

Table 4.5: Antibacterial activity of plant extracts against *Ralstonia solanacearum*

Plant species	Extraction solvent	
	Ethanol	Acetone
	Inhibition zone (mm)	Inhibition zone (mm)
Plant extracts Conc. 100 mg/mL		
*Pelargonium zonale*leaves	18.73 ± 0.31 a	18.60 ± 0.20 a
Psidium guajava leaves	14.27 ± 0.12 b	14.13 ± 0.12 b
Tarchonanthus camphoratus leaves	8.40 ± 0.00 c	8.73 ± 0.30 c
Moringa oleifera leaves	7.37 ± 0.15 d	7.47 ± 0.12 d
Moringa oleifera seeds	7.33 ± 0.12 d	7.33 ± 0.06 d
Positive controls at commercial rates		
ENRICH BM (Bronopol 27%w/w)	18.13 ± 0.46 a	18.13 ± 0.46 a
KOBE 1.2 SL (Chrysophanol 12g/l)	8.67 ± 0.12 c	8.67 ± 0.12 c
Negative controls		
Distilled water	0.00 ± 0.00 e	0.00 ± 0.00 e
1% DMSO	0.00 ± 0.00 e	0.00 ± 0.00 e
Mean	10.20	9.24
MSD	0.62	0.60
CV	2.11	2.25

The values are the average growth inhibition zones (mm) ± standard deviation from triplicates of ethanol and acetone extracts of each of the four plant materials and controls. Means within the same column having the same letter(s) do not differ significantly at p ≤0.05, DMSO = Dimethyl sulfoxide, MSD = mean square displacement, CV = coefficient of variation.



PA; *Pelargonium zonale* leaves acetone, **PE;** *Pelargonium zonale* leaves ethanol, **PGA**; *Psidium guajava* leaves acetone, **PGE**; *Psidium guajava* leaves ethanol; **MA**; *Moringa oleifera* leaves acetone, **ME**; *Moringa oleifera* leaves ethanol, **SA;** *Moringa oleifera* seeds acetone, **SE**; *Moringa oleifera* seeds ethanol, **TA**; *Tarchonanthus camphoratus* leaves acetone, **TE**; *Tarchonanthus camphoratus* leaves ethanol, **EN**; ENRICH BM (Bronopol 27%w/w) postive control, **KB**; KOBE 1.2 SL (Chrysophanol 12g/l) positive control, **DM**; 1% Dimethlysulfoxide (DMSO) negative control, **DW**; Distilled water negative control

Plate 4. 4: Growth inhibition zones of various plant extracts and controls against *R. solanacearum*.

4.3.5 Minimum inhibitory concentration (MIC) of effective plant extracts

The minimum inhibitory concentration (MIC) of *Pelargonium zonale* and *Psidium guajava* leaf extracts are illustrated in (Table 4.6). The inhibitory effect of *P. zonale* against *R. solanacearum* started at 6.25 mg/mL of 1% DMSO with inhibition zones of 7.67 and 8.0 mm for ethanol and acetone extracts while *P. guajava* exhibited inhibitory effect against the same pathogen at 6.25 mg/mL of 1% DMSO with inhibition zones of 7.67 and 8.0 mm for ethanol and acetone extracts respectively.

Table 4.6: Minimum inhibitory concentration (MIC) of effective plant extracts against *R. solanacearum*.

Plant extract	Conc. Mg/mL	Inhibition zones (mm)	
		Ethanol	Acetone
Pelargonium *zonale* leaves	50.00	16.17 ± 0.76	16 .00 ± 0.00
	25.00	12.83 ± 0.29	12.17 ± 0.29
	12.50	10.00 ± 0.87	10.33 ± 0.29
	6.25	7.67 ± 0.29	8.00 ± 0.50
	3.13	0.00 ± 0.00	0.00 ± 0.00
Psidium guajava leaves	50.00	11.17 ± 0.29	11.83 ± 0.29
	25.00	8.67 ± 0.29	8.67 ± 0.58
	12.50	8.00 ± 0.00	8.17 ± 0.29
	6.25	7.67 ± 0.58	8.00 ± 0.00
	3.13	0.00 ± 0.00	0.00 ± 0.00

The values are average growth inhibition zones (mm) ± standard deviation from triplicates of ethanol and acetone extracts of each concentration of the two effective plant extracts.

4.4 Discussion

The isolated bacteria exhibited irregular, round, and white fluidal colonies with pink centers on triphenyl tetrazolium chloride (TZC) media which was consistent with *Ralstonia solanacearum* characteristics on this medium as described by Kelman (1954). Both the Gram stain and KOH solubility tests confirmed that the isolated bacterium was gram negative, and this agreed with the research findings by Khasabulli *et al.* (2017), Rahman *et al.* (2010), and She *et al.* (2017). The isolated bacterium oxidized all the disaccharide sugars and hexose alcohols. Similar results were reported by various scientists who classified the isolated *R. solanacearum* with similar characteristics as biovar III race 1 [*Ralstonia solanacearum* (phylotype I)] (Boschi *et al.,* 2017; Khasabulli *et al.,* 2017; Popoola *et al.,* 2015; Rahman *et al.,* 2010). The pathogenicity test of the isolated *R. solanacearum* to susceptible potato seedlings *in-vivo* produced similar wilt symptoms to those diagnosed in the field. Similarly, morphological characteristics of the re-isolated bacterium from these test plants were identical to those of the original pathogen on TZC media. Similar results were reported in other studies (Khasabulli *et al.,* 2017; Popoola *et al.,* 2015; Rahman *et al.,* 2010; She *et al.,* 2017).

Ethanol as an extraction solvent recorded significantly higher percent extract yields from all the leaves compared to acetone but this was different with the *M. oleifera* seed extract in which acetone recorded slightly higher yield. Ethanol is a polar solvent thus extracts more diverse secondary metabolites from various plant parts as opposed to acetone which is non-polar (Snehlata *et al.,* 2018; Yusnawan, 2013). Even though polar solvents are documented for high extract yields, the yielded extracts are always low in phenolic and flavonoid content in comparison to extracts from non-polar solvents (Nawaz *et al.,* 2020). High yields of *M. oleifera* seed extracts from acetone solvent can be attributed to high oil content observed after extract concentration. It is argued that oils are easily extracted by non-polar solvents (Nwabueze & Okocha, 2008). Similarly, *M. oleifera* seed extracts might have contained high proportions of phenolic and flavonoid compounds in addition to oils which are highly soluble in non-polar solvents (Nawaz *et al.,* 2020).

Results from *in-vitro* screening of antibacterial activity of the four plant extracts against *R. solanacearum* revealed that *P. zonale* leaves were the most effective extract followed by *P. guajava* leaves while *Moringa oleifera* seeds were the least. The varied antibacterial activity between the plant extracts can be attributed to diversity and/or difference in concentrations of secondary metabolites per plant extract (Yihune & Yemata, 2019). These *in-vitro* results were in accordance with those of Biswal (2015) and Oboo *et al.* (2014) who reported antibacterial activity of these plant extracts against *R. solanacearum in-vitro*. However, *P. zonale* and *P. guajava* leaf extracts exhibited high growth inhibition zones which contrasted the findings of Biswal (2015), and this can be attributed to difference in plant species, plant parts, adopted extraction method, varied agro-climatic conditions and diverse abiotic factors during the plant growth (Gololo, 2018; Kumar *et al.,* 2017; Liu *et al.,* 2016; Mutimawurugo *et al.,* 2020). Oboo *et al.* (2014) reported high antibacterial activity of *T. camphoratus* against *R. solanacearum* both *in-vitro* and *in-vivo*. In this study, *T. camphoratus* extract was the third best extract and this contrasted their findings. Its dismal performance can be attributed to failure of the extracted paste to dissolve in 1% DMSO.

KOBE 1.2 SL (Chrysophanol 12g/l) and ENRICH BM (Bronopol 27%w/w) are documented to control bacterial wilt pathogen through induction of host plant resistance (*https://agroduka.com/enrich-bm;* Liu *et al.,* 2016). Stretton and Manson (1973) reported *in-vitro* efficacy of bronopol against different strains of bacteria and this was confirmed by the findings of this study which demonstrated its *in-vitro* antibacterial activity against *R. solanacearum*. The study results also revealed *in-vitro* antibacterial potency of Chrysophanol against *R. solanacearum*. The

direct mode of action of bronopol against various bacteria is assumed to be through oxidation of thiols within the bacterial cell and generation of free radicals leading to cell death (Shepherd *et al.*, 1988; Stretton & Manson, 1973).

The minimum inhibitory concentration (MIC) results revealed that the antibacterial activity of the *P. zonale* and *P. guajava* leaf extracts decreased with decreasing extract concentration. For both extraction solvents, the antibacterial activity of *P. zonale* and *P. guajava* leaf extracts against *R. solanacearum* started at 6.25 mg/mL of 1% DMSO. These results were in accordance with the findings of Mutimawurugo *et al.* (2020), who reported varied MICs ranging from 6.25 to 12.5 mg/mL for different plant extracts against *R. solanacearum*. Similarly, Mutimawurugo *et al.*, (2020) also reported decreased antibacterial activity of different plant extracts against *Ralstonia solanacearum* with decreased extract concentrations and this can be attributed to reduced toxicity levels of bioactive compounds due to dilution effect.

Numerous researchers have investigated the antibacterial efficacy of different plant extracts and their respective bioactive compounds against *R. solanacearum* both *in-vitro* and *in-vivo* (Biswal 2015; Din *et al.,* 2016; Hassan *et al.,* 2009; Mutimawurugo *et al.,* 2020; Oboo *et al.,* 2014; Wamani, 2020). Some of the documented bioactive compounds against *R. solanacearum* include flavonoids and alkaloids (Mutimawurugo *et al.,* 2020), 5-(3-buten-1-ynyl)-2,2'-bithienyl (BBT) and 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (BBTOAc) from *Tagetes patula* (Terblanche & Villiers 1998). These bioactive compounds are reported to demonstrate bactericidal effects through interaction with enzymes and proteins of the target bacterial cell membrane causing disruption. Additionally, the bioactive compounds with hydrophobic characteristics can react with proteins of the target cell membrane and mitochondria of the target bacteria thereby changing its membrane permeability (Gonelimali *et al.,* 2018; Mostafa *et al.,* 2018; Sánchez *et al.,* 2010).

4.5 Conclusion and Recommendation

The study results revealed varied antibacterial potency of the tested plant extracts against *R. solanacearum in-vitro*. *P. zonale* and *P. guajava* leaf extracts displayed significantly high antibacterial activity compared to other extracts. Further research studies should be conducted to assess the antibacterial potency of these two plant extracts against *R. solanacearum* both *in-vivo* and under field conditions. Similarly, phytochemical analysis studies should be carried out on these two extracts to identify the bioactive compounds against *R. solanacearum.*

CHAPTER FIVE

DETERMINATION OF BIOACTIVE COMPOUNDS AGAINST BACTERIAL WILT OF POTATO (*Ralstonia solanacearum*) IN *PSIDIUM GUAJAVA* AND *PELARGONIUM ZONALE* LEAF EXTRACTS

Abstract

Common guava *(Psidium guajava)* and Pelargonium *(Pelargonium zonale)* have shown *in-vitro* antibacterial activity against *Ralstonia solanacearum* in previous studies. However, their phytochemical constituents and bioactive compounds against the pathogen have not been identified. The present study investigated the phytochemical components of *P. guajava* and *P. zonale* leaf extracts by phytochemical screening and gas chromatography-mass spectrometry (GC-MS). Phytochemical screening was done using different solvents while 100 mg of the dried ethanolic extract pastes from each plant sample was subjected to GC-MS analysis. Automated mass spectral deconvolution and identification system software (AMDIS, US) was used to analyze chromatograms and spectra representing individual compounds. Compound identification was performed by comparing each of the mass spectra with the database of NIST 11 (Gaithersburg, MD, USA), Wiley 7N (John Wiley, NY, USA) and by comparing the calculated Kovats linear retention indices using retention times of n-alkane series against the values in the NIST webbook. Flavonoids, phenols, alkaloids, saponins, terpenoids and tannins were detected in both plant samples. GC-MS analysis revealed presence of 36 and 26 compounds from *P. zonale* and *P. guajava* respectively. Both *P. zonale* and *P. guajava* had 7 similar compounds with antibacterial properties; Fumaric acid, Phytol, Pyrogallol, 4-Hydroxybenzoic acid, Shikimic acid, Protocatechuic acid, 3, 4, 5-Trihydroxybenzoic acid ethyl ester but *P. zonale* had one additional antibacterial compound; Lactic acid. In both cases, Shikimic acid had the highest percent peak areas of 3.2% for *P. zonale* and 6.8% *P. guajava* respectively. Therefore, *P. zonale* and *P. guajava* can serve as alternative sources of active ingredients for formulation of commercial botanicals for management of bacterial wilt of potato.

5.1 Introduction

Bacterial wilt of potato caused by different species of gram-negative bacterium *Ralstonia solanacearum* mainly *Ralstonia pseudosolanacearum* sp. nov. (*R. solanacearum* (phylotypes I and III) is one of the major biotic constraints to potato production worldwide (Boschi *et al.,* 2017; Safni *et al.,* 2014). To date there is no satisfactory management option available for complete

eradication of the disease hence affected farmers have relied on integrated disease management (IDM) options. Conversely, efficiency of integrated diseases management has been challenging due to its site-specific nature (Priou *et al.,* 1999). This limitation has propelled adoption and extensive use of conventional pesticides for its management in potato fields (Biswal & Dhal 2018; Sarkar & Chaudhuri, 2016). However, improper use of these chemical pesticides poses human and environmental health risks especially in developing countries where most farmers use poor quality personal protective equipment (PPE) and deploy limited good agricultural practices (GAPs) (Mulugeta *et al.,* 2020). These pesticides can also infiltrate into the soil and spill into water bodies causing both terrestrial and aquatic health hazards (Mulugeta *et al.,* 2020; Rahman *et al.,* 2012).

Efforts have consequently been focused on developing botanicals (plant extracts) as eco-friendly management options against bacterial wilt pathogen (Rahman *et al.,* 2012). Plant extracts contain numerous bioactive compounds with bioactivity against various plant pathogens (fungi, bacteria and nematodes). For instance, various researchers have used raw plant extracts and oils for the management of fungi, bacteria, and nematodes (Borges *et al.,* 2018). Some of the plant-derived bioactive compounds with biopesticide activity include chitosan, salicylic acid, benzoic acid, benzothiadiazole, saponins, alkaloids, flavonoids, terpenes, proteins, peptides, blasticidin, mildiomycin, cyanogenic glycosides, glucosinolates, lipids, polyacetylenes, polythienyls, polyoxins, tannins and phenolic compounds (Din *et al.,* 2016; Mutimawurugo *et al.,* 2020; Singh *et al.,* 2020). These compounds have shown good antimicrobial efficacy both *in-vitro* and *in-vivo* (under greenhouse) conditions (Isman, 2000; Zaker, 2016). However, with few exceptions, these efficacy results have not been reproduced in the field and this phenomenon has been attributed to rapid degradation and volatilization of their bioactive compounds under field condition due to varied abiotic factors (Borges *et al.,* 2018).

In-vitro and *in-vivo* studies by different research scientists have reported antibacterial activity of various plant extracts against bacterial wilt disease pathogen (Din *et al.,* 2016; Hassan *et al.,* 2009; Mutimawurugo *et al.,* 2020; Oboo *et al.,* 2014; Wamani 2020). Examples of reported plant extracts with phytobiocidal effect against bacterial wilt pathogen include; onion (*Allium cepa* L.), garlic (*Allium sativum* L.), lemongrass (*Cymbopogon citratus* Stapf), castor bean (*Ricinus communis* L.), rosemary (*Rosmarinus officinalis* L.), lion's ear (*Leonotis nepetifolia* R.Br.), African basil (*Ocimum gratissimum* L.), tobacco (*Nicotiana tabacum* L.), wild marigold (*Tagetes minuta* L.), stinging nettle (*Urtica massaica* Mildbr), moringa (*Moringa oloifera*), guava (*Psidium*

guajava), geranium (*Bauhinia recimosa*), camphor brush (*Tarchonanthus camphoratus*) and French marigold (*Tagetes patula*) among others (Biswal, 2015; Mutimawurugo *et al.,* 2020; Oboo *et al.,* 2014; Terblanche & Villiers, 1998).

In-vitro study in chapter four revealed high antibacterial activity of *Pelargonium zonale* and *Psidium guajava* against bacterial wilt of potato (*R. pseudosolanacearum* sp. nov. [*R. solanacearum* (phylotype I)]. However, phytochemical profiling nor identification of the specific bioactive compounds with antibacterial effect against the target pathogen was not conducted. Therefore, the present study investigated the phytochemical components in ethanolic leaf extracts of *P. zonale* and *P. guajava* by quantitative phytochemical screening and gas chromatography-mass spectrometry (GC-MS).

5.2 Materials and methods

5.2.1 Sample collection and identification

Sample collection and identification is explained in section 4.3.5.

5.2.2 Preparation of the plant materials

Pelargonium zonale and *Psidium guajava* leaf samples were dried and crushed to fine powder as outlined in section 4.3.5 respectively. Twenty grams (20 g) of each powdered sample was weighed and stored separately for phytochemical screening. For Gas Chromatography spectrometry (GC-MS) analysis, 20 g of fine powder of each plant material were used to prepare plant extracts using 98% ethanol as extraction solvent as outlined in section 4.3.5.

5.2.3 Phytochemical screening

Phytochemical screening of *Pelargonium zonale* and *Psidium guajava* leaf extracts was conducted at the animal nutrition laboratory, department of animal production at the University of Nairobi. Quantitative phytochemical profiling of *P. zonale* and *P. guajava* powdered leaf samples was carried out to determine the presence of alkaloids, flavonoids, saponins, phenols, terpenoids and tannins (Harborne, 1973; Indumathi *et al.,* 2014; Obdoni & Ochuko, 2002; Padma *et al.,* 2013; Quettier-Deleu *et al.,* 2000; Shah & Yadav, 2015).

5.2.4 Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of *P. zonale* and *P. guajava* leaf extracts was conducted at the Mycotoxin and Nutrition Platform laboratory of the International Livestock Research Institute (ILRI), Nairobi Kenya. One hundred milligrams of the dried paste per plant sample was transferred into 2 ml eppendorf tubes containing 2000 µl of absolute

methanol and each tube vortexed for 2 minutes for total dissolution. After complete dissolution, 150 µl aliquot per sample was trasfered into a 1.5 ml eppendorf tube and vacuum dried in a vacuum concentrator at room temperature. Vacuum dried samples were derivatized according to Lisec *et al.* (2006). Briefly, vacuum dried samples were transferred into dried sample vials followed by addition of 100 µl of pyridine and 75 µl of methoxyamination reagents respectively and the sample vials were tightly capped. The samples were heated at 37°C on a heating block for 2 hours with regular vortexing after every 15 minutes. After 2 hours, 75 µl of silylation reagent was added in each sample and heated at 70°C on a heating block for 1 hour with regular vortexing after every 15 minutes. The derivatized samples were cooled and transferred into 250 µl glass inserts loaded in GC vials and the lids capped.

The derivatized samples were analyzed by GC–MS. A portion (1 µl) of the derivatized sample solution was injected in to a 7890A GC system (Agilent Technologies, USA) coupled with a 240-ion trap mass spectrometer detector (Agilent Technologies) using the Agilent 7693A automatic liquid sampler at a split ratio of 10:1. A VF5-MS (5% phenyl methylpolysiloxane, 30.0 m × 0.25 mm, 0.25 µm) film capillary column was used with the injector port set at 280 °C. Helium was used as carrier gas at a flow rate of 1 mL/min. The oven temperature was held at 50 °C followed by an increase of 4 °C/min to 180 °C and finally followed by an increase to 250 °C at 3 °C/min. The ion trap mass spectrometer parameters were as follows: scan range 50–450 (m/z), ionization mode EI, filament delay time 8 min. The transfer line temperature, manifold temperature and trap temperature of 250 °C, 100 °C and 150 °C, respectively. The total run time was 56 minutes.

5.2.5 Compound identification

A homologous n-alkane series was analyzed alongside the derivatized sample and used to compute the Kovats Linear retention index. Chromatograms and spectra representing individual compounds were analyzed using the automated mass spectral deconvolution and identification system software (AMDIS, US). The identification of the individual compounds was performed by comparing each of the mass spectra with the database of NIST 11 (Gaithersburg, MD, USA) and Wiley 7N (John Wiley, NY, USA) consisting of more than 62,000 patterns of known compounds and also by comparing the calculated Kovats linear retention indices using retention times of n-alkane series against the values obtained in the NIST webbook (https://webbook.nist.gov/chemistry/) for the same capillary column stationary phase (Strehmel *et*

al., 2008). The Compounds were identified as their corresponding Silyl and/or Oxime derivatives. Absolute compound identity was assigned for Matches within +/- 5 of the database Kovats linear retention index. The quantification of individual compounds was performed by the peak area percentage method. The identified compound concentrations were expressed as the percentage of each individual compound to the total of all compounds detected in the derivatized sample.

5.2.6 Data analysis

Data obtained from quantitative phytochemical profiling was first tested for normality using the Shapiro-Wilk tests and the difference in the mean compositions compared using Mann-Whitney U test at 5% probability level (Mann &Whitney, 1947; Wilcoxon, 1945, 1992) in R software, version 4.2.2 (R Studio Team 2020).

5.3 Results

5.3.1 Quantitative phytochemical screening

Quantitative phytochemical profiling of *Pelargonium zonale* and *Psidium guajava* leaves revealed the presence of all the six tested phytochemicals; flavonoids, phenols, alkaloids, saponins, terpenoids and tannins in both *P. zonale* and *P. guajava* leaves as illustrated in Table 5.1. A comparison between *P. guajava* and *P. zonale* leaf extracts showed that there was no significant difference at $p \leq 0.05$ in their phytochemical composition. Tannins were the most abundant phytochemical component in P. *zonale* and *P. guajava* leaves. Alkaloids were the least abundant in P. *zonale leaves* while *terpenoids* were the least abundant in P. *guajava* leaves.

Table 5.1: Phytochemical components of *Psidium guajava* and *Pelargonium zonale* leaf extracts

Plant species	Relative abundance					
	Flavonoids (mg Q.E/g)	Phenols (mg G.A.E/g)	Alkaloids (%)	Saponins (%)	Terpenoids (%)	Tannins (mg T.A.E/g)
Psidium guajava	34.44±0.01[a]	46.22 ±0.02[a]	1.26 ± 0.02[a]	6.08 ± 0.02[a]	0.67 ± 0.01[a]	106.40 ± 0.02[a]
Pelargonium zonale	16.24±0.01[a]	47.62 ± 0.02[a]	1.93 ± 0.01[a]	9.41 ± 0.02[a]	2.27 ± 0.01[a]	190.30 ± 0.02[a]
p-value ($\alpha = 0.05$)	0.08	0.10	0.08	0.10	0.08	0.10

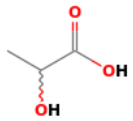
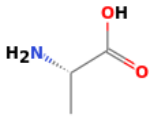
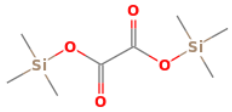
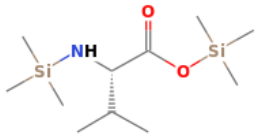
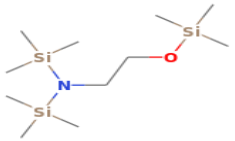
The values are presented as average means ± standard deviation. Q.E = Quercetin Equivalent, G.A.E = Gallic Acid Equivalent, T.A.E= Tannic Acid Equivalent.

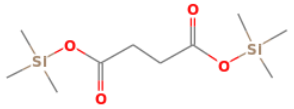
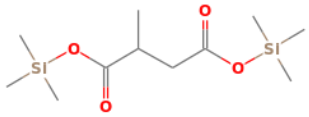
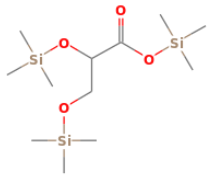
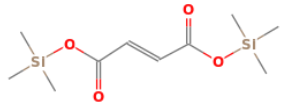
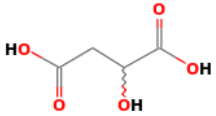
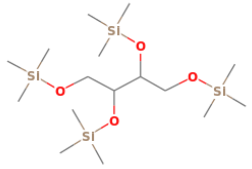
5.3.2 Gas chromatography-mass spectrometry (GC-MS) analysis

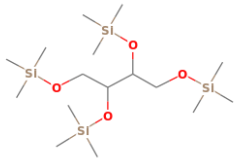
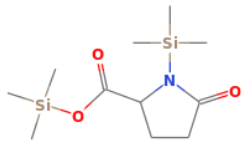
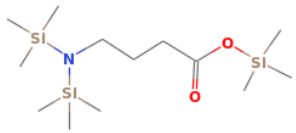
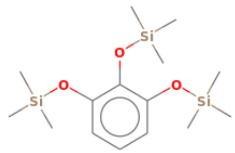
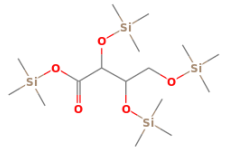
A total of 36 and 26 compounds were identified from GC-MS analysis of ethanolic leaf extracts of *Pelargonium zonale* and *Psidium guajava* respectively. These phytochemical constituents, their retention time (RT), molecular formula, molecular weight (g/mol), percent peak areas and structures of detected compounds are presented in Tables 5.2 and 5.3.

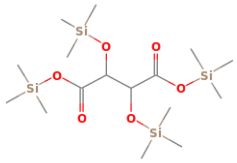
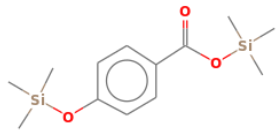
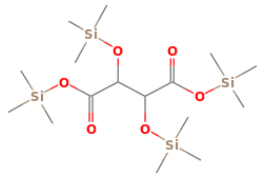
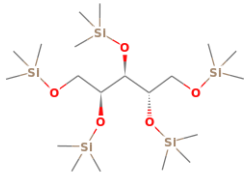
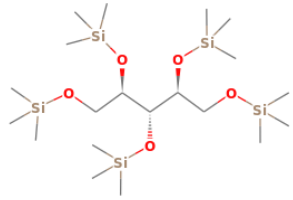
The chromatograms are presented in Figures 5.1 and 5.2. *P. zonale* had 8 compounds with antibacterial properties: Shikimic acid (3.15 %), Phytol (0.59 %), 3, 4, 5-Trihydroxybenzoic acid ethyl ester (0.34 %), Protocatechuic acid (0.21 %), Pyrogallol (0.12 %), Lactic acid (0.08 %), 4-Hydroxybenzoic acid (0.04 %) and Fumaric acid (0.03%). *P. guajava* had 7 compounds with antibacterial properties; Shikimic acid (6.77 %), Phytol (0.54%), Protocatechuic acid (0.14 %), Pyrogallol (0.09 %), Fumaric acid (0.09 %), 3,4,5-Trihydroxybenzoic acid ethyl ester (0.04 %) and 4-Hydroxybenzoic acid (0.02 %).

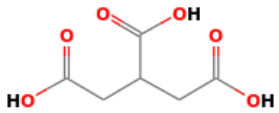
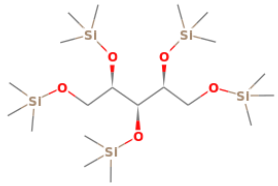
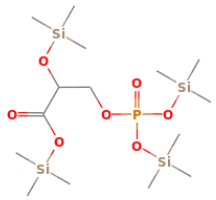
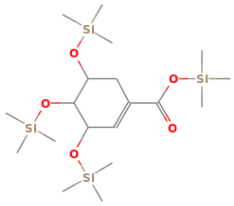
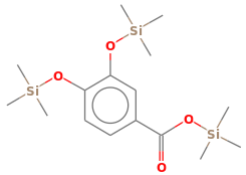
Table 5.2: Bioactive compounds in aqueous ethanolic fraction of *Pelargonium zonale*

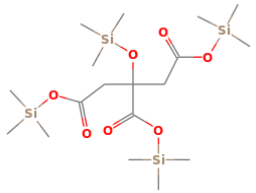
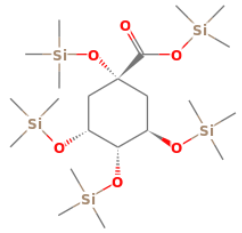
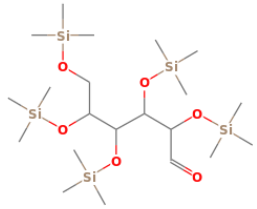
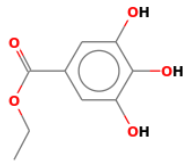
Peak No.	RT (min)	Compound ID	Molecular formula	MW g/mol	Peak area (%)	Structure of detected compounds
2	15.45	Propane-1,3-diol	$C_3H_8O_2$	76.09	0.04	
3	15.63	Lactic acid	$C_3H_6O_3$	90.08	0.08	
6	17.18	Alanine	$C_3H_7NO_2$	89.09	0.06	
8	18.53	Oxalic acid	$C_8H_{18}O_4Si_2$	234.40	0.47	
11	21.31	Valine	$C_{11}H_{27}NO_2Si_2$	261.51	0.09	
13	23.13	Ethanolamine	$C_{11}H_{31}NOSi_3$	277.63	0.07	

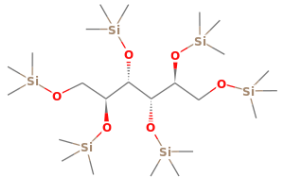
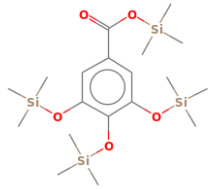
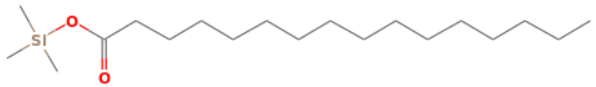
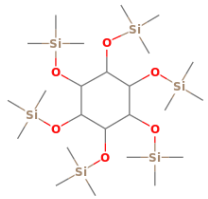
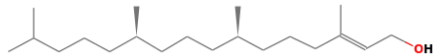
16	24.54	Succinic acid	$C_{10}H_{22}O_4Si_2$	262.45	0.16	
17	24.98	Methylsuccinic acid	$C_{11}H_{24}O_4Si_2$	276.48	0.03	
18	25.34	Glyceric acid	$C_{12}H_{30}O_4Si_3$	322.62	0.11	
21	25.68	Fumaric acid	$C_{10}H_{20}O_4Si_2$	260.43	0.03	
23	30.49	Malic acid	$C_4H_6O_5$	134.09	2.47	
26	31.03	Theitol	$C_{16}H_{42}O_4Si_4$	410.84	0.12	

27	31.31	Erythritol	$C_{16}H_{42}O_4Si_4$	410.84	2.24	
29	31.57	Oxoproline	$C_{11}H_{23}NO_3Si_2$	273.48	0.4	
30	31.81	Aminobutyric acid,	$C_{13}H_{33}NO_2Si_3$	319.66	0.25	
34	32.35	Pyrogallol	$C_{15}H_{30}O_3Si_3$	342.65	0.12	
38	32.96	Threonic acid	$C_{16}H_{40}O_5Si_4$	424.83	0.08	

46	34.23	Tartaric acid	$C_{16}H_{38}O_6Si_4$	438.81	0.16	
47	34.84	4-Hydroxybenzoic acid	$C_{13}H_{22}O_3Si_2$	282.48	0.04	
50	35.46	Tartaric acid	$C_{16}H_{38}O_6Si_4$	438.81	1.81	
55	37.53	Arabitol	$C_{20}H_{52}O_5Si_5$	513.05	0.31	
58	37.87	Ribitol	$C_{20}H_{52}O_5Si_5$	513.05	0.35	

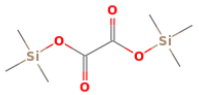
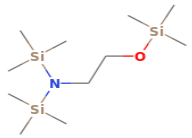
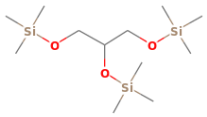
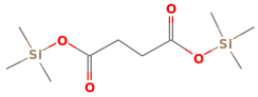
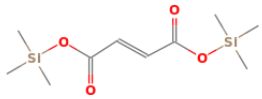
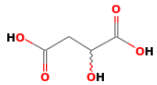
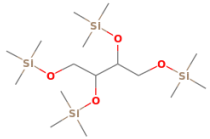
59	37.9	Tricarballylic acid	$C_6H_8O_6$	176.12	0.36	
64	38.27	Xylitol	$C_{20}H_{52}O_5Si_5$	513.05	9.94	
69	39.42	Glycerol-3-phosphate	$C_{15}H_{39}O_7PSi_4$	474.78	0.35	
74	40.73	Shikimic acid	$C_{19}H_{42}O_5Si_4$	462.88	3.15	
76	41.03	Protocatechuic acid	$C_{16}H_{30}O_4Si_3$	370.66	0.21	

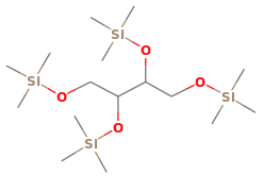
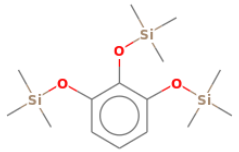
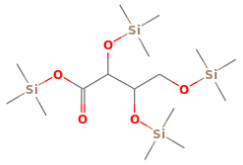
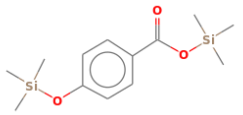
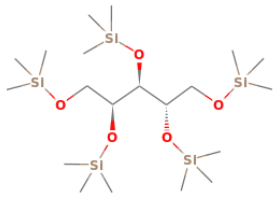
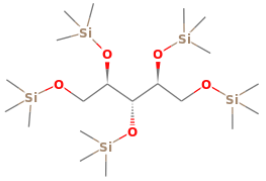
78	41.21	Citric acid	$C_{18}H_{40}O_7Si_4$	480.85	0.33	
83	42.77	Quinic acid	$C_{22}H_{52}O_6Si_5$	553.07	1.81	
99	44.05	Glucose	$C_{21}H_{52}O_6Si_5$	541.06	2.32	
102	44.84	3,4,5-Trihydroxybenzoic acid ethyl ester	$C_9H_{10}O_5$	198.17	0.34	

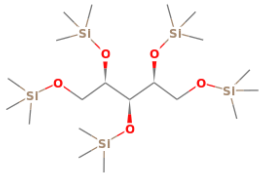
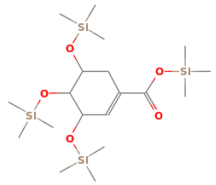
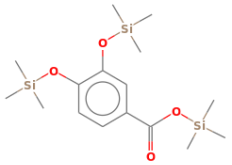
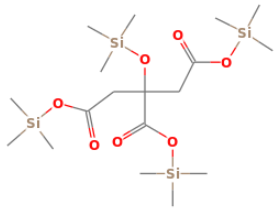
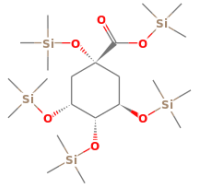
104	45.13	Mannitol	$C_{24}H_{62}O_6Si_6$	615.26	10.59	
112	45.49	Gallic acid	$C_{19}H_{38}O_5Si_4$	458.84	48.96	
121	47.65	Palmitic acid	$C_{19}H_{40}O_2Si$	328.61	0.76	
129	49.99	Myo-Inositol	$C_{24}H_{60}O_6Si_6$	613.24	0.43	
134	51.58	Phytol	$C_{20}H_{40}O$	296.53	0.59	

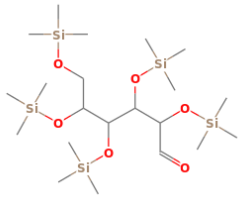
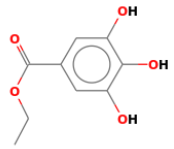
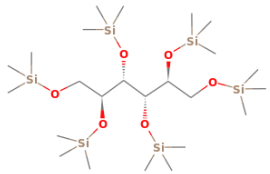
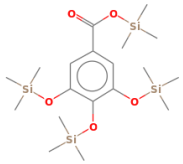
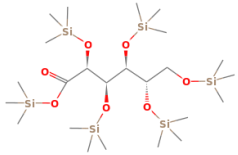
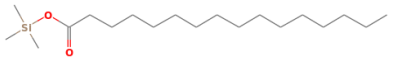
RT= Retention time. Structures, molecular formulas and molecular weights were sourced from NIST webbook.

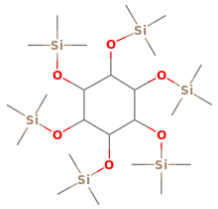
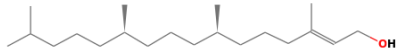
Table 5. 3: Bioactive compounds in aqueous ethanolic fraction of *Psidium guajava*

Peak No.	RT (min)	Compound ID	Molecular formula	MW g/mol	Peak area (%)	Structure of detected compounds
1	18.52	Oxalic acid	$C_8H_{18}O_4Si_2$	234.40	0.40	
3	23.13	Ethanolamine	$C_{11}H_{31}NOSi_3$	277.63	0.02	
4	23.39	Glycerol	$C_{12}H_{32}O_3Si_3$	308.64	6.00	
6	24.56	Succinic acid	$C_{10}H_{22}O_4Si_2$	262.45	0.03	
7	25.68	Fumaric acid	$C_{10}H_{20}O_4Si_2$	260.43	0.09	
10	30.50	Malic acid	$C_4H_6O_5$	134.09	0.06	
11	31.05	Theitol,	$C_{16}H_{42}O_4Si_4$	410.84	0.09	

12	31.31	Erythritol	$C_{16}H_{42}O_4Si_4$	410.84	0.73	
14	32.37	Pyrogallol	$C_{15}H_{30}O_3Si_3$	342.65	0.09	
16	32.97	Threonic acid	$C_{16}H_{40}O_5Si_4$	424.83	0.08	
18	34.85	4-Hydroxybenzoic acid	$C_{13}H_{22}O_3Si_2$	282.48	0.02	
23	37.53	Arabitol	$C_{20}H_{52}O_5Si_5$	513.05	0.14	
24	37.88	Ribitol	$C_{20}H_{52}O_5Si_5$	513.05	0.04	

25	38.27	Xylitol	$C_{20}H_{52}O_5Si_5$	513.05	2.01	
31	40.75	Shikimic acid	$C_{19}H_{42}O_5Si_4$	462.88	6.77	
32	41.05	Protocatechuic acid	$C_{16}H_{30}O_4Si_3$	370.66	0.14	
33	41.18	Citric acid	$C_{18}H_{40}O_7Si_4$	480.85	0.03	
37	42.79	Quinic acid	$C_{22}H_{52}O_6Si_5$	553.07	10.04	

45	44.06	Glucose	$C_{21}H_{52}O_6Si_5$	541.06	11.15	
48	44.86	3,4,5-Trihydroxybenzoic acid ethyl ester	$C_9H_{10}O_5$	198.17	0.04	
49	45.11	Mannitol	$C_{24}H_{62}O_6Si_6$	615.26	1.66	
52	45.46	Gallic acid	$C_{19}H_{38}O_5Si_4$	458.84	21.78	
60	47.36	Gluconic acid	$C_{24}H_{60}O_7Si_6$	629.24	0.10	
61	47.65	Palmitic acid	$C_{19}H_{40}O_2Si$	328.61	0.77	

63	50.00	Myo-Inositol	$C_{24}H_{60}O_6Si_6$	613.24	7.24	
68	51.60	Phytol	$C_{20}H_{40}O$	296.53	0.54	

RT= Retention time. Structures, molecular formulas and molecular weights were sourced from NIST webbook.

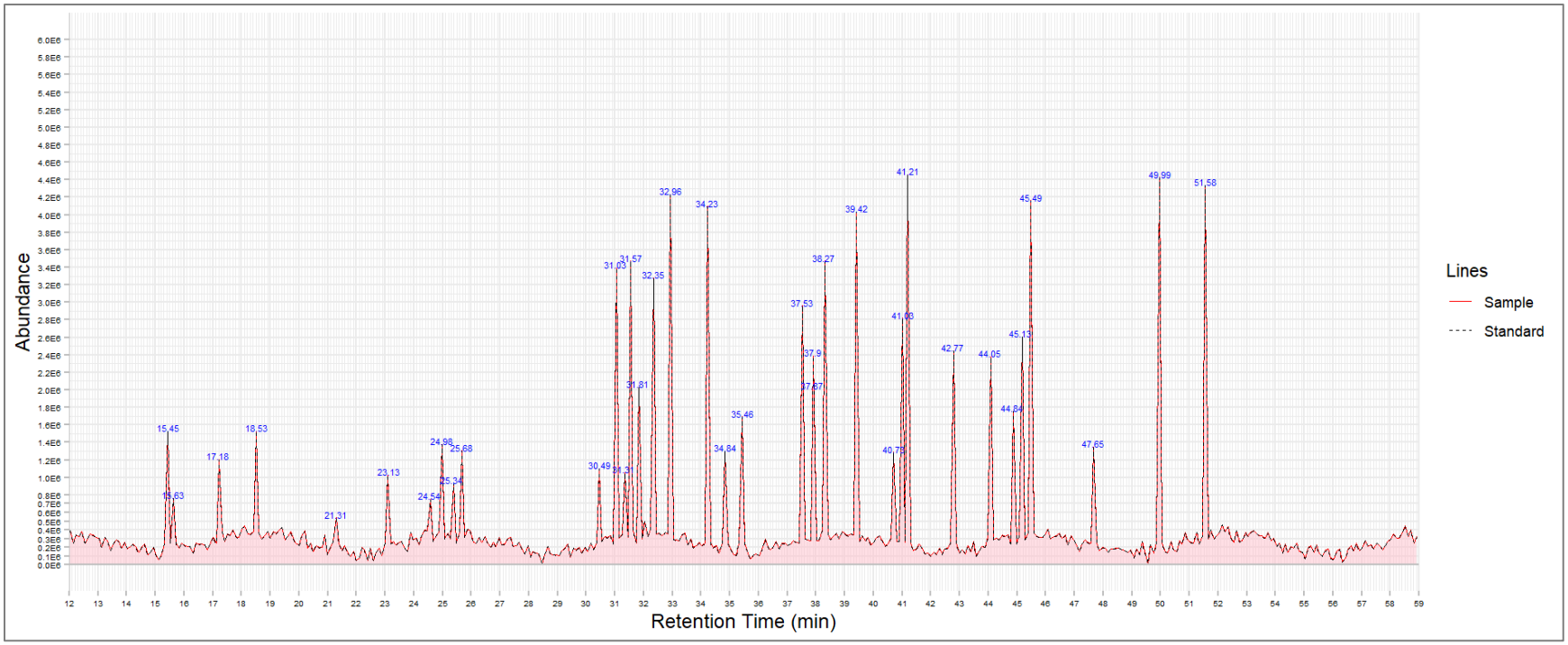


Figure 5. 1: GC-MS chromatogram of ethanolic extracts of *Pelargonium zonale*

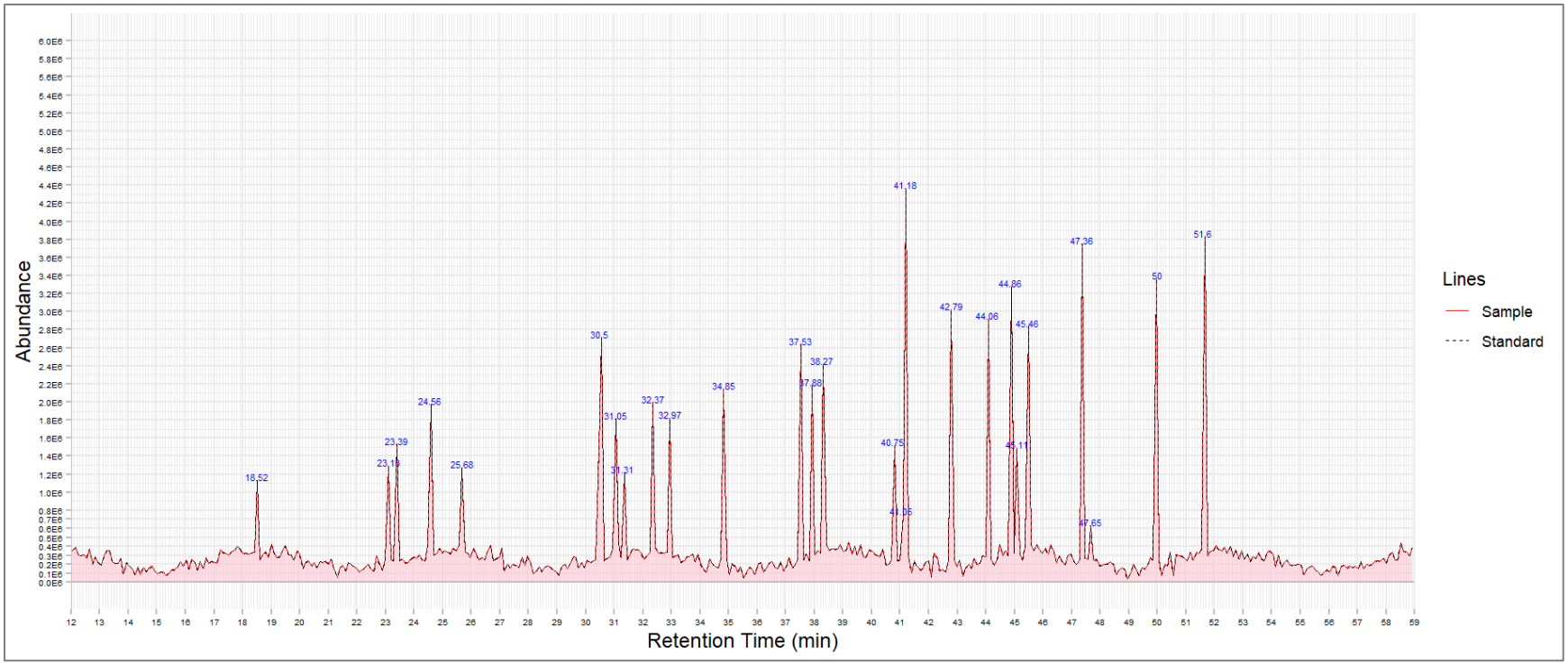


Figure 5. 2: GC-MS chromatogram of ethanolic extracts of *Psidium guajava*

5.4 Discussion

Results from phytochemical profiling of *P. zonale* and *P. guajava* leaf extracts revealed the presence of six phytochemicals namely flavonoids, phenols, alkaloids, saponins, terpenoids and tannins. Flavonoids are polyphenolic compounds (secondary antioxidants) synthesized by plants in response to infections from various abiotic and biotic stresses (Karak, 2019; Kumar & Pandey, 2013). They are reported to function as antibacterial agents against both gram-negative and gram-positive bacteria (Adamczak *et al.,* 2020; Karim & Hossain, 2018). Karim and Hossain (2018) and Mutimawurugo *et al.* (2020), reported efficacy of flavonoids against bacterial wilt of potato. Flavonoids control plant pathogenic bacteria through coagulation of bacterial cell proteins as well as affecting different amino acid synthesis enzymes (Al-Obaidi, 2014; Din *et al.,* 2s016). Alkaloids have antibacterial and antifungal properties which have propelled their adoption and use in the plant protection field (Adamski *et al.,* 2020). *In-vitro* and *in-vivo* studies have revealed their antibacterial properties against bacterial wilt of solanaceous plants (Abd-Elrahim *et al.,* 2021; Din *et al.,* 2016; Mutimawurugo *et al.,* 2020). Some alkaloids act through inhibition of topoisomerase enzymes while others such as bisindole monoterpenoids act as DNA intercalating agents and hence poisoning the target bacteria (Tanaka *et al.,* 2006).

Saponins protect plants against infection by different microbes as well as infestation by insect pests (Desai *et al.,* 2009). Steroidal saponins react with bacterial membrane sterols inhibiting cell growth and hence overall bacterial growth (Wang *et al.,* 2000). Some groups of terpenoids are used as pesticides and fungicides due to their insecticidal and antimicrobial properties (Martin-Smith & Khatoon, 1963). They exhibit antibacterial activity by acting on the phospholipid bilayers of bacterial cells affecting electron transport, phosphorylation process, protein translocation and other enzyme dependent reactions leading to cell mortality (Dorman & Deans, 2000). Phenols aid adaptation of plant species to abiotic and biotic stresses (Cosme *et al.,* 2020). Some phenols such as thymol and carvacrol are documented to have antibacterial activity against bacterial wilt of solanaceous plants (Abd-Elrahim *et al.,* 2021). Tannins also have antimicrobial properties (Din *et al.,* 2016). They can directly kill the target bacteria by damaging the cell membrane and/or can bind to adhesins in the host tissue preventing attachment of the bacterial inoculum, disease establishment and spread (Mainasara *et al.,* 2012; Wang, 2014).

The GC-MS analysis results revealed that *P. zonale* had 8 compounds documented to have antibacterial activity while *P. guajava* had 7. The 7 compounds were similar for the two plant

extracts, but their percentage concentration varied per plant. *P. zonale* had one additional antibacterial compound. These compounds comprised of Fumaric acid, Pyrogallol, 4-Hydroxybenzoic acid, Shikimic acid, Protocatechuic acid, 3, 4,5-Trihydroxybenzoic acid ethyl ester (Gallic acid ethyl ester), Phytol for both the two plant extracts and Lactic acid for *P. zonale* (Aldulaimi *et al.,* 2019; Amalia & Ramon, 2012; Cynthia *et al*., 2018; He *et al.,* 2011; Imade *et al.,* 2021; Islam *et al.,* 2018; Khan *et al.,* 2015). In both plant extracts, Shikimic acid had the highest peak area while the other compounds had less than 1% concentration and this can be an indication that Shikimic acid was the main bioactive component against *Ralstonia solanacearum* in the two plant extracts. Shikimic acid is a common precursor for the synthesis of various phenolic compounds (Santos-Sánchez *et al.,* 2019). component in the However, the antibacterial effect might have also resulted from any of the detected compounds and/or from the synergistic effect between either or all the identified antibacterial compounds per plant extract.

Shikimic acid has shown *in-vitro* antibacterial activity against different bacteria but with higher inhibition activity against gram-negative bacteria as opposed to gram-positive bacteria (Bai *et al.,* 2022; Tripathi *et al.,* 2015). The high inhibitory activity against gram-negative bacteria can be attributed to thinner peptidoglycan layer in gram-negative bacteria as opposed to gram-positive bacteria (Tripathi *et al.,* 2015). Shikimic acid is assumed to exhibit different modes of action against pathogenic bacteria; disrupts oxidative phosphorylation pathway, inhibits membrane fluidity by changing glycerophospholipid and fatty acid levels, disturbs the normal functions of potassium and calcium channels, dishevels protein synthesis through influenced ribosome function and aminoacyl-tRNA synthesis upon penetration of the bacteria cell membrane and finally, it interferes with the pyruvate metabolic pathway (Bai *et al.,* 2022). The un-dissociated form of Fumaric acid and Lactic acid passes freely through the bacterial cell membrane into cytoplasm. Upon entry, the acid dissociates to release protons which acidify the cytoplasm leading to cell mortality (Lu et *al.,* 2011; Tango *et al.,* 2015). Even though pyrogallol has shown antibacterial activity against different bacterial pathogens, its mechanism of action and toxicity have not been studied (Kharouf *et al.,* 2022; Tinh *et al.,* 2016).

Protocatechuic acid exhibits antibacterial activity through depolarization of the cell membrane, reduction of intracellular pH and adenosine triphosphate (ATP) as well as leakage of cell content and destruction of cell morphology. Additionally, Protocatechuic acid affects energy metabolism and amino acid biosynthesis of the target bacteria (Wu *et al.,* 2022). The 4-

Hydroxybenzoic acid affects the fluidity of the bacterial cell membrane (Patra, 2012). Gallic acid ethyl ester induces permanent changes in the cell membrane such as increased hydrophobicity, alters the surface charge as well as increased pore formation in the cell membrane resulting to leakage of essential intracellular constituents and hence mortality of the target bacteria (Aldulaimi *et al.*, 2019; Borges *et al.*, 2013). Phytol induces intracellular reactive oxygen species (ROS) accumulation in the bacterial cell leading to imbalance between intracellular ROS and the antioxidant defense system hence reducing glutathione (GSH) cell content. The low GSH exposes the cell to detrimental effects from the action of low pH, chlorine compounds, as well as oxidative and osmotic stresses. Phytol also causes DNA damage of affected bacteria (Lee *et al.*, 2016).

5.5 Conclusion and Recommendation

The study revealed that *Pelargonium zonale* and *Psidium guajava* leaf extracts have various secondary metabolites with different bioactivities. GC-MS analysis showed a total of 8 and 7 antibacterial compounds from ethanolic leaf extracts of *P. zonale* and *P. guajava* respectively and most of the antibacterial compounds were phenols. In both plants, Shikimic acid had the highest percent peak area among the detected antibacterial compounds and hence could be the main bioactive component against *Ralstonia solanacearum* in the two plant extracts. Further screening should be done with each compound to confirm their singular and/or synergistic antibacterial activity as well as their mode of action against *R. solanacearum*.

CHAPTER SIX

THE EFFICACY OF *Pelargonium zonale* and *Psidium guajava* AGAINST BACTERIAL WILT OF POTATO UNDER GREENHOUSE CONDITION

Abstract

Pelargonium zonale and *Psidium guajava* are plant extracts with effective *in-vitro* antibacterial activity against *Ralstonia solanacearum.* However, their *in-vivo* antibacterial activity against *R. solanacearum* has not been fully explored. This study explored the *in-vivo* efficacy of ethanolic leaf extracts of *P. zonale* and *P. guajava* in the management of *R. solanacearum*. of potatoes. The greenhouse experiment was laid down in a completely randomized design (CRD) of 2*6 factorial arrangements [2 varieties (Shangi (Highly susceptible) and Sherekea (Moderately resistant) and 6 treatments] with 3 replicates. The treatments comprised; 2 ethanolic leaf extracts (*P. guajava* and *P. zonale*) at 50 mg/mL, 2 positive controls [ENRICH BM (Bronopol 27%w/w) and KOBE 1.2 SL (Chrysophanol 12g/l)] at commercial rates and 2 negative controls (Untreated control and 1% DMSO). Data was collected on plant emergence, disease incidence, disease severity, plant heights, number of stems, yield (t/ha) and number of bacterial wilt colony counts. The study results revealed that all the treated plants exhibited significantly low disease incidences, low area under disease progress curves (AUDPCs), high number of stems, high stem heights, low bacterial wilt colony counts, and high yield (Kgs) compared to negative controls. However, the efficacy results were dependent on resistant levels of each variety against *R. solanacearum*. Potato variety Sherekea exhibited the highest efficacy from the two plant extracts demonstrating the synergistic effect of host plant tolerance and botanicals in management of *R. solanacearum*. The results were comparable to those of Enrich BM (Bronopol 27%w/w) and KOBE SL (Chrysophanol 12g/l). Further studies are needed to assess the efficacy of the 2 extracts against the target pathogen under field conditions.

6.1 Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important food crop globally after wheat, rice and maize, providing nourishment to over one billion people (Gautam *et al.*, 2021; Harahagazwe *et al.*, 2018; Taiy *et al.*, 2017). In Kenya, potato is ranked second after maize in terms of production volume and it plays a major role in national food and nutrition security. Its tubers are the major source of carbohydrates and other key dietary nutrients such as potassium, vitamin C and fiber (Beals, 2019). Apart from its nutritional values, the crop also acts as a source

of employment and income generation to various stakeholders along its value chain (Taiy *et al.,* 2017). However, among other biotic factors mainly from field infestation, bacterial wilt pathogen has affected its optimal production worldwide and has resulted in approximately 50 – 100% yield losses in infested potato fields (Kromann *et al.,* 2014; Muthoni *et al.,* 2014).

Bacterial wilt pathogen caused by *Ralstonia solanacearum* is a destructive disease with worldwide distribution mainly in the tropical, subtropical and temperate regions (Boschi *et al.,* 2017; Shimelis & Melis, 2014). The pathogen is both seed and soil borne, and it overwinters in infested soils, plant debris and alternate hosts such as solanaceous weeds (Boschi *et al.,* 2017; Kromann *et al.,* 2014). The mode of spread of bacterial wilt pathogen is through latently infected tubers, infested water (irrigation water and or run-offs), infested soil adhering to tools, farm handlers' shoes and through mechanical activities in the farm which causes wounds to potato crop (Kwambai *et al.,* 2011; Shimelis & Melis, 2014). Due to lack of satisfactory management options available for complete eradication of bacterial wilt pathogen, farmers have been advised to adopt integrated disease management (IDM) option as an ecofriendly management option against the disease (Sharma *et al.,* 2017; Uwamahoro *et al.,* 2018). But the site-specific nature and diversity of bacterial wilt pathogen has constantly affected the effectiveness of integrated disease management as a management option (Karim & Hossain, 2018; Priou *et al.,* 1999).

There is limited adoption of integrated disease management against bacterial wilt pathogen hence the extensive use of conventional pesticides for the management of bacterial wilt disease in potato fields (Biswal & Dhal, 2018; Sarkar & Chaudhuri 2016). However, this has posed human health and environmental risks mainly in developing countries where most farmers use poor quality personal protective equipment (PPE) coupled with limited practice of good agricultural practices (GAPs) (Mulugeta *et al.,* 2020). Due to improper use, these pesticides can infiltrate into the soil as well as spill into water bodies causing both terrestrial and aquatic health hazards (Mulugeta *et al.,* 2020; Rahman *et al.,* 2012). The negative impacts of conventional pesticides have consequently shifted the focus of various scientists to research on development of eco-friendly management options against bacterial wilt pathogen (Rahman *et al.,* 2012).

In-vitro and *in-vivo* studies by different researchers have confirmed antibacterial activity of various plant extracts against bacterial wilt pathogens (Abo-Elyousr *et al.,* 2009). Apart from commercialized botanicals (formulated plant extracts), plant extracts have shown efficacy against bacterial wilt pathogen when used in various forms; aqueous form, dried powder form and/or green

manure form (Abd-Elrahim *et al.,* 2021; Chen *et al.,* 2020; Din *et al.,* 2016). Use of plant extracts in dried powder form and/or as green manure is advantageous since in addition to their antibacterial activity, they also improve biological, physical and chemical properties of the soil as well as plant growth and development (Chen *et al.,* 2020). When used as dried powder (organic amendment), the powder mixes with soil water to release water-soluble secondary metabolites with antibacterial activity against bacterial wilt pathogen and the mode of action against the pathogen is compound dependent (Din *et al.,* 2016).

The antibacterial screening of various plant extracts against plant pathogenic bacteria usually begins with *in-vitro* studies to confirm their antibacterial activity before proceeding to *in-vivo* studies respectively (Abd-Elrahim *et al.,* 2021). In-vitro antibacterial activity of *P. zonale* and *P. guajava* against *Ralstonia solanacearum* was first tested and confirmed in chapter four before they were used in this study. Thus, this study was conducted to investigate the efficacy of ethanolic leaf extracts of *P. zonale* and *P. guajava* against *Ralstonia solanacearum* of potatoes under greenhouse conditions.

6.2 Materials and methods

6.2.1 Bacterial isolation, identification and inoculum preparation

Bacteria was isolated from infected potato plant tissues on Kelma's TZC agar media as described by Kelman, (1954) and the isolated bacteria was identified as outlined in section 3.3.4. The virulent colonies were multiplied in several plates containing Casamino Acid-Peptone-Glucose (CPG) medium at 28 ± 1^0C for 48 hours as described by Kelman (1954). Forty-eight hours old bacterial cells were harvested in sterile distilled water and the suspension adjusted to attain the optical density (OD) of 600 nm (approximately 10^7 to 10^8 CFU/mL) using a spectrophotometer as described by Chen *et al.* (2020) and Mihovilovich *et al.* (2017).

6.2.2 Preparation of plant materials

Pelargonium zonale and *Psidium guajava* leaf extracts which exhibited high antibacterial activity against *R. solanacearum* from chapter four experiment were used in this study. *P. zonale* and *P. guajava* leaf samples were dried and ground into fine powders using sterile mortars and pestles as outlined in section 4.3.5. The ground powders were passed through 1 mm sieves to remove coarse particles. One kilogram of fine powder of each plant material was soaked in 10L of 98% ethanol in the ratio of 1:10 (w/v) with regular stirring for 48 hours. After 48 hours, the solutions were filtered, and the filtrates were concentrated to pastes as outlined in section 4.3.5.

The pastes were air-dried overnight, weighed and stored at 4^0C awaiting the greenhouse experiment.

6.2.3 Experimental design

The greenhouse experiment was laid down in a completely randomized design (CRD) of 2*6 factorial arrangements (2 varieties and 6 treatments) with 3 replicates. The first level of treatments comprised two potato varieties (Shangi and Sherekea) which according to chapter three results above were highly susceptible and moderately resistant respectively. The second level of treatments consisted of ethanolic leaf extracts (*Psidium guajava* and *Pelargonium zonale*) tested at a concentration of 50 mg/mL, two commercial bactericides as positive controls [ENRICH BM (Bronopol 27%w/w) a conventional bactericide and KOBE 1.2 SL (Chrysophanol 12g/l) a botanical bactericide] applied at their commercial rates, 1% Dimethyl sulfoxide (DMSO) as a negative control treatment and untreated control (Table 6.1). The experiment was repeated once.

Table 6.1: Treatment description of greenhouse experiment

Treatments	Verities	
	Shangi (Highly susceptible)	Sherekea (Moderately resistant)
Treatment 1	Untreated control	Untreated control
Treatment 2	*Psidium guajava*	*Psidium guajava*
Treatment 3	*Pelargonium zonale*	*Pelargonium zonale*
Treatment 4	ENRICH BM (Bronopol 27%w/w)	ENRICH BM (Bronopol 27%w/w)
Treatment 5	KOBE 1.2 SL (Chrysophanol 12g/l)	KOBE 1.2 SL (Chrysophanol 12g/l)
Treatment 6	1% DMSO	1% DMSO

DMSO = Dimethyl sulfoxide.

6.2.4 Greenhouse experiment and treatment application

The greenhouse experiment was conducted at the University of Nairobi field station, upper Kabete campus. Forest soil was collected from tree forest (*Eucalyptus spp.)* which has been out of cultivation for over ten years and autoclaved for 1 hour at 121°C as described by Mahmood *et al.* (2014). Upon cooling, 4 Kg sterile plastic pots with a surface area of 0.03 M^2 were half filled with the cooled sterile soil. Five pots were used per treatment translating to 60 pots per replicate. Uniform wounds/physical injuries were created on each of the well sprouted certified seed potato tubers using a sterile knife. The pots were inoculated with 10 mL of bacterial suspension adjusted to 600 nm OD (approximately 10^7 to 10^8 CFU/mL) using a syringe as described by Mihovilovich

et al. (2017). The injured tubers were planted at the centre of each half-filled plastic pot (one tuber per pot). The tubers were then covered with sterile soil to about ¾ full.

Each treatment [ethanolic leaf extracts (*P. guajava* and *P. zonale*), ENRICH BM (Bronopol 27%w/w), KOBE 1.2 SL (Chrysophanol 12g/l) and 1% DMSO] was mixed with sterile distilled water in different containers at recommended rates (Table 6.1) and the mixed solutions applied as soil drench to their target treatment pots at the rate of 90 ml per pot translating to (3 L/M^2) as described by Kumar (2021). Two weeks after plant emergence, 2 foliar applications of the above treatments were applied on the emerged plants at 2 weeks interval. Each treatment was mixed with sterile distilled water at recommended rates and the solution applied to respective test plants as foliar spray using hand sprayers. Maximum plant coverage was observed during foliar treatment applications. All potato agronomic practices (watering, fertilization, insect pest and disease control) except application of additional bactericides were conducted according to potato optimal production requirements. The experiment was repeated once and in each experimental period, the experiment was terminated once the test plant exhibited senescence symptoms.

6.2.5 Determination of *Ralstonia solanacearum* population in the soil at harvest

During the experiment termination phase, soil samples were collected around the rhizosphere at 15-20 cm depth from each treatment and taken to the laboratory to evaluate the effect of each treatment on the *R. solanacearum* population. A sterile trowel was used to collect soil samples per pot in each treatment and the samples mixed thoroughly to form a composite sample from which 10 g of soil sample was sub-sampled and placed in plastic bags. The bags were labeled and taken to the laboratory for microbial analysis. Three samples were collected per treatment. In the laboratory, 1 g of soil per sample was suspended in 10 mL of sterile distilled water and shaken for 30 minutes. Ten-fold serial dilution was then conducted on each sample to attain 10^{-3} CFU/mL from which 1 mL/sample was cultured on Kelma's TZC agar media as described by Kelman (1954) and Karim and Hossain (2018) using pour plate method. The TZC plates were incubated at 28 ± 1^0C for 48 hours. After 48 hours, the number of bacterial colonies were counted, and bacterial population determined using the following formula;

$$CFU/mL = \frac{Total\ number\ of\ colonies}{Plated\ volume\ (mL)} * dilution\ factor$$

(10)

6.2.6 Data collection

Agronomic data was collected on plant emergence, plant heights, number of stems, tuber grade and yield (Kgs). The number of bacterial wilt colony forming units were counted at the end of the experiment. Disease severity and incidence data was recorded after plant emergence at two weeks intervals. Disease severity scale of 1-3 based on the degree of wilting of the affected plant whereby 1 = healthy plant, 2 = ≤50% of the plant foliage wilted and 3 = ≥50% of plant foliage wilted was used. Percent disease severity (%) was calculated using the following formula;

$$S = 100(\frac{\sum n}{N * Max.score\ in\ the\ scale})$$

(11)

Whereby S = percent disease severity, Σn = summation of wilt scores, and N = total number of plants evaluated per treatment (Mihovilovich *et al.,* 2017).

Disease incidence was recorded by assessing the number of symptomatic plants per treatment at two weeks interval from which Percent disease incidence [PDI (%)] was calculated using the following formula (Okeyo *et al.,* 2018);

$$PDI(\%) = \frac{\text{Number of symptomatic plants}}{\text{Total number of plants assessed}} * 100$$

(12)

Percent disease incidence control [PDIC (%)] achieved in treated plots compared to untreated control was calculated using the following formula;

$$\text{PDIC } (\%) = \frac{PDI\ in\ UCP - PDI\ in\ TC}{PDI\ in\ UCP} * 100$$

(13)

Whereby PDI= Percentage Disease Incidence, UCP=Untreated Control Plot and TC= Treated Control.

Percent reduction in number of bacterial colonies [PRNVBC (%)] achieved in treated plots compared to untreated control was calculated using the following formula;

$$\text{PRNVBC (\%)} = \frac{NC\ in\ UCP - NC\ in\ TC}{NC\ in\ UCP} * 100 \quad (14)$$

Whereby NC= Number of bacterial colonies, UCP=Untreated Control Plot and TC= Treated Control.

The harvested tubers were graded as symptomatic tubers (for tubers showing either externally visible and/or internal bacterial wilt symptoms) and asymptomatic tubers (for tubers without both external and internal bacterial wilt symptoms). The harvested tubers were weighed and recorded in kilograms (Kgs).

6.2.7 Data analysis

All the data collected from the two experiments except disease severity was subjected to Levene tests to test for equal variance (Levene, 1960) before testing for mean differences using Mann-Whitney U test at 5% probability level (Mann & Whitney, 1947; Wilcoxon, 1945, 1992) using R software, version 4.2.2 (R Studio Team 2020). The data was then pooled together since there were no significant differences between the treatments and the pooled data was subjected to normality tests using Shapiro-Wilk tests (Shapiro & Wilk, 1965) at $p<0.05$ in R software, version 4.2.2 (R Studio Team 2020). Where the data lacked equal variance as well as normal distribution, the data were transformed using the formula;

$$\text{Log}\ (X + 1) \quad (15)$$

The pooled data was then subjected to two-way analysis of variance (ANOVA) using R software, version 4.2.2 (R Studio Team 2020). The treatment means were separated using Tukey's Honestly Significant Difference (HSD) at $p \leq 0.05$ with the agricolae package. The following statistical model was fitted for green house and laboratory experiment:

$$Yij = \mu + Ti + \varepsilon ij \quad (16)$$

Whereby Means μ= general mean, $T_{i=}$ Treatment effect and ε_{ij} = effects due to random error.

The percent disease severity data per treatment was used to calculate area under disease progress curve (AUDPC) values using the following formula;

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

(17)

Whereby yi= percent severity score at the ith observation, ti = time in days at the ith observation and n= total number of observations.

6.3 Results

6.3.1 Efficacy of ethanolic leaf extracts of *P. zonale* and *P. guajava* against *Ralstonia solanacearum* of potatoes under greenhouse condition

The two-way analysis of variance (ANOVA) results revealed highly significant interactions between treatments and varieties (Treatment x Variety) among all the tested parameters except for percent plant emergence (% PE), number of stems (NS), stem heights (SH), final wilt incidence (FWI) and proportion of symptomatic tuners (PST) (Table 6.2). ANOVA results also revealed highly significant effects among treatments and varieties across all the response values.

Table 6.2: F-test statistics of two-way ANOVA of percent plant emergence, number of stems, stem heights, colony counts, final wilt incidence, symptomatic tubers, number of tubers and weight (Kgs)

Source of variation	Df	% PE	NS	SH	CC	FWI	PST	NAST	Weight (t/ha)
Treatment	5	1	5.61***	3.95**	10447.81***	4.77***	1	155.88***	66.20***
Variety	1	1	42.20***	50.48***	2773.66***	140.48***	1	962.17***	469.04***
Treatment * variety	5	1	0.63	2.02	10.50***	1.63	1	49.67***	12.46***
Residuals	60								

Significance codes: 0 '***', 0.001 '**', 0.01 '*' 0.05 's' 0.1 '' 1. Df = Degree of freedom, % PE = Percent plant emergence, NS= Number of stems, SH = Stem heights, CC = colony counts, FWI = Final wilt incidence, PST = Proportion of symptomatic tubers and NAST = Number of asymptomatic tubers.

6.3.2 Effect of ethanolic leaf extracts of *P. zonale* and *P. guajava* treatment on mean percent plant emergence and number of stems for Shangi and Sherekea varieties

The mean percent plant emergence was not significantly different at $p \leq 0.05$ across treatments and varieties. All the varieties recorded 100% plant emergence per treatment. The mean number of stems were significantly different at $p \leq 0.05$ across treatments and varieties. However, there was no significant interaction effect at $p \leq 0.05$ between treatments and varieties (Treatment x variety) (Table 6.2). KOBE 1.2 SL recorded the highest numbers of stems in Shangi (0.93) and Sherekea (0.88) while untreated controls and 1% DMSO recorded the least for both varieties respectively (Table 6.3). Shangi had the highest overall mean number of stems (0.89) while Sherekea had the least (0.81).

Table 6. 3: Effect of *P. zonale* and *P. guajava* treatments on mean percent plant emergence and number of stems for Shangi and Shareke potato varieties

Treatments	Mean percent emergence and Mean number of stems			
	Percent emergence		Number of stems	
	Shangi	Sherekea	Shangi	Sherekea
Untreated control	100 ± 0.00a	100 ± 0.00a	0.86 ± 0.02 abcde	0.77 ± 0.04 e
Psidium guajava	100 ± 0.00a	100 ± 0.00a	0.91 ± 0.04 ab	0.80 ± 0.04 cde
Pelargonium zonale	100 ± 0.00a	100 ± 0.00a	0.88 ± 0.03 abc	0.80 ± 0.07 cde
ENRICH BM	100 ± 0.00a	100 ± 0.00a	0.87 ± 0.07 abcd	0.83 ± 0.07 bcde
KOBE 1.2 SL	100 ± 0.00a	100 ± 0.00a	0.93 ± 0.05 a	0.88 ± 0.04 abc
1% DMSO	100 ± 0.00a	100 ± 0.00a	0.86 ± 0.03 abcde	0.78 ± 0.03 de
Grand mean	100.00	100.00	0.89	0.81
MSD	0.00	0.00	0.09	0.09
CV	0.00	0.00	0.27	0.36

The values are the average percent plant emergence ± standard deviations and number of stems ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$, CV = coefficient of variation, MSD = mean square displacement.

6.3.3 Effect of ethanolic leaf extracts of *P. zonale* and *P. guajava* treatment on mean stem heights for Shangi and Sherekea varieties

The transformed mean stem heights were significantly different at $p \leq 0.05$ across varieties. The transformed mean stem heights of Shangi were not significantly different at $p \leq 0.05$ across all treatments except for untreated control while those of Sherekea exhibited insignificant differences at $p \leq 0.05$ across all the treatments. Sherekea recorded the highest average mean stem heights while Shangi recorded the least (Table 6.4).

Table 6. 4: Transformed mean stem heights recorded per treatment for Shangi and Sherekea potato varieties

Treatments	Transformed mean stem heights	
	Shangi	Sherekea
Untreated control	1.69 ± 0.09 c	1.82 ± 0.03 a
Psidium guajava	1.78 ± 0.03 ab	1.85 ± 0.02 a
Pelargonium zonale	1.78 ± 0.04 ab	1.83 ± 0.03 a
ENRICH BM	1.78 ± 0.06 ab	1.83 ± 0.04 a
KOBE 1.2 SL	1.78 ± 0.01 ab	1.83 ± 0.04 a
1% DMSO	1.72 ± 0.03 bc	1.81 ± 0.03 a
Grand mean	1.76	1.83
MSD	0.08	0.08
CV	0.15	0.10

The values are the average stem heights ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$, CV = coefficient of variation, MSD = mean square displacement.

6.3.4 Effect of ethanolic leaf extracts of *P. zonale* and *P. guajava* treatments on potato bacterial wilt incidence symptom expression and area under disease progress curves (AUDPCs) on Shangi and Sherekea potato varieties

Bacterial wilt incidence (BWI) varied across treatments and varieties and were significantly different at $p \leq 0.05$ at 38 days after planting (DAP), 52 DAP and 66 DAP. Both Shangi and Sherekea varieties showed significantly increased BWIs from 38 DAP to 66 DAP, but the increase was variety dependent. For Shangi, BWI commenced at 38 DAP and increased rapidly to 66 DAP. Apart from *Pelargonium zonale* leaf extract which exhibited BWI at 38 DAP, most

treatments expressed BWIs at 52 DAP for Sherekea and the BWI progressed at a slow rate to 66 DAP (Table 6.5). In general, Shangi exhibited high final bacterial wilt incidences (FBWIs) at all treatment levels compared to Sherekea. Area under disease progress curves (AUDPCs) varied across treatments and varieties. For both varieties, untreated control and 1% DMSO recorded the highest AUDPCs of 2155.68 (untreated control) and 2154.28 (1% DMSO) for Shangi and 1236.55 (untreated control) and 1237.04 (1% DMSO) for Sherekea respectively. In general, Shangi exhibited high AUDPCs at all treatment levels compared to Sherekea (Figure 6.1).

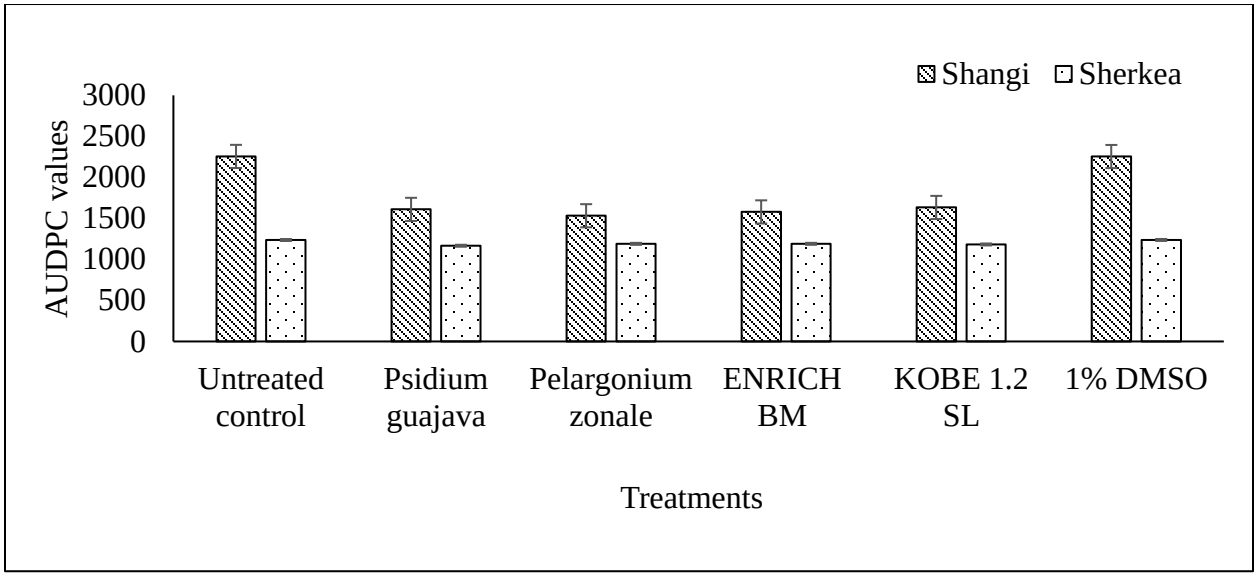


Figure 6. 1: Effect of *Psidium guajava* and *Pelargonium zonale* plant extracts on Area under disease progress curves (AUDPCs) on Shangi and Sherekea potato varieties. Bars represent standard deviation.

Table 6. 5: Effect of *Psidium guajava* and *Pelargonium zonale* plant extracts against bacterial wilt incidence symptom expression on Shangi and Sherekea potato varieties

Treatments	38 DAP		52 DAP		66 DAP	
	Shangi	Sherekea	Shangi	Sherekea	Shangi	Sherekea
Untreated control	1.30 ± 0.65 a	0.00 ± 0.00 c	1.91 ± 0.00 ab	0.44 ± 0.68 cd	2.00 ± 0.00 a	0.93 ± 0.73 bcd
Psidium guajava	0.66 ± 0.72 abc	0.00 ± 0.00 c	1.23 ± 0.63 abc	0.00 ± 0.00 d	1.79 ± 0.12 ab	0.00 ± 0.00 e
Pelargonium zonale	0.71 ± 0.78 abc	0.22 ± 0.54 bc	1.52 ± 0.15 ab	0.22 ± 0.54 d	1.67 ± 0.09 ab	0.27 ± 0.66 de
ENRICH BM	0.71 ± 0.78 abc	0.00 ± 0.00 c	1.45 ± 0.20 ab	0.00 ± 0.00 d	1.67 ± 0.09 ab	0.49 ± 0.76 cde
KOBE 1.2 SL	0.54 ± 0.83 abc	0.00 ± 0.00 c	1.39 ± 0.72 ab	0.00 ± 0.00 d	1.74 ± 0.16 ab	0.44 ± 0.68 cde
1% DMSO	1.20 ± 0.60 ab	0.00 ± 0.00 c	1.92 ± 0.08 a	1.10 ± 0.54 bc	2.00 ± 0.00 a	1.20 ± 0.60 abc
Grand mean	0.85	0.04	1.57	0.29	1.57	0.56
MSD	1.06	1.06	0.81	0.81	0.88	0.88
CV	5.13	1.13	13.5	6.07	0.29	6.13

The values are the average bacterial wilt incidence ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$, CV = coefficient of variation, MSD = Mean square displacement, DAP = days after planting.

6.3.5 Effect of ethanolic leaf extracts of *P. zonale* and *P. guajava* treatment on potato yield parameters for Shangi and Sherekea varieties

6.3.5.1 Number of asymptomatic tubers

The mean number of asymptomatic tubers by visual assessment were significantly different at $p \leq 0.05$ across treatments and varieties. KOBE 1.2 SL (Chrysophanol 12g/l) of Sherekea recorded the highest number of asymptomatic tubers (1.87) while untreated control of Shangi recorded the least (1.41) (Table 6.6). Sherekea had the highest overall mean number of asymptomatic tubers (1.76) while Shangi had the least (1.59). However, dissection of all the sampled asymptomatic tubers per treatment per variety revealed brown discoloration of the vascular ring and the adjacent tissues extending to the pith and/or tuber cortex (Plate 6.1).

Table 6. 6: Transformed mean number of asymptomatic tubers recorded per treatment from Shangi and Sherekea potato varieties

Treatments	Transformed mean No. of asymptomatic tubers	
	Shangi	Sherekea
Untreated control	1.41 ± 0.04 g	1.71 ± 0.02 cd
Psidium guajava	1.68 ± 0.04 def	1.78 ± 0.01 b
Pelargonium zonale	1.67 ± 0.03 def	1.75 ± 0.01 bc
ENRICH BM	1.67 ± 0.01 ef	1.75 ± 0.01 bc
KOBE 1.2 SL	1.65 ± 0.03 f	1.87 ± 0.02 a
1% DMSO	1.43 ± 0.02 g	1.70 ± 0.02 de
Grand mean	1.59	1.76
MSD	0.05	0.05
CV	0.11	0.05

The values are the average number of asymptomatic tubers ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$, CV = coefficient of variation, MSD = mean square displacement.

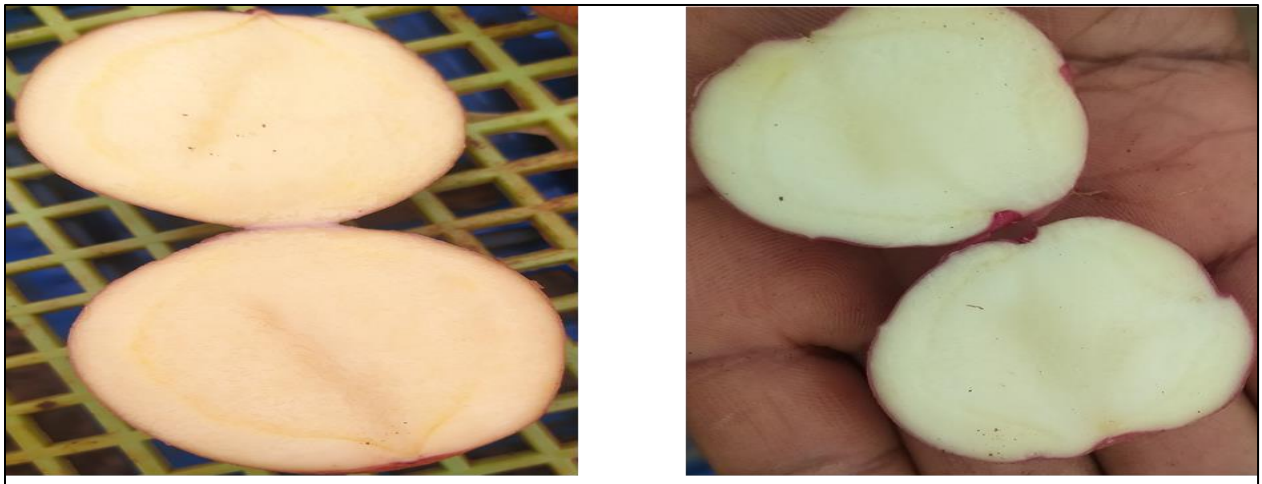


Plate 6. 1: A) Vascular ring symptoms on tubers sampled from Shangi and B) Vascular ring symptoms on tubers sampled from Sherekea.

6.3.5.2 Tuber weights

Mean tuber weights (Kgs) were significantly different at $p \leq 0.05$ across treatments and varieties. KOBE 1.2 SL (Chrysophanol 12g/l) of Sherekea recorded the highest transformed tuber weights (0.31) while untreated control and 1% DMSO of Shangi recorded the least (0.17) (Table 6.7). Sherekea had the highest overall transformed mean tuber weights (0.29) while Shangi had the least (0.22).

Table 6. 7: Transformed mean tuber wights (Kgs) recorded per treatment from Shangi and Sherekea potato varieties

Treatments	Transformed mean tuber weights (Kgs)	
	Shangi	Sherekea
Untreated control	0.17 ± 0.01 g	0.26 ± 0.02 def
Psidium guajava	0.24 ± 0.01 f	0.29 ± 0.01 abc
Pelargonium zonale	0.25 ± 0.01 ef	0.28 ± 0.01 bcd
ENRICH BM	0.25 ± 0.01 ef	0.30 ± 0.02 ab
KOBE 1.2 SL	0.25 ± 0.01 ef	0.31 ± 0.01 a
1% DMSO	0.17 ± 0.01 g	0.27 ± 0.01 cde
Grand mean	0.22	0.29
MSD	0.02	0.02
CV	0.27	0.28

The values are the average transformed tuber weights (Kgs) ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$, CV = coefficient of variation, MSD = mean square displacement.

6.3.6 Effect of ethanolic leaf extracts of *P. zonale* and *P. guajava* treatments against *Ralstonia solanacearum* colony counts

The transformed mean bacterial wilt colony counts from soils sampled around the rhizosphere of the two potato varieties were significantly different at $p \leq 0.05$ across treatments and varieties. Untreated control and 1% DMSO recorded the highest mean transformed colony counts of 3.15 and 3.16 for Shangi and 3.06 and 3.05 for Sherekea respectively. *Pelargonium zonale* (Treatment 3) recorded the least mean transformed colony counts of 2.70 for Shangi and 2.63 for Sherekea (Table 6.8).

Table 6. 8: Transformed mean bacterial wilt colony counts per treatment recorded from the soil samples sampled around the rhizosphere of Shangi and Sherekea potato varieties

Treatments	Transformed mean bacterial wilt colony counts	
	Shangi	Sherekea
Untreated control	3.15 ± 0.01 a	3.06 ± 0.00 b
Psidium guajava	2.78 ± 0.01 d	2.69 ± 0.00 f
Pelargonium zonale	2.70 ± 0.00 f	2.63 ± 0.01 g
ENRICH BM	2.80 ± 0.01 c	2.73 ± 0.00 e
KOBE 1.2 SL	2.78 ± 0.01 d	2.70 ± 0.01 f
1% DMSO	3.16 ± 0.00 a	3.05 ± 0.00 b
Grand mean	2.9	2.81
MSD	0.01	0.01
CV	0.01	0.01

The values are the average transformed mean bacterial wilt colony counts ± standard deviations. Means within the same column having the same letter(s) do not differ significantly at $p \leq 0.05$, CV = coefficient of variation, MSD = mean square displacement.

6.4 Discussion

The bacterial isolate completely oxidized all the disaccharide sugars and hexose alcohols within 5 days of incubation. These results confirmed the findings of Boschi *et al.* (2017), Khasabulli *et al.* (2017), Popoola *et al.* (2015) and Rahman *et al.* (2010) who classified *Ralstonia pseudosolanacearum* sp. nov. bacterial isolate with similar traits as biovar III race 1 *(Ralstonia solanacearum* (phylotype I)). The mean plant emergence was not significantly different at $p \leq 0.05$ across all treatments and varieties. All the two varieties recorded 100% plant emergence per treatment which could be an indication that the applied treatments and soil inoculation with bacterial wilt pathogen did not affect plant emergence. The mean number of stems varied across treatments and varieties. All the treated pots exhibited a higher mean number of stems per variety compared to untreated controls. The soil drench of various treatments (plant extracts and conventional bactericide) used in this study might have suppressed apical dominance on the planted tubers resulting in increased number of lateral buds, sprouts and hence increased number of stems (Biruk-Masrie *et al.,* 2015). Similarly, Biruk-Masrie *et al.* (2015) also reported a high number of stems from potato tubers treated with extracts from essential oils. KOBE 1.2 SL

(Chrysophanol 12g/l) (Treatment 5) recorded the highest mean number of stems for both varieties, and this could be an indication that KOBE 1.2 SL had high phytochemical composition compared to other treatments. In general, Shangi recorded the highest mean number of stems per treatment compared to Sherekea and this can be attributed to difference in their genetic traits as well as high number of tuber eyes recorded on Shangi variety at planting (Nielson *et al.,* 1989).

The mean stem heights were significantly different at $p \leq 0.05$ across varieties. The mean stem heights of Shangi were not significantly different across all treatments except for untreated control (Treatment 1) while those of Sherekea exhibited insignificant differences across all the treatments. Both varieties exhibited insignificantly higher stem heights from pots with various treatment applications compared to negative control pots. The high stem heights from treated pots can be attributed to low disease indices scored on potato plants planted in these pots (Chen *et al.,* 2020; Priou *et al.,* 1999). Additionally, the research findings in chapter five reported presence of Succinic acid (one of the major components used in the manufacture of bio-stimulants) in low concentrations from the ethanolic leaf extracts of *Psidium guajava* and *Pelargonium zonale* and this could have resulted to slight increase in stem heights in pots treated with the two extracts compared to untreated pots (Levchyk *et al.,* 2017; Zeikus *et al.,* 1999). Liu *et al.* (2016) also reported the bio-stimulant effect of Chrysophanol on potato plants and this can explain the high stem height in pots treated with Chrysophanol. Sherekea exhibited the highest average stem heights compared to Shangi and this can be attributed to differences in genetic traits for plant height expression from the two varieties.

The final bacterial wilt incidences (FBWIs) and area under disease progress curves (AUDPCs) varied across treatments and varieties. All the treated pots recorded low FBWIs and AUDPCs compared to the negative control pots. This agreed with the research findings of Abd-Elrahim *et al.* (2021), Chen *et al.* (2020) and Oboo *et al.* (2014) who reported in-vivo efficacy of plant extracts (essential oils) against bacterial wilt of potatoes with regards to disease indices (disease incidence and disease severity). Both KOBE 1.2 SL (Chrysophanol 12g/l) and ENRICH BM (Bronopol 27%w/w) are known to control bacterial wilt pathogen through induced host plant resistance (*https://agroduka.com/enrich-bm;* Liu *et al.,* 2016), while the mode of action of both *P. guajava* and *P. zonale* against this pathogen is still unknown. In general, Shangi exhibited high FBWIs and AUDPCs at all treatment levels compared to Sherekea and this can be attributed to their varied degree of resistance to bacterial wilt pathogen (Muthoni *et al.,* 2014; Patil *et al.,* 2012).

Both the mean number of asymptomatic tubers and tuber weight (kgs) were significantly different across treatments and varieties. All the treated pots recorded a significantly higher number of asymptomatic tubers and tuber weights (Kgs) compared to negative control pots. These results were similar to those of Abd-Elrahim *et al.* (2021) who reported increased yield parameters from bacterial wilt inoculated potato plants treated with plant extracts *in-vivo*. Sherekea variety recorded the highest yield parameters per treatment compared to Shangi and this can be attributed to high bacterial wilt resistance level of Sherekea compared to Shangi. KOBE 1.2 SL (Chrysophanol 12g/l) recorded the highest yield parameters per variety, and this can be attributed to its high concentration of antibacterial compounds compared to other treatments. Additionally, chapter five experimental results revealed that the overall proportion of antibacterial compounds per plant extract was less than 10% for both *P. zonale* and *P. guajava* and hence the lower yields compared to KOBE 1.2 SL (Chrysophanol 12g/l). Dissection of all the sampled asymptomatic tubers per treatment per variety revealed 100% bacterial wilt incidence (brown discoloration of the vascular ring and the adjacent tissues extending to the pith and/or tuber cortex). This is an indication that all the asymptomatic tubers harvested from all the treated pots had latent infection symptoms and therefore cannot be used as seed potato tubers (Patil *et al.,* 2012). Hundred percent wilt incidence on the sampled asymptomatic tubers can be attributed to wounds created on the tubers at planting to avoid disease escape.

Bacterial wilt (*Ralstonia solanacearum*) colony counts from the soils sampled around the rhizosphere revealed significantly low *Ralstonia solanacearum* population from all treated pots compared to negative control pots. These results confirm the findings of Abd-Elrahim *et al.* (2021) and Chen *et al.* (2020) who reported reduced bacterial wilts population from soils sampled from pots treated with various plant extracts *in-vivo*. *P. zonale* recorded the least mean bacterial wilt population among all the treated pots for both the two varieties (Shangi and Sherekea). This confirmed the *in-vitro* study results from chapter four which reported *P. zonale* as the most effective plant extract against *Ralstonia solanacearum* of potato. In general, Shangi had the highest bacterial wilt population per treatment, and this can be attributed to high disease indices (disease incidence and disease severity) scored per treatment for Shangi compared to Sherekea.

6.5 Conclusion and Recommendation

The present study revealed *in-vivo* antibacterial efficacy of *P. zonale* and *P. guajava* leaf extracts against soil inoculated *R. solanacearum*. However, the efficacy results were dependent on

resistant levels of varieties used in the study against *R. solanacearum* Sherekea variety exhibited the highest efficacy from the two plant extracts demonstrating the synergistic effect of host plant resistance and botanicals in management of bacterial wilt of potatoes. The results were comparable to those of Enrich BM (Bronopol 27%w/w) a conventional bactericide and KOBE SL (Chrysophanol 12g/l) a botanical bactericide. Further studies should be conducted to assess the efficacy of *P. zonale* and *P. guajava* against the target pathogen under field conditions.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

Ralstonia solanacearum infestation has affected optimal potato production in various fields leading to yield losses ranging between 30-100% in potato fields (Longwe *et al.*, 2023; Muthoni *et al.*, 2014). Various management options such as cultural (crop rotation, field sanitation and planting of disease-free materials), chemical control and biological control (use of antagonistic microbes and botanicals) have been proposed against this pathogen (Charkowski *et al.,* 2020; Longwe *et al.,* 2023; Wang *et al.,* 2023). However, most of the proposed management options have myriads of drawbacks which have hindered their success. Additionally, there is limited development of efficient eco-friendly management strategies against *R. solanacearum* pathogen (Wang *et al.,* 2023). Chapter three of this study revealed the ability of host-plant resistance in the management of *R. solanacearum* pathogen in the field. The field efficacy of host-plant resistance against bacterial wilt pathogen was dependent on genotype, bacterial wilt population in the soil and other abiotic factors such as temperature and rainfall (Muthoni *et al.,* 2014; Patil *et al.,* 2012). In general, highly susceptible genotype recorded the highest bacterial wilt disease indices (bacterial wilt incidence and severity) while highly resistant genotypes recorded the least.

In-vitro screening of antibacterial activity of ethanolic and acetonic extracts of four different plants revealed that the two extraction solvents had no significant effect on antibacterial activity of the four plant extracts against bacterial wilt pathogen. However, the total amount of extracted paste varied per solvent and plant part. Apart from *Moringa oleifera* seeds, ethanol as a polar extraction solvent recorded significantly higher percentage extract yields from all the leaves compared to acetone, a non-polar solvent. Among the four plant extracts, *Pelargonium zonale* leaves were the most effective extract against *R. solanacearum* while *Moringa oleifera* seeds were the least effective. The results of *P. zonale* were comparable to those of ENRICH BM (Bronopol 27%w/w) (conventional bactericide) but was significantly higher than that of KOBE 1.2 SL (Chrysophanol 12g/l) (botanical bactericide). Further screening of most effective plant extracts to determine their minimum inhibitory concentration (MIC) revealed that the antibacterial activity of the *P. zonale* and *P. guajava* leaf extracts decreased with decreasing extract concentration. Both ethanolic and acetonic leave extracts of *P. zonale* and *P. guajava* exhibited MICs of 6.25mg/mL of 1% DMSO against *R. solanacearum*. These *in-vitro* results confirmed the findings of Biswal

(2015) and Oboo *et al.* (2014) who reported antibacterial activity of these plant extracts against *R. solanacearum in-vitro*. However, some plant extracts like *P. zonale* and *P. guajava* leaf extracts exhibited high growth inhibition zones which contrasted the findings of Biswal (2015) and this can be attributed to difference in plant species, plant parts used in the study, adopted extraction method and varied agro-climatic conditions coupled with diverse abiotic factors during the plant growth (Gololo, 2018; Kumar *et al.*, 2017; Liu *et al.*, 2016; Mutimawurugo *et al.*, 2020). The decrease in antibacterial activity of plant extracts against *R. solanacearum* with decreased extract concentration can be attributed to reduced toxicity levels of bioactive compounds due to dilution effect (Mutimawurugo *et al.*, 2020).

Phytochemical profiling study on *Pelargonium zonale* and *Psidium guajava* leaf extracts revealed the presence of six phytochemicals namely flavonoids, phenols, alkaloids, saponins, terpenoids and tannins. This confirmed the findings of Amel *et al.* (2022), Barbalho *et al.* (2012), Krishnarathi *et al.* (2014), Oncho *et al.* (2021), Porwal *et al.* (2012), Saraswathi *et al.* (2011), and Thenmozhi and Rajan (2015) who reported flavonoids, phenols, alkaloids, saponins, terpenoids and tannins as the main phytochemical components in *P. zonale* and *P. guajava* respectively. The GC-MS analysis revealed that *P. zonale* and *P. guajava* had seven similarly antibacterial compounds; Shikimic acid, Phytol, Protocatechuic acid, Pyrogallol, Fumaric acid, 3,4,5-Trihydroxybenzoic acid ethyl ester and 4-Hydroxybenzoic acid but *P. zonale* had one additional antibacterial compound, Lactic acid. The concentration of these antibacterial compounds varied per plant. In both cases, shikimic acid exhibited the highest concentration (peak areas) and hence could be the main antibacterial compound against *R. solanacearum* in the two plant extracts.

In-vivo screening of antibacterial activity of ethanolic leaf extracts of the most effective plant extracts (*P. zonale* and *P. guajava*) revealed that the two extracts effectively managed *R. solanacearum* population and bacterial wilt disease indices (incidence and severity) leading to increased yields from treated pots and the results were comparable to those obtained from pots treated with positive controls [ENRICH BM (Bronopol 27%w/w) (conventional bactericide) and KOBE 1.2 SL (Chrysophanol 12g/l) (botanical bactericide)]. However, the efficacy results were dependent on resistant levels of varieties used in the study. Sherekea exhibited the highest efficacy from the two plant extracts demonstrating the synergistic effect of host plant resistance and botanicals in management of bacterial wilt of potatoes.

7.2 Conclusions

The present study aimed at evaluation of the efficacy of host plant resistance and plant extracts in the management of bacterial wilt (R*alstonia solanacearum*) of potato and to contribute to increased food security in Kenya through improved potato production. From the results, the following conclusions were made:

i. Genotypes CIP 515004.535 and CIP 515008.561 were ranked as highly resistant, CIP 515008.521 as resistant, Sherekea, CIP 515002.516, CIP 515008.530, CIP 515008.503, CIP 515008.555, CIP 515014.567 and CIP 515013.558 as moderately resistant, Chulu, Kenya Karibu, Wanjiku and Lenana as moderately susceptible, Kenya Mpya, Nyota, Unica, Dutch Robyjin, Konjo, Purple gold, CIP 515002.528, CIP 515011.555 and CIP 515006.507 as susceptible while Shangi, Asante, Tigoni, Arka and CIP 515008.535 were highly susceptible. Additionally, all the highly resistant, resistant, moderately resistant and moderately susceptible genotypes except CIP: 515008.561 displayed internal bacterial wilt symptoms from dissected tubers and hence are only suitable for ware potato production as opposed to seed potato production.

ii. *Pelargonium zonale* and *Psidium guajava* leaf extracts had the highest *in-vitro* antibacterial activity against *Ralstonia solanacearum* among the four tested plants. The antibacterial activity of *P. zonale* and *P. guajava* leaf extracts was not affected by extraction solvents.

iii. Phytochemical profiling revealed the presence of all the six tested phytochemicals: flavonoids, phenols, alkaloids, saponins, terpenoids and tannins in both *P. zonale* and *P. guajava* leaves. GC-MS analysis revealed 8 antibacterial compounds (Shikimic acid, Phytol, Protocatechuic acid, Pyrogallol, Fumaric acid, 3,4,5-Trihydroxybenzoic acid ethyl ester, 4-Hydroxybenzoic acid and Lactic acid) from ethanolic leaf extracts of *P. zonale* and 7 antibacterial compounds (Shikimic acid, Phytol, Protocatechuic acid, Pyrogallol, Fumaric acid, 3,4,5-Trihydroxybenzoic acid ethyl ester and 4-Hydroxybenzoic acid) from ethanolic leaf extracts of *P. guajava*. In both plant extracts, Shikimic acid had the highest percent peak area among the detected antibacterial compounds and hence could be the main bioactive component against *R. solanacearum*.

iv. *In-vivo* studies revealed *in-vivo* antibacterial efficacy of *P. zonale* and *P. guajava* leaf extracts against soil inoculated *R. solanacearum* but the efficacy results were dependent on resistant levels of varieties used in the study. Sherekea (moderately resistant commercial

variety) exhibited the highest efficacy from the two plant extracts demonstrating the synergistic effect of host plant resistance and botanicals in the management of bacterial wilt (*R. solanacearum*) of potatoes.

7.3 Recommendations

i. Highly resistant, resistant, moderately resistant and moderately susceptible genotypes should be promoted for adoption and commercialization by farmers. They can also be used in potato breeding programs to enhance the resistance of already released potato varieties. However, due to their latent infection symptoms, they should only be used for ware potato production as opposed to seed potato production.

ii. *In-vitro* studies revealed antibacterial potency of *Pelargonium zonale* and *Psidium guajava* leaf extracts against *R. solanacearum* and hence are recommended for bacterial wilt management.

iii. Shikimic acid, Phytol, Protocatechuic acid, Pyrogallol, Fumaric acid, 3,4,5-Trihydroxybenzoic acid ethyl ester, 4-Hydroxybenzoic and Lactic acid were detected as the bioactive compounds against *R. solanacearum* and hence should be formulated for commercialization as botanical bactericides to reduce environmental hazards associated with conventional bactericides.

iv. *In-vivo* screening of antibacterial activity of ethanolic leaf extracts of *P. zonale* and *P. guajava* against *R. solanacearum* revealed effective synergistic effect of botanicals and host-plant resistance in the management of *R. solanacearum* and hence should be promoted as an integrated disease management (IDM) option.

7.4 Areas for further studies

i. Most of the highly resistant, resistant, moderately resistant and moderately susceptible genotypes recorded very low yields which could affect their future adoption and commercialization by farmers and hence further research should be done to integrate high yielding traits in these genotypes.

ii. Further screening should be conducted to determine the singular and/or synergistic antibacterial activity of the detected antibacterial compounds against *R. solanacearum*.

iii. The detected compound should also be tested under different formulations to identify the most effective formulation for commercialization.

iv. Advanced studies should also be conducted to determine the mode of action of each of the detected antibacterial compounds against *R. solanacearum* as well as their physiological interactions with the test plants.

v. Further studies should be conducted to assess the efficacy of *P. zonale* and *P. guajava* against the target pathogen under field conditions.

vi. Further studies should be undertaken to determine both invitro and in-vivo efficacy of *P. zonale* and *P. guajava* leaf extracts against other potato pathogens.

REFERENCES

Abd-Elrahim, R., Tohamy, M. R., Atia, M. M., Elashtokhy, M. M., & Ali, M. A. (2021). Bactericidal activity of some plant essential oils against *Ralstonia solanacearum* infection. *Saudi Journal of Biological Sciences*, *29*(4), 2163-2172. https://doi.org/10.1016/j.sjbs.2021.11.045.

Abdurahman, A., Parker, M. L., Kreuze, J., Elphinstone, J. G., Struik, P. C., Kigundu, A., & Sharma, K. (2019). Molecular epidemiology of *Ralstonia solanacearum* species complex strains causing bacterial wilt of potato in Uganda. *Phytopathology*, *109*(11), 1922-1931. https://doi.org/10.1094/PHYTO-12-18-0476-R.

Abo-Elyousr, K. A. M., & Asran, M. R. (2009). Antibacterial activity of certain plant extracts against bacterial wilt of tomato. *Archives of Phytopathology and Plant Protection*, *42*(6), 573-578. https://doi.org/10.1080/03235400701284740.

Adamczak, A., Ożarowski, M., & Karpiński, T. M. (2020). Antibacterial activity of some flavonoids and organic acids widely distributed in plants. *Journal of Clinical Medicine*, *9*(1), 109-125. https://doi.org/10.3390/jcm9010109.

Adamski, Z., Blythe, L. L., Milella, L., & Bufo, S. A. (2020). Biological activities of alkaloids: from toxicology to pharmacology. *Toxins*, *12*(4), 210-213. https://doi.org/10.3390/toxins12040210.

Adhikari, T. B., & Basnyat, R. C. (1998). Effect of crop rotation and cultivar resistance on bacterial wilt of tomato in Nepal. *Canadian Journal of Plant Pathology*, *20*(3), 283-287. https://doi.org/10.1080/07060669809500394.

Adolf, B., Andrade-Piedra, J., Bittara Molina, F., Przetakiewicz, J., Hausladen, H., Kromann, P., & Secor, G. A. (2020). Fungal, oomycete, and plasmodiophorid diseases of potato. In H. Campos & O. Ortiz (Eds.), *The potato crop* (pp. 307-350). Springer, Cham. https://link.springer.com/chapter/10.1007/978-3-030-28683-5_9.

Agrios, G. N. (2005). Plant Pathology (5th ed.). Elsevier Academic Press.

Agutaa, A. A. (2015). *Effect of crop rotation pattern on soil bacterial wilt (Ralstonia solanacearum) population and potato (Solanum tuberusum L.) yield in Njoro, Kenya* [Master's thesis, Egerton University]. http://41.89.96.81:8080/xmlui/handle/123456789/1988.

Ahmed, W., Yang, J., Tan, Y., Munir, S., Liu, Q., Zhang, J., & Zhao, Z. (2022). *Ralstonia solanacearum*, a deadly pathogen: Revisiting the bacterial wilt biocontrol practices in tobacco and other Solanaceae. *Rhizosphere*, *21*, 100479. https://doi.org/10.1016/j.rhisph.2022.100479.

Aldulaimi, O., Drijfhout, F., Uche, F. I., Horrocks, P., & Li, W. W. (2019). Discovery, synthesis and antibacterial evaluation of phenolic compounds from *Cylicodiscus gabunensis*. *BMC Complementary and Alternative Medicine*, *19*(1), 1-11. https://doi.org/10.1186/s12906-019-2589-2.

Alemayehu, M., Jemberie, M., Yeshiwas, T., & Aklile, M. (2020). Integrated application of compound NPS fertilizer and farmyard manure for economical production of irrigated potato (*Solanum tuberosum* L.) in highlands of Ethiopia. *Cogent Food & Agriculture*, *6*(1), 1724385. https://doi.org/10.1080/23311932.2020.1724385.

Aliye, N., Dilbo, C., & Pillay, M. (2015). Understanding reaction of potato (*Solanum tuberosum*) to *Ralstonia solanacearum* and relationship of wilt incidence to latent Infection. *Journal of Phytopathology*, *163*(6), 444-455. https://doi.org/10.1111/jph.12341.

Allen, C., Prior, P., & Hayward, A. C. (2005). Bacterial wilt disease and the *Ralstonia solanacearum* species complex. *American Phytopathological Society* (APS Press).

Al-Obaidi, O. (2014). Studies on antibacterial and anticancer activity of *Nerium oleander* extracts. *European Chemical Bulletin*, *3*(3), 259-262.

Amalia M. E., & Ramon J. E. (2012). A short overview on the medicinal chemistry of (—)-shikimic acid. *Mini Reviews in Medicinal Chemistry*, *12*(14), 1443-1454. https://doi.org/10.2174/138955712803832735.

Amel, H. A., Kamel, H., Meriem, F., & Abdelkader, K. (2022). Traditional Uses, Botany, Phytochemistry, and Pharmacology of *Pelargonium graveolens*: A Comprehensive Review. *Tropical Journal of Natural Product Research*, *6*(10), 1547-1569. http://dx.doi.org/10.26538/tjnpr/v6i10.2.

Anton, H. (2021). *Plant products to control Ralstonia solanacearum causing bacterial wilt in tomatoes (Solanum lycopersicum) in Kenya* [Master's Thesis, University of Embu, Kenya]. https://stud.epsilon.slu.se/16498/3/hampl_a_210301.pdf.

Aslam, M. N., Mukhtar, T., Hussain, M. A., & Raheel, M. (2017). Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm. *Journal of Plant Diseases and Protection*, *124*, 585-590. https://doi.org/10.1007/s41348-017-0100-1.

Ateka, E. M., Mwang'Ombe, A. W., & Kimenju, J. W. (2001). Reaction of potato cultivars to *Ralstonia solanacearum* in Kenya. *African Crop Science Journal*, *9*(1), 251-256. https://doi.org/10.4314/acsj.v9i1.27646.

Atieno, E. O., Kilwinger, F. B. M., Almekinders, C. J., & Struik, P. C. (2023). How Kenyan potato farmers evaluate the seed: implications for the promotion of certified seed potato. *Potato Research*, *66*(3), 811-829. https://doi.org/10.1007/s11540-022-09602-8.

Aung, K., Jiang, Y., & He, S. Y. (2018). The role of water in plant–microbe interactions. *The Plant Journal*, *93*(4), 771-780. https://doi.org/10.1111/tpj.13795.

Bai, J., Wu, Y., Bu, Q., Zhong, K., & Gao, H. (2022). Comparative study on antibacterial mechanism of shikimic acid and quinic acid against *Staphylococcus aureus* through transcriptomic and metabolomic approaches. *LWT-Food Science and Technology*, *153* (112441), 1-10. https://doi.org/10.1016/j.lwt.2021.112441.

Barbalho, S. M., Farinazzi-Machado, F. M., de Alvares Goulart, R., Brunnati, A. C. S., Otoboni, A. M., & Ottoboni, B. J. M. A. P. (2012). *Psidium guajava* (Guava): A plant of multipurpose medicinal applications. *Medicinal and Aromatic Plants*, *1*(4), 1-6. http://dx.doi.org/10.4172/2167-0412.1000104.

Beals, K. A. (2019). Potatoes, nutrition and health. *American Journal of Potato Research*, *96*(2), 102-110. https://doi.org/10.1007/s12230-018-09705-4.

Beattie, G. A. (2011). Water relations in the interaction of foliar bacterial pathogens with plants. *Annual review of phytopathology*, *49*, 533-555. https://doi.org/10.1146/annurev-phyto-073009-114436.

Bereika, M. F. F., Moharam, M. H. A., Abo-elyousr, K. A. M., & Asran, M. R. (2020). Control of potato brown rot and wilt disease caused by *Ralstonia solanacearum* using some water plant extracts. *Journal of Sohag Agriscience*, *5*(1), 30-47.

Berg, T., Tesoriero, L., & Hailstones, D. L. (2005). PCR-based detection of *Xanthomonas campestris* pathovars in Brassica seed. *Plant Pathology*, *54*(3), 416-427. https://doi.org/10.1111/j.1365-3059.2005.01186.x.

Bhanwar, R. (2022). Effect of temperature and pH level on growth of bacterial wilt causing *R. solanacearum* under in vitro condition. *The Pharma Innovation Journal*, *11*(1), 1656-1659.

Birch, P. R., Bryan, G., Fenton, B., Gilroy, E. M., Hein, I., Jones, J. T., & Toth, I. K. (2012). Crops that feed the world 8: Potato: are the trends of increased global production sustainable?. *Food Security*, *4*(4), 477-508. https://doi.org/10.1007/s12571-012-0220-1.

Biruk-Masrie, Z., Nigussie-Dechassa, R., Alemayehu, Y. I. B. E. K. A. L., Abebie, B., & Tana, T. A. M. A. D. O. (2015). Influence of treatment of seed potato tubers with plant crude essential oil extracts on performance of the crop. *African Crop Science Journal*, *23*(3), 295-304. https://www.ajol.info/index.php/acsj/article/view/121506.

Biswal, G. (2015). Studies on antibacterial activity of some aqueous plant extracts against *Ralstonia solanacearum* causing bacterial wilt and brown rot of potato. *International Journal of Plant Sciences (Muzaffarnagar)*, *10*(1), 1-6. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20153172023 .

Biswal, G., & Dhal, N. K. (2018). Management of bacterial wilt disease of potato in coastal plains of Odisha. *African Journal of Microbiology Research*, *12*(12), 284–289. http://dx.doi.org/10.5897/AJMR2017.8714.

Black, V. (2008). Hot Potato: GM Potatoes in South Africa, a Critical Analysis. African Center for Biosafety.

Bocsanczy, A. M., Achenbach, U. C., Mangravita-Novo, A., Yuen, J. M., & Norman, D. J. (2012). Comparative effect of low temperature on virulence and twitching motility of *Ralstonia solanacearum* strains present in Florida. *Phytopathology*, *102*(2), 185-194. https://doi.org/10.1094/PHYTO-05-11-0145.

Borges, A., Ferreira, C., Saavedra, M. J., & Simões, M. (2013). Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microbial Drug Resistance*, *19*(4), 256-265. https://doi.org/10.1089/mdr.2012.0244.

Borges, D. F., Lopes, E. A., Moraes, A. R. F., Soares, M. S., Visôtto, L. E., Oliveira, C. R., & Valente, V. M. M. (2018). Formulation of botanicals for the control of plant-pathogens: A review. *Crop Protection*, *110*, 135-140. https://doi.org/10.1016/j.cropro.2018.04.003.

Bornventure, M. I., Muthomi, J. W., Narla, R. D., Nyongesa, M., & Olubayo, F. M. (2017). Effect of seed potato tuber storage methods on occurrence of potato diseases. *International Journal of Research in Agricultural Sciences*, 4(4), 2348 –3997.

Boschi, F., Schvartzman, C., Murchio, S., Ferreira, V., Siri, M. I., Galván, G. A., & Dalla-Rizza, M. (2017). Enhanced bacterial wilt resistance in potato through expression of Arabidopsis EFR and introgression of quantitative resistance from *Solanum commersonii*. *Frontiers in Plant Science*, 8 (1642), 1-11. https://doi.org/10.3389/fpls.2017.01642.

Boshou, L. (2005) A Broad Review and Perspective on Breeding for Resistance to Bacterial Wilt. In: Allen, C., Prior, P. and Hayward, A.C., (Eds), *Bacterial wilt disease and the Ralstonia solanacearum species complex*, (pp. 225-238). *American Phytopathological Society (APS Press).*

Brockett, B. F., Prescott, C. E., & Grayston, S. J. (2012). Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biology and Biochemistry*, *44*(1), 9-20. https://doi.org/10.1016/j.soilbio.2011.09.003.

Buddenhagen, I. W. (1985). Bacterial wilt revisited. *Bacterial wilt disease in Asia and the South Pacific*, (13), 126-143.

Caitlin, A. P. (2014). Local-level appraisal of benefits and barriers affecting adoption of climate-smart agricultural practices: Ghana (p. 37) [Technical report for the CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS)]. https://hdl.handle.net/10568/35688.

Cao, Y., Thomashow, L. S., Luo, Y., Hu, H., Deng, X., Liu, H., & Shen, Q. (2022). Resistance to bacterial wilt caused by *Ralstonia solanacearum* depends on the nutrient condition in soil and applied fertilizers: a meta-analysis. *Agriculture, Ecosystems & Environment*, *329*, 107874. https://doi.org/10.1016/j.agee.2022.107874.

Cardoso, S. C., Soares, A. C. F., Brito, A. D. S., Laranjeira, F. F., Ledo, C. A. S., & Santos, A. P. D. (2006). Control of tomato bacterial wilt through the incorporation of the aerial part of pigeon pea and crotalaria to soil. *Summa Phytopathologica*, *32*(1), 27-33. https://doi.org/10.1590/S0100-54052006000100004.

Cavagnaro, T. R. (2016). Soil moisture legacy effects: impacts on soil nutrients, plants and mycorrhizal responsiveness. *Soil Biology and Biochemistry*, *95*, 173-179. https://doi.org/10.1016/j.soilbio.2015.12.016.

Centre for Agriculture and Bioscience International (CABI). (2020). *Ralstonia solanacearum* (bacterial wilt of potato). https://doi.org/10.1079/cabicompendium.45009. Date accessed: 18/10/2022.

Céspedes, C. L., Salazar, J. R., Ariza-Castolo, A., Yamaguchi, L., Ávila, J. G., Aqueveque, P., & Alarcón, J. (2014). Biopesticides from plants: *Calceolaria integrifolia* sl. *Environmental Research*, *132*, 391-406. https://doi.org/10.1016/j.envres.2014.04.003.

Champoiseau, G. (2008). Ralstonia solanacearum race 3 biovar 2: detection, exclusion and analysis of a Select Agent Educational modules. *The United States Department of Agriculture-National Research Initiative Program (2007-2010)*. https://plantpath.ifas.ufl.edu/rsol/RalstoniaPublications_PDF/RsolanacearumR3b2_PDF.pdf. Date accessed: 14/10/2022.

Champoiseau, P. G., Jones, J. B., & Allen, C. (2009). *Ralstonia solanacearum* race 3 biovar 2 causes tropical losses and temperate anxieties. *Plant Health Progress*, *10*(1), 35-44.

Charkowski, A., Sharma, K., Parker, M. L., Secor, G. A., & Elphinstone, J. (2020). Bacterial diseases of potato. In H. Campos & O. Ortiz, (Eds), *The potato crop* (pp. 351-388). Springer, Cham. https://doi.org/10.1007/978-3-030-28683-5_10.

Chen, K., Khan, R. A. A., Cao, W., & Ling, M. (2020). Sustainable and ecofriendly approach of managing soil born bacterium *Ralstonia solanacearum* (Smith) using dried powder of Conyza canadensis. *Pathogens*, *9*(5), 327-444. https://doi.org/10.3390/pathogens9050327.

Chen, M. M., Zhu, Y. G., Su, Y. H., Chen, B. D., Fu, B. J., & Marschner, P. (2007). Effects of soil moisture and plant interactions on the soil microbial community structure. *European Journal of Soil Biology*, *43*(1), 31-38. https://doi.org/10.1016/j.ejsobi.2006.05.001.

Choudhary, D. K., Nabi, S. U., Dar, M. S., & Khan, K. A. (2018). *Ralstonia solanacearum*: A widespread and global bacterial plant wilt pathogen. *Journal of Pharmacognosy and Phytochemistry*, *7*(2), 85-90.

Cook, J. R. & Baker, K. F. (1983). The Nature and Practice of Biological Control of Plant Pathogens. *American Phytopathological Society*, St. Paul.

Cosme, P., Rodríguez, A. B., Espino, J., & Garrido, M. (2020). Plant phenolics: Bioavailability as a key determinant of their potential health-promoting applications. *Antioxidants*, *9*(12), 1263-1282. https://doi.org/10.3390/antiox9121263.

Cynthia, I. F., Hery, S., & Akhmad, D. (2018). Antibacterial and antioxidant activities of pyrogallol and synthetic pyrogallol dimer. *Research Journal of Chemistry and Environment*, 22(2), 39-48.

Dahal, K., Li, X. Q., Tai, H., Creelman, A., & Bizimungu, B. (2019). Improving potato stress tolerance and tuber yield under a climate change scenario–a current overview. *Frontiers in Plant Science*, *10*(563), 1-16. https://doi.org/10.3389/fpls.2019.00563.

Dalsing, B. L., Truchon, A. N., Gonzalez-Orta, E. T., Milling, A. S., & Allen, C. (2015). *Ralstonia solanacearum* uses inorganic nitrogen metabolism for virulence, ATP production, and detoxification in the oxygen-limited host xylem environment. *MBio*, *6*(2), 10-1128. https://doi.org/10.1128/mbio.02471-14.

de Haan, S., & Rodriguez, F. (2016). Potato origin and production. In J. Singh & L. Kaur, (Eds), *Advances in potato chemistry and technology* (pp. 1-32). Academic Press.

De Haan, S., Forbes, A., Amoros, W., Gastelo, M., Salas, E., Hualla, V., & Bonierbale, M. (2014). *Procedures for standard evaluation and data management of advanced potato clones. Module 2: Healthy Tuber Yield Trials. International Cooperators Guide*. Lima, Peru: International Potato Center. http://cipotato.org/wp-content/uploads/2014/08/003875.pdf.

Deberdt, P., Perrin, B., Coranson-Beaudu, R., Duyck, P. F., & Wicker, E. (2012). Effect of *Allium fistulosum* extract on *Ralstonia solanacearum* populations and tomato bacterial wilt. *Plant Disease*, *96*(5), 687-692. https://doi.org/10.1094/pdis-07-11-0601.

Demissie, Y. T. (2019). Integrated Potato (Solanum Tuberosum L.) Late Blight (*Phytophthora Infestans*) Disease Management in Ethiopia. *American Journal of BioScience*, *7*(6), 123-130. http://dx.doi.org/10.11648/j.ajbio.20190706.16.

Desai, S. D., Desai, D. G., & Kaur, H. (2009). Saponins and their biological activities. *Pharma Times*, *41*(3), 13-16.

Devaux, A., Goffart, J. P., Kromann, P., Andrade-Piedra, J., Polar, V., & Hareau, G. (2021). The potato of the future: opportunities and challenges in sustainable agri-food systems. *Potato Research*, *64*(4), 681-720. https://doi.org/10.1007/s11540-021-09501-4.

Din, N., Ahmad, M., Siddique, M., Ali, A., Naz, I., Ullah, N., & Ahmad, F. (2016). Phytobiocidal management of bacterial wilt of tomato caused by *Ralstonia solanacearum* (Smith) Yabuuchi. *Spanish Journal of Agricultural Research*, *14*(3), 22-34. http://dx.doi.org/10.5424/sjar/2016143-9012.

Ding, J., Wang, N., Liu, P., Liu, B., Zhu, Y., Mao, J., & Ding, G. C. (2023). Bacterial wilt suppressive composts: Significance of rhizosphere microbiome. *Waste Management*, *169*, 179-185. https://doi.org/10.1016/j.wasman.2023.07.011.

Dorman, H. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, *88*(2), 308-316. https://doi.org/10.1046/j.1365-2672.2000.00969.x.

Dossoumou, M. E., Sikirou, R., Zannou, A., Paret, M., & Baba-Moussa, L. (2023). Bacterial wilt and dissemination factors in tomato production areas of Benin, West Africa. *Archives of Phytopathology and Plant Protection*, *56*(18), 1427-1449. http://dx.doi.org/10.1080/03235408.2023.2291842.

Dubey, N. K., Srivastava, B., & Kumar, A. (2008). Current status of plant products as botanical pesticides in storage pest management. *Journal of Biopesticides*, *1*(2), 182-186. http://dx.doi.org/10.57182/jbiopestic.1.2.182-186.

Durr, G., & Lorenzl, G. (1980). *Potato production and utilization in Kenya* (No. 338.17521 D8). Lima, Peru: CIP.

EFSA, Panel, O. P. H., Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P. (2019). Pest categorization of the *Ralstonia solanacearum* species complex. *EFSA Journal*, *17*(2), e05618. https://doi.org/10.2903/j.efsa.2019.5618.

Elphinstone, J. G. (2005). The current bacterial wilt situation: a global overview. In: Allen, C., Prior, P. and Hayward, A.C., (Eds), *Bacterial wilt disease and the Ralstonia solanacearum species complex*, (pp. 9-28). *American Phytopathological Society (APS Press)*.

EPPO (European and Mediterranean Plant Protection Organization). (2023). EPPO Global Database. Available online: https://gd.eppo.int/taxon/RALSSL/distribution.

Esteves, I., Maleita, C., & Abrantes, I. (2015). Root-lesion and root-knot nematodes parasitizing potato. *European Journal of Plant Pathology*, *141*, 397-406. https://doi.org/10.1007/s10658-014-0551-1.

Farag, N. S., Lashin, S. M., Abdel-All, R. S., Shatta, H. M., & Seif-Elyazal, A. M. (1982). Antibiotics and control of potato black leg and brown rot diseases. *Agricultural Research Review*, *60*(2), 149-166. https://api.semanticscholar.org/CorpusID:83366131.

Felix, R., Onyango, O. J., & Eliazer, O. M. (2010). Assessment of Irish potato cultivars' field tolerance to bacterial wilt (*Ralstonia solanacearum*) in Kenya. *Plant Pathology Journal (Faisalabad)*, *9*(3), 122-128. https://doi.org/10.3923/ppj.2010.122.128.

Fock, I., Collonnier, C., Purwito, A., Luisetti, J., Souvannavong, V., Vedel, F., & Sihachakr, D. (2000). Resistance to bacterial wilt in soOkeyomatic hybrids between *Solanum tuberosum* and *Solanum phureja*. *Plant Science*, *160*(1), 165-176. https://doi.org/10.1016/s0168-9452(00)00375-7.

French, E. R., Anguiz, R. & Aley, P. (1998). The usefulness of potato resistance to *Ralstonia solanacearum*, for the integrated control of bacterial wilt. In: Prior, P., Allen, C. and Elphinstone, J. (Eds.), *Bacterial wilt disease* (pp. 381–385). Berlin: Springer. https://doi.org/10.1007/978-3-662-03592-4_58.

Gakuya, D. W., Itonga, S. M., Mbaria, J. M., Muthee, J. K., & Musau, J. K. (2013). Ethnobotanical survey of biopesticides and other medicinal plants traditionally used in Meru central district of Kenya. *Journal of Ethnopharmacology*, *145*(2), 547-553. https://doi.org/10.1016/j.jep.2012.11.028.

García, R. O., Kerns, J. P., & Thiessen, L. (2019). *Ralstonia solanacearum* Species Complex: A Quick Diagnostic Guide. *Plant Health Progress*, *20*(1), 7-13. http://dx.doi.org/10.1094/PHP-04-18-0015-DG.

Gautam, U., Poudel, S., & Rajbhandari, B. P. (2021). The impact of potato-based cropping system on livelihood and income generation at Tamankhola rural municipality, Baglung. *Nepalese Journal of Agricultural Sciences*,20,108-114.

Gelaye, Y. (2023). Effect of combined application of organic manure and nitrogen fertilizer rates on yield and yield components of potato: A review. *Cogent Food & Agriculture*, *9*(1), 2217603. https://doi.org/10.1080/23311932.2023.2217603.

Genin, S. (2010). Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. *New Phytologist*, *187*(4), 920-928.

Gobena, S. T. (2020). *Understanding and managing bacterial wilt and late blight of potato in Ethiopia: Combining an innovation systems approach and a collective action*

perspective [Doctoral dissertation, Wageningen University and Research]. https://edepot.wur.nl/527063.

Gololo, S. S. (2018). Effects of Environmental Factors on the Accumulation of Phytochemicals in Plants. In C. Egbuna, J. Chinenye, S. Kumar, & N. Sharif (Eds.), *Phytochemistry* (pp. 267–278). Apple Academic Press. https://doi.org/10.1201/9780429426155.

Gomes, A. T., Smania Júnior, A., Seidel, C., Smania, E. D. F. A., Honda, N. K., Roese, F. M., & Muzzi, R. M. (2003). Antibacterial activity of orsellinates. *Brazilian Journal of Microbiology*, *34*(3), 194-196. https://doi.org/10.1590/S1517-83822003000300002.

Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., & Hatab, S. R. (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in Microbiology*, 9(1639), 1-9. https://doi.org/10.3389/fmicb.2018.01639.

Gorissen, A., Van Overbeek, L. S., & Van Elsas, J. D. (2004). Pig slurry reduces the survival of *Ralstonia solanacearum* biovar 2 in soil. *Canadian Journal of Microbiology*, *50*(8), 587-593. https://doi.org/10.1139/w04-042.

Gu, Y., Wang, X., Yang, T., Friman, V. P., Geisen, S., Wei, Z., & Shen, Q. (2020). Chemical structure predicts the effect of plant-derived low-molecular weight compounds on soil microbiome structure and pathogen suppression. *Functional Ecology*, *34*(10), 2158-2169. https://doi.org/10.1111/1365-2435.13624.

Guleria, S., & Tiku, A. K. (2009). Botanicals in pest management: current status and future perspectives. In Peshin, R., Dhawan, A.K. (eds). *Integrated pest management: innovation-development process* (pp. 317-329). Springer, Dordrecht. https://link.springer.com/chapter/10.1007/978-1-4020-8992-3_12#citeas .

Gutarra, L., Herrera, J., Fernandez, E., Kreuze, J., & Lindqvist-Kreuze, H. (2017). Diversity, pathogenicity, and current occurrence of bacterial wilt bacterium *Ralstonia solanacearum* in Peru. *Frontiers in Plant Science, 8*, 1221. https://doi.org/10.3389/fpls.2017.01221.

Harahagazwe, D., Condori, B., Barreda, C., Bararyenya, A., Byarugaba, A. A., Kude, D. A., & Quiroz, R. Q. (2018). How big is the potato (*Solanum tuberosum* L.) yield gap in Sub-Saharan Africa and why? A participatory approach. *Open Agriculture*, *3*(1), 180-189. https://doi.org/10.1515/opag-2018-0019.

Hase, S., Shimizu, A., Nakaho, K., Takenaka, S., & Takahashi, H. (2006). Induction of transient ethylene and reduction in severity of tomato bacterial wilt by *Pythium oligandrum*. *Plant Pathology*, *55*(4), 537-543. https://doi.org/10.1111/j.1365-3059.2006.01396.x.

Hassan, M. A. E., Bereika, M. F. F., Abo-Elnaga, H. I. G., & Sallam, M. A. A. (2009). Direct antimicrobial activity and induction of systemic resistance in potato plants against bacterial wilt disease by plant extracts. *The Plant Pathology Journal*, *25*(4), 352-360. http://dx.doi.org/10.5423/PPJ.2009.25.4.352.

Haverkort, A. J., Franke, A. C., Engelbrecht, F. A., & Steyn, J. M. (2013). Climate change and potato production in contrasting South African agro-ecosystems 1. Effects on land and water use efficiencies. *Potato Research*, *56*, 31-50. https://doi.org/10.1007/s11540-013-9230-4.

Hayward, A. C. (1991). Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annual Review of Phytopathology*, *29*(1), 65-87. https://doi.org/10.1146/annurev.py.29.090191.000433.

Hayward, A. C., & Hartman, G. L. (1994). *Bacterial wilt: the disease and its causative agent, Pseudomonas solanacearum.* CAB International, Wallingford, UK. https://www.cabidigitallibrary.org/doi/full/10.5555/19952311190.

He, C. L., Fu, B. D., Shen, H. Q., Jiang, X. L., & Wei, X. B. (2011). Fumaric acid, an antibacterial component of Aloe vera L. *African Journal of Biotechnology*, *10*(15), 2973-2977. https://doi.org/10.5897/AJB10.1497.

He, L. Y., Sequeira, L., & Kelman, A. (1983). Characteristics of strains of *Pseudomonas solanacearum* from China. *Plant Disease, 67(12), 1357- 1361.*

He, W., Wang, B., Huang, M., Meng, C., Wu, J., Du, J., & Chen, H. (2024). Screening for Resistance Resources against Bacterial Wilt in Wild Potato. *Plants*, *13*(2), 220. https://doi.org/10.3390/plants13020220.

Hijmans, R. J. (2003). The effect of climate change on global potato production. *American Journal of Potato Research, 80,* 271-279. https://doi.org/10.1007/BF02855363.

Hong, J. C., Norman, D. J., Reed, D. L., Momol, M. T., & Jones, J. B. (2012). Diversity among *Ralstonia solanacearum* strains isolated from the southeastern United States. *Phytopathology*, *102*(10), 924-936. https://doi.org/10.1094/phyto-12-11-0342.

https://agroduka.com/enrich-bm. Date accessed: 11/11/2022.

https://webbook.nist.gov/chemistry/. Date accessed: 24/01/2022.

https://www.pcpb.go.ke/biopesticides-on-crops/. Date accessed: 08/10/2021

Huamán, Z., Golmirzaie, A., & Amoros, W. (1997). The potato. *Biodiversity in trust: conservation and use of plant genetic resources in CGIAR Centres* (pp. 21-28). Cambridge University Press.

Huang, J., Wei, Z., Tan, S., Mei, X., Yin, S., Shen, Q., & Xu, Y. (2013). The rhizosphere soil of diseased tomato plants as a source for novel microorganisms to control bacterial wilt. *Applied Soil Ecology*, *72*, 79-84. https://doi.org/10.1016/j.apsoil.2013.05.017.

Ichang'i, G.N. (1987). *The use of remote sensing in resource (fuelwood) planning in Tetu division Nyeri District, Kenya* [Doctoral dissertation, University of Nairobi]. http://erepository.uonbi.ac.ke:8080/xmlui/handle/123456789/28418.

Imade, E. E., Omonigho, S. E., Babalola, O. O., & Enagbonma, B. J. (2021). Lactic acid bacterial bacteriocins and their bioactive properties against food-associated antibiotic-resistant bacteria. *Annals of Microbiology*, *71*(1), 1-14. https://doi.org/10.1186/s13213-021-01652-6 .

Indumathi, C., Durgadevi, G., Nithyavani, S., & Gayathri, P. K. (2014). Estimation of terpenoid content and its antimicrobial property in Enicostemma litorrale. *International Journal of Chem Tech Research*, *6*(9), 4264-4267.

Innocent, U., Balekye, A., Prossy, N., Mwesige, R., George, K., & Benon, M. (2021). Effects of fertilizer application on yield and yield related parameters of low yielding potato varieties in Uganda. *African Journal of Agricultural Research*, *17*(12), 1540-1546.

Islam, M. T., Ali, E. S., Uddin, S. J., Shaw, S., Islam, M. A., Ahmed, M. I., & Atanasov, A. G. (2018). Phytol: A review of biomedical activities. *Food and Chemical Toxicology*, *121*, 82-94. https://doi.org/10.1016/j.fct.2018.08.032.

Islam, T. M., & Toyota, K. (2004). Effect of moisture conditions and pre-incubation at low temperature on bacterial wilt of tomato caused by *Ralstonia solanacearum*. *Microbes and Environments*, *19*(3), 244-247. http://dx.doi.org/10.1264/jsme2.19.244.

Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop Protection*, *19*(8), 603-608. https://doi.org/10.1016/S0261-2194(00)00079-X.

Ito, S., Ushuima, Y., Fujii, T., Tanaka, S., Kameya-Iwaki, M., Yoshiwara, S., & Kishi, F. (1998). Detection of viable cells of *Ralstonia solanacearum* in soil using a semiselective medium

and a PCR technique. *Journal of Phytopathology*, *146*(8-9), 379-384. https://doi.org/10.1111/j.1439-0434.1998.tb04769.x.

Jaetzold, R. & Schmidt, H. (1983). *Farm Management Handbook of Kenya*, Vol. 2 (2nd ed.). Ministry of Agriculture, Kenya, in Cooperation with the Germany Agency for Technical Cooperation (GTZ). https://edepot.wur.nl/487562.

Jaetzold, R., Ira, F., Schmidt, H., Hornet, B., & Shisanya, C. (2007). *Farm Management Handbook of Kenya*, Vol. 2 (2nd ed.). Ministry of Agriculture/GTZ, Nairobi Kenya.

Janssens, S. R. M., Wiersema, S. G., Goos, H. J. T., & wiersma, W. (2013). *The value chain for seed and ware potatoes in Kenya: Opportunities for development*. (Memorandum / LEI Wageningen UR; No. 13-080). LEI. https://edepot.wur.nl/279729.

Jiang, G., Wang, N., Zhang, Y., Zhang, Y., Yu, J., Zhang, Y., & Shen, Q. (2021). The relative importance of soil moisture in predicting bacterial wilt disease occurrence. *Soil Ecology Letters*, 1-11. https://doi.org/10.1007/s42832-021-0086-2.

Jiang, G., Wei, Z., Xu, J., Chen, H., Zhang, Y., She, X., & Liao, B. (2017). Bacterial wilt in China: history, current status, and future perspectives. *Frontiers in Plant Science*, 8 (1549), 1-10. https://doi.org/10.3389/fpls.2017.01549.

Jiang, Y., Huang, M., Zhang, M., Lan, J., Wang, W., Tao, X., & Liu, Y. (2018). Transcriptome analysis provides novel insights into high-soil-moisture-elevated susceptibility to *Ralstonia solanacearum* infection in ginger (*Zingiber officinale* Roscoe cv. Southwest). *Plant Physiology and Biochemistry*, *132*, 547-556. https://doi.org/10.1016/j.plaphy.2018.10.005.

Kaguongo, W. P., Ng'ang'a, N. M., Muthoka, N., & Maingi, G. (2010). Seed potato sub-sector master plan for Kenya (2009–2014). Seed potato study sponsored by GTZ-PSDA, USAID, CIP and Government of Kenya. Ministry of Agriculture Kenya. *Plant Pathology Journal*, *32*(1), 58-64.

Kakuhenzire, R. O. G. E. R. S., Lemaga, B. E. R. G. A., Kashaija, I. M. E. L. D. A., Ortiz, O. S. C. A. R., & Mateeka, B. E. N. O. N. (2013). Effect of *Crotalaria falcata* in crop rotation and fallowing on potato bacterial wilt incidence, disease severity and latent infection in tubers and field soil. *Biopesticides International*, *9*(2), 182-194.

Kang, M. J., Lee, M. H., Shim, J. K., Seo, S. T., Shrestha, R., Cho, M. S., & Park, D. S. (2007). PCR-based specific detection of *Ralstonia solanacearum* by amplification of cytochrome

c1 signal peptide sequences. *Journal of Microbiology and Biotechnology*, *17*(11), 1765-1771.

Karak, P. (2019). Biological activities of flavonoids: an overview. *International Journal of Pharmaceutical Sciences and Research*, *10*(4), 1567-1574. http://dx.doi.org/10.13040/IJPSR.0975-8232.10(4).1567-74 .

Karim, Z., & Hossain, M. S. (2018). Management of bacterial wilt (*Ralstonia solanacearum*) of potato: focus on natural bioactive compounds. *Journal of Biodiversity Conservation and Bioresource Management*, *4*(1), 73-92. http://dx.doi.org/10.3329/jbcbm.v4i1.37879.

Kassa, B. (2016). Potato bacterial wilt management in the Central Highlands of Ethiopia. *Ethiopian Journal of Agricultural Sciences*, *26*(2), 83-97. https://doi.org/10.20372/eiar-rdm/WPKRCI.

Katafiire, M., E. Adipala, B. Lemaga, M. Olanya, & R. El-bedewy. (2005). Management of bacterial wilt of potato using one-season rotation crops in southwestern Uganda. In C. Allen, P. Prior and A.C. Hayward (Eds.), *Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex* (pp. 197–203). American Phytopathological Society Press, St. Paul, MN. https://www.cabidigitallibrary.org/doi/full/10.5555/20053172615.

Kelman, A. (1954). The relationship of pathogenicity of *Pseudomonas solanacearum* to colony appearance in a tetrazolium medium. *Phytopathology*, *44*(12), 693-695.

Khakbazan, M., Mohr, R. M., Huang, J., Xie, R., Volkmar, K. M., Tomasiewicz, D. J., & Nelson, A. (2019). Effects of crop rotation on energy use efficiency of irrigated potato with cereals, canola, and alfalfa over a 14-year period in Manitoba, Canada. *Soil and Tillage Research*, *195*, 104357. https://doi.org/10.1016/j.still.2019.104357.

Khalid, W., Khalid, M. Z., Aziz, A., Tariq, A., Ikram, A., Rehan, M., & Fatima, A. (2020). Nutritional composition and health benefits of potato: A review. *Advance Food & Nutrition Science*, *5*, 7-16. https://dx.doi.org/10.21065/25631640.5.7.

Khan, A. K., Rashid, R., Fatima, N., Mahmood, S., Mir, S., Khan, S. & Murtaza, G. (2015). Pharmacological activities of protocatechuic acid. *Acta Polonise Pharmaceutica*, *72*(4), 643-650. https://doi.org/10.1155/2015/593902.

Kharouf, N., Sauro, S., Hardan, L., Fawzi, A., Suhanda, I. E., Zghal, J., & Mancino, D. (2022). Impacts of Resveratrol and Pyrogallol on Physicochemical, Mechanical and Biological

Properties of Epoxy-Resin Sealers. *Bioengineering*, *9*(3), 85-101. http://dx.doi.org/10.3390/bioengineering9030085.

Khasabulli, B. D., Musyimi, D. M., Miruka, D. M., Opande, G. T., & Jeruto, P. (2017). Isolation and characterisation of *Ralstonia solanacearum* strains of tomato wilt disease from Maseno, Kenya. *Journal of Asian Scientific Research*, *7*(9), 404-420. http://dx.doi.org/10.18488/journal.2.2017.79.404.420.

Khatri-Chhetri, A., Aggarwal, P. K., Joshi, P. K., & Vyas, S. (2017). Farmers' prioritization of climate-smart agriculture (CSA) technologies. *Agricultural Systems*, *151*, 184-191. https://doi.org/10.1016/j.agsy.2016.10.005.

Kinyua, Z. M., Miller, S. A., Ashlina, C., & Subedi, N. (2014). Bacterial wilt disease *Ralstonia solanacearum*: Standard operating procedure for use in diagnostic laboratories. *Version: EA-SOP-RS1*. https://ipmil.cired.vt.edu/wp-content/uploads/2014/06/SOP-Ralstonia-solanacerum-EastAfricaFinal-Apr2014-2.pdf.

Kinyua, Z. M., Smith, J. J., Lung'aho, C., Olanya, M., & Priou. S. (2001). On-farm success and challenges of producing bacterial wilt free tubers in seed plots in Kenya. *African Crop Science Journal*, 9, 279-285. http://dx.doi.org/10.4314/acsj.v9i1.27649.

Kirigo, M. P. (2019). *Status of potato bacterial wilt in Nakuru County (Kenya) and its management through crop rotation and soil amendments* [Doctoral dissertation, Egerton University]. http://irlibrary.egerton.ac.ke/jspui/bitstream/123456789/1961/1/Status%20of%20potato%20bacterial%20wilt%20in%20Nakuru%20County%20%28Kenya%29%20and%20its%20management%20through%20crop%20rotation%20and%20soil%20amendments.PDF.

Koch, M., Naumann, M., Pawelzik, E., Gransee, A., & Thiel, H. (2020). The importance of nutrient management for potato production Part I: Plant nutrition and yield. *Potato Research*, *63*(1), 97-119. https://doi.org/10.1007/s11540-019-09431-2.

Kooman, P. L., Fahem, M., Tegera, P., & Haverkort, A. J. (1996). Effects of climate on different potato genotypes 2. Dry matter allocation and duration of the growth cycle. *European Journal of Agronomy*, *5*(3-4), 207-217. https://doi.org/10.1016/S1161-0301(96)02032-1.

Koparde, A. A., Doijad, R. C., & Magdum, C. S. (2019). Natural products in drug discovery. In P. Shagufta & A. Areej (Eds.), *Pharmacognosy-medicinal plants* (pp.269-287). IntechOpen, London, UK.

Kramer, P. J., & Boyer, J. S. (1995). Water relations of plants and soils. Academic press. https://books.google.co.ke/books?hl=en&lr=&id=H6aHAwAAQBAJ&oi=fnd&pg=PP1&dq=Water+Relations+of+Plants+by+Kramer,+P.J.,&ots=BZFh5YV86M&sig=CW4sLH4fOklDMNumb9YOHehKgi0&redir_esc=y#v=onepage&q=Water%20Relations%20of%20Plants%20by%20Kramer%2C%20P.J.%2C&f=false.

Kreuze, J. F., Souza-Dias, J. A. C., Jeevalatha, A., Figueira, A. R., Valkonen, J. P. T., & Jones, R. A., C. (2020). Viral diseases in potato. In H. Campos & O. Ortiz (Eds.), *The potato crop* (pp. 389-430). Springer, Cham. https://doi.org/10.1007/978-3-030-28683-5_11.

Krishnarathi, A., Issac, S. S., & Kannan, M. (2014). Phytochemical analysis of selected plant leaves consumed by the folivory bats in Tirunelveli. *Journal of Environmental and Applied Bioresearch*, *31*, 14-18.

Kromann, P., Miethbauer, T., Ortiz, O., & Forbes, G. A. (2014). Review of potato biotic constraints and experiences with integrated pest management interventions. In D. Pimentel & R. Peshin (Eds.), *Integrated pest management* (pp. 245-268). Springer, Dordrecht. https://doi.org/10.1007/978-94-007-7796-5_10.

Kroschel J., & Schaub, B. (2013). Biology and ecology of potato tuber moths as major pests of potato. In P. Giordanengo, C. Vincent, & A. Alyokhin, (Eds.), *Insect pests of potato: global perspectives on biology and management*. (pp. 165-192). Academic Press, Oxford.

Kroschel, J., Mujica N., Okonya, J., & Alyokhin A. (2020). Insect pests affecting potatoes in tropical, subtropical, and temperate regions. In H. Campos & O. Ortiz (Eds.), *The potato crop* (pp. 251-306). Springer, Cham. https://doi.org/10.1007/978-3-030-28683-5_8.

Kumar, D., Minhas, J. S., & Singh, B. (2003). Abiotic stress and potato production. *khurana, SMP; Minhas; JS, Pandey, SK (Eds). The potato: production and utilization in sub-tropics. Mehta Publishers, New Delhi (India)*.

Kumar, S. (2021, October 31). Soil application methods of fungicides: Fundamental information. *Just Agriculture*. https://justagriculture.in/files/newsletter/2021/october/051.pdf.

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal*, *2013(*62750), 1-16. http://dx.doi.org/10.1155/2013/162750.

Kumar, S., Yadav, A., Yadav, M., & Yadav, J. P. (2017). Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of *Aloe*

vera (L.) Burm. f. *BMC Research Notes*, *10*(1), 1-12. https://doi.org/10.1186/s13104-017-2385-3.

Kwambai, T. K., Omunyin, M. E., Okalebo, J. R., Kinyua, Z. M., & Gildemacher, P. (2011). Assessment of potato bacterial wilt disease status in North Rift Valley of Kenya: A Survey. In B. Andre, W. Boaz, J. Okeyo, F. Maina & J. Kihara (Eds.), I*nnovations as key to the green revolution in Africa* (pp. 449-456). Springer, Dordrecht. https://doi.org/10.1007/978-90-481-2543-2_46.

Kyamanywa, S., Kashaija I., Getu E., Amata R., Senkesha N., & Kullaya A. (2011). Enhancing food security through improved seed systems of appropriate varieties of cassava, potato and sweetpotato resilient to climate change in Eastern Africa. https://cgspace.cgiar.org/bitstream/handle/10568/10817/Project2_Cassava.pdf?sequence=6

Lal, M., Arora, R. K., Maheshwari, U., Rawal, S., & Yadav, S. (2016). Impact of late blight occurrence on potato productivity during 2013-14. *International Journal of Agricultural and Statistical Sciences*, *12*(1), 187-192.

Lamichhane, J. R., Dürr, C., Schwanck, A. A., Robin, M. H., Sarthou, J. P., Cellier, V., & Aubertot, J. N. (2017). Integrated management of damping-off diseases. A review. *Agronomy for Sustainable Development*, *37*(2), 1-25. https://doi.org/10.1007/s13593-017-0417-y.

Larson, J. E., & Funk, J. L. (2016). Seedling root responses to soil moisture and the identification of a belowground trait spectrum across three growth forms. *New Phytologist*, *210*(3), 827-838. https://doi.org/10.1111/nph.13829.

Lee, S. M., Kong, H. G., Song, G. C., & Ryu, C. M. (2021). Disruption of Firmicutes and Actinobacteria abundance in tomato rhizosphere causes the incidence of bacterial wilt disease. *The ISME Journal*, *15*(1), 330-347. https://doi.org/10.1038/s41396-020-00785-x.

Lee, W., Woo, E. R., & Lee, D. G. (2016). Phytol has antibacterial property by inducing oxidative stress response in *Pseudomonas aeruginosa*. *Free Radical Research*, *50*(12), 1309-1318. https://doi.org/10.1080/10715762.2016.1241395.

Lemaga, B. (1997). Integrated control of potato bacterial wilt. Literature Review and work plan 1995-1997. The African highlands Initiative Technical report. Series No. 3, Nairobi. *International Center for Research on Agroforestry (ICRAF)*. https://agris.fao.org/search/en/providers/122507/records/64775cb8f2e6fe92b3668d61.

Levchyk, N. Y., Liubinska, A. V., Skrypchenko, N. V., Dziuba, O. I., Kachalova, N. M., & Mironov, O. L. (2017). Biological activity of aqueous solution of amber. *Biotechnologia Acta*, *10*(6), 53-60. https://doi.org/10.15407/biotech10.06.053.

Levene, H. (1960). In *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling*, I. Olkin et al. (Ed.), (pp. 278-292). Stanford University Press.

Li, S., Liu, Y., Wang, J., Yang, L., Zhang, S., Xu, C., & Ding, W. (2017a). Soil acidification aggravates the occurrence of bacterial wilt in South China. *Frontiers in Microbiology*, *8*, 1-12. https://doi.org/10.3389/fmicb.2017.00703.

Li, S., Xu, C., Wang, J., Guo, B., Yang, L., Chen, J., & Ding, W. (2017b). Cinnamic, myristic and fumaric acids in tobacco root exudates induce the infection of plants by *Ralstonia solanacearum. Plant and Soil*, *412*, 381-395. https://doi.org/10.1007/s11104-016-3060-5.

Ling, N., Xue, C., Huang, Q., Yang, X., Xu, Y., & Shen, Q. (2010). Development of a mode of application of bioorganic fertilizer for improving the biocontrol efficacy to Fusarium wilt. *Biocontrol*, *55*, 673-683. https://doi.org/10.1007/s10526-010-9290-1.

Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., & Fernie, A. R. (2006). Gas chromatography mass spectrometry–based metabolite profiling in plants. *Nature Protocols*, *1*(1), 387-396. https://doi.org/10.1038/nprot.2006.59.

Liu, S. Y., Liao, C. K., Lo, C. T., Yang, H. H., Lin, K. C., & Peng, K. C. (2016). Chrysophanol is involved in the biofertilization and biocontrol activities of *Trichoderma*. *Physiological and Molecular Plant Pathology*, *96*, 1-7. https://doi.org/10.1016/j.pmpp.2016.06.003.

Liu, W., Yin, D., Li, N., Hou, X., Wang, D., Li, D., & Liu, J. (2016). Influence of environmental factors on the active substance production and antioxidant activity in *Potentilla fruticosa* L. and its quality assessment. *Scientific Reports*, *6*(1), 1-18. https://doi.org/10.1038/srep28591.

Long, H. H., Furuya, N., Kurose, D., Yamamoto, I., Takeshita, M., & Takanami, Y. (2004). Identification of the endophytic bacterial isolates and their in vitro and in vivo antagonism against *Ralstonia solanacearum*. *Journal of the Faculty of Agriculture, Kyushu University*, *49*(2), 233-241. http://dx.doi.org/10.5109/4583.

Longwe, K., Akiniwale, G., Mwenye, O. J., van Vugt, D., Chiipanthenga, M., & Phiri, A. T. (2023). Effects of soil amendments on bacterial wilt incidences and potato tuber yield across

different environments in Malawi. *Resources, Environment and Sustainability*, *13*, 100116. https://doi.org/10.1016/j.resenv.2023.100116.

Lu, H. J., Breidt Jr, F., Pérez-Díaz, I. M., & Osborne, J. A. (2011). Antimicrobial effects of weak acids on the survival of *Escherichia coli* O157: H7 under anaerobic conditions. *Journal of Food Protection*, *74*(6), 893-898. https://doi.org/10.4315/0362-028X.JFP-10-404.

Lung'aho, C., M'makwa, C., & Kidanemariam, H. M. (1997). Effect of source of mother plant, variety, and growing conditions on the production of stem cuttings and subsequent yield of mini-tubers in the Kenyan potato programme. In Proceedings of the 4th Triennial Congress of the African Potato Association, 23-28 February 1997, Pretoria, South Africa (pp. 72-91). African Potato Association, Kampala, Uganda.

Machmud, M., & Suryadi, Y. (2008). Detection and identification of *Ralstonia solanacearum* strains using the indirect ELISA technique. *Indonesian Journal of Agriculture*, *1*(1), 13-21. https://doi.org/10.1023/A:1008892400077.

Mahdy, A. M., El-Habbaa, G. M., Mohamed, F. G., & Badr, A. A. (2012). Virulence of *Ralstonia solanacearum* the causal of potato brown rot disease under Egyptian conditions. *Annals of Agricultural Science Moshtohor*, *50*(1), 59-67.

Mahmood, T., Mehnaz, S., Fleischmann, F., Ali, R., Hashmi, Z. H., & Iqbal, Z. (2014). Soil sterilization effects on root growth and formation of rhizosheaths in wheat seedlings. *Pedobiologia*, *57*(3), 123-130. https://doi.org/10.1016/j.pedobi.2013.12.005.

Maina, M., Wambu, E., & Lusweti, J. K. (2020). Analysis of fluoride in kales (*Brassicca oleracea*) and tomatoes (*Lycopersicum esculentum*) from Nakuru County, Kenya. *Africa Environmental Review Journal*, 4(1), 35-42.

Mainasara, M. M., Aliero, B. L., Aliero, A. A., & Yakubu, M. (2012). Phytochemical and antibacterial properties of root and leaf extracts of *Calotropis procera*. *Nigerian Journal of Basic and Applied Sciences*, *20*(1), 1-6.

Mainiero, R., & Kazda, M. (2005). Effects of *Carex rostrata* on soil oxygen in relation to soil moisture. *Plant and Soil*, *270*, 311-320. https://doi.org/10.1007/s11104-004-1724-z.

Mamphogoro, T. P., Babalola, O. O., & Aiyegoro, O. A. (2020). Sustainable management strategies for bacterial wilt of sweet peppers (*Capsicum annuum*) and other Solanaceous crops. *Journal of Applied Microbiology*, *129*(3), 496-508. https://doi.org/10.1111/jam.14653.

Manda, R. R., Addanki, V. A., & Srivastava, S. (2020). Bacterial wilt of solanaceous crops. *International Journal of Chemical Studies*, *8*(6), 1048-1057.

Mann, H. B., & Whitney, D. R. (1947). On a test of whether one of two random variables is stochastically larger than the other. *The Annals of Mathematical Statistics*, 18, 50-60.

Marković, S., Milovanović, T. P., Jelušić, A., Iličić, R., Medić, O., Berić, T., & Stanković, S. (2023). Biological control of major pathogenic bacteria of potato by *Bacillus amyloliquefaciens* strains SS-12.6 and SS-38.4. *Biological Control*, *182*, 105238. https://doi.org/10.1016/j.biocontrol.2023.105238.

Martin-Smith, M., & Khatoon, T. (1963). Biological Activity of the Terpenoids and Their Derivatives. In: Jucker, E. (eds) Progress in Drug Research / Fortschritte der Arzneimittelforschung / Progrès des recherches pharmaceutiques. Progress in Drug Research / Fortschritte der Arzneimittelforschung / Progrès des Recherches Pharmaceutiques, vol 6. Birkhäuser Basel. https://doi.org/10.1007/978-3-0348-7050-4_4.

Mereu, V., Santini, M., Cervigni, R., Augeard, B., Bosello, F., Scoccimarro, E., Spano, D., & Valentini, R. (2018). Robust Decision Making for a Climate-Resilient Development of the Agricultural Sector in Nigeria. In L. Lipper, N. McCarthy, D. Zilberman, S. Asfaw, & G. Branca (Eds.), *Climate Smart Agriculture: Building Resilience to Climate Change* (pp. 277–306). Springer International Publishing.

Mihovilovich, E., Lopes, C., Gutarra, L., Lindqvist-Kreuze, H., Aley, P., Priou, S., Bonierbale M. (2017). *Protocol for assessing bacterial wilt resistance in greenhouse and field conditions. International cooperators' guide. Lima (Peru).* International Potato Center. ISBN 978-92-9060-214-9. 35 p. https://doi.org/10.4160/9789290602149.

Milijasevic-Marcic, S., Todorovic, B., Potočnik, I., Rekanovic, E., Stepanovic, M., Mitrovic, J., & Duduk, B. (2015). *Ralstonia solanacearum*–a New Threat to Potato Production in Serbia. *Pesticides and Phytomedicine*, 28(4), 229-237. http://dx.doi.org/10.2298/PIF1304229M.

Mohamed, B. F., Sallam, N. M., Alamri, S. A., Abo-Elyousr, K. A., Mostafa, Y. S., & Hashem, M. (2020). Approving the biocontrol method of potato wilt caused by *Ralstonia solanacearum* (Smith) using *Enterobacter cloacae* PS14 and *Trichoderma asperellum* T34. *Egyptian Journal of Biological Pest Control*, *30*, 1-13. https://doi.org/10.1186/s41938-020-00262-9.

Mohr, R. M., Volkmar, K., Derksen, D. A., Irvine, R. B., Khakbazan, M., McLaren, D. L., & Tomasiewicz, D. J. (2011). Effect of rotation on crop yield and quality in an irrigated potato system. *American Journal of Potato Research*, *88*, 346-359. https://doi.org/10.1007/s12230-011-9200-9.

Mondal, B., Bhattacharya, I., & Khatua, D. C. (2014). Incidence of bacterial wilt disease in West Bengal, India. *Academia Journal of Agricultural Research*, 2, 139-146.

Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, *25*(2), 361-366. https://doi.org/10.1016/j.sjbs.2017.02.004.

Muchiri, F. N., Narla, R. D., Olanya, O. M., Nyankanga, R. O., & Ariga, E. S. (2009). Efficacy of fungicide mixtures for the management of *Phytophthora infestans* (US-1) on potato. *Phytoprotection*, *90*(1), 19-29. http://dx.doi.org/10.7202/038983ar.

Mujica, N., & Kroschel, J. (2013). Pest intensity-crop loss relationships for the leaf miner fly *Liriomyza huidobrensis* (Blanchard) in different potato (*Solanum tuberosum* L.) varieties. *Crop Protection*, *47*, 6-16. http://dx.doi.org/10.1016/j.cropro.2012.12.019.

Mulugeta, T., Muhinyuza, J. B., Gouws-Meyer, R., Matsaunyane, L., Andreasson, E., & Alexandersson, E. (2020). Botanicals and plant strengtheners for potato and tomato cultivation in Africa. *Journal of Integrative Agriculture*, *19*(2), 406-427. https://doi.org/10.1016/S2095-3119(19)62703-6.

Murakoshi, S., & Takahashi, M. (1984). Trials of some control of tomato bacterial wilt caused by *Pseudomonas solanacearum*. *Bulletin of the Kanagawa Horticultural Experiment Station*, 31, 50-56. https://www.cabdirect.org/cabdirect/abstract/19851311537.

Muthoni, J., & Nyamongo, D. O. (2009). A review of constraints to ware Irish potatoes production in Kenya. *Journal of Horticulture Forestry*, *1*(7), 98-102.

Muthoni, J., Hussein, S., & Melis, R. (2012). Management of bacterial wilt [*Ralstonia solanacearum* Yabuuchi et al., 1995] of potatoes: Opportunity for host resistance in Kenya. *Journal of Agricultural Science, 4*(9), 64-78. http://dx.doi.org/10.5539/jas.v4n9p64.

Muthoni, J., Kabira, J., Shimelis, H., & Melis, R. (2014). Spread of bacterial wilt disease of potatoes in Kenya: Who is to blame?. *International Journal of Horticulture*, *4*(3), 10-15. http://dx.doi.org/10.5376/ijh.2014.04.0003.

Muthoni, J., Mbiyu, M. W., & Nyamongo, D. O. (2010). A review of potato seed systems and germplasm conservation in Kenya. *Journal of Agricultural & Food Information*, *11*(2), 157-167. http://dx.doi.org/10.1080/10496501003680565.

Muthoni, J., Shimelis, H., & Melis, R. (2015). Genotype x environment interaction and stability of potato tuber yield and bacterial wilt resistance in Kenya. *American Journal of Potato Research*, *92*, 367-378. http://dx.doi.org/10.1007/s12230-015-9442-z.

Muthoni, J., Shimelis, H., & Melis, R. (2020). Conventional breeding of potatoes for resistance to bacterial wilt (*Ralstonia solanacearum*): any light in the horizon? *Australian Journal of Crop Science*, *14*(3), 485-494. http://dx.doi.org/10.21475/ajcs.20.14.03.p2144.

Muthoni, J., Shimelis, H., Melis, R., & Kinyua, Z. M. (2014). Response of potato genotypes to bacterial wilt caused by *Ralstonia solanacearum* (Smith) (Yabuuchi *et al*.) in the tropical highlands. *American Journal of Potato Research*, *91*(2), 215-232. http://dx.doi.org/10.1007/s12230-013-9340-1.

Mutimawurugo, M. C., Ogweno, J. O., Muhinyuza, J. B., & Wagara, I. N. (2020). In-vitro antibacterial activity of selected plant extracts against potato bacterial wilt (*Ralstonia solanacearum* Smith) in Rwanda. *Journal of Applied Horticulture*, *22*(3), 202– 208. http://dx.doi.org/10.37855/jah.2020.v22i03.36.

Mutimawurugo, M. C., Ogweno, J. O., Wagara, N. I., Muhinyuza, J. B., Habimana, S., & Mukamuhirwa, A. (2023). Biological Control of Potato Bacterial Wilt Using Selected Plant Extracts. *Journal of Horticultural Research*, *31*(2), 129-140. https://doi.org/10.2478/johr-2023-0031.

Mutimawurugo, M. C., Wagara, I. N., Muhinyuza, J. B., & Ogweno, J. O. (2019). Virulence and characterization of isolates of potato bacterial wilt caused by *Ralstonia solanacearum* (Smith) in Rwanda. *African Journal of Agricultural Research*, *14*(6), 311-320. https://doi.org/10.5897/AJAR2018.13686.

Mwakidoshi, E. R., Gitari, H. H., Maitra, S., & Muindi, E. M. (2021). Economic Importance, Ecological Requirements and Production Constraints of Potato (*Solanum tuberosum* L.) in Kenya. *International Journal of Bioresource Science*, 08(02), 61-68. http://dx.doi.org/10.30954/2347-9655.02.2021.1.

Mwaniki, P. K., Wagara, I. N., Birech, R., Kinyua, Z. M., Schulte-Geldermann. E., & Freyer, B. (2017). Impact of crop rotation sequences on potato in fields inoculated with bacterial wilt

caused by *Ralstonia solanacearum*. *African Journal of Agricultural Research, 12(14),* 1226-1235. https://doi.org/10.5897/AJAR2016.11769.

Mwankemwa, Z. (2015). *Occurrence and distribution of potato bacterial wilt disease and variability of its causal agent in southern highlands of Tanzania* [Doctoral dissertation, Sokoine University of Agriculture]. https://www.suaire.sua.ac.tz/handle/123456789/1371.

Naeem, M., Maqbool, A., & Aksoy, E. (2023). Potato taxonomy and wild relatives. In M. E. Calıskan, A. Bakhs & K. Jabran (Eds.), *Potato Production Worldwide* (pp. 19-55). Academic Press. https://doi.org/10.1016/B978-0-12-822925-5.00003-7.

Namanda, S., Olanya, O. M., Adipala, E., Hakiza, J. J., El-Bedewy, R., Baghsari, A. S., & Ewell, P. (2004). Fungicide application and host-resistance for potato late blight management: benefits assessment from on-farm studies in SW Uganda. *Crop Protection*, *23*(11), 1075-1083. https://doi.org/10.1016/j.cropro.2004.03.011.

Nawaz, H., Shad, M. A., Rehman, N., Andaleeb, H., & Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences*, *56*, 1-9. http://dx.doi.org/10.1590/s2175-97902019000417129.

Nesmith, W. C., & Jenkins, J. S. F. (1985). Influence of antagonists and controlled matric potential on the survival of *Pseudomonas solanacearum* in four North Carolina soils. *Phytopathology*, *75*(11), 1182-1187.

Nielson, M., Iritani, W. M., & Weiler, L. D. (1989). Potato seed productivity: Factors influencing eye number per seed piece and subsequent performance. *American Potato Journal*, *66*(3), 151-160. https://doi.org/10.1007/BF02853676.

Njoroge, A. W., Andersson, B., Lees, A. K., Mutai, C., Forbes, G. A., Yuen, J. E., & Pelle, R. (2019). Genotyping of *Phytophthora infestans* in Eastern Africa reveals a dominating invasive European lineage. *Phytopathology*, *109*(4), 670-680. http://dx.doi.org/10.1094/PHYTO-07-18-0234-R.

Nwabueze, T. U., & Okocha, K. S. (2008). Extraction performances of polar and non-polar solvents on the physical and chemical indices of African breadfruit (*Treculia africana*) seed oil. *African Journal of Food Science*, *2*(10), 119-125.

Nyankanga, R. O., Wien, H. C., Olanya, O. M., & Ojiambo, P. S. (2004). Farmers' cultural practices and management of potato late blight in Kenya highlands: implications for

development of integrated disease management. *International Journal of Pest Management*, *50*(2), 135-144. https://doi.org/10.1080/09670870410001691812.

Obadoni, B. O., & Ochuko, P. O. (2002). Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, *8*(2), 203-208. http://dx.doi.org/10.4314/gjpas.v8i2.16033.

Oboo, H., Muia, A. W., & Kinyua, Z. M. (2014). Effect of selected essential oil plants on bacterial wilt disease development in potatoes. *Journal of Applied Biosciences*, *78*, 6666-6674. http://dx.doi.org/10.4314/jab.v78i1.2.

Okeyo, G. O., Sharma, K., Atieno, E., Narla, R. D., Miano, D. W., & Schulte-Geldermann, E. (2018). Effectiveness of positive selection in managing seed-borne potato viruses. *Journal of Agricultural Science10(3)*, 71-80. http://dx.doi.org/10.5539/jas.v10n3p71.

Okwute, S. K. (2012). Plants as Potential Sources of Pesticidal Agents: A Review. In R. P., Soundararajan (Ed.), *Pesticides advances in chemical and botanical pesticides* (pp. 207-232). https://doi.org/10.5772/46225.

Oncho, D. A., Ejigu, M. C., & Urgessa, O. E. (2021). Phytochemical constituent and antimicrobial properties of guava extracts of east Hararghe of Oromia, Ethiopia. *Clinical Phytoscience*, *7*, 1-10. http://dx.doi.org/10.1186/s40816-021-00268-2.

Onditi, J., O., Nderitu, S.,W., K., Landeo, J., A., Abong, G., O., Sikinyi, E., O., & Kabira, J., N., (2012). Release of three improved varieties for the expanded potato market in Kenya. *Agriculture and Biology Journal of North America*, 3, 192-197. http://dx.doi.org/10.5251/abjna.2012.3.5.192.197.

Otipa, M. J., Wakahiu, M. W., Kinyae, P. M., Thuo, D. M., & Kinoti, J. I. (2003). Survey of the bacterial wilt of potatoes caused by *Ralstonia solanacearum* and its spread in major potato growing areas of Kenya. *Task Force Report. KARI, Nairobi, Kenya*.

Ovchinnikova, A., Krylova, E., Gavrilenko, T., Smekalova, T., Zhuk, M., Knapp, S., & Spooner, D. M. (2011). Taxonomy of cultivated potatoes (Solanum section Petota: Solanaceae). *Botanical Journal of the Linnean Society*, *165*(2), 107-155. https://doi.org/10.1111/j.1095-8339.2010.01107.x.

Padma, R., Parvathy, N. G., Renjith, V., Kalpana, P. R., & Rahate, P. (2013). Quantitative estimation of tannins, phenols, and antioxidant activity of methanolic extract of *Imperata cylindrica*. *International Journal of Research in Pharmaceutical Sciences*, *4*(1), 73-77.

Parker, M. L., Low, J. W., Andrade, M., Schulte-Geldermann, E., & Andrade-Piedra, J. (2019). Climate change and seed systems of roots, tubers and bananas: The cases of potato in Kenya and Sweet potato in Mozambique. In T. S. Rosenstock, A. Nowak, & E. Girvetz (Eds.), *The climate-smart agriculture papers: Investigating the Business of a productive, resilient and Low emission future* (pp. 99–111). Springer International Publishing.

Patel, R., Mitra, B., Vinchurkar, M., Adami, A., Patkar, R., Giacomozzi, F., & Baghini, M. S. (2022). A review of recent advances in plant-pathogen detection systems. *Heliyon*, *8*(12), 1-12. https://doi.org/10.1016/j.heliyon.2022.e11855.

Patil, V. U., Gopal, J., & Singh, B., P. (2012). Improvement for bacterial wilt resistance in potato by conventional and biotechnological approaches. *Agricultural Research*, *1*(4), 299-316. https://doi.org/10.1007/s40003-012-0034-6.

Patra, A. K. (2012). An Overview of Antimicrobial Properties of Different Classes of Phytochemicals. In A. K. Patra (Ed.), *Dietary phytochemicals and microbes* (pp. 1-32). Springer, Dordrecht. https://doi.org/10.1007/978-94-007-3926-0_1.

Peralta, I. E., Clausen, A. M., Zorrilla, C., Ames, M., Digilio, A., & Rodriguez, F. (2021). Wild and Cultivated Potato Species Diversity, Taxonomy, and Conservation. In D. Carputo, R. Aversano, & M. R. Ercolano (Eds), *The Wild Solanums Genomes* (pp. 51-94). Compendium of Plant Genomes. Springer, Cham. https://doi.org/10.1007/978-3-030-30343-3_4.

Placide, R., Ndacyayisenga, T., Ntizo, S., Kirimi, S., & Nshimiyima, J. C. (2019). Yield and yield components of CIP advanced potato clones under Rwandan agro-ecologies. *Journal of Applied Biosciences*, *136*, 13909-13920. http://dx.doi.org/10.4314/jab.v136i1.7.

Placide, R., Theophile, N., Vandamme, E., Claude, N. J., & Thiago, M. (2022). Yield performance, adaptability and processing qualities of pre-release potato clones under different Rwandan agro-ecologies. *CABI Agriculture and Bioscience*, *3*(1), 1-10. http://dx.doi.org/10.1186/s43170-022-00105-7.

Popoola, A. R., Ganiyu, S. A., Enikuomehin, O. A., Bodunde, J. G., Adedibu, O. B., Durosomo, H. A., & Karunwi, O. A. (2015). Isolation and characterization of *Ralstonia solanacearum*

causing bacterial wilt of tomato in Nigeria. *Nigerian Journal of Biotechnology*, *29*, 1-10. http://dx.doi.org/10.4314/njb.v29i1.1.

Porwal, V., Singh, P., & Gurjar, D. (2012). A comprehensive study on different methods of extraction from guajava leaves for curing various health problem. *International Journal of Engineering Research and Applications*, *2*(6), 490-496.

Pradhanang, P. M., Elphinstone, J. G., & Fox, R. T. V. (2000). Identification of crop and weed hosts of *Ralstonia solanacearum* biovar 2 in the hills of Nepal. *Plant Pathology*, *49*(4), 403-413. http://dx.doi.org/10.1046/j.1365-3059.2000.00480.x.

Prior, P., Bart, S., Leclercq, S., Darrasse, A., & Anais, G. (1996). Resistance to bacterial wilt in tomato as discerned by spread of *Pseudomonas* (*Burholderia*) *solanacearum* in the stem tissues. *Plant Pathology*, *45*(4), 720-726. https://doi.org/10.1046/j.1365-3059.1996.d01-9.x.

Priou, S., Aley, P., Chujoy, E., Lemaga, B. A., French, E. R., & French, E. (1999). Integrated control of bacterial wilt of potato. In *CIP Slide Training Series IV-3. International Potato*. http://cipotato.org/wp-content/uploads/2014/10/guiaing.pdf. Date accessed: 25/01/2022.

Priou, S., Gutarra, L., & Aley, P. (2006). An improved enrichment broth for the sensitive detection of *Ralstonia solanacearum* (biovars 1 and 2A) in soil using DAS–ELISA. *Plant Pathology*, *55*(1), 36-45. https://doi.org/10.1111/j.1365-3059.2005.01293.x.

Priou, S., Gutarra, L., Aley, P., De Mendiburu, F., & Llique, R. (2010). Detection of *Ralstonia solanacearum* (biovar 2A) in stems of symptomless plants before harvest of the potato crop using post-enrichment DAS-ELISA. *Plant Pathology*, *59*(1), 59-67. https://doi.org/10.1111/j.1365-3059.2009.02155.x.

Priou, S., Torres, R., Villar, A., Gutarra, L., & De Mendiburu, F. (2001). Optimisation of sample size for the detection of latent infection by *Ralstonia solanacearum* in potato seed tubers in the highlands of Peru. *Potato research*, *44*(4), 349-358. http://dx.doi.org/10.1007/BF02358595.

Pule, B. B., Meitz, J. C., Thompson, A. H., Linde, C. C., Fry, W. E., Langenhoven, S. D., & McLeod, A. (2013). *Phytophthora infestans* populations in central, eastern and southern African countries consist of two major clonal lineages. *Plant pathology*, *62*(1), 154-165. https://doi.org/10.1111/j.1365-3059.2012.02608.x.

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., & Trotin, F. (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of Ethnopharmacology*, *72*(1-2), 35-42. https://doi.org/10.1016/S0378-8741(00)00196-3.

Quimby, P. C., King, L. R., & Grey, W. E. (2002). Biological control as a means of enhancing the sustainability of crop/land management systems. *Agriculture, Ecosystems & Environment*, *88*(2), 147-152. http://dx.doi.org/10.1016/S0167-8809(01)00251-1.

Quiroz, R., Ramírez, D. A., Kroschel, J., Andrade-Piedra, J., Barreda, C., Condori, B., & Perez, W. (2018). Impact of climate change on the potato crop and biodiversity in its center of origin. *Open Agriculture*, *3*(1), 273-283. http://dx.doi.org/10.1515/opag-2018-0029.

Radcliffe, E. B., & Lagnaoui, A. (2007). Insect Pests in Potato. In D. Vreugdenhil, J. Bradshaw, C. Gebhardt, F. Govers, D.K.L. Mackerron, M.A. Taylor & H.A. Ross, (Eds.), *Potato Biology and Biotechnology (*pp. 543–567). Elsevier Science B.V., Amsterdam.

Rahman, M. F., Islam, M. R., Rahman, T., & Meah, M. B. (2010). Biochemical characterization of *Ralstonia solanacearum* causing bacterial wilt of brinjal in Bangladesh. *Progressive Agriculture*, *21*(1-2), 9-19. http://dx.doi.org/10.3329/pa.v21i1-2.16744.

Rahman, M. M., Khan, A. A., Ali, M. E., Mian, I. H., Akanda, A. M., & Abd Hamid, S. B. (2012). Botanicals to control soft rot bacteria of potato. *The Scientific World Journal*, 2,796472. http://dx.doi.org/10.1100/2012/796472.

Ravelomanantsoa, S., Vernière, C., Rieux, A., Costet, L., Chiroleu, F., Arribat, S., & Prior, P. (2018). Molecular epidemiology of bacterial wilt in the Madagascar highlands caused by Andean (Phylotype IIB-1) and African (Phylotype III) brown rot strains of the *Ralstonia solanacearum* species complex. *Frontiers in Plant Science*, *8*, 2258. https://doi.org/10.3389/fpls.2017.02258.

Riungu, C. (2011). No easy walk for potatoes. *Horticultural News. The East African Fresh Produce Journal*, *19*, 16-17.

Rivard, C. L., O'connell, S., Peet, M. M., Welker, R. M., & Louws, F. J. (2012). Grafting tomato to manage bacterial wilt caused by *Ralstonia solanacearum* in the southeastern United States. *Plant Disease*, *96*(7), 973-978. https://doi.org/10.1094/pdis-12-10-0877.

Robinson, R. A., & Ramos, A. H. (1964). Bacterial wilt of potatoes in Kenya. *East African Agricultural and Forestry Journal*, *30*(1), 59-64. https://doi.org/10.1080/00128325.1964.11661963.

Robison, R. S., Starkey, R. L., & Davidson, O. W. (1954). Control of bacterial wilt of chrysanthemums with streptomycin. *Phytopathology*, *44*(11), 646-650.

Rodríguez, F., Ghislain, M., Clausen, A. M., Jansky, S. H., & Spooner, D. M. (2010). Hybrid origins of cultivated potatoes. *Theoretical and Applied Genetics*, *121*, 1187-1198. https://doi.org/10.1007/s00122-010-1422-6.

Rostand, R. C., Joel, M., Viviene, M., & Steven, R. (2018). Isolation and characterization of *Ralstonia solanacearum* strains causing bacterial wilt of potato in Nakuru County of Kenya. *African Journal of Biotechnology*, *17*(52), 1455-1465.

RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Safni, I., Cleenwerck, I., De Vos, P., Fegan, M., Sly, L., & Kappler, U. (2014). Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex: proposal to emend the descriptions of *Ralstonia solanacearum* and *Ralstonia syzygii* and reclassify current *R. syzygii* strains as *Ralstonia syzygii* subsp. *syzygii* subsp. *nov*., *R. solanacearum* phylotype IV strains as *Ralstonia syzygii* subsp. *indonesiensis* subsp. *nov.*, banana blood disease bacterium strains as *Ralstonia syzygii* subsp. *celebesensis* subsp. *nov*. and *R. solanacearum* phylotype I and III strains as. *International Journal of Systematic and Evolutionary Microbiology*, *64*(9), 3087-3103. https://doi.org/10.1099/ijs.0.066712-0.

Sagar, V., Somani, A. K., Arora, R. K., Sharma, S., Chakrabarti, S. K., Tiwari, S. K., & Singh, B. P. (2013). Status of bacterial wilt of potato in the Malwa region of Madhya Pradesh in India. *Journal of Plant Pathology*, 95(2), 321-328.

Sahu, P. K., Gupta, A., Kedarnath, Kumari, P., Lavanya, G., & Yadav, A. K. (2017). Attempts for Biological Control of *Ralstonia solanacearum* by Using Beneficial Microorganisms. In: Meena, V., Mishra, P., Bisht, J., Pattanayak, A. (Eds), *Agriculturally Important Microbes for Sustainable Agriculture* (pp. 315-342). Springer, Singapore. https://doi.org/10.1007/978-981-10-5343-6_11.

Sánchez, E., García, S., & Heredia, N. (2010). Extracts of edible and medicinal plants damage membranes of *Vibrio cholerae*. *Applied and Environmental Microbiology*, *76*(20), 6888-6894. https://doi.org/10.1128/aem.03052-09.

Sandilya, V. K., Rangare, N. R., Rangare, S. B., Kumar, V., Rohit, T. S., Dehari, B., & Sahu, P. (2023). Potato production usages and nutrition: A review. *The Pharma Innovation*, *12*(7), 1357-1365.

Sangoyomi, T. E., Owoseni, A. A., Adebayo, O. S., & Omilani, O. A. (2011). Evaluation of some botanicals against bacterial wilt of tomatoes. *International Research Journal of Microbiology*, *2*(9), 365-369.

Santos-Sánchez, N. F., Salas-Coronado, R., Hernández-Carlos, B., & Villanueva-Cañongo, C. (2019). Shikimic acid pathway in biosynthesis of phenolic compounds. In M. Soto-Hernández, R. García-Mateos & M. Palma-Tenango (Eds.), *Plant physiological aspects of phenolic compounds* (1st Ed., Vol. 1, pp. 1–15). IntechOpen London, UK. http://dx.doi.org/10.5772/intechopen.83815.

Saraswathi, J., Venkatesh, K., Nirmala Baburao, N. B., Hilal, M. H., & Rani, A. R. (2011). Phytopharmacological importance of Pelargonium species. *Journal of Medicinal Plants Research*, 5(13), 2587-2598.

Sarkar, S., & Chaudhuri, S. (2016). Bacterial wilt and its management. *Current Science*, *110*(8), 1439-1445. https://www.jstor.org/stable/24908099.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, *8*(1), 1-10. http://dx.doi.org/10.4314/ajtcam.v8i1.60483.

Schönfeld, J., Heuer, H., Van Elsas, J. D., & Smalla, K. (2003). Specific and sensitive detection of *Ralstonia solanacearum* in soil on the basis of PCR amplification of fliC fragments. *Applied and Environmental Microbiology*, *69*(12), 7248-7256. https://doi.org/10.1128%2FAEM.69.12.7248-7256.2003.

Shah, R. K., & Yadav, R. N. S. (2015). Qualitative phytochemical analysis and estimation of total phenols and flavonoids in leaf extract of *Sarcochlamys pulcherrima*. *Global Journal of Bio-science and Biotechnology*, *4*(1), 81-4.

Shakeel, F. (2023). Analysis of Natural Bioactive Compounds in Plant, Food, and Pharmaceutical Products Using Chromatographic Techniques. *Separations*, *10*(10), 541. https://doi.org/10.3390/separations10100541.

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, *52*(3/4), 591-611. https://doi.org/10.1093/biomet/52.3-4.591.

Sharma, K., Iruegas-Bocardo, F., Abdurahman, A., Alcalá-Briseño, R. I., Garrett, K. A., Goss, E. M., & Munguti, F. (2022). *Ralstonia* Strains from Potato-Growing Regions of Kenya Reveal Two Phylotypes and Epidemic Clonality of Phylotype II Sequevar 1 Strains. *Phytopathology®*, *112*(10), 2072-2083. https://doi.org/10.1094/PHYTO-11-21-0455-R.

Sharma, K., Shawkat, B., Miethbauer, T., & Schulte-Geldermann, E. (2017). Strategies for bacterial wilt (*Ralstonia solanacearum*) management in potato field: Farmers' guide. https://cgspace.cgiar.org/bitstream/handle/10568/97844/CIP-Strategies-for-Bacterial-Wilt-Management.pdf?sequence=1. Date accessed: 04/04/2022.

She, X., Yu, L., Lan, G., Tang, Y., & He, Z. (2017). Identification and genetic characterization of *Ralstonia solanacearum* species complex isolates from *Cucurbita maxima* in China. *Frontiers in Plant Science*, *8*(1794), 1-11. https://doi.org/10.3389/fpls.2017.01794.

Shepherd, J. A., Waigh, R. D., & Gilbert, P. E. T. E. R. (1988). Antibacterial action of 2-bromo-2-nitropropane-1, 3-diol (bronopol). *Antimicrobial Agents and Chemotherapy*, *32*(11), 1693-1698. https://doi.org/10.1128%2Faac.32.11.1693.

Shimelis, H., & Melis, R. (2014). Spread of bacterial wilt disease of potatoes in Kenya: who is to blame?. *International Journal of Horticulture*, *4*(3), 10-15. http://dx.doi.org/10.5376/ijh.2014.04.0003.

Silas, M. N. E., Murungi, J. I., & Wanjau, R. N. (2012). The pH of leaf water extracts and amount of acid required lowering the pH of leaf water extracts to 5.0. *American International Journal of Contemporary Research*, *2*(11), 72-78.

Singh, D., Sinha, S., Yadav, D. K., & Chaudhary, G. (2014). Detection of *Ralstonia solanacearum* from asymptomatic tomato plants, irrigation water, and soil through non-selective enrichment medium with hrp gene-based bio-PCR. *Current Mcrobiology*, *69*(2), 127-134. https://doi.org/10.1007/s00284-014-0566-z.

Singh, D., Yadav, D. K., Sinha, S., & Choudhary, G. (2014). Effect of temperature, cultivars, injury of root and inoculums load of *Ralstonia solanacearum* to cause bacterial wilt of

tomato. *Archives of Phytopathology and Plant Protection*, *47*(13), 1574-1583. https://doi.org/10.1080/03235408.2013.851332.

Singh, N., Phukan, T., Sharma, P. L., Kabyashree, K., Barman, A., Kumar, R., & Ray, S. K. (2018). An innovative root inoculation method to study *Ralstonia solanacearum* pathogenicity in tomato seedlings. *Phytopathology*, *108*(4), 436-442. https://doi.org/10.1094/phyto-08-17-0291-r.

Singh, S., Kumar, V., Datta, S., Dhangial, D. S., & Singh, J. (2020). Plant disease management by bioactive natural products. In J. Singh & A. N., Yadav (Eds.), *Natural Bioactive Products in Sustainable Agriculture* (pp. 15-19). Springer, Singapore. http://dx.doi.org/10.1007/978-981-15-3024-1_2.

Sinha, R., Gupta, A., & Senthil-Kumar, M. (2016). Understanding the impact of drought on foliar and xylem invading bacterial pathogen stress in chickpea. *Frontiers in Plant Science*, *7*, 205582. https://doi.org/10.3389/fpls.2016.00902.

Siri, M. I., Sanabria, A., & Pianzzola, M. J. (2011). Genetic diversity and aggressiveness of *Ralstonia solanacearum* strains causing bacterial wilt of potato in Uruguay. *Plant Disease*, *95*(10), 1292-1301. https://doi.org/10.1094/PDIS-04-15-0384-RE.

Smith, E. F. (1896). A bacterial disease of the tomato, eggplant and Irish potato. *United States Department of Agriculture, National agricultural Library. https://archive.org/details/bacterialdisease12smit/page/4/mode/2up*.

Smith, E. F. (1911). *Bacteria in relation to plant diseases* (No. 27). Carnegie Institution of Washington.

Snehlata, K., Sheel, R., & Kumar, B. (2018). Evaluation of phytochemicals in polar and nonpolar solvent extracts of leaves of *Aegle marmelos* (L.). *Journal of Biotechnology and Biochemistry*, *4*(5), 31-38. https://www.iosrjournals.org/iosr-jbb/papers/Volume%204,%20Issue%205/Version-1/E0405013138.pdf.

Soare, E., & Chiurciu, I. A. (2021). Study on the dynamics of potato production and worldwide trading during the period 2012-2019. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, *21*(4), 527-532. https://www.researchgate.net/profile/Ia-Chiurciu/publication/357328970_STUDY_ON_THE_DYNAMICS_OF_POTATO_PRODUCTION_AND_WORLDWIDE_TRADING_DURING_THE_PERIOD_2012-

2019/links/61c78e32b6b5667157a6d7be/STUDY-ON-THE-DYNAMICS-OF-POTATO-PRODUCTION-AND-WORLDWIDE-TRADING-DURING-THE-PERIOD-2012-2019.pdf.

Soesanto, L., Mugiastuti, E., & Khoeruriza, K. (2019). Granular Formulation Test of *Pseudomonas fluorescens* P60 for Controling Bacterial Wilt (*Ralstonia solanacearum*) of Tomato in Planta. *AGRIVITA, Journal of Agricultural Science, 41*(3), 513-523. http://dx.doi.org/10.17503/agrivita.v41i3.2318.

Sofo, A., Zanella, A., & Ponge, J. F. (2022). Soil quality and fertility in sustainable agriculture, with a contribution to the biological classification of agricultural soils. *Soil Use and Management, 38*(2), 1085-1112. https://doi.org/10.1111/sum.12702.

Spooner, D. M. (2016). Species delimitations in plants: lessons learned from potato taxonomy by a practicing taxonomist. *Journal of Systematics and Evolution*, *54*(3), 191-203. http://dx.doi.org/10.1111/jse.12203.

Spooner, D. M., Ghislain, M., Simon, R., Jansky, S. H., & Gavrilenko, T. (2014). Systematics, diversity, genetics, and evolution of wild and cultivated potatoes. *The Botanical Review, 80*, 283-383. http://dx.doi.org/10.1007/s12229-014-9146-y.

Strehmel, N., Hummel, J., Erban, A., Strassburg, K., & Kopka, J. (2008). Retention index thresholds for compound matching in GC–MS metabolite profiling. *Journal of Chromatography B, 871*(2), 182-190. https://doi.org/10.1016/j.jchromb.2008.04.042.

Stretton, R. J., & Manson, T. W. (1973). Some Aspects of the Mode of Action of the Antibacterial Compound Bronopol (2-bromo-2-nitropropan-1, 3-diol). *Journal of Applied Bacteriology*, *36*(1), 61-76. https://doi.org/10.1111/j.1365-2672.1973.tb04073.x.

Sukhotu, T., & Hosaka, K. (2006). Origin and evolution of Andigena potatoes revealed by chloroplast and nuclear DNA markers. *Journal of Genomics, 49*(6), 636-647. https://doi.org/10.1139/g06-014.

Sun, Y., Su, Y., Meng, Z., Zhang, J., Zheng, L., Miao, S., & Yu, G. (2023). Biocontrol of bacterial wilt disease in tomato using Bacillus subtilis strain R31. *Frontiers in Microbiology*, *14*, 1281381. https://doi.org/10.3389%2Ffmicb.2023.1281381.

Swanson, J. K., Yao, J., Tans-Kersten, J., & Allen, C. (2005). Behavior of *Ralstonia solanacearum* race 3 biovar 2 during latent and active infection of geranium. *Phytopathology, 95*(2), 136-143. https://doi.org/10.1094/phyto-95-0136.

Tahat, M. M., Sijam, K., & Othman, R. (2012). The potential of endomycorrhizal fungi in controlling tomato bacterial wilt *Ralstonia solanacearum* under glasshouse conditions. *African Journal of Biotechnology*, *11*(67), 13085-13094. http://dx.doi.org/10.5897/AJB11.3629.

Taiy, R. J., Onyango, C., Nkurumwa, A., & Ngetich, K. (2017). Socio-economic characteristics of smallholder potato farmers in Mauche Ward of Nakuru County, Kenya. *Universal Journal of Agricultural Research*, *5*(5), 257-266. http://dx.doi.org/10.13189/ujar.2017.050502.

Tanaka, J. C. A., Da Silva, C. C., De Oliveira, A. J. B., Nakamura, C. V., & Dias Filho, B. P. (2006). Antibacterial activity of indole alkaloids from *Aspidosperma ramiflorum*. *Brazilian Journal of Medical and Biological Research*, *39*, 387-391. https://doi.org/10.1590/S0100-879X2006000300009.

Tango, C. N., Mansur, A. R., & Oh, D. H. (2015). Fumaric acid and slightly acidic electrolyzed water inactivate gram positive and gram-negative foodborne pathogens. *Microorganisms*, *3*(1), 34-46. https://doi.org/10.3390%2Fmicroorganisms3010034.

Terblanche, J., & De Villiers, D. A. (1998). The suppression of *Ralstonia solanacearum* by marigolds. In P. Prior, C. Allen, & J. Elphinstone (Eds), *Bacterial wilt disease* (pp. 325-331). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-03592-4_49.

Tessema, G. L., & Seid, H. E. (2023). Potato bacterial wilt in Ethiopia: history, current status, and future perspectives. *PeerJ*, *11*, 14661. https://doi.org/10.7717/peerj.14661.

Thenmozhi, S., & Rajan, S. (2015). GC-MS analysis of bioactive compounds in *Psidium guajava* leaves. *Journal of Pharmacognosy and Phytochemistry*, *3*(5), 162-166.

Thomas-Sharma, S., Abdulrahman, A., Ali, S., Andre Piedra, L. J., Bao, S., Charkowski, A.O., Crook, D., Kadian, M. K. M., Kromann, P., Struik, C.P., L. Torrence, L., Garett, K. A., & Forbes, G. A. (2016). Seed degeneration in potato: The need for an integrated seed health strategy to mitigate the problem in developing countries. *Plant Pathology*, *65*(1), 3 –16. https://doi.org/10.1111/ppa.12439.

Tinh, T. H., Nuidate, T., Vuddhakul, V., & Rodkhum, C. (2016). Antibacterial activity of pyrogallol, a polyphenol compound against *Vibrio parahaemolyticus* isolated from the central region of Thailand. *Procedia Chemistry*, *18*, 162-168. https://doi.org/10.1016/j.proche.2016.01.025.

Todd, J. M. (1969). A prospect of potato growing in Kenya. *Annual Report. Ministry of Overseas Development, London*.

Tripathi, P., Rawat, G., Yadav, S., & Saxena, R. K. (2015). Shikimic acid, a base compound for the formulation of swine/avian flu drug: statistical optimization, fed-batch and scale up studies along with its application as an antibacterial agent. *Antonie Van Leeuwenhoek*, *107*(2), 419-431. https://doi.org/10.1007/s10482-014-0340-z.

Tung, P. X. (1992). Genetic variation for bacterial wilt resistance in a population of tetraploid potato. *Euphytica*, *61*, 73–80. https://doi.org/10.1007/BF00035549.

Tung, P. X., Hermsen, J. G. T., Vander Zaag, P., & Schmiediche, P. (1992). Effects of resistance genes, heat tolerance genes and cytoplasms on expression of resistance to *Pseudomonas solanacearum* (EF Smith) in potato. *Euphytica*, *60*, 127-138. https://doi.org/10.1007/BF00029668.

Tung, P. X., Rasco, E. T., Vander Zaag, P., & Schmiediche, P. (1990). Resistance *to Pseudomonas solanacearum* in the potato: I. Effects of sources of resistance and adaptation. *Euphytica*, *45*, 203-210. https://doi.org/10.1007/BF00032987.

Upreti, R., & Thomas, P. (2015). Root-associated bacterial endophytes from *Ralstonia solanacearum* resistant and susceptible tomato cultivars and their pathogen antagonistic effects. *Frontiers in Microbiology*, *6*, 129679. https://doi.org/10.3389/fmicb.2015.00255.

Uwamahoro, F., Berlin, A., Bucagu, C., Bylund, H., & Yuen, J. (2020). *Ralstonia solanacearum* causing potato bacterial wilt: host range and cultivars' susceptibility in Rwanda. *Plant Pathology*, *69*(3), 559-568. https://doi.org/10.1111/ppa.13140.

Uwamahoro, F., Berlin, A., Bucagu, C., Bylund, H., & Yuen, J. (2018). Potato bacterial wilt in Rwanda: occurrence, risk factors, farmers' knowledge and attitudes. *Food Security*, *10*, 1221-1235. http://dx.doi.org/10.1007/s12571-018-0834-z.

Van Bniggen, A. H., & Termorskuizen, A. J. (2003). Integrated approaches to root disease management in organic farming systems. *Australasian Plant Pathology*, *32*(2), 141-156. https://doi.org/10.1071/AP03029.

van Elsas, J. D., Kastelein, P., van Bekkum, P., van der Wolf, J. M., de Vries, P. M., & van Overbeek, L. S. (2000). Survival of *Ralstonia solanacearum* biovar 2, the causative agent of potato brown rot, in field and microcosm soils in temperate

climates. *Phytopathology*, *90*(12), 1358-1366. https://doi.org/10.1094/phyto.2000.90.12.1358.

van Elsas, J. D., van Overbeek, L. S., Bailey, M. J., Schönfeld, J., & Smalla, K. (2005). Fate of Ralstonia solanacearum biovar 2 as affected by conditions and soil treatments in temperate climate zones. In C. Allen, P. Prior, & A. C. Hayward (Eds.), *Bacterial wilt disease and the Ralstonia solanacearum species complex* (pp. 39-49). APS Press.

Vanitha, S. C., Niranjana, S. R., Mortensen, C. N., & Umesha, S. (2009). Bacterial wilt of tomato in Karnataka and its management by *Pseudomonas fluorescens*. *Biocontrol*, *54*(5), 685-695. http://dx.doi.org/10.1007/s10526-009-9217-x.

Velásquez, A. C., Castroverde, C. D. M., & He, S. Y. (2018). Plant–pathogen warfare under changing climate conditions. *Current biology*, *28*(10), R619-R634. https://doi.org/10.1016/j.cub.2018.03.054.

Waaswa, A., Oywaya Nkurumwa, A., Mwangi Kibe, A., & Ngeno Kipkemoi, J. (2022). Climate-Smart agriculture and potato production in Kenya: review of the determinants of practice. *Climate and Development*, *14*(1), 75-90. https://doi.org/10.1080/17565529.2021.1885336.

Wamani, A. O. (2020). *Screening of Microbial Antagonists and Plant Extracts Against Selected Tomato Pathogens and Their Potential in the Management of Bacterial Wilt* [Doctoral dissertation, University of Nairobi]. http://erepository.uonbi.ac.ke/bitstream/handle/11295/154678/Wamani_Screening%20of%20Microbial%20Antagonists%20and%20Plant%20Extracts%20Against%20Selected%20Tomato%20Pathogens%20and%20Their%20Potential%20in%20the%20Management%20of%20Bacterial%20Wilt.pdf?sequence=1&isAllowed=y. Date accessed: 24/01/2022.

Wang, L. (2014, March). The antibacterial effect of secondary compounds in plants. In *50th Annual Southeastern Michigan Junior Science and Humanities Symposium. March* (pp. 6-7). https://dx.doi.org/10.3923/rjmp.2014.204.213.

Wang, R., Zhang, H., Sun, L., Qi, G., Chen, S., & Zhao, X. (2017). Microbial community composition is related to soil biological and chemical properties and bacterial wilt outbreak. *Scientific Reports*, *7*(1), 343. https://doi.org/10.1038/s41598-017-00472-6.

Wang, Y., McAllister, T. A., Yanke, L. J., & Cheeke, P. R. (2000). Effect of steroidal saponin from *Yucca schidigera* extract on ruminal microbes. *Journal of Applied Microbiology*, *88*(5), 887-896. https://doi.org/10.1046/j.1365-2672.2000.01054.x.

Wang, Z., Luo, W., Cheng, S., Zhang, H., Zong, J., & Zhang, Z. (2023). *Ralstonia solanacearum*–a soil borne hidden enemy of plants: research development in management strategies, their action mechanism and challenges. *Frontiers in Plant Science*, *14*, 1141902. https://doi.org/10.3389/fpls.2023.1141902.

Wang'ombe, J. G., & van Dijk, M. P. (2013). Low potato yields in Kenya: do conventional input innovations account for the yields disparity?. *Agriculture & Food Security*, *2*(1), 1-11. http://dx.doi.org/10.1186/2048-7010-2-14.

Wei, Z., Gu, Y., Friman, V. P., Kowalchuk, G. A., Xu, Y., Shen, Q., & Jousset, A. (2019). Initial soil microbiome composition and functioning predetermine future plant health. *Science Advances*, *5*(9), 1-11. https://doi.org/10.1126/sciadv.aaw0759.

Wei, Z., Hu, J., Yin, S., Xu, Y., Jousset, A., Shen, Q., & Friman, V. P. (2018). *Ralstonia solanacearum* pathogen disrupts bacterial rhizosphere microbiome during an invasion. *Soil Biology and Biochemistry*, *118*, 8-17. https://doi.org/10.1016/j.soilbio.2017.11.012.

Wen, T., Zhao, M., Liu, T., Huang, Q., Yuan, J., & Shen, Q. (2020). High abundance of *Ralstonia solanacearum* changed tomato rhizosphere microbiome and metabolome. *BMC Plant Biology*, *20*, 1-11. https://doi.org/10.1186/s12870-020-02365-9.

Were, H. K., Kabira, J. N., Kinyua, Z. M., Olubayo, F. M., Karinga, J. K., Aura, J., and Torrance, L. (2013). Occurrence and distribution of potato pests and diseases in Kenya. *Potato Research*, *56*(4), 325-342. http://dx.doi.org/10.1007/s11540-013-9246-9.

Whipps, J. M. (2001). Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany*, *52*(suppl_1), 487-511. https://doi.org/10.1093/jexbot/52.suppl_1.487.

Whipps, J. M. (2007). Biological pesticides for control of seed-and soil-borne plant pathogen. In J.D.van Elsas, J.K. Jansson & J.T. Tr evors (ed.), *Modern soil microbiology* (2nd ed, pp. 479–501). Marcel Dekker, New York, USA.

Wicker, E., Lefeuvre, P., De Cambiaire, J. C., Lemaire, C., Poussier, S., & Prior, P. (2012). Contrasting recombination patterns and demographic histories of the plant pathogen

Ralstonia solanacearum inferred from MLSA. *The ISME Journal*, *6*(5), 961-974. https://doi.org/10.1038%2Fismej.2011.160.

Wilcoxon, F. (1945). Individual comparisons by ranking methods. Biometrics Bulletin, 1, 80–83.

Wilcoxon, F. (1992). Individual comparisons by ranking methods. In *Breakthroughs in statistics* (pp. 196-202). Springer, New York, NY.

Wu, M., Tian, L., Fu, J., Liao, S., Li, H., Gai, Z., & Gong, G. (2022). Antibacterial mechanism of Protocatechuic acid against *Yersinia enterocolitica* and its application in pork. *Food Control*, *133*, 108573. https://doi.org/10.1016/j.foodcont.2021.108573.

Wu, X., Li, H., Wang, Y., & Zhang, X. (2020). Effects of bio-organic fertilizer fortified by *Bacillus cereus* QJ-1 on tobacco bacterial wilt control and soil quality improvement. *Biocontrol Science and Technology*, *30*(4), 351-369. https://doi.org/10.1080/09583157.2020.1711870.

Xue, Q. Y., Chen, Y., Li, S. M., Chen, L. F., Ding, G. C., Guo, D. W., & Guo, J. H. (2009). Evaluation of the strains of Acinetobacter and Enterobacter as potential biocontrol agents against *Ralstonia* wilt of tomato. *Biological Control*, *48*(3), 252-258. http://dx.doi.org/10.1016/j.biocontrol.2008.11.004.

Ya, N., & Toyota, K. (2008). Suppression of bacterial wilt of tomato by a *Burkholderia nodosa* strain isolated from Kalimantan soils, Indonesia. *Microbes and Environments*, *23(2)*, 134-141. https://doi.org/10.1264/jsme2.23.134.

Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H., & Nishiuchi, Y. (1995). Transfer of Two *Burkholderia* and An *Alcaligenes* Species to *Ralstonia* Gen. Nov. Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) Comb. Nov., *Ralstonia solanacearum* (Smith 1896) Comb. Nov. and *Ralstonia eutropha* (Davis 1969) Comb. Nov. *Microbiology and Immunology*, *39*(11), 897-904.

Yakhin, O. I., Lubyanov, A. A., Yakhin, I. A., & Brown, P. H. (2017). Biostimulants in plant science: a global perspective. *Frontiers in Plant Science*, 7(2049), 1-32. https://doi.org/10.3389/fpls.2016.02049.

Yan, L., & Khan, R. A. A. (2021). Biological control of bacterial wilt in tomato through the metabolites produced by the biocontrol fungus, *Trichoderma harzianum*. *Egyptian Journal of Biological Pest Control*, *31*, 1-9. http://dx.doi.org/10.1186/s41938-020-00351-9.

Yeon, J., Le, N. T., & Sim, S. C. (2022). Assessment of temperature-independent resistance against bacterial wilt using major QTL in cultivated tomato (*Solanum lycopersicum* L.). *Plants*, *11*(17), 2223. https://doi.org/10.3390/plants11172223.

Yihune, E., & Yemata, G. (2019). Antibacterial activity of medicinal plant extracts against *Ralstonia solanacearum* (Smith) that causes bacterial wilt in hot pepper (*Capsicum annuum* L.). *Acta Scientiarum. Biological Sciences*, *41*(1), 1-11. https://periodicos.uem.br/ojs/index.php/ActaSciBiolSci/article/view/45402.

Yuliar., Nion, Y. A., & Toyota, K. (2015). Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. *Microbes and Environments*, *30*(1), 1-11. https://doi.org/10.1264%2Fjsme2.ME14144.

Yusnawan, E. (2013). The effectiveness of polar and non-polar fractions of *Ageratum conyzoides* l. to control peanut rust disease and phytochemical screenings of secondary metabolites. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, *13*(2), 159-166. http://dx.doi.org/10.23960/j.hptt.213159-166.

Zaker, M. (2016). Natural plant products as eco-friendly fungicides for plant diseases control-A review. *The Agriculturists*, *14*(1), 134-141. http://dx.doi.org/10.3329/agric.v14i1.29111.

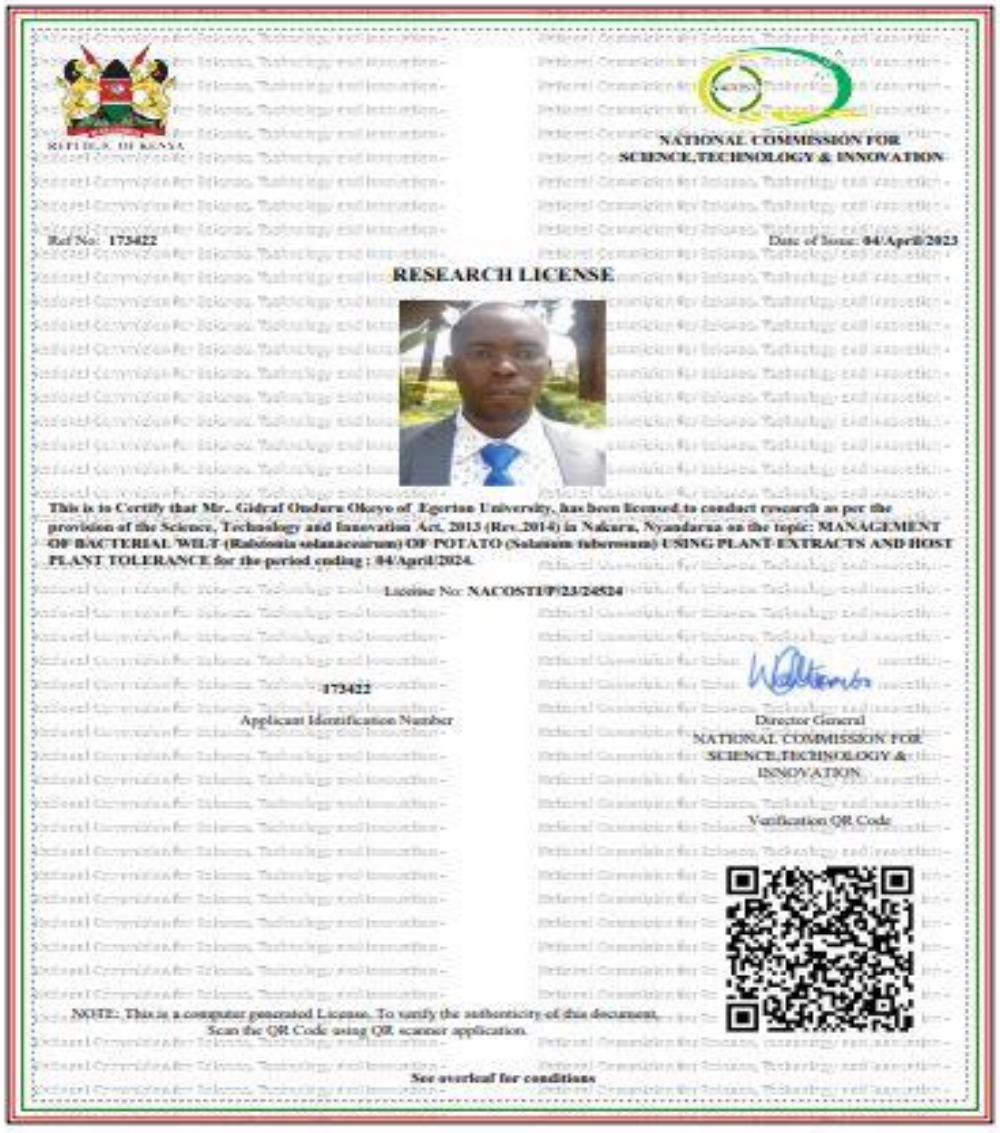
Zeikus, J. G., Jain, M. K., & Elankovan, P. (1999). Biotechnology of succinic acid production and markets for derived industrial products. *Applied Microbiology and Biotechnology*, *51*(5), 545-552. http://dx.doi.org/10.1007/s002530051431.

Zhou, Y., Choi, Y. L., Sun, M., & Yu, Z. (2008). Novel roles of *Bacillus thuringiensis* to control plant diseases. *Applied Microbiology and Biotechnology*, *80*(4), 563-572. https://doi.org/10.1007/s00253-008-1610-3.

Zinnat, K., Hossain, M. S., & Begum, M. M. (2018). *Ralstonia solanacearum*: a threat to potato production in Bangladesh. *Fundamental and Applied Agriculture*, *3*(1), 407-421. http://dx.doi.org/10.5455/faa.280361.

APPENDICES

Appendix A: Research permit



NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 173422

Date of Issue: 04/April/2023

RESEARCH LICENSE

This is to Certify that Mr.. Gidraf Ondura Okuye of Egerton University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nakuru, Nyandarua on the topic: MANAGEMENT OF BACTERIAL WILT (Ralstonia solanacearum) OF POTATO (Solanum tuberosum) USING PLANT EXTRACTS AND HOST PLANT TOLERANCE for the period ending : 04/April/2024.

License No: NACOSTI/P/23/24524

173422

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

Appendix B: List of publications

HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

SAUDI BIOLOGICAL SOCIETY

Original article

Antibacterial activity of guava, moringa, camphor bush and pelargonium extracts against bacterial wilt (*Ralstonia pseudosolanacearum* sp. nov.) of potato

Gidraf Onduru Okeyo [a,*], Miriam K. Charimbu [a], Jane Nyaanga [a], Thiago Mendes [b]

[a] Department of Crops, Horticulture and Soil Sciences, Egerton University, Njoro, Kenya
[b] The CGIAR Research Program on Roots, Tubers and Bananas (RTB), International Potato Center, Regional Office Sub-Saharan Africa, ILRI Campus, Nairobi, Kenya

ARTICLE INFO

Article history:
Received 4 January 2022
Revised 7 August 2022
Accepted 1 September 2022
Available online 6 September 2022

Keywords:
Antibacterial activity
Bacterial wilt
Bio-control
Plant extracts

ABSTRACT

Bacterial wilt (*Ralstonia pseudosolanacearum* sp. nov.) is a major disease devastating global potato production. Proposed management options are mostly expensive and ineffective. This has necessitated efforts to develop cheaper and eco-friendly management options such as use of botanicals. Antibacterial activity of ethanol and acetone plant extracts from guava (*Psidium guajava*), drumstick (*Moringa oleifera*), camphor bush (*Tarchonanthus camphoratus*) and pelargonium (*Pelargonium zonale*) against *R. pseudosolanacearum* sp. nov. was evaluated in-vitro at a concentration of 100 mg/mL of 1 % Dimethlysulfoxide (DMSO) using disk diffusion technique. The *R. pseudosolanacearum* sp. nov was isolated from infected haulms collected from potato growing field at the University of Nairobi. The most effective extracts were subjected to further screening at different concentrations to determine their minimum inhibitory concentrations (MICs). All the four plant extracts showed varied antibacterial efficacy. *P. zonale* leaves extract was the most effective with growth inhibition zone of 18.73 mm and 18.60 mm for ethanol and acetone solvents respectively. The average of growth inhibition zones for each plant extract was not significantly different at $p \leq 0.05$ among extraction solvents. The minimum inhibitory concentration (MIC) results showed that antibacterial activity of *P. zonale* and *P. guajava* leaf started at 6.25 mg/mL with growth inhibition zones of 7.67 and 8.0 mm for ethanol and acetone solvents respectively. *P. zonale* and *P. guajava* leaf extracts exhibited significantly higher antibacterial activity at $p \leq 0.05$ compared to other extracts. Thus, further research should be conducted to assess their antibacterial potency against *R. pseudosolanacearum* sp. nov. both in-vivo and under field condition.

Journal of Agricultural Science; Vol. 14, No. 11; 2022
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Determination of Bioactive Compounds Against Bacterial Wilt of Potato (*Ralstonia pseudosolanacearum* sp. nov.) in *Psidium guajava* and *Pelargonium zonale* Leaf Extracts

Gidraf Onduru Okeyo[1], Miriam Karwitha Charimbu[1], Jane Nyaanga[1] & Thiago Mendes[2]

[1] Department of Crops, Horticulture and Soil Sciences, Egerton University, Njoro, Kenya

[2] The CGIAR Research Program on Roots, Tubers and Bananas (RTB), International Potato Center, Sub-Saharan Africa Regional Office, ILRI Campus, Nairobi, Kenya

Correspondence: Gidraf Onduru Okeyo, Department of Crops, Horticulture and Soil Sciences, Egerton University, Njoro, Kenya. E-mail: ogidraf@gmail.com

Received: August 6, 2022 Accepted: September 14, 2022 Online Published: October 15, 2022

doi:10.5539/jas.v14n11p64 URL: https://doi.org/10.5539/jas.v14n11p64

Abstract

Common guava (*Psidium guajava*) and Pelargonium (*Pelargonium zonale*) have shown *in-vitro* antibacterial activity against *Ralstonia pseudosolanacearum* sp. nov. in previous studies. However, their phytochemical constituents and bioactive compounds against the pathogen have not been identified. The present study investigated the phytochemical components of *P. guajava* and *P. zonale* leaf extracts by phytochemical screening and gas chromatography-mass spectrometry (GC-MS). Phytochemical screening was done using different solvents while 100 mg of the dried ethanolic extract pastes from each plant sample was subjected to GC-MS analysis. Automated mass spectral deconvolution and identification system software (AMDIS, US) was used to analyze chromatograms and spectra representing individual compounds. Compound identification was performed by comparing each of the mass spectra with the database of NIST 11 (Gaithersburg, MD, USA), Wiley 7N (John Wiley, NY, USA) and by comparing the calculated Kovats linear retention indices using retention times of n-alkane series against the values in the NIST webbook. Flavonoids, phenols, alkaloids, saponins, terpenoids and tannins were detected in both plant samples. GC-MS analysis revealed presence of 35

Appendix C: Mixed model analysis of variance of percent emergence, final bacterial wilt incidence and yield parameters [proportion of symptomatic tubers, number of symptomatic tubers and weights (t/ha)]

Source of variation	Df	%PE	FWI	PST	NAST	Weight (t/ha)
Genotypes	29	3.71***	27.92***	35.03***	15.00***	21.84***
Site	1	14.35***	67.73***	2.83^{s}	29.96***	373.73***
Season	1	13.53***	0.11	15.06***	42.93***	3.18^{s}
Genotype x Site	27	2.71***	4.20***	5.00***	2.24***	2.58***
Genotype x Season	19	2.76***	4.38***	3.41***	5.10***	7.14***
Site x Season	1	0.04	34.48***	30.08***	0.39	5.15*
Genotype x Site x Season	17	2.17**	4.65***	2.91***	2.86***	5.48***
Residuals	192					

Significance codes: 0 '***', 0.001 '**', 0.01 '*' 0.05 '^{s}' 0.1 '' 1. Df = Degree of freedom, PE = Percent plant emergence, FWI = Final wilt incidence, PST = Proportion of symptomatic tubers and NAST = Number of asymptomatic tubers.

Appendix D: One way analysis of variance of *in-vitro* antibacterial activity of ethanolic leaf and seed extracts against R*alstonia solanacearum* based on growth inhibition zones

	Df	Sum sq	Mean sq	F-value	Pr(>F)
Ethanol_data $`Plant extrat	9	1386.0	154.00	3324	<2e-16 ***
Residuals	20	0.9	0.05		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Df = degree of freedom, sq = su ms of squares.

Appendix E: One way analysis of variance of *in-vitro* antibacterial activity of acetone leaf and seed extracts against R*alstonia solanacearum* based on growth inhibition zones

	Df	Sum sq	Mean sq	F-value	Pr(>F)
Acetone_data $`Plant extrat	8	1105.2	138.15	3188	<2e-16 ***
Residuals	18	0.8	0.04		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Df = degree of freedom, sq = su ms of squares.

Appendix F: Two-way analysis of variance of *in-vivo* antibacterial activity of ethanolic leaf extracts of *Pelargonium zonale* and *Psidium guajava* against R*alstonia solanacearum* based on plant emergence

	Df	Sum sq	Mean sq	F-value	Pr (>F)
Treatment	5	$1.98e^{-25}$	$3.96e^{-26}$	1	0.4256
Variety	1	$3.96e^{-26}$	$3.96e_{-26}$	1	0.3213
Treatment:variety	5	$1.98e^{-25}$	$3.96e^{-26}$	1	0.4256
Residuals	60	$2.37e^{-24}$	$3.96e^{-26}$		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Df = degree of freedom, sq = su ms of squares.

Appendix G: Two-way analysis of variance of *in-vivo* antibacterial activity of ethanolic leaf extracts of *Pelargonium zonale* and *Psidium guajava* against R*alstonia solanacearum* based on number of stems

	Df	Sum sq	Mean sq	F-value	Pr (>F)
Treatment	5	0.065082	0.013016	5.6078	0.000264 ***
Variety	1	0.097944	0.097944	42.1963	$1.8e^{-08}$ ***
Treatment:variety	5	0.007275	0.001455	0.6269	0.679868
Residuals	60	0.139269	0.002321		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Df = degree of freedom, sq = su ms of squares.

Appendix H: Two-way analysis of variance of *in-vivo* antibacterial activity of ethanolic leaf extracts of *Pelargonium zonale* and *Psidium guajava* against R*alstonia solanacearum* based on stem heights

	Df	Sum sq	Mean sq	F-value	Pr (>F)
Treatment	5	0.035121	0.007024	3.9483	0.003675 **
Variety	1	0.089804	0.089804	50.4787	1.657e-09 ***
Treatment:variety	5	0.018007	0.003601	2.0244	0.087982 .
Residuals	60	0.106743	0.001779		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. . Df = degree of freedom, sq = s ums of squares.

Appendix I: Two-way analysis of variance of *in-vivo* antibacterial activity of ethanolic leaf extracts of *Pelargonium zonale* and *Psidium guajava* against R*alstonia solanacearum* based on bacterial wilt incidence at 38 days after planting

	Df	Sum sq	Mean sq	F-value	Pr (>F)
Treatment	5	1.4115	0.2823	0.9619	0.4484
Variety	1	11.9642	11.9642	40.7673	$2.772e^{-08}$ ***
Treatment:variety	5	1.7883	0.3577	1.2187	0.3115
Residuals	60	17.6086	0.2935		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Df = degree of freedom, sq = su ms of squares.

Appendix J: Two-way analysis of variance of *in-vivo* antibacterial activity of ethanolic leaf extracts of *Pelargonium zonale* and *Psidium guajava* against R*alstonia solanacearum* based on bacterial wilt incidence at 52 days after planting

	Df	Sum sq	Mean sq	F-value	Pr (>F)
Treatment	5	7.1867	1.4373	8.4906	4.007e-06 ***
Variety	1	29.2324	29.2324	172.6786	$< 2.2e^{-16}$ ***
Treatment:variety	5	0.8749	0.1750	1.0336	0.4063
Residuals	60	10.1573	0.1693		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Df = degree of freedom, sq = su ms of squares.

Appendix K: Two-way analysis of variance of *in-vivo* antibacterial activity of ethanolic leaf extracts of *Pelargonium zonale* and *Psidium guajava* against R*alstonia solanacearum* based on bacterial wilt incidence at 66 days after planting

	Df	Sum sq	Mean sq	F-value	Pr (>F)
Treatment	5	4.8379	0.9676	4.7685	0.0009811 ***
Variety	1	28.5054	28.5054	140.4846	$< 2.2e^{-16}$ ***
Treatment:variety	5	1.6575	0.3315	1.6338	0.1649157
Residuals	60	12.1745	0.2029		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Df = degree of freedom, sq = su ms of squares.

Appendix L: Two-way analysis of variance of *in-vivo* antibacterial activity of ethanolic leaf extracts of *Pelargonium zonale* and *Psidium guajava* against R*alstonia solanacearum* based on yields (Kg)

	Df	Sum sq	Mean sq	F-value	Pr (>F)
Treatment	5	0.051896	0.010379	66.202	$< 2.2e^{-16}$ ***
Variety	1	0.073537	0.073537	469.043	$< 2.2e^{-16}$ ***
Treatment:variety	5	0.009769	0.001954	12.462	$2.632e^{-08}$ ***
Residuals	60	0.009407	0.000157		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Df = degree of freedom, sq = su ms of squares.

Appendix M: Two-way analysis of variance of *in-vivo* antibacterial activity of ethanolic leaf extracts of *Pelargonium zonale* and *Psidium guajava* against R*alstonia solanacearum* based on bacterial wilt colony counts

	Df	Sum sq	Mean sq	F-value	Pr (>F)
Treatment	5	2.39376	0.47875	10447.814	$< 2.2e^{-16}$ ***
Variety	1	0.12710	0.12710	2773.656	$< 2.2e^{-16}$ ***
Treatment:variety	5	0.00241	0.00048	10.499	$2.86e^{-07}$ ***
Residuals	60	0.00275	0.00005		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Df = degree of freedom, sq = su ms of squares.