STATUS OF BACTERIAL WILT OF TOMATO IN KAJIADO AND KIRINYAGA COUNTIES AND ITS MANAGEMENT BY HOST RESISTANCE AND BIOCONTROL AGENT

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(B.Sc Agriculture and Biotechnology-MMUST)

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN CROP PROTECTION

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

2023

DECLARATION

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

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Course Name: **MASTER OF SCIENCE CROP PROTECTION**

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DEDICATION

I dedicate this thesis to my mother Jane Luvai Shitiavai, my wife, Jemimah Matinde, and my children, Adel Nicah Kanaga, Fidel Feisal Shitiavai and Adah Nicah Luvai.

ACKNOWLEDGEMENTS

I am grateful to the almighty God for granting me the opportunity to undertake this work with financial support from the Kenya Climate Smart Agriculture Project.

The technical support provided by the Kenya Agricultural and Livestock Research Organization (KALRO-Narl) Kabete Centre Nairobi was invaluable in conducting my research in the laboratories, and I express my appreciation for their contribution. Throughout the project, Prof. James W. Muthomi provided me with encouragement, advice, and mentorship, for which I am sincerely grateful.

Additionally, I am thankful for the efforts and guidance of Prof. John W. Kimenju during the project. I would also like to acknowledge the technical assistance provided by Research Scientists from KALRO-Narl Dr. Paddy Likhayo, Dr. Ruth Amata, Ms. Miriam Otipa, Laboratory Technicians, Ms. Bornice Langat, Ms. Faith Imari, Mr. Eliud Wakoli, and Mr. Henry Onzere, and the support I received from farmers in Mwea and Oloitoktok, namely Mr. Edward Mwea and Mr. Martin Kirasi. Finally, I extend my gratitude to my colleagues Joe Hebert, Stacey Odunga, Celestine Okinda, and Simon Vudiya for their support and encouragement.

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ABBREVIATIONS AND ACRONYMS

AVRDC:	Asian Vegetable Research and Development Centre
BCA:	Biological Control Agent
BW:	Bacterial Wilt
CABI:	Centre for Agriculture and Bioscience International
CFU:	Colony forming Units
CRD:	Completely Randomized Design
CPG:	Casamino acid Peptone Glucose
FAO:	Food and Agriculture Organization
GDP:	Gross Domestic Product
HCDA:	Horticultural Crops Development Authority
Inc:	Incidence
KALRO:	Kenya Agricultural Livestock Research Organization
KARI:	Kenya Agricultural Research Institute
Kelman's T.T.C.:	Kelman's Triphenyl Tetrazolium Chloride
NA:	Nutrient Agar
NARL:	National Agricultural Research Laboratory
PCR:	Polymerase Chain Reaction
RCBD:	Randomized Complete Block Design
RS*:*	*Ralstonia solanacearum*
Sev:	Severity
SMSA:	Semi- Selective Medium, South Africa

ABSTRACT

Bacterial wilt, caused by *Ralstonia solanacearum,* is responsible for yield losses of up to 64 to 100% in tomato grown under open field and greenhouse conditions, respectively. Additionally, the disease has resulted in a decrease in farmer's income. Therefore, the study determined (i) the current status of bacterial wilt of tomato in Kajiado and Kirinyaga counties, (ii) the susceptibility and resistance of newly introduced tomato cultivars to bacterial wilt, and (iii) determined the efficacy of *Bacillus subtilis* in managing bacterial wilt in tomato.

A survey was conducted in tomato growing farms in Oloitoktok and Mwea in Kajiado and Kirinyaga counties in May and June 2021, using a semi-structured questionnaire. Symptomatic tomato plants and soil samples were collected from the farmers' fields and taken to Kenya Agricultural and Livestock Research Organization, National Agricultural Research Laboratory for isolation, identification, and quantification of *R. solanacearum*. Data was collected on the size of land under tomato production, duration of tomato production, diseases, and pest levels, and sources of water for irrigation. Susceptibility of newly introduced tomato cultivars to bacterial wilt were evaluated in the field in Oloitoktok, Mwea, and greenhouse at the Field Station, Kabete campus. Experimental treatments were laid out in a Randomized Complete Block Design (RCBD) in the field and a complete randomized design (CRD) in the greenhouse with three replications. In the greenhouse, the root system of each cultivar was clipped and dipped into an inoculum concentration of 1 x 10^7cfu/ml before transplanting. Data was collected on both the number of wilted and dead plants from the emergence up to the maturity stage.

The efficacy of *Bacillus subtilis* on bacterial wilt was conducted in the greenhouse at the Field station, Kabete campus, and open field in Oloitoktok and Mwea regions. Experimental treatments were laid out in a Randomized Complete Block Design (RCBD) in the field and the greenhouse with three replications. Before transplanting in the field and greenhouse, the root systems of each cultivar were dipped into 200 ml of *Bacillus subtilis* and after one week, the cultivars in the greenhouse were inoculated with an inoculum concentration of 1 x 10^7cfu/ml. Data was collected on the number of wilted plants, plant height, root length, stem discoloration, biomass, and yield data was recorded during harvesting of mature fruits.

The survey indicated that more than 90 % of the farmers in both regions were able to identify bacterial wilt of tomatoes in their farms. In Mwea, over 70 % of the famers considered bacterial

wilt to be highly significant while in Oloitoktok, 90% of the respondents and 40% in Mwea reported experiencing bacterial wilt experienced in the past two years of cropping seasons. The management practices for bacterial wilt varied between the two regions, with more than 50% in Mwea and 60% in Oloitoktok employing rouging as a control method. Field experiments revealed that tomato cultivars Cal J and Rio Grande were highly susceptible to bacterial wilt, each exhibiting 100% incidence score. On the other hand, Big rock variety displayed tolerance with a 30% incidence score eight weeks after inoculation. Furthermore, Cal J variety had the highest severity score, exceeding 4.9 on the disease index. The least affected varieties were Big rock, TO 135, and Crown, with incidence scores of 1.0, 3.0, and 3.1 respectively days after inoculation.

Significant difference ($P \leq 0.05$) were observed among the eighteen varieties in the management of bacterial wilt in the green house in Kabete. In Mwea and Kajiado the disease levels were lower compared to those observed during the screening process. Therefore, the study provided insights into the current status of bacterial wilt in Kajiado and Kirinyaga regions, tolerant varieties, and the efficacy of *Bacillus subtilis* to bacterial wilt.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Tomato (*Solanum lycopersicum* L) is an important vegetable that is widely consumed worldwide (Ben *et al.,* 2021). They are grown for their fruits and are used in salads and cooking. They belong to *solanacea*e family and are native to South America. In Kenya, tomato is grown in greenhouses under irrigation or in open fields to meet the increasing demand (Eviness *et al*., 2022). However, greenhouse cultivation can create optimal conditions for the growth of pathogens, leading to increased disease incidences such as bacterial wilt caused by *Ralstonia solanacearum*, which can cause massive plant deaths and reduced yields and income for farmers (Yuliar *et al*., 2015). Kenya is estimated to have produced over 539,000tons of tomatoes in 2012, but poor practices such as continuous cropping without rotation, poor field hygiene, infected seedlings and furrow irrigation can lead to increased disease incidence and reduced productivity (FAO, 2013).

Tomato plants are affected by over 200 diseases (Singh *et al*., 2017). The diseases are caused by viruses, fungi, and bacteria. Some of the diseases include bacterial canker, *Verticillium* wilt, bacterial speck, and bacterial wilt. Bacterial wilt is one of the most important tomato diseases in tropical and subtropical regions (Konappa *et al.,* 2020). Bacterial wilt is ranked second among the bacterial diseases causing vascular wilts (Balamurugan *et al.,* 2020). *Ralstonia solanacearum* lives in the soil and causes bacterial wilt to many plants. It continues to infect other new hosts' worldwide (Verburg *et al.,* 2018). The pathogen can stay in water reservoirs or soil for longer periods of up to 8 years (Balamurugan *et al*., 2020). It enters the host via lateral root emergent points or wounds (Xue *et al.,* 2020). The pathogen then colonizes the xylem vessels that transport water and mineral salts and thrives there. They produce exopolymeric substances that block the xylem vessels which causes the plants to wilt, become stunted, get discolored, and then die.

Biological controls have been effectively used to manage the bacterial wilt of tomatoes. *Pseudomonas* spp isolated from the rhizosphere of tomato were shown to produce enzymes that inhibited *R. solanacearum* (Mohammed *et al*., 2020). *Bacillus subtilis* has been tested against *Ralstonia solanacearum* in previous studies and has proven to be effective in bacterial wilt management (Mohammed *et al.,* 2020). Many people prefer to use biological controls because the other methods of controlling bacterial wilt in tomatoes give varied results. *Candida ethanolica* and *Pichia guillermondii* were tested against bacterial wilt and showed a high disease suppression

potential (Abd-El-Khair, 2019). *Trichoderma harzianum* has been reported to reduce bacterial wilt by up to 61% (Kariuki *et al.,* 2020).

1.2 Statement of the problem

In Kenya, tomato production is affected by pests and diseases such as bacterial wilt that has adverse effects on the yield and income to farmers (Kemboi *et al.,* 2022). A study by Wang *et al*., (2022) showed there is inadequate information about the mechanisms of plant resistance although some tomato cultivars have been reported to show resistance to bacterial wilt. The long term survival of *Ralstonia solanacearum* in the soil even in the absence of a host plant poses a serious challenge in its management especially in an infected field. Tomatoes infected with *R. solanacearum*, grown under green houses and open field's amount to 100% and 64% losses respectively (Kariuki *et al.*, 2020).

In a survey conducted among six sub counties of Kiambu, over 60% of the respondents reported bacterial wilt of tomatoes as a problem (Ireri *et al*,. 2018). *Ralstonia solanacearum* has been isolated in Kajiado, Kiambu, Kirinyaga, and Bomet counties where it is endemic. Tomatoes grown in high tunnels are mostly affected by bacterial wilt (Kariuki *et al.,* 2020). Different approaches have been used to manage bacterial wilt like chemicals such as Metam-sodium, 1, 3-dichloropropene (1, 3-D), and chloropicrin as soil fumigants and physical approaches like soil solarization but in Kenya, they are unavailable and expensive. Grafting of tomato rootstock with a resistant variety, breeding for resistance, and crop rotation have been used and their effectiveness is limited by the genetic diversity of *R solanacearum*, its wide host range, and its ability to survive in absence of a host (Aloyce *et al.,* 2017). Breeders have developed varieties conferring resistance to bacterial wilt which are not stable across different environmental conditions (Abebe *et al.,* 2020). Some of the resistant varieties lack stability and durability (Ramesh *et al*., 2021). Biological control agents (BCA) and resistant varieties are effective against bacterial wilt of tomatoes (Le *et al*., 2020), however, many farmers have not adopted them due to few resistant varieties and costly. Due to changing environmental conditions and the complexity of soil-plant systems, it is not easy to achieve the desired disease control using biological control agents alone (Peng *et al.*, 2017). The available information on integrated disease management of bacterial wilt using resistant tomato varieties and biological control agents is not commonly adopted due to technical support to farmers and insufficient training (Parsa *et al*., .2014). The prevalence and distribution of bacterial wilt of

tomatoes in Kenya is being studied but not well understood. The resistance to bacterial wilt by the varieties is also not been well studied.

1.3 Justification

Tomato is an important crop grown by farmers, especially in Kirinyaga and Kajiado counties for domestic and commercial purposes. However, bacterial wilt is a major problem in tomato production because it causes significant losses in yields. The use of tolerant varieties combined with biological control agents is a promising method to manage bacterial wilt. Farmers have inadequate knowledge of the resistance of existing tomato varieties and BCA in the market. There is a need to continuous screen available germplasm in the market to identify those with resistance against bacterial wilt. The synergy of host resistance and bio control enhances the effectiveness of the individual methods. Understanding the status of bacterial wilt in Kenya and the resistance levels of different tomato cultivars will enable the development of targeted disease management strategies. The use of *B. subtilis* has shown to have effects on bacterial wilt, in addition it is known to be environmentally friendly. By identifying locally adapted resistant varieties, farmers can be recommended suitable cultivars that reduce the risk of disease and minimize on the cost of management. Integrated disease management of bacterial wilt using both tolerant varieties and *B. subtilis* will increase tomato production of large and small scale farmers in Kenya.

1.4 Objectives

The main objective was to improve tomato production by determining the prevalence of bacterial wilt and its management through host resistance and bio control agent.

The following were the specific objectives of the study:-

i. To determine the prevalence, incidence and severity of bacterial wilt in Kajiado and Kirinyaga counties.
ii. To screen newly introduced tomato cultivars for resistance to bacterial wilt.
iii. To determine the efficacy of *Bacillus subtilis* in managing bacterial wilt in tomato.

1.5 Hypotheses

i. Bacterial wilt is widespread in tomato-producing farms causing significant losses in Kajiado and Kirinyaga Counties due to mono-cropping and over-cultivation of crops in the same family.

ii. Newly introduced tomato cultivars show resistance to bacterial wilt leading to an increase in tomato production in Kajiado and Kirinyaga counties.

iii. The use of *Bacillus subtilis* is effective in the management of bacterial wilt by colonizing the root system of tomato through microbial interactions.

CHAPTER TWO: LITERATURE REVIEW

2.1 Tomato production in Kenya

Tomato (*Solanum lycopersicum* L) is one of the most widespread horticultural crops in the world (Abera *et al.,* 2020). The tomato value chain provided employment opportunities in rural and peri-urban residents being a good source of income (Apreku, 2020).The horticultural industry is a thriving sub-sector of agriculture in Kenya, making a significant contribution to the economy. It accounts for 36% of the agricultural GDP and 8% of the country's GDP. Over the past decades, horticulture has been growing at a rate of 15-20 % (Gok, 2012). The industry encompasses various crops such as vegetables, nuts, flowers, and fruits. Among these crops, tomato is a promising commodity for horticultural expansion and development in Kenya, making up 6.72% of the horticultural crops and 14% of the total vegetable production (Gok, 2012). Tomato is grown using either greenhouse technology or open field, with greenhouse technology accounting for 5% and open field production accounting for 95 % (Mwangi *et al.*, 2020). Kenya is a leading producer of tomato in Africa and ranks 6th in the continent, with a total production of 397,007 tons (FAO, 2013).Counties producing tomatoes in Kenya include; Kirinyaga with 14%, Taita Taveta with 7%, and Kajiado with 9%. Among the counties that produce tomatoes in Kenya, Kirinyaga ranks highest with 54,524 tonnes per year, while Kajiado County follows with 36,460 tonnes per year (Geoffrey *et al*, 2014**).** Tomatoes in Kenya can be grown both in the field and in the greenhouse. Some of the varieties grown in the greenhouse include; Chonto F1, Prostar F1, Tylka F1, and Anna F1. In the field, Rio grande and Cal j can be grown (Thomas *et al.,* 2020).

2.2 Economic importance of tomato

The main tomato producing counties in Kenya are Kirinyaga, Bungoma, Kajiado, Nakuru and Homabay among others (Table 2.1). The total production of tomatoes in Kenya was 397,007 tons under an area of 18,613 hectares (Table 2.1). Tomatoes can be sold in open-air markets, middlemen's groceries, and supermarkets, and they are versatile enough to be used either fresh or processed into products with a longer shelf life (Wiersinga *et al*., 2008). Fresh tomatoes can be used in a variety of meals, eaten raw as a salad, or processed into other forms like dried fruits, powder, and flakes, as well as tomato-based foods such as ketchup, juice, soup, and chili. Other processed tomato products includes paste, whole peeled, pickled tomatoes, juice, puree, and pulp

(Geetha and Rani, 2020). Tomatoes are a nutritious food, containing vitamins A and C, lycopene, and retinol which have antioxidant properties that are important for human health (Geetha and Rani, 2020). The tomato industry has also created job opportunities for people, such as those working at Centrofoods Industries that produce chilies and tomato sauce.

Table 2.1: Annual tomato production in Kenya

Counties	Areas (Ha)	Quantity (Tonnes)	Value (Kshs) Millions	Share by Quantity (%)
Bungoma	1,022	21,720	887	5.5
Homabay	803	13,120	638	3.3
Machakos	314	10,240	357	2.6
Kirinyaga	1,978	54,524	1,070	13.9
Taita Taveta	548	27,400	959	6.9
Kiambu	930	20,972	884	5.2
Kajiado	1,551	36,460	990	9.1
Migori	1,068	18,429	910	4.6
Meru	420	22,214	468	5.6
Makueni	403	17,552	682	4.4
Nakuru	580	10,990	257	2.7
Others	8,996	143,386	4738	36.2
Total	**18,613**	**397,007**	**12,840**	**100**

Source HCDA 2013

However, tomato production is challenged by disease caused by fungi, viruses, and bacteria. These disease include early blight, late blight, septoria leaf spot, anthracnose, cucumber mosaic virus, tobacco mosaic virus, tomato spotted wilt virus, bacterial spot, bacterial speck, bacterial canker, and bacterial wilt. In Kirinyaga region, bacterial wilt is a prevalent disease affecting tomatoes, with a prevalence rate of 60% (Kago *et al*., 2016).

2.3 Tomato production constraints

In Africa, about 60% of the people live in rural areas that are of great agricultural activities, however, they cannot realize this potential because of the challenges that come with farming (Larson *et al*., 2020). Some of the challenges may include pests, diseases, drought, perishability syndrome, and agronomic constraints which result in low productivity (Thomas *et al*., 2020). The prices of tomatoes are not stable in the market across the whole year which makes planning difficult (Geoffrey *et al*, 2014). Production techniques and periodic monitoring can be used as good tools to develop a sustainable, productive tomato value chain. Farmers produce a lot of

tomatoes in rural areas but cannot access good markets that can guarantee them good returns on their investments. In developing countries, affordable and better transportation is very essential for the improvement of commercialization, but rural areas in Kenya are served by poor road networks making tomato marketing difficult (Zeleke *et al.,* 2020). In the last decades, the population has been increasing putting pressure on the available agricultural land and this has adversely affected tomato production as the crop is now grown in smaller units of land. Degraded lands give lower tomato yields than expected resulting in losses (Thomas *et al*., 2020). Most farmers are poor and cannot access the factors of production like capital which enables them to fully utilize the available technologies like greenhouses (Ochilo *et al.*, 2019).

2.4 Tomato diseases

Tomato is affected by several diseases whose occurrence is determined by the susceptibility of the host and environmental factors like temperature and humidity (Sharma *et al*., 2023). The causal agents of the diseases include, fungi, viruses, bacteria, and plant parasitic nematodes which affect the quality and quantity of the produce. Important fungal diseases include *Fusarium* wilt, septoria, anthracnose root rot, early and late blight (Koike *et al*., 2007). The different diseases produce distinct symptoms which can be easily diagnosed and infected plants exhibit leaf spot, wilts, and rots. Tomatoes are also affected by viral diseases including tomato spotted wilt and cucumber mosaic virus. Viral diseases lead to streaking, yellowing, and leaf mottling which leads to leaf defoliation. Several bacterial diseases affect tomatoes like bacterial spot, bacterial canker, bacterial speck, and bacterial wilt (Panno *et al.,* 2021). Bacterial infection on tomatoes leads to the development of cankers, white halos, and spots that reduces the market quality (Ally *et al*., 2023).

2.5 Bacterial wilt of tomato

2.5.1 Occurrence, distribution, and host range of *Ralstonia solanacearum*

Ralstonia solanacearum has more than 400 host plants in more than 50 plant families (Sowndarya *et al.,* 2020) and can survive in the soil without a host. Some soil types can suppress *R. solanacearum*. Race one of *Ralstonia solanacearum* is mostly found in tropical and sub-tropical regions and finds it difficult to survive in regions that are cooler (Potnis, 2021) and are mostly found in the South Eastern States of the United States of America. Race two is found in tropical environments and Race three is found everywhere in the world. Some races are specifically found

in some parts of the world where they evolve with the host and hence are not found everywhere. In Kenya bacterial wilt affects tomatoes in Nakuru, Nyeri, Embu, Kiambu, Kirinyaga Nyandarua, and Murang'a counties. The diseases occur in the tropics which are wet, humid subtropics, and may be found in some temperate regions (Jibat and Alo, 2020). In Africa, bacterial wilt has been recorded in Burundi, Egypt, Zambia, South Africa, and Libya.

2.5.2 Causal agent of bacterial wilt and its characteristics

Ralstonia solanacearum, the causal agent of bacterial wilt in solanaceous plants, is a gram-negative plant pathogenic bacteria and grows in aerobic conditions. The bacteria live in the soil and are motile, it has a polar tuft flagellum and colonizes the xylem tissues of the plant which causes bacterial wilt. It affects many crops that are of economic importance in the solanaceous family. The plants affected include tomato, pepper, eggplant, potato, and groundnut (Fufa *et al.*, 2009). The pathogen is classified into races according to the host range they affect and which can be identified by polymerase chain reaction (PCR). Tomatoes are affected by race 3 while bananas are affected by race 2 (Behiry *et al.*, 2018). The species can also be subdivided into biovars based on alcohols, cellobiose, maltose, and lactose (Singh *et al.*, 2021).

Inoculum sources for *Ralstonia solanacearum* are weeds, infested soil, irrigation water, and latently infected vegetative materials. Dissemination is carried out by irrigation water and contaminated farm equipment. The pathogen gains entry into the plants via the roots and is trans-located via the xylem to the aerial part of the plant (Xue *et al.*, 2020). The bacteria multiply in the plant when they reach the large xylem element, they spread and establish blocking the xylem vessels which results in the wilting of the plant. They also can enter the parenchyma cells in the cellular spaces and prefer the vascular system (Xue *et al.*, 2020). Slimy pockets of cell debris are formed when the cells are dissolved by the bacteria. The youngest leaves of the tomato are the first parts to be affected especially during the warmest time of the day making the cells to be flaccid.

2.5.3 Symptoms of bacterial wilt

The symptoms begin with the younger leaves and gradually progress to the older ones which then turns yellow (Osdaghi *et al*., 2020). The symptoms progress rapidly after the plant is infected and the affected plants appear to wilt during the day and seem healthy during the sunset. The affected plants have brown vascular tissue which extends 10 inches above the ground. Bacterial wilt does

not form leaf spots but kills the whole plant (Choi *et al*., 2020). When affected plants are cut, bacteria ooze from the stems and they become hollow in the later stages of the disease because the pith decays. The disease causes the roots to decay and changes their color to dark brown. Moist soils make the roots slimy and soft. The bacterial wilt of tomatoes is favored by higher temperatures of 29-35^{0}C. When the condition favors the disease, tomato crops remain latently infected showing no symptoms of bacterial wilt for an extended period.

2.5.4 Epidemiology of bacterial wilt of tomatoes

There are very many strains within the species that vary significantly. The types are determined according to their ancestral origin. Races that are capable of causing bacterial wilt in tomatoes are race one and race three (Potnis, 2021). Infection occurs through wounded roots and stems. The avenues are created by parasitic nematodes like *Meloidogyne incognita*. Plant-to-plant infection occurs via irrigation water. It has the ability to stay in water at a temperature of 20-25^{o}C for 40 years (Planas-Marquès *et al.,* 2019). More tomato bacterial wilt incidence is experienced during warmer weather of 29-35^0 C. When conditions are favorable, tomatoes may have the bacteria but not show the symptoms. The symptoms become latent but continue to spread. *Ralstonia Solanacearum* has the ability to stay in infected planting materials for a period ranging from days to years (Manda *et al*., 2020). It can also survive on weeds, favorable soils, or in irrigation water. The spread between fields is enhanced by machinery and surface runoff water. When temperatures are lower than 39.2^{0}F, the population of *Ralstonia solanacearum* in the soil falls rapidly (Manda *et al*., 2020). Both Plants showing symptoms and those not showing symptoms shade huge amounts of the bacterium to the soil which is then taken by weeds. Many virulent factors are required for the pathogenicity of *R. solanacearum* (De Pedro-Jové *et al.,* 2021). The virulent factors help the pathogen to sense, invade and colonize the host roots (De Pedro-Jové *et al.*, 2021). The pathogen with higher virulence had more extracellular plant cell walls, higher swimming motility, and chemotaxis. Metabolomics and transcriptomics have improved people's knowledge of how bacteria change from saprophytic to parasitic lifestyles (Lowe-Power *et al.*, 2020). Virulence has been determined by models of affected plants to test the suggested hypothesis. The models should be quantitative, replicable, and relevant biologically. Many protocols have been developed and adapted that assess the interaction of *R. solanacearum* and tomatoes. To identify traits that aid in the pathogen's success, a petiole inoculation assay can be used (Planas-Marquès *et al.,* 2019).

2.5.5 Management of bacterial wilt in tomatoes

Ralstonia solanacearum affects a wide range of crops (Jibot and Alo, 2020), and was formerly known as *Pseudomonas solanacearum*. Initially, *Ralstonia solanacearum* was placed into five races based on their host range, and five biovars according to their ability to produce acid from carbohydrates (De Pedro-Jové *et al.*, 2021). The races of *R. solanacearum* are distributed across different geographical areas. Bacterial wilt is a very serious disease in tomatoes and potatoes. During summertime in India, bacterial wilt showed 10 to 100 percent incident (Pratap *et al.*, 2020). The control of bacterial wilt has proven to be difficult as the different strategies used in the management have not shown 100% efficiency. Different farmers have used copper-based bactericides with little efficiency and have contributed to environmental destruction. Antibiotics like tetracycline and ampicillin are expensive and therefore a combination of a wide range of methods of control needs to be applied. Some of them include; cultural practices, the use of biological control, and host resistance in an integrated management approach. There are resistant and moderately resistant tomato cultivars that confer resistance to bacterial wilts like FL7514 and BHN 466. Some of the resistant cultivars have undesirable traits that are not required in the market like small fruit size. Resistance in other tomato cultivars is not stable and varies with temperature and location because the strains are different (Huang *et al.,* 2016). Grafting susceptible tomato cultivars onto resistant rootstocks has been used to control bacterial wilt in the Philippines and Japan among others. This method however has not been tested against all *Ralstonia solanacearum* biovars. Some virulent biovars have the ability to affect disease-resistant tomato cultivars (Farias *et al.*, 2013).

Solarization and hot water treatment are effective methods used in the control of *Ralstonia solanacearum* (Dai *et al.,* 2020*).* Transparent plastic mulches applied for 60 days before planting tomatoes reduces bacterial wilt incidence. When ginger rhizomes are solarized for a period of between 2 and 4 hours, bacterial wilt is reduced by 90 to 100 percent. Bacterial wilt in ginger was also controlled by discontinuous microwaving at 45^0 C which reduced the disease incidence by 100 percent. Solarization reduces the soil pH, sodium ions, potassium ions, and boron (Sangma, 2020). Solarization also affects microbial respiration and microbial biomass. Chemical properties are however not affected. The population of bacteria in the soil can be reduced by heating the soil

at a minimum temperature of 60 degrees Celsius for 2 hours or 45 degrees Celsius for 2 days which reduces the bacteria population by 60 to 97 percent.

Plant diseases can best be controlled by growing resistant cultivars because the method is economical and environmentally friendly (Richard *et al*., 2021). Breeders have directed most of their efforts towards crops of economic importance like tomatoes, pepper, and tobacco. The process is influenced by factors like the availability of resistant sources, genetic linkage, diversity, and other agronomic traits. Selection methodology in breeding has been used to enhance resistance to bacterial wilt. Other breeders use somatic hybrids, especially in Irish potatoes to control up to 90 % of disease incidence. Some breeders produce resistant cultivars by electrical fusion of the mesophyll protoplast to come up with tolerant cultivars (Sangma, 2020). Tomato cultivars that are resistant to bacterial wilt can be heavily invaded by the bacteria without displaying wilt symptoms (Planas-Marquès *et al.,* 2019). The bacteria multiplication is limited in resistant cultivars because the movement of the pathogen from the primary xylem to other xylem tissues is suppressed. Tomato cultivars with resistance to bacterial wilt often exhibit low yields. In the future farmers are expecting tomato cultivars that are resistant to bacterial wilt and at the same time high yielding.

It is challenging to control the bacterial wilt of tomatoes using chemicals as the pathogen stays in the xylem tissues of the plant. The presence of *R. solanacearum* in the soil is mainly reduced by fumigation. Common chemicals used include carbendazim, chloropicrin, and metam sodium, 1, 3-dichloropropene (Mamphogoro *et al.,* 2020). Some other chemicals are used as plant activators inducing systemic resistance against bacterial wilt which includes, validoxylamine and validamycin A. After the roguing of diseased plants, the holes left behind are treated with sodium hypochlorite however, this is expensive.

Crop rotation has several benefits like maintaining the structure of the soil and increasing the organic matter. Soil erosion is also reduced when continuous row crops are grown. When a susceptible cultivar is continuously grown in an area they establish a pathogenic population that has a detrimental effect and this can be solved by crop rotation (Yao *et al*., 2013). A study to test rotation between sweet potato, maize, carrots, wheat, beans, and sorghum reduced the incidence of wilt by 64 to 94 percent.

Fertilizers have been shown to reduce the incidence of bacterial wilt in tomatoes (Wei *et al*., 2011). Among the fertilizers that are well known to suppress bacterial wilt, calcium is the best. The

population of *Ralstonia solanacearum* in the tomato stems can be reduced by increasing the calcium concentrations which also lowers the severity of bacterial wilt. A combination of nitrogenous fertilizer with phosphorous reduces bacterial wilt by 29% (Hu *et al*., 2021). The use of soil amendments like rock dust and commercial organic fertilizer reduces bacterial wilt incidence in tomatoes by affecting the soil pH and calcium concentration (Hacisalihoglu *et al*., 2007). The susceptibility or resistance of plants to diseases can be influenced by several elements in the cell wall. Silicon is considered an important element to plants and animals (Kiirika *et al*., 2013). It works well in combination with chitosan in the management of bacterial wilt.

Integrated pest management has five goals which are; elimination or reduction of initial inoculum, reduction of the effectiveness of initial inoculum, increasing the resistance of the host, delaying the disease onset, and slowing down the secondary cycles according to (Singh and Gupta, 2016). IPM combines cultural, chemical, and biological methods to control bacteria. The organic mixture which consisted of agricultural waste and industrial waste like bagasse, oyster shell powder, and mineral ash decreased the incidence of bacterial wilt by 32%. When the mixture was combined with Actigard, bacterial wilt incidence was reduced by 53 %(Anith *et al*., 2004). The two methods when combined with biological control agents suppressed bacterial wilt more. Organic compounds improve the colonization of the roots by the BCAs. Integrated pest management methods to be adopted should be profitable, easy, and practical. They should increase yields and have minimal effects on the environment.

2.6 Use of resistant varieties in the management of bacterial wilt of tomatoes

The use of resistant varieties is one of the methods that can be used to control bacterial wilt of tomatoes, it is environmentally friendly and the least cost to the farmer (Ddamulira *et al*., 2021). Breeding programs have been developed to improve resistant lines against bacterial wilt of tomatoes by incorporating resistance from various sources (Abebe *et al*., 2020). Studies on the grafting of tomatoes have revealed that resistance to bacterial wilt is found in the roots therefore the disease can be controlled by grafting resistant rootstock to susceptible scions (Keatinge *et al* 2014).

Histological studies show that plants that are susceptible to bacterial wilt have a different distribution of *Ralstonia solanacearum* when compared to the resistant cultivar. Resistance to bacterial wilt was correlated to the colonization of bacteria in the mid-stem (Kim et al., 2016).

Farmers in Kenya grow varieties like Mavuno and Ansal F1 which confer resistance to bacterial wilt and other diseases of tomatoes. Extensive trials have been conducted on the Ansal varieties and confirmed to be a solution to bacterial wilt among tomato farmers in Kenya.

2.7 Bio control agents in the management of soil-borne diseases

Bacillus subtilis is a ubiquitous gram positive bacterium that is isolated from the soil and formulated as a bio-control. It possess qualities that promotes plant growth and is therefore classified as bio-fertilizer (Chauhan *et al*., 2021). Bio-control agents play an important role in suppressing the amount of *R. solanacearum* in the soil (Rostand *et al*., 2018). A number of bio control agents have been studied but few are commercially available. Studies show that some bio control agents are not effective in managing bacterial wilt (Marian *et al.*, 2019). Some of the bio-control agents used to control bacterial wilt in tomatoes is phage PE 204 which can be used in a wide range of pH and temperatures (Bae *et al*., 2012). *Pseudomonas fluorescence* HRA32 has strong oxidizing power and provides enzymes for plant growth. It also secretes hydrogen cyanide and antibiotics which inhibits pathogen growth. The siderophores produced by *Pseudomonas* chelate with the soil making it difficult for the pathogens to proliferate. They also produce growth substances that contribute to the vigorous growth of tomatoes. *Trichoderma* has also been effectively used to manage the bacterial wilt of tomatoes. The *Trichoderma* species produces different secondary metabolites that have anti-microbial activity against *Ralstonia solanacearum*. They also suppress the bacterial population in the soil reducing the severity of the disease and their use is eco-friendly and cost-effective (Guo *et al*., 2021). *Trichoderma* strains can adapt to harsh environmental conditions (Kariuki *et al*., 2020).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Determination of the status of bacterial wilt in Kajiado and Kirinyaga counties

3.1.1 Description of study areas

The experiment was conducted in farmers' fields in Oloitoktok and Mwea sub-counties in Kajiado and Kirinyaga Counties respectively. These areas were selected because they are among the main tomato-producing Counties in Kenya, (HCDA, 2013). The high incidences of bacterial wilt are occasioned by the continuous production of tomatoes throughout the year. Kajiado County lies in the southwest part of Kenya (2.0981^0S, 36.7820^0E) (Magogo *et al.,* 2020) and in agro-ecological zone five. It borders Nairobi, Machakos, Taita Taveta, and Makueni. It receives minimum rainfall of about 500 mm annually due to its location and the temperature ranges between 16- 20^0 C. Oloitoktok has black cotton soil (Kinyanjui *et al.,* 2021). Kirinyaga County is located in central Kenya (0.6897^0S, 37.3400^0E) (Kihoro *et al.,* 2013) with rainfall patterns ranging from 800-2200 mm annually with a temperature range of about 9.0- 21.0^0 C. Kirinyaga County has pellic vertisols which are imperfectly drained, dark grey, deep and black in color. Other parts have verto-eutric nitisols that are well-drained, friable, very deep, and dark reddish brown. Kirinyaga County is also found in agro-ecological zone two and lies at an altitude of between 1000- 1100 meters above sea level.

3.1.2 Determination of the production practices

Sampling was conducted in Kirinyaga and Kajiado counties during the month of May and June 2021. Purposive sampling was used to select the sample size in both Mwea and Oloitoktok regions (Mugenda and Mugenda, 2003). Based on information from county and sub county extension officers, 170 tomato growing farmers, managed by individuals or groups, were selected and out of these, 60 farms who had practiced tomato growing in the last 5 years were picked for survey. The distance of 1 km from each other and challenges farmers experienced with pests and diseases in tomato production was also considered. A total of 30 farms each from both Mwea and Oloitoktok regions were identified of which 25 were from the open field while 5 were greenhouse farmers. The selected farms comprised of male and female farmers who were interviewed using a semi-structured questionnaire (Appendix 1). The survey covered both the open field and greenhouse systems of tomato production to help with the identification of the diseases. Agro-ecological zones,

gender, size of land under tomato production, duration under which tomato has been produced, diseases and pest levels, and sources of water for irrigation were used for determination of prevalence of bacterial wilt.

3.1.3 Determination of the occurrence of bacterial wilt

Symptoms of bacterial wilt of tomato was observed by visually examining tomato plants within the farmers' fields and the presence of leaf wilting during hot periods of the day, leaf yellowing and browning of the vascular tissue signified the existence of the disease . The number of plants showing bacterial wilt symptoms were counted and divided by the number of plants within the farms. The results were converted to a percentage by multiplying by 100(Mensah *et al*., 2021).

$$\text{Percentage incidence} = \frac{\text{Number of plants wilted}}{\text{Total number of plants examined}} \times 100\%$$

Disease severity was scored as described by (Bashir *et al.*, 2017) using a disease rating scale of 0-5(Table 3.1).

Table 3.1: A disease rating scale for severity of bacterial wilt of tomato

Rating	Reaction
0	No plant wilting
1	1-12% plant wilted
2	13-25% plant wilted
3	26-50% wilted plant
4	51-75% plant wilted
5	>75% plant wilted

In addition to visual examination, a vascular flow detection technique was utilized to confirm the presence of the pathogen at vegetative stage of the tomato. To conduct the test, 5-10 cm of the stem of three symptomatic tomato plants were cut and immersed in a clear glass filled with water. The vascular brown tissues of the infected plant part were suspended in water to observe whitish bacterial exudates coming out of the xylem.

3.1.4 Collection of plant and soil samples

Samples of plant materials and soils were collected from farmer's fields in Mwea and Oloitoktok regions. Purposive random sampling technique was used for collection of five diseased plant samples using a 5 m x 5 m quadrant from five random spots. Vascular flow detection technique was used to identify diseased plant samples while grid soil sampling technique was implemented to sample soils, where each quadrant was subdivided into four quarters (Mallarino, 2001). One kilogram of soil at a depth of 15 cm was collected using a soil auger from five random spots in each quadrant and thoroughly mixed in a plastic container. Two hundred and fifty grams of the composite soil sample was packed in khaki bags and labeled with details on collection date, host and the locality before being placed in a cool box.

A total of 300 plant materials and 60 soil samples were collected from 30 farms per region and transported in a cool box to the National Agricultural Research Laboratory (NARL), Pathology Laboratories, where they were refrigerated at 5^{o}C before isolation and identification of pathogens.

3.1.5 Isolation and identification of *Ralstonia solanacearum*

Prior to isolation, stem samples from infected tomato plants, were washed with running tap water for 5 minutes then cut into 2 cm pieces. Surface sterilization was done using 2% sodium hypochlorite (NaOCl) to remove saprophytic bacteria from plant surfaces. The samples were then rinsed three times with sterile distilled water and then blot dried. The plant tissue was then crushed and put in the universal bottles with 1000 µl of sterile distilled water, the bacteria suspension was then used for streaking on Kelman's triphenyl tetrazolium chloride (TZC or TTC) medium composing of Casamino Acids 1.0 g, Bacto-Peptone 10.0 g, Dextrose 5.0 g Bacto-Agar 15.0 g (Kelman, 1954; Masanga *et al*., 2018). The plates were then incubated at 30^{0} c for 2 to 3 days and monitored for colony formation. The individual distinct colony was streaked onto a new TZC medium to obtain pure cultures. Identification, therefore, was carried out by morphological characteristics including; color with a fluidal pinkish colony, rod shaped cells and gram staining (Seleim *et al.,* 2014). The resulting isolates were given codes based on the collection regions and preserved in 25% (v/v) glycerol solution at -20%.

Isolation from the soil samples were done where 10 g of each sample was weighed and homogenized, put in a conical flask, then 30 ml of sterile distilled water was added and agitated

for 220 revolutions per minute. Serial dilution of the suspension is carried out to get the concentration to allow separate colonies to grow. Then this concentration was plated on a semi-selective medium for *R. solanacearum* (SMSA) and then incubated for 24 hrs. at 30. To identify *Ralstonia solanacearum,* a creamy white, and a mucoid colony was formed upon plating and observation. Additionally, this bacterium produces a water-soluble pigment when grown on nutrient agar.

3.2 Screening of newly introduced tomato cultivars for resistance to bacterial wilt

3.2.1 Determination of pathogenicity of isolated *Ralstonia solanacearum*

Selected tomato varieties including Rio Grande, Commando F1, Cal J, Big rock F1 and Shanty F1 were used to conduct pathogenicity of *Ralstonia solanacearum,* in the green house at Kabete site. The varieties under test were considered because they were commonly grown by farmers in Kirinyaga and Kajiado counties. The *Ralstonia* isolates obtained from the field were used to prepare the inoculum of concentration 10^7 CFU/ml. Bacterial inoculum was prepared in Casamino acid Peptone Glucose medium composed of 0.1% Casamino acid, 1% peptone, 0.5% glucose, following Wei *et al.*, (2017). The bacterial suspension was streaked on the casamino acid peptone glucose agar (CPG) media for it to grow. The CPG media was used for the cultivation of the colonies incubated at 30^0C for 24hrs (Vanitha *et al.,* 2009).

The distilled water was added to bacterial colonies on the CPG media, then using a microscope slide to dislodge the colonies. The suspension was then put in the conical flask (stock solution). Nine ml of distilled water was added to seven universal bottles and labeled from 10^{-1} to 10^{-7}. Ten ml of the stock solution was then put in a separate universal bottle and labeled 10^{-0}. Using a micropipette 1ml of the stock solution was drawn and added to the 10^{-1} universal bottle and serially diluted to 10^{-7}. Therefore, 0.5 ml from 10^{-4} and 10^{-7} was plated on the CPG media using the pour plate method and incubated at 30^0C for 24hrs.

The number of colonies grown on the media was counted and used to determine the concentration of the stock solution. This was done by using a formula:

$$stock\ solution\ concentration = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{volume used}}$$

The inoculation was carried out using root dipping and soil drenching techniques on 30 day old healthy seedlings as described by Kusajima *et al*., (2022). After 14 days of inoculation, the pathogenic interactions were assessed by observing wilt symptoms, with uninoculated seedlings serving as the control. Data was recorded on the disease incidence and severity for two weeks days after inoculation.

3.2.2 Description of the experimental materials

The tomato varieties were sourced from different companies in Kenya, and they included the F1s and the non-hybrid (Table 3.2). The varieties were chosen according to their agronomic characteristics and reaction to various known diseases of tomato.

Table 3.2: Varieties, sources, agronomic characteristics, and their reaction to various diseases

No.	Variety	Source	Agronomic characteristics	Known reaction to various disease
1	Onyx F1	Kenya highland seed	Determinate, excellent shelf life, and yields up to 18 tons/acre	Tolerant to Verticillium and Fusarium Wilt
2	Nyati F1	Ultravetis seed company	Yields potential of up to 100-150fruits /m^2 and 30-35tons/ acre	High tolerance to bacterial wilt.
3	TO 135 F1	Africasia seed company	Fruit weight 130-135g and very high yielding	Tolerant to bacterial wilt and late blight
4	Zara F1	Syngenta	Has a fruit weight of about 100-120grams	Intermediate resistance to bacterial wilt
5	Commando F1	Continental seeds	Yield potential of about 30–35 Tons/acre under good agronomic practice.	Tolerant to bacterial wilt and Fusarium wilt
6	Rambo F1	Planttech Kenya	Yield potential of 30tonnes/acre	High bacterial wilt tolerant
7	Raja F1	Advanta seed international	Yield of about 30-35tons /acre	Tolerant to bacterial wilt
8	Rio Grande	Kenya seed company	Yield potential of up to 12-16tons/acre	Resistance to Fusarium wilt
9	Ranger F1	Continental seeds	Yield potential of 30-33tons/acre	Strong observable field tolerance to Bacterial wilt
10	President F1	Safari seeds company	Yield potential of 7-9kgs/m^2	Tolerant to Verticillium wilt
11	Big rock F1	Prime seeds company	Early maturing in 65 days after planting	Tolerant to leaf curl virus
12	Sandokan	Royal seeds company	Yield potential of 30 tons/acre	Tolerant to bacterial spot
13	Bashaer F1.	Syngenta	Yields of up to 30-3tons/acre	Resistant to Verticillium wilt
14	Assila F1	Semenis vegetable seeds	Yields up to 23tons/ acre	Tolerant to leaf curl virus
15	Cal J	Simlaw seeds	Yield potential 15000-20000kgs/acre	Tolerant to Fusarium wilt
16	Nyota F1	Simlaw seeds	Yield potential of 38tons/acre	Resistant to bacterial wilt
17	Crown F1	Royal seeds	Yields potential of 9-11kgs /plant in GH and up to 50tons/acre in field	Intermediate resistance to bacterial wilt
18	Tylka F1	Syngenta seeds	Average yield of 70-80tons/acre	Resistant to Fusarium wilt

3.2.3 Production of tomato seedlings

The 18 tomato varieties sourced from certified seed distributors were raised in seedling trays containing cocopeat planting media obtained from the local agrovets. Macronutrients, micronutrients and water was added as required by the plant. The plants were monitored in the green house for 30 days and controlled from pests and diseases using recommended fungicides and insecticides.

3.2.4 *Ralstonia solanacearum* inoculum preparation

The *Ralstonia* isolates obtained from the field were used to prepare the inoculum of concentration 10^7 CFU/ml. The bacterial suspension was streaked on the casamino acid peptone glucose agar (CPG) media for it to grow. The CPG media was used for the cultivation of the colonies incubated at 30^0C for 24hrs (Vanitha *et al.,* 2009).

The distilled water was added to bacterial colonies on the CPG media, then using a microscope slide to dislodge the colonies. The suspension was then put in the conical flask (stock solution). Nine ml of distilled water was added to seven universal bottles and labeled from 10^{-1} to 10^{-7}. Ten ml of the stock solution was then put in a separate universal bottle and labeled 10^{-0}. Using a micropipette 1ml of the stock solution was drawn and added to the 10^{-1} universal bottle and serially diluted to 10^{-7}. Therefore, 0.5 ml from 10^{-4} and 10^{-7} was plated on the CPG media using the pour plate method and incubated at 30^0C for 24hrs.

The number of colonies grown on the media was counted and used to determine the concentration of the stock solution. This was done by using a formula:

$$\text{Stock solution concetration} = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{volume used}}$$

3.2.5 Experimental design and treatments

The experimental treatments consisted of varieties obtained from agro shops and local markets (Table 3.2). In the field experiment, tomato seedlings were planted in plots measuring 1.2 M x 1.8 M at a spacing of 90 cm apart between plants and 45 cm between rows with a plant density of 2 plants per M^2. One meter space was left between plots and the guard rows. The experiment was laid out in a randomized complete block design (RCBD), with three replications. During planting, 150 Kg per hectare of DAP was applied followed by top dressing with 200 Kg per hectare of CAN

after two weeks of transplanting. A foliar feed of 50 ml/20L of water was applied at weeks four, six, and eight, consecutively.

In the greenhouse experiment, seedlings of the different varieties were planted in pots measuring 255 x 356 x 356 mm. The planting media in the pots composed of sterile sand, silt, and compost in the ratio 3:1:1 respectively, making 5 kg of the media per pot. The treatments were laid out in a randomized complete block design with three replications. Each block was randomized independently to increase precision and also reduce biasness and this was carried out by random numbers and repeated in the second and third blocks. During transplanting, the root systems of each cultivar were slightly clipped with a sterilized scalpel of about 2 cm to facilitate bacterium penetration. Two weeks after transplanting, bacterial culture containing 1x 10^7 cfu/ml was added through soil drenching with 30 ml of inoculum per pot. Watering of the plants was done as required by the plants during growing season.

3.2.6 Assessment of incidence and severity of bacterial wilt

A weekly assessment of incidence and severity was carried out days after transplanting the seedlings. The crops were evaluated for visible disease symptoms such as wilting and yellowing. Plants showing bacterial symptoms were tagged for subsequent monitoring. Bacterial wilt incidences were determined by counting the number of wilted plants in each plot over the total number of plants examined using the following formula below:

$$\text{Percentage incidence: } = \frac{\text{Number of plants wilted}}{\text{Total number of plants examined}} \times 100\%$$

On the other hand, severity was determined through visual observation of the plant parts affected by bacterial wilt. A disease rating scale of 0-5 was used to determine the disease severity as described by Bashir *et al*. (2017) in (Table 3.1).

3.3 Evaluation of the efficacy of *Bacillus subtilis* in managing bacterial wilt in tomato

3.3.1 Multiplication of *Bacillus subtilis* inoculum and inoculation

Bacillus subtilis was isolated using serial dilution method, which involved the use of nutrient agar as the growth medium (Beef extract: 1.5 g/l, agar: 20g/l, NaCl: 5 g/l, Peptic digest of animal tissue: 5 g/l, yeast extract: 1.5 g/l.). Soil samples were taken from a cultivated land and one gram of soil sample was suspended in 9 milliliters of sterile distilled water and vortexed for 5 minutes (Yendyo *et al*., 2018). The soil suspension was diluted serially to a concentration of 10^{-6}. The pour plate method was used and this involved mixing 1 ml of the diluted soil suspension in 3 nutrient agar plates for each sample and incubated at 27° C for 48 h. The strains were purified using sub-culture technique on the nutrient agar plates.

The *Bacillus subtilis* isolates were sub-cultured on nutrient agar, and the inoculum was produced on nutrient broth incubated at 30±1°C for 72 h. The incubated culture promoted the growth of *Bacillus subtilis*, formation and multiplication of colonies on the culture medium. The bacteria was then harvested and 10 ml of concentration (1.2×10^5 cells/ml) was used. Thirty day-old, well-established seedlings were used for the inoculation of *R. solanacearum* and bio control agents. The inoculation was done by carefully removing the surface soil and *Bacillus subtilis* inoculum was uniformly poured around roots and the soil replaced (Khan and Siddiqui, 2020). In control pots and plots, a similar amount of water was poured in the same way around the roots.

3.3.2 Culturing of *Ralstonia solanacearum*

Ralstonia solanacearum isolates were obtained from the plant materials collected from the fields in Mwea and Oloitoktok regions as described in section 3.1.5. The isolates were then grown on SMSA agar to support bacterial growth, and on the bacterial culture, streaking was done on the surface with a wire loop and incubated at 30^0C for 2-3 days (Vanitha *et al.,* 2009). The colonies appeared small, round, smooth, and shiny with a yellowish-white color. Then single colony of *R solanacearum* was transferred to a fresh medium on a new plate and the culture stored at -80^0C.

3.3.3 Experimental design and treatments

To determine the effect of bio control agent in the management, the experiment was conducted in the greenhouse in the Kabete campus field station and the open fields in Kirinyaga and Kajiado Counties. In the field, experimental layout was done as descried in section 3.2.5. The plots were

randomized with three replications each, and the same design was maintained as in the first season. Prior to transplanting, seedlings from the nursery were treated with a drench of 200 ml of *Bacillus subtilis*, a bio control agent, at a concentration of 10g/2L of distilled water (Almoneafy *et al*., 2014), before planting in each plot. Holes were dug, DAP was added, and the exercise was carried out randomly on all the varieties in the fields for the three blocks. The agronomic practices were carried out as described in section 3.2.5. Green house experiment layout was implemented as described in section 3.2.5 whereby, the seedlings were drenched with 200 ml of *Bacillus subtilis*, and planted, then inoculum was added thereafter, and periodic watering followed to ensure soil moisture levels were maintained. The parameters that were recorded included the number of wilted plants, plant height, root length, measurement of stem discoloration, fresh and dry weights, and yields.

3.3.4 Determination of incidence and severity of bacterial wilt

Numbers of wilted and dead plants were counted and recorded on weekly basis to determine the incidence of the disease (Bashir *et al*. 2017). The evaluation of the disease severity involved cutting the stems of plants that were either withered or dead and then assessing the degree of stem browning. This was carried out by uprooting, cleaning, and dissecting the region between the roots and stems. The length of the vascular discoloration was measured with a ruler and in total 4 symptomatic plants per treatment was recorded at the 8^{th} week during destructive sampling. The severity of browning was scored on a scale of 0-3, which was based on the scoring systems described by (Elphinstone *et al*., 1998; Kariuki *et al.*, 2020).

Where:

Table 3. 3: Stem discoloration scale

Score	Reaction
0.	No browning
1.	Light brown color restricted to 2cm from base of the stem
2.	Light brown color spread more than 2cm from the base of the stem
3.	Dark brown color on the vascular tissue

The percentage of stem discoloration was calculated using the following formulae

$$\frac{Length\ of\ the\ brown\ discolored\ stem}{Total\ lenght\ of\ the\ stem} \times 100\%$$

3.3.5 Assessment of agronomic parameters

The agronomic parameters recorded included plant height, root lengths, biomass and the yield after harvest. The height was measured using a ruler by placing it alongside the plant and reading the height from the tip to the base of the plant per treatment on a weekly basis after one week of inoculation up to setting of the first fruit.

Shoot and root, fresh and dry weights were measured at the 8th week after inoculation, shoots of 4 plants per treatment were cut at the base and weighed immediately by using the electronic balance, to measure the fresh weight of the shoot. The chopped shoot was dried at 70^0 C for 72 h and dry weight was measured. The roots of the same plants were washed thoroughly with running tap water to remove soil debris, dried using filter paper, and weighed to measure the fresh weight of the root. The root samples were used for the determination of root dry weight after drying in the oven at 70^0 C for 72 h till constant weight is obtained.

3.4 Data analysis

Survey data were analyzed through the Statistical Package for Social Science version 20 (SPSS) (Dube *et al*., 2020) through computation of percentages, means and the frequencies where the frequency of the various parameters was analyzed to generate the outcomes of the disease in the two regions. Data from both the greenhouse and the open field in Kajiado and Kirinyaga counties respectively were subjected to ANOVA using GENSTAT® version 15.0, 15th edition, and the means were separated by Duncan's multiple range test, LSD at 5% significance level.

CHAPTER FOUR: RESULTS

4.1 Current status of bacterial wilt in Kajiado and Kirinyaga counties

4.1.1 Knowledge of tomato bacterial wilt among farmers

More than 90% of the farmers in Oloitoktok and Mwea were able to identify bacterial wilt of tomatoes in their farms (Figure 4.1). In Oloitoktok, the proportion of farmers who were able to identify the disease was four percent higher than in the Mwea region. Additionally, less than 8% of the farmers were unable to identify the bacterial wilt of tomatoes.

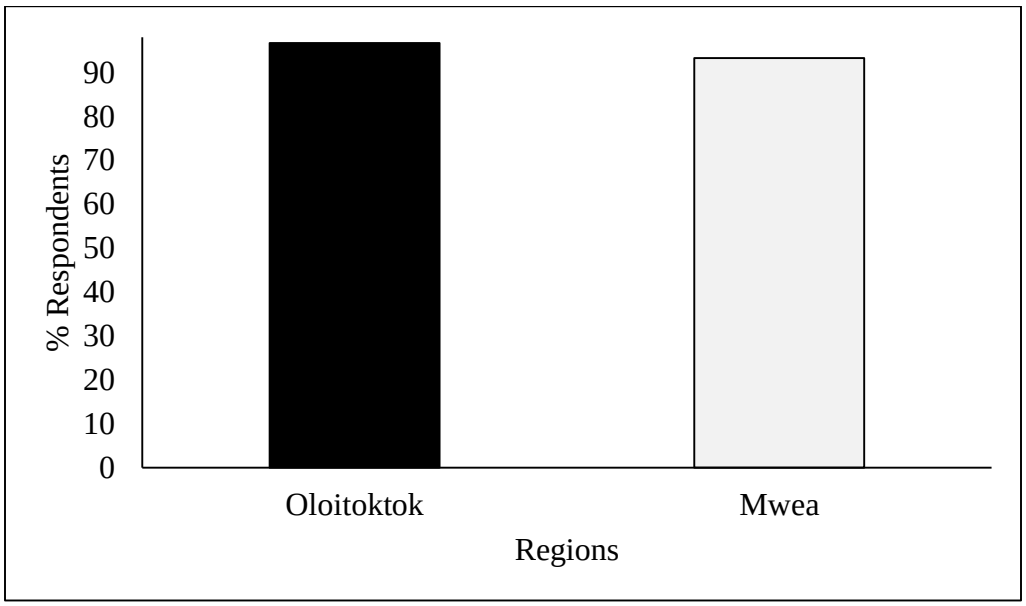

Figure 4.1: Percentage of farmers who were able to identify bacterial wilt of tomatoes in Oloitoktok and Mwea

4.1.2 Level of importance of tomato bacterial wilt

The presented table outlined the level of importance of tomatoes attributed to bacterial wilt in both the Oloitoktok and Mwea regions. According to the results, over 70% of the respondents in the Mwea region regarded bacterial wilt as a highly significant disease. Similarly, in both the Oloitoktok and Mwea regions, more than 40% and 20% of the respondents, respectively, considered bacterial wilt to be moderately important. Nevertheless, the results revealed that more than 50% and 5% of the respondents in the Oloitoktok and Mwea regions, respectively, did not view bacterial wilt of tomatoes as a serious disease, as indicated in (Table 4.1).

Table 4.1: Percentage response on the significance of bacterial wilt of tomato in Mwea and Oloitoktok regions

level of importance	Oloitoktok	Mwea
Important	0.0	73.3
Moderately important	46.7	20.0
Not important	53.3	6.7
Total	100.0	100.0

4.1.3 The duration farmers have experienced tomato bacterial wilt

In both the Oloitoktok and Mwea regions, tomato bacterial wilt has been reported by farmers for six years. According to the results, more than 90% of the respondents in Oloitoktok and 40% of the respondents in Mwea have reported observing bacterial wilt in their fields in the last two years (Table 4.2). However, at least 6% and 40% of respondents in Oloitoktok and Mwea, respectively, reported seeing the disease between three to six years ago. Only respondents in the Mwea region reported observing bacterial wilt in tomatoes between seven to more than ten years.

Table 4. 2: Percentage of farmers and duration over which they have experienced bacterial wilt in Mwea and Oloitoktok

No. of years	Oloitoktok	Mwea
0-2	93.3	46.7
3-6	6.7	43.3
7-9	0.0	6.7
>10	0.0	3.3
Total	100.0	100.0

4.1.4 Sources of tomato seeds for planting

Farmers in Oloitoktok and Mwea regions reported having different sources of tomato seeds for their farms. For instance, in the Mwea region, most farmers preferred to save their own seeds for the next planting, while in Oloitoktok, farmers had alternative preference for saving seeds (Figure 4.2). In both regions, farmers tended to buy seeds from agro shops, with over 90% and 80%, respectively. Other alternative sources of acquiring seeds was noted in Mwea region, with over 95%, which was not the case as in the Oloitoktok region.

Figure 4. 2: Percentage responses by tomato farmers in Oloitoktok and Mwea on the occurrence of bacterial wilt in the different sources of seeds

The majority of farmers in both Oloitoktok and Mwea regions used seeds for planting and reported the occurrence of bacterial wilt of tomatoes on their farms. Over 60% and 80% of farmers in both regions, respectively, reported using seeds for planting (Figure 4.3). Alternatively, less than 40 percent of the respondents in both regions reported not adapting seeds for planting. Despite using the seeds for planting, the disease was also observed in both regions with over 7 percent.

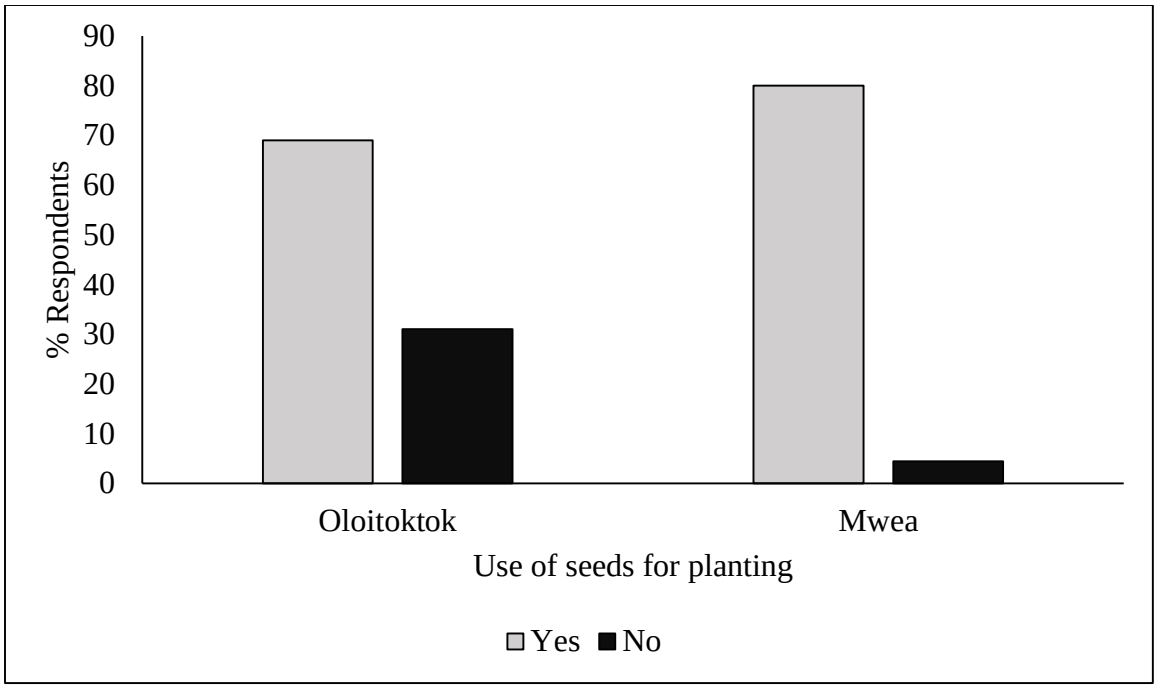

Figure 4. 3: Percentage responses of farmers on the use of seed for planting and bacterial wilt observation

The same trend was observed in the use of seedlings, as depicted in (Figure 4.4), where farmers in both regions responded positively, with over 40% and 60% reporting having seen the disease. However, in Oloitoktok, 45% of the farmers responded positively to the use of seedlings, while in the Mwea region, at least 60 % positive.

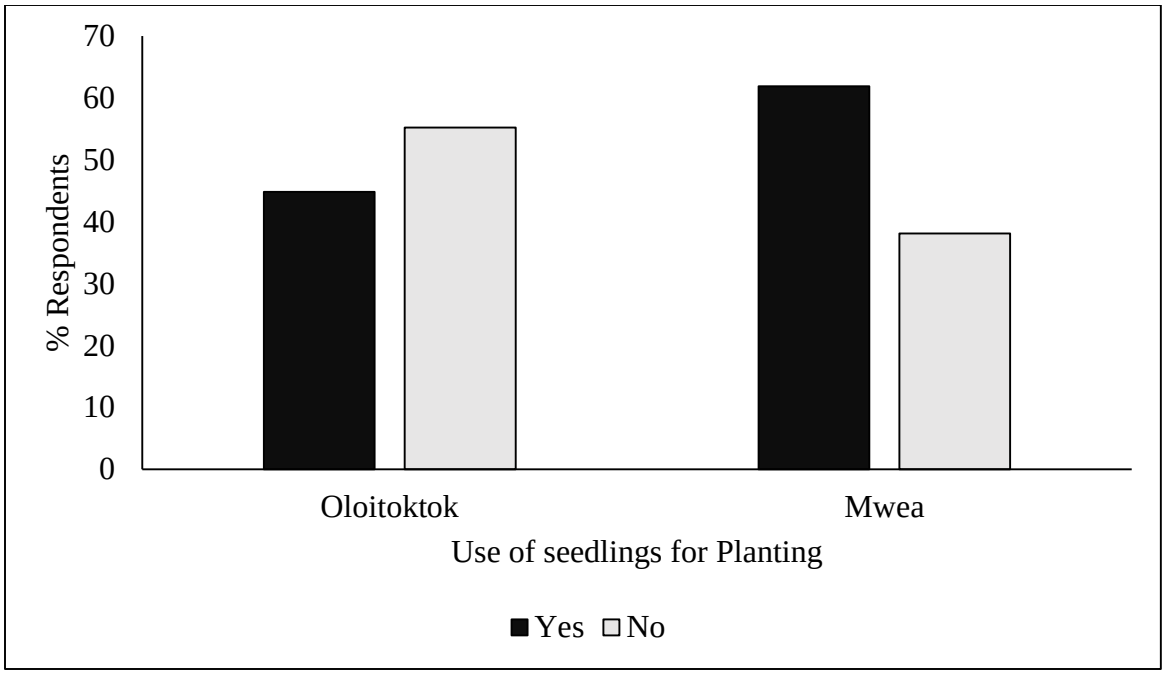

Figure 4. 4: Percentage responses of farmers on the use of seedlings for planting and bacterial wilt observation

4.1.5 Management practices against bacterial wilt of tomatoes

Different management methods were used in both Oloitoktok and Mwea regions to control the bacterial wilt of tomatoes (Figure 4.5). Farmers in Oloitoktok adopted both management practices of chemical application and rouging as opposed to the farmers in Mwea. In Oloitoktok, at least 10% of farmers depended entirely on chemical application to manage the disease which was not the case in the Mwea region. Both regions, however, had over 60% and 50%, in Oloitoktok and Mwea respectively, practicing rouging to manage the disease. A good proportion of farmers, over 20 and 50 percent respectively did nothing once they spotted the disease in their farms.

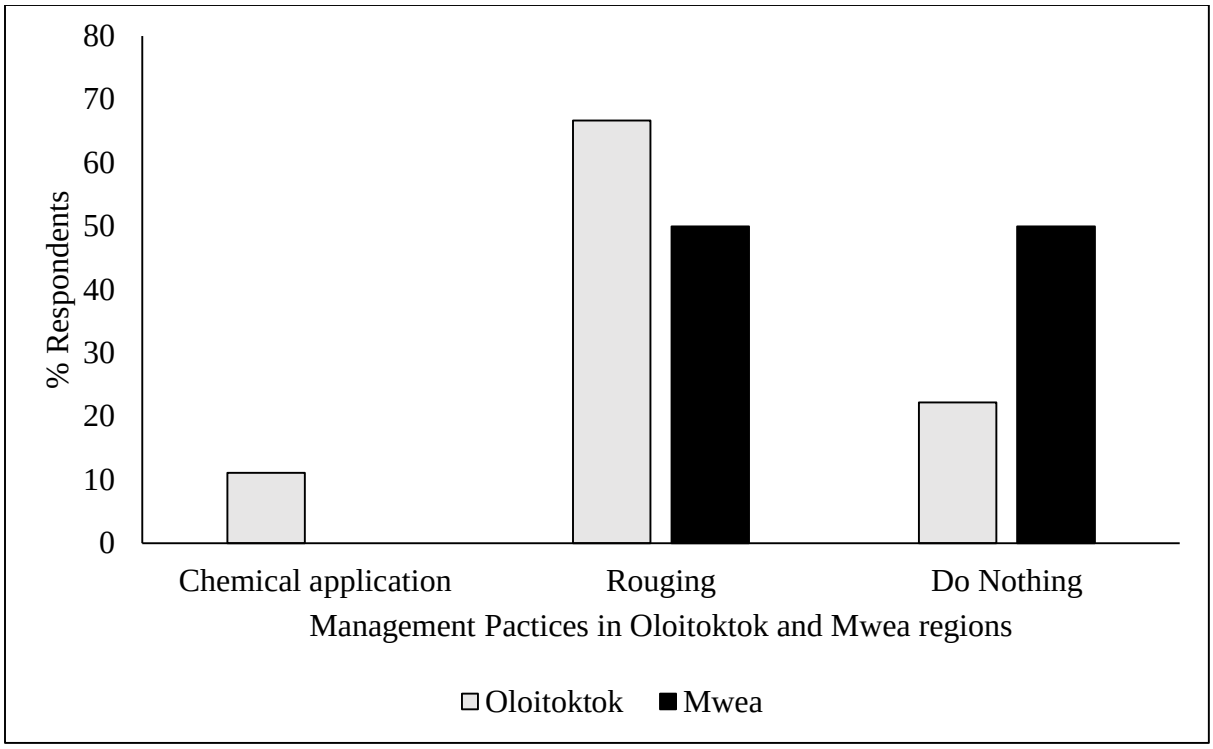

Figure 4. 5: Percentage responses by tomato farmers in Oloitoktok and Mwea regions on different management practices of bacterial wilt

Significant difference was noted in the farmer's practices in management response in both regions. In the Mwea region, at least ten percent of the farmers responded positively to crop rotation in green houses, while over 80% preferred not to conduct rotation in the greenhouse, even after observing the disease (Figure 4.6).

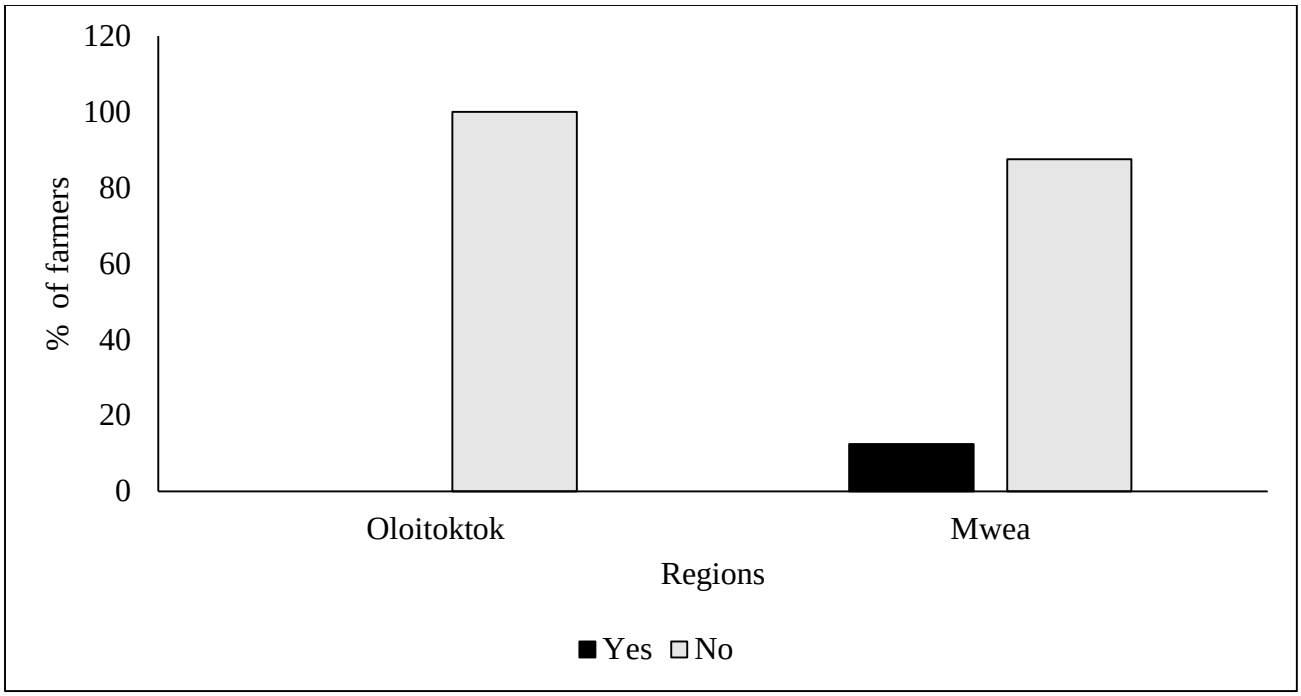

Figure 4.6: Percentage response of farmers on whether they practiced crop rotation in the greenhouse in Oloitoktok and Mwea regions

4.1.6 Production systems used by tomato farmers

Farmers in the Mwea region adapted to both production systems in tomato farming, unlike the farmers in Oloitoktok who largely depended on open field systems. There was no significant difference in the two regions in terms of the application of an open field production system, which was relied upon by over 90% of farmers (Figure 4.7). However, at least 5% of farmers in the Mwea region preferred greenhouse production systems.

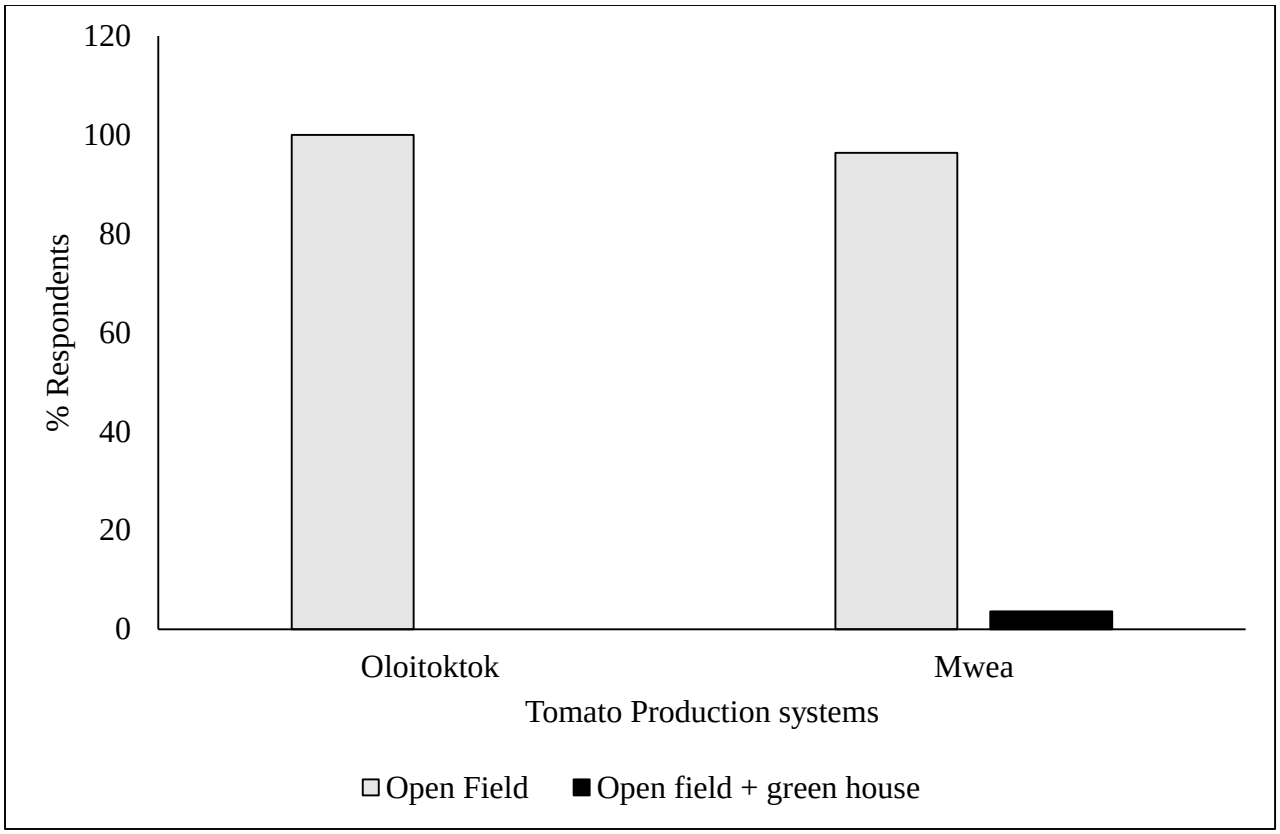

Figure 4.7: Percentage response of the production systems used by tomato farmers in Oloitoktok and Mwea regions

4.1.7 Isolation and identification of *Ralstonia solanacearum*

The results show presence of *Ralstonia solanacearum* in both the Mwea and Oloitoktok regions. The colonies were slimy, watery appearance and had a beige to pinkish color. In the Mwea region, twenty-one out of the thirty samples had colonies of *Ralstonia solanacearum*, while nine farms had no bacterial colonies (Table 4.3). Conversely, in the Oloitoktok region, only nine farms showed the presence of *Ralstonia solanaceaerum.* The samples collected from different AEZs had significant differences in Mwea and Oloitoktok regions. Moreover, the Mwea region had a larger population of CFUs compared to Oloitoktok, and there was a significant difference between the two regions.

Table 4.3: Mean number of colony forming units in Mwea and Oloitoktok regions

Mwea				Oloitoktok			
AEZ	Farming Practices	Mean		AEZ	Farming Practices	Mean	
LM 4	Crop rotation	789.0	ab	LM 6	Spraying Herbicide	84.5	ab
LM 5	Roguing	911.0	ab	LM 6	Roguing	86.7	ab
LM 4	Roguing	1,544.3	ab	LM 7	Roguing	118.9	abc
LM 4	Roguing	2,122.3	abc	LM 7	Crop rotation	138.9	bcd
LM 5	Fallowing	3,189.0	abc	LM 5	Crop rotation	222.2	cde
LM 4	Roguing	3,211.0	abc	LM 6	Roguing	227.8	cde
LM 4	Crop rotation	3,355.7	abc	LM 6	Roguing	234.4	cde
LM 4	Roguing	3,499.7	abc	LM 5	Roguing	262.2	de
LM 4	Crop rotation	3,522.0	abc	LM 7	Roguing	284.4	e
LM 4	Roguing	3,611.0	abc				
LM 4	Roguing	3,700.0	abc				
LM 4	Crop rotation	4,066.7	abc				
LM 5	Fallowing	4,489.0	abc				
LM 4	Crop rotation	4,833.3	abc				
LM 4	Crop rotation	5,533.3	abc				
LM 5	Fallowing	5,555.7	abc				
LM 5	Roguing	6,633.3	abc				
LM 4	Roguing	7,044.3	bc				
LM 4	Crop Rotation	8,688.7	c				
LM 5	Crop Rotation	16,855.7	d				
LM 4	Roguing	17,011.0	d				
Mean		3,672.2				55.3	
LSD		3,436.8				69.6	
CV%		100.6				135.2	
P value		<.001				<.001	

Means with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); Means with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation; AEZ= Agro ecological zones; LM= lower midland

4.2 Susceptibility of newly introduced tomato cultivars to bacterial wilt in the greenhouse, Oloitoktok and Mwea regions

4.2.1 Pathogenicity of *Ralstonia solanacearum* on five tomato varieties under greenhouse conditions

Incidence and severity scores were recorded for five different tomato varieties that were inoculated with *Ralstonia solanacearum*. Rio Grande exhibited the highest susceptibility, followed by Cal J variety. Shanty and Big rock varieties were the least susceptible in comparison to the other varieties, with scores of less than 4.5. Furthermore, there was a sharp increase of infection as from day six to day nine for most of the varieties. Incidence was present from day six up to day eighteen after inoculation, and severity of the infection progressed as time passed in all of the affected tomato varieties tested in the green house.

Table 4.4: Incidence and severity scores of bacterial wilt of tomato

	Incidence (Days)					Severity (Days)				
Varieties	3	6	9	15	18	3	6	9	15	18
Rio Grande	0.0	0.1	0.7	1.0	1.0	0.0	0.1	0.8	4.5	4.7
Commando F1	0.0	0.2	0.6	0.9	1.0	0.0	0.2	0.7	3.9	4.4
Cal J	0.0	0.2	0.7	0.9	0.9	0.0	0.2	0.9	3.9	4.5
Big rock F1	0.0	0.1	0.5	0.5	0.9	0.0	0.1	0.8	2.5	3.7
Shanty F1	0.0	0.1	0.6	0.8	0.9	0.0	0.1	0.7	3.0	3.9

The values shows scores of incidence and severity of bacterial week from day 3 to 18

4.2.2 Percentage incidence scores of bacterial wilt of tomato

A significant difference was observed in the percentage incidence scores from the second week up to the 8th week days after inoculation. Tomato cultivars Cal J and Rio Grande had the highest percentage scores of 100 each after the third week, while Big rock had the least percentage incidence score of 30 by week 8. However, there was no significant difference among Commando, Nyati, Nyota, Onyx, President, Raja, Rambo, and Ranger. The results also indicated a variation in the first two weeks days after inoculation, and the incidence level was noted to be uniform after the third week days after inoculation (Table 4.5).

Table 4.5: Incidence (%) of bacterial wilt in different tomato cultivars under greenhouse conditions in Kabete

	Weeks after inoculation							
Varieties	1	2	3	4	5	6	7	8
Assila	3.3cd	13.3cd	96.7a	96.7a	96.7a	96.7a	96.7a	96.7a
Bashier	23.3abcd	43.3abcd	96.7a	96.7a	96.7a	96.7a	96.7a	96.7a
Big rock	0.0d	3.3d	30.0d	30.0d	30.0d	30.0d	30.0d	30.0d
Cal j	36.7ab	80.0a	100.0a	100.0a	100.0a	100.0a	100.0ab	100.0a
Commando	10.0bcd	23.3bcd	93.3a	93.3a	93.3a	93.3a	93.3a	93.3a
Crown	10.0bcd	30.0abcd	66.7c	66.7c	66.7c	66.7c	66.7c	66.7c
Nyati	16.7abcd	63.3abc	96.7a	96.7a	96.7a	96.7a	96.7a	96.7a
Nyota	10.0bcd	30.0abcd	86.7ab	86.7ab	86.7ab	86.7ab	86.7ab	86.7ab
Onyx	20.0abcd	43.3abcd	86.7ab	86.7ab	86.7ab	86.7ab	86.7ab	86.7ab
President	23.3abcd	60.0abc	83.3a	96.7a	96.7a	96.7a	96.7a	96.7a
Raja	13.3abcd	26.7abcd	93.3a	93.3a	93.3a	93.3a	93.3a	93.3a
Rambo	16.7abcd	23.3bcd	96.7a	96.7a	96.7a	96.7a	96.7a	96.7a
Ranger	23.3abcd	46.7abcd	93.3a	93.3a	93.3a	93.3a	93.3a	93.3a
Rio grande	40.0a	70.0ab	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
Sandokan	30.0abc	63.3abc	93.3a	93.3a	93.3a	93.3a	93.3a	93.3a
TO 135	10.0bcd	43.3abcd	73.3bc	73.3bc	73.3bc	73.3bc	73.3bc	73.3bc
Tylka	16.7abcd	26.7abcd	90.0ab	90.0ab	90.0ab	90.0ab	90.0ab	90.0ab
Zara	6.7cd	33.3abcd	96.7a	96.7a	96.7a	96.7a	96.7a	96.7a
Grand means	17.2	40.2	88.5	88.1	88.1	88.1	88.1	88.1
LSD	24.5	45.8	17.2	16.3	16.3	16.3	16.3	16.3
CV %	85.2	68.8	11.7	11.1	11.1	11.1	11.1	11.1
P Value	0.107	0.096	<.001	<.001	<.001	<.001	<.001	<.001

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

The varietal scores for the percentage incidence of bacterial wilt of tomato was not significantly different among the varieties. Incidence of bacterial wilt was noted on only eight varieties. From the third week, the incidence was persistent up to the last week (Table 4.6). The percentage incidence recorded showed low symptoms of the disease from the first week of transplanting, and only Zara variety had a score of over 5 in the first week. However, President and Zara varieties had more than 10 percent scores in the last week as compared to the rest of the varieties.

Table 4. 6: Percentage incidence scores of bacterial wilt of tomato varieties in the Oloitoktok region

	Weeks after transplanting							
Varieties	1	2	3	4	5	6	7	8
Assila	0.0b	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a
Bashier	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Big rock	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Cal j	0.0b	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a
Commando	0.0b	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a
Crown	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Nyati	0.0b	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a
Nyota	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Onyx	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
President	3.3ab	10.0a	10.0a	10.0a	10.0a	10.0a	10.0a	10.0a
Raja	3.3ab	6.7a	6.7a	6.7a	6.7a	6.7a	6.7a	6.7a
Rambo	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Ranger	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Rio grande	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Sandokan	3.3ab	6.7a	6.7a	6.7a	6.7a	6.7a	6.7a	6.7a
TO 135	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Tylka	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Zara	6.7a	10.0a	10.0a	10.0a	10.0a	10.0a	10.0a	10.0a
Grand means	0.9	2.6	2.6	2.6	2.6	2.6	2.6	2.6
LSD	4.5	9.3	9.3	9.3	9.3	9.3	9.3	9.3
CV %	293.9	215.7	215.7	215.7	215.7	215.7	215.7	215.7
P Value	0.16	0.32	0.32	0.32	0.32	0.32	0.32	0.32

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

In Mwea region, various tomato varieties showed percentage scores of incidence of bacterial wilt. Cal j and Tylka were among the two varieties with highest scores of bacterial wilt incidence compared to the rest of the varieties. Additionally, some varieties, such as Commando, Crown, Nyota, Onyx, Rambo, Ranger, and Rio grande, had zero incidence scores. The incidence of bacterial wilt of tomatoes also progressed across the weeks in the affected varieties. In the 3rd, 4^{th,} and 5th weeks, Cal j, President and Tylka varieties experienced an increase in the incidence of bacterial wilt respectively (Table 4.7)

Table 4. 7: Percentage incidence scores of bacterial wilt of tomato in the Mwea region

	Weeks after transplanting							
Varieties	1	2	3	4	5	6	7	8
Assila	3.3a	3.3ab	3.3ab	3.3a	3.3a	3.3a	3.3a	3.3a
Bashier	3.3a	6.7ab	6.7ab	6.7a	6.7a	6.7a	6.7a	6.7a
Big rock	0.0a	0.0a	3.3ab	3.3a	3.3a	3.3a	3.3a	3.3a
Cal j	3.3a	13.3b	16.7b	16.7a	16.7a	16.7a	16.7a	16.7a
Commando	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Crown	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Nyati	0.0a	3.3ab	3.3ab	3.3a	3.3a	3.3a	3.3a	3.3a
Nyota	0.0a	0.0a	0.0a	6.7a	6.7a	6.7a	6.7a	6.7a
Onyx	0.0a	0.0a	0.0a	3.3a	3.3a	3.3a	3.3a	3.3a
President	0.0a	10.0ab	10.0ab	10.0a	13.3a	13.3a	13.3a	13.3a
Raja	0.0a	3.3ab	3.3ab	3.3a	3.3a	3.3a	3.3a	3.3a
Rambo	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Ranger	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Rio grande	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Sandokan	3.3a	3.3ab	3.3ab	3.3a	3.3a	3.3a	3.3a	3.3a
TO 135	0.0a	3.3ab	3.3ab	6.7a	6.7a	6.7a	6.7a	6.7a
Tylka	0.0a	13.3b	13.3ab	16.7a	16.7a	16.7a	16.7a	16.7a
Zara	0.0a	3.3ab	6.7ab	6.7a	6.7a	6.7a	6.7a	6.7a
Grand means	0.7	3.5	4.1	5.0	5.2	5.2	5.2	5.2
LSD	4.1	10.8	12.1	14.5	14.5	14.5	14.5	14.5
CV %	333.4	183.6	179.5	174.5	168.9	168.9	168.9	168.9
P Value	0.48	0.17	0.22	0.44	0.36	0.36	0.36	0.36

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

4.2.3 Severity scores of bacterial wilt of tomato

In the greenhouse experiment, there significant differences among the 18 varieties evaluates. Out of the 18 tomato varieties screened, Cal J was the most susceptible with a severity score of over 4.9 in the eighth week after inoculation. Rio grande also exhibited a high severity score of over 4.8, followed closely by Sandokan with a severity score of 4.5. All of the tomato varieties showed symptoms of the disease after inoculation, but some were less affected, such as Big rock, TO 135, Crown, and Nyota, with severity scores of less than 3.6. Disease severity was noted to increase as

days progressed across all screened varieties, and the difference was significant in all weeks across all the varieties. (Table 4.8)

Table 4. 8: Severity, overtime of bacterial wilt in different tomato cultivars under greenhouse conditions at Upper Kabete Campus

	Weeks after inoculation							
Varieties	1	2	3	4	5	6	7	8
Assila	0.0defg	0.2gh	2.5cd	3.1efg	3.5c	3.7bcd	3.9bc	3.9bc
Bashier	0.2abcde	0.9cdef	2.9bc	3.5cdef	3.9bc	4.1bcd	4.2abc	4.3abc
Big rock	0.0eg	0.0h	0.5e	0.7j	0.9e	0.9g	0.9g	1.0f
Cal j	0.4ab	1.5ab	4.5a	4.8a	4.8a	4.9a	4.9a	5.0a
Commando	0.1cdefg	0.4fgh	2.6bcd	3.5cdef	3.7bc	3.9bcd	4.0bc	4.1bc
Crown	0.1cdefg	0.5defgh	2.0d	2.4gi	2.7d	2.9ef	3.0df	3.1de
Nyati	0.2abcdefg	1.0bcd	3.1bc	3.6cdef	3.9bc	4.0bcd	4.2abc	4.3abc
Nyota	0.1cdefg	0.4efgh	2.5cd	3.1defg	3.5c	3.5def	3.6cdef	3.6cde
Onyx	0.2abcdefg	0.8cdef	3.0bc	3.4cdef	3.6c	3.7bcd	3.8bcd	3.9bc
President	0.3abcd	1.0cde	3.3b	4.0bc	4.3abc	4.4abc	4.4abc	4.4abc
Raja	0.1cdefg	0.5defgh	2.4cd	3.0fghi	3.5c	3.6cde	3.6cdef	3.9bc
Rambo	0.2abcdefg	0.6defg	3.3b	3.8bcde	4.1abc	4.2abcd	4.3abc	4.4abc
Ranger	0.2abcdef	0.8cdef	2.7bcd	3.2defg	3.6c	3.7bcd	3.8bcde	3.8cd
Rio grande	0.4a	1.6a	4.5a	4.9a	4.9a	4.9a	4.9a	4.9a
Sandokan	0.3abc	1.3abc	4.1a	4.4ab	4.4ab	4.5ab	4.5ab	4.5ab
TO 135	0.1cdefg	0.6defg	2.0d	2.3i	2.7d	2.8f	2.9f	3.0e
Tylka	0.2bcdefg	0.4efgh	2.4cd	3.1defgh	3.7bc	3.8bcd	4.0bc	4.0bc
Zara	0.1cdefg	0.6defgh	3.1bc	3.9bcd	4.2abc	4.3abcd	4.4abc	4.4abc
Grand means	0.2	0.7	2.9	3.4	3.7	3.8	3.9	3.9
LSD	0.2	0.5	0.6	0.7	0.7	0.7	1.0	0.7
CV %	212.6	130.6	44.4	39.8	37.4	36.3	35.7	35.3
P Value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

The disease was not so severe in Oloitoktok region as no variety showed more than 50 percent of the total plant wilt (Table 4.9). In the first two weeks after transplanting, the disease was very low in all the varieties that were tested. Seven varieties started showing wilt symptoms as from the third week of transplanting. Zara variety was the most affected with a severity score in the first

week while the majority started showing symptoms in the second and third weeks. President and Zara were also the most affected variety with a severity score higher than the rest of the varieties in the eight weeks (Table 4.9). Additionally, the results indicated that both the percentage incidences and severity scores increased progressively with the days after transplanting.

Table 4. 9: Severity score means of bacterial wilt of tomato varieties in Oloitoktok region

	Weeks after transplanting							
Varieties	1	2	3	4	5	6	7	8
Assila	0.0b	0.0b	0.0b	0.1ab	0.1ab	0.1ab	0.1ab	0.1abc
Bashier	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0c
Big rock	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0c
Cal j	0.0b	0.0b	0.1b	0.1ab	0.2ab	0.2ab	0.2ab	0.2abc
Commando	0.0b	0.0b	0.1b	0.1ab	0.1ab	0.1ab	0.2ab	0.2abc
Crown	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.00c
Nyati	0.0b	0.0b	0.1ab	0.1ab	0.1ab	0.1ab	0.2ab	0.2abc
Nyota	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0c
Onyx	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0c
President	0.0ab	0.1ab	0.2ab	0.2ab	0.3ab	0.4a	0.5a	0.5ab
Raja	0.0ab	0.1ab	0.1ab	0.2ab	0.2ab	0.3ab	0.3ab	0.3abc
Rambo	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0ac
Ranger	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0abc
Rio grande	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0abc
Sandokan	0.0ab	0.1ab	0.2ab	0.2ab	0.2ab	0.3ab	0.3ab	0.3abc
TO 135	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0abc
Tylka	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0abc
Zara	0.1a	0.2a	0.3a	0.3a	0.4a	0.4a	0.4a	0.5a
Grand means	0	0	0.1	0.1	0.1	0.1	0.1	0.1
LSD	0.1	0.1	0.2	0.2	0.3	0.4	0.4	0.4
CV %	1032.4	671.3	642.1	637.1	621.9	612.2	612.2	609.1
P Value	0.26	0.09	0.06	0.1	0.11	0.07	0.09	0.09

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

Severity scores were measured across the weeks, and different tomato varieties exhibited varying levels of disease. Cal j and Tylka varieties had the highest severity scores compared to other varieties. The first two weeks of disease monitoring revealed that the severity of bacterial wilt of tomato was not severe, with varieties exhibiting lower scores than in the 7^{th} and 8^{th} week (Table

4.10). Significant differences were observed among the tomato varieties in the Mwea region during weeks 4, 5, and 6. In the first week, all tomato varieties had a severity score of less than 0. However, the severity score progressed across the weeks, (Table 4.10).

Table 4. 10: Severity score means of bacterial wilt of tomato in Mwea region

	Weeks after transplanting							
Varieties	1	2	3	4	5	6	7	8
Assila	0.0a	0.1ab	0.1abc	0.1bc	0.1cd	0.1bc	0.2bc	0.2bc
Bashier	0.0a	0.1ab	0.1abc	0.1bc	0.2cd	0.2bc	0.2abc	0.3abc
Big rock	0.0a	0.0b	0.0bc	0.1c	0.1cd	0.1bc	0.1bc	0.1bc
Cal j	0.0a	0.1a	0.2ab	0.6a	0.7a	0.7a	0.8a	0.8a
Commando	0.0a	0.0b	0.0c	0.0c	0.0d	0.0c	0.0c	0.0c
Crown	0.0a	0.0b	0.0c	0.0c	0.0d	0.0c	0.0c	0.0c
Nyati	0.0a	0.0ab	0.1abc	0.1c	0.1cd	0.1bc	0.2bc	0.2bc
Nyota	0.0a	0.0b	0.0c	0.1bc	0.2cd	0.2abc	0.3abc	0.3abc
Onyx	0.0a	0.0b	0.0c	0.0c	0.1cd	0.1bc	0.1bc	0.2bc
President	0.0a	0.1ab	0.1abc	0.2bc	0.5abc	0.5ab	0.6abc	0.6abc
Raja	0.0a	0.0ab	0.1abc	0.1c	0.1cd	0.1bc	0.1bc	0.1bc
Rambo	0.0a	0.0b	0.0bc	0.0c	0.0d	0.0c	0.0c	0.0c
Ranger	0.0a	0.0b	0.0bc	0.0c	0.0cd	0.0c	0.0c	0.0c
Rio grande	0.0a	0.0b	0.0bc	0.0c	0.0cd	0.0c	0.0c	0.0c
Sandokan	0.0a	0.1ab	0.1abc	0.1bc	0.1cd	0.1bc	0.2bc	0.2bc
TO 135	0.0a	0.0ab	0.0bc	0.2bc	0.2bcd	0.3abc	0.3abc	0.3abc
Tylka	0.0a	0.1a	0.2a	0.4ab	0.6ab	0.6ab	0.7ab	0.7ab
Zara	0.0a	0.0ab	0.1abc	0.2bc	0.2bcd	0.2abc	0.3abc	0.3abc
Grand means	0.0	0.0	0.1	0.1	0.2	0.2	0.2	0.2
LSD	0.0	0.1	0.2	0.3	0.4	0.4	0.5	0.5
CV %	1155.2	538.7	513.9	459.6	427.7	427.1	423.5	423.1
P Value	0.66	0.11	0.11	0	0	0.01	0.02	0.03

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

Stem discoloration was observed in all the varieties tested under the green house, six varieties in Oloitoktok and a total of 13 varieties in Mwea region. From the varieties tested, Zara had the highest score amongst all other varieties (Table 4.11). There was a significant difference in all the sites where stem discoloration data was taken. In Oloitoktok the highest recorded score was in

President and Zara varieties while in Mwea region, was in Cal J and Tylka varieties. Big rock on the other hand performed well with the least score against all other varieties in the three sites.

Table 4. 11: Length (cm) of stem discoloration in different tomato cultivars infected with bacterial wilt under greenhouse conditions

	Stem discoloration (cm)		
Varieties	Kabete (Greenhouse)	Oloitoktok	Mwea
Assila	1.6abc	0.0b	0.1a
Bashier	1.6abc	0.0b	0.1ab
Big rock	0.4d	0.0b	0.1a
Cal j	1.9ab	0.0ab	0.4b
Commando	1.4bc	0.1ab	0.0a
Crown	1.3bc	0.0b	0.0a
Nyati	1.6abc	0.1ab	0.1a
Nyota	1.4bc	0.0b	0.1ab
Onyx	1.5bc	0.0b	0.1a
President	1.7abc	0.2a	0.3ab
Raja	1.9ab	0.1ab	0.1a
Rambo	1.5bc	0.0b	0.0a
Ranger	1.5bc	0.0b	0.0a
Rio grande	1.6abc	0.0b	0.0a
Sandokan	1.5bc	0.1ab	0.1a
TO 135	1.2c	0.0b	0.1ab
Tylka	1.4bc	0.0b	0.4b
Zara	2.2a	0.2a	0.1ab
Grand means	1.5	0.1	0.1
LSD	0.3	0.2	0.2
CV %	43.9	644.7	425.9
P Value	<.001	0.03	0.01

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

4.2.4 Growth parameter scores of bacterial wilt of tomato

In the greenhouse, significant variations were noted among the various tomato varieties in terms of plant height, fresh, and dry weight. Out of the eighteen varieties, only three had plant heights above 40 centimeters, while majority of them averaged around 30 centimeters. Additionally, it was

noted that all of the tomato varieties had root lengths between 20 to 30 centimeters (Table 4.12). In terms of both fresh and dry weights of the eighteen varieties, significant differences were only observed when the tomato plants were dried, with only one variety, Raja, weighing above 23 kilograms. Nevertheless, the majority of the varieties did not show any significant difference as noted during the observation.

Table 4.12: Growth parameters scores of bacterial wilt of tomato varieties in the greenhouse experiment in Kabete

Varieties	Plant height(cm)	Root Length(cm)	Fresh weight(kg)	Dry weight(kg)
Assila	34.8d	26.1a	48.8a	20.9a
Bashier	35.5d	26.8a	49.2a	21.0a
Big rock	46.4a	26.2a	48.4a	20.3a
Cal j	37.0cd	26.8a	48.5a	20.6a
Commando	35.6d	25.0a	49.6a	20.1a
Crown	43.7ab	27.1a	54.6a	22.7a
Nyati	36.1d	26.9a	50.0a	21.0a
Nyota	38.4bcd	27.5a	48.4a	20.5a
Onyx	38.2bcd	26.7a	51.8a	20.6a
President	38.2bcd	27.5a	53.0a	21.4a
Raja	36.6cd	25.8a	53.5a	23.7a
Rambo	34.5d	25.9a	49.2a	20.1a
Ranger	35.2d	25.4a	52.2a	21.1a
Rio grande	33.1d	25.8a	48.6a	20.1a
Sandokan	36.4cd	27.4a	51.9a	21.2a
TO 135	42.2abc	26.1a	51.9a	21.1a
Tylka	36.1d	27.3a	49.0a	20.0a
Zara	35.9d	26.3a	51.1a	22.9a
Grand means	37.5	26.5	50.5	21.1
LSD	3.2	2.4	4.8	2.1
CV %	17.1	17.8	18.8	19.2
P Value	<.001	0.709	0.212	0.01

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

The growth parameter scores displayed significant variations among varieties in Oloitoktok region alternatively, plant heights and root lengths did not show a significant difference (Table 4.13). For instance, all the varieties except Assila and Tylka had more than 54 cm, and on the root length,

Crown had the highest score of more than 26. The weight levels of both fresh and dry matter among the varieties also showed significant differences, with the Tylka variety recording the highest weight when measured in fresh and dried form.

Table 4. 13: Growth parameters scores of bacterial wilt of tomato in Oloitoktok region

Varieties	Plant height(cm)	Root Length(cm)	Fresh weight(kg)	Dry weight(kg)
Assila	54.2a	25.8ab	65.8ab	30.3abc
Bashier	52.4a	23.9ab	63.8ab	29.1bcde
Big rock	52.9a	24.4ab	64.3abcd	29.9abcd
Cal j	52.7a	25.5ab	63.8bcd	28.7cdef
Commando	53.8a	24.8ab	66.1ab	31.0ab
Crown	53.5a	26.2a	65.5abc	29.7abcd
Nyati	52.3a	23.8ab	63.7bcd	29.2bcde
Nyota	52.7a	24.7ab	63.0bcd	28.6cdef
Onyx	52.2a	24.2ab	64.1abcd	28.1def
President	51.2a	24.5ab	62.9bcd	28.5cdef
Raja	51.4a	24.0ab	62.4cd	26.8f
Rambo	52.6a	23.8ab	64.0bcd	28.8cdef
Ranger	51.5a	23.7ab	63.2bcd	28.8cdef
Rio grande	53.4a	24.3ab	64.4abcd	29.8abcd
Sandokan	52.5a	24.4ab	62.9bcd	28.8cdef
TO 135	52.2a	24.2ab	62.8bcd	28.4cdef
Tylka	54.4a	25.1ab	67.2a	31.56a
Zara	51.2a	23.6b	61.5d	27.4ef
Grand means	52.6	24.5	64	29.1
LSD	2.6	2	2.7	1.7
CV %	9.7	16.1	8.2	11.8
P Value	0.39	0.43	0	<.001

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

The growth parameters significantly differed among the varieties. Plant heights showed significant differences among the varieties with Cal J and President having scores of less than 60 cm while, Rambo variety was the tallest compared to the rest (Table 4.14). The root lengths also showed significant differences with Cal J having also the least scores while Sandokan, Ranger, and Rambo

having scores more than 30 cm. Additionally, the biomass of the tomato varieties showed a significant difference, with Rambo having the highest fresh weight while Cal j had the least with scores ranging from 80 to 67 respectively. Most of the tomato varieties had substantial average heights, but Rambo was the tallest, while Cal j was the shortest (Table 4.14).

Table 4. 14: Growth parameters scores of bacterial wilt of tomato in the Mwea region

Varieties	Plant height	Root Length	Fresh weight	Dry weight
Assila	67.6ef	29.8defg	77.5ef	30.1abc
Bashier	63.2cde	28.5cdef	72.1bcd	26.6def
Big rock	65.8cdef	29.7defg	75.7cdef	27.9cdef
Cal j	56.8a	23.8a	66.7a	25.3f
Commando	67.1def	29.8defg	77.1def	31.6ab
Crown	64.7cdef	28.7cdef	73.6cde	28.0cdef
Nyati	66.6def	29.7defg	76.4def	28.2cde
Nyota	64.2cde	28.5cdef	74.6cde	28.2cde
Onyx	64.4cde	28.3cdef	74.2cde	27.9cdef
President	58.5ab	24.7ab	67.5ab	26.1ef
Raja	66.0cdef	28.5cdef	76.6def	30.0abc
Rambo	69.4f	31.8g	80.1f	32.2a
Ranger	67.3ef	30.9fg	76.6def	29.0bcde
Rio grande	66.1cdef	29.6defg	76.3def	29.3bcd
Sandokan	66.5def	30.0efg	76.9def	29.2bcd
TO 135	62.8bcde	27.5cde	72.9cde	28.4cde
Tylka	61.4bc	27.0bcd	71.1abc	27.3cdef
Zara	62.2bcd	26.5bc	73.1cde	29.0bcde
Grand means	64.5	28.5	74.4	28.6
LSD	4.1	2.4	4.4	2.4
CV %	12.5	16.8	11.6	16.8
P Value	<.001	<.001	<.001	<.001

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

4.3 Efficacy of *Bacillus subtilis* in managing bacterial wilt in tomatoes in the greenhouse, Oloitoktok and Mwea regions

4.3.1 Percentage incidence scores of bacterial wilt of tomato management

Among the varieties tested in the greenhouse, Cal j and Sandokan had high incidence scores throughout the weeks (Table 4.15). Crown had the least incidence score of less than 30% in the 8th week. Majority of the varieties had scores averaging between 50 and 80%, while the disease consistently progressed days after inoculation. These results also indicated significant differences among the varieties across the weeks (Table 4.15).

Table 4. 15: Percentage incidence scores of bacterial wilt of tomato management in the greenhouse experiment in Kabete

	Weeks after inoculation							
Varieties	1	2	3	4	5	6	7	8
Assila	3.3d	43.3def	53.3ef	53.3ef	53.3ef	53.3ef	53.3ef	53.3ef
Bashier	20.0bcd	60.0cd	80.0abc	80.0abc	80.0abc	80.0abc	80.0abc	80.0abc
Big rock	3.3d	26.7ef	43.3f	43.3f	43.3f	43.3f	43.3f	43.3f
Cal j	50.0a	90.0ab	96.7a	96.7a	96.7a	96.7a	96.7a	96.7a
Commando	10.0cd	53.3cde	70.0cde	70.0cde	70.0cde	70.0cde	70.0cde	70.0cde
Crown	3.3d	23.3f	23.3g	26.7g	26.7g	26.7g	26.7g	26.7g
Nyati	6.7d	63.3cd	80.0abc	80.0abc	80.0abc	80.0abc	80.0abc	80.0abc
Nyota	10.0cd	63.3bcd	70.0cde	70.0cde	70.0cde	70.0cde	70.0cde	70.0cde
Onyx	16.7bcd	63.3bcd	66.7cde	66.7cde	66.7cde	66.7cde	66.7cde	66.7cde
President	16.7bcd	63.3bcd	73.3bcd	73.3bcd	73.3bcd	73.3bcd	73.3bcd	73.3bcd
Raja	6.7d	53.3cde	70.0cde	70.0cde	70.0cde	70.0cde	70.0cde	70.0cde
Rambo	6.7d	56.7cd	60.0def	60.0def	60.0def	60.0def	60.0def	60.0def
Ranger	3.3d	63.3bcd	63.3cde	63.3cde	63.3cde	63.3cde	63.3cde	63.3cde
Rio grande	30.0bc	76.7abc	90.0ab	90.0ab	90.0ab	90.0ab	90.0ab	90.0ab
Sandokan	33.3ab	93.3a	93.3a	93.3a	93.3a	93.3a	93.3a	93.3a
TO 135	0.0d	53.3cde	53.3ef	53.3ef	53.3ef	53.3ef	53.3ef	53.3ef
Tylka	10.0cd	50.0cdef	53.3ef	53.3ef	53.3ef	53.3ef	53.3ef	53.3ef
Zara	13.3bcd	60.0cd	63.3cde	63.3cde	63.3cde	63.3cde	63.3cde	63.3cde
Grand means	13.5	58.7	66.9	67.0	67.0	67.0	67.0	67.0
LSD	18.3	25.0	15.8	16.1	16.1	16.1	16.1	16.1
CV %	81.5	25.6	14.3	14.5	14.5	14.5	14.5	14.5
P Value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

Incidence scores were observed among eight varieties of tomato with bio-control agent against bacterial wilt of tomato in Oloitoktok. President and Zara varieties had the highest scores in the eighth week while the majority of the varieties recorded low scores. Furthermore, the incidence of bacterial wilt was observed to progress across the weeks, with consistency from the second week (Table 4.16). Based on the results, there was no significant difference among the varieties with a bio-control agent in Oloitoktok.

Table 4. 16: Percentage incidence scores of bacterial wilt of tomato management in Oloitoktok

	Weeks after transplanting							
Varieties	1	2	3	4	5	6	7	8
Assila	0.0b	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a
Bashier	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Big rock	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Cal j	0.0b	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a
Commando	0.0b	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a
Crown	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Nyati	0.0b	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a	3.3a
Nyota	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Onyx	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
President	3.3ab	10.0a	10.0a	10.0a	10.0a	10.0a	10.0a	10.0a
Raja	3.3ab	6.7a	6.7a	6.7a	6.7a	6.7a	6.7a	6.7a
Rambo	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Ranger	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Rio grande	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Sandokan	3.3ab	6.7a	6.7a	6.7a	6.7a	6.7a	6.7a	6.7a
TO 135	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Tylka	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Zara	6.7a	10.0a	10.0a	10.0a	10.0a	10.0a	10.0a	10.0a
Grand means	0.9	2.6	2.6	2.6	2.6	2.6	2.6	2.6
LSD	4.5	9.3	9.3	9.3	9.3	9.3	9.3	9.3
CV %	293.9	215.7	215.7	215.7	215.7	215.7	215.7	215.7
P Value	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

In Mwea region, percentage incidence scores were reported among different tomato varieties after management against bacterial wilt. Tylka and Cal j had the highest percentage scores of more than 16, while Commando, Crown, Rambo, Ranger, and Rio grande recorded zero incidence scores

(Table 4.17). The incidence was also observed to progress from the third week after inoculation across the different varieties. However, there was no significant difference observed among the varieties across the weeks after the management of bacterial wilt of tomatoes.

Table 4. 17: Percentage incidence scores of bacterial wilt of tomato management in Mwea region

	Weeks after transplanting							
Varieties	1	2	3	4	5	6	7	8
Assila	3.3a	3.3ab	3.3ab	3.3a	3.3a	3.3a	3.3a	3.3a
Bashier	3.3a	6.7ab	6.7ab	6.7a	6.7a	6.7a	6.7a	6.7a
Big rock	0.0a	0.0b	3.3ab	3.3a	3.3a	3.3a	3.3a	3.3a
Cal j	3.3a	13.3a	16.7a	16.7a	16.7a	16.7a	16.7a	16.7a
Commando	0.0a	0.0b	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a
Crown	0.0a	0.0b	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a
Nyati	0.0a	3.3ab	3.3ab	3.3a	3.3a	3.3a	3.3a	3.3a
Nyota	0.0a	0.0b	0.0b	6.7a	6.7a	6.7a	6.7a	6.7a
Onyx	0.0a	0.0b	0.0b	3.3a	3.3a	3.3a	3.3a	3.3a
President	0.0a	10.0ab	10.0ab	10.0a	13.3a	13.3a	13.3a	13.3a
Raja	0.0a	3.3ab	3.3ab	3.3a	3.3a	3.3a	3.3a	3.3a
Rambo	0.0a	0.0b	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a
Ranger	0.0a	0.0b	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a
Rio grande	0.0a	0.0b	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a
Sandokan	3.3a	3.3ab	3.3ab	3.3a	3.3a	3.3a	3.3a	3.3a
TO 135	0.0a	3.3ab	3.3ab	6.7a	6.7a	6.7a	6.7a	6.7a
Tylka	0.0a	13.3a	13.3ab	16.7a	16.7a	16.7a	16.7a	16.7a
Zara	0.0a	3.3ab	6.7ab	6.7a	6.7a	6.7a	6.7a	6.7a
Grand means	0.7	3.5	4.1	5.0	5.2	5.2	5.2	5.2
LSD	4.1	10.7	12.1	14.5	14.5	14.5	14.5	14.5
CV %	333.4	183.6	179.5	174.5	168.9	168.9	168.9	168.9
P Value	0.5	0.2	0.2	0.4	0.4	0.4	0.4	0.4

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

4.3.2 Severity scores of bacterial wilt of tomato management

It has been observed from the results that every tomato variety in the Kabete greenhouse had a severity score (Table 4.18). It was also noticed that during the first two weeks after the control of bacterial wilt of tomato, there were lower levels of severity score of up to 1.2. In addition, Cal j

was severely affected with scores of more than 4.5, while the least affected variety was Crown, with less than 0.9. Most of the varieties were highly affected during the 6th, 7th, and 8th weeks after inoculation. There was a significant difference among the varieties across the weeks, and the disease progressed over time.

Table 4. 18: Severity score means of bacterial wilt of tomato management in the greenhouse experiment in Kabete

	Weeks after inoculation							
Varieties	1	2	3	4	5	6	7	8
Assila	0.0de	0.5bcd	0.9def	1.1de	1.4ef	1.6de	1.9cd	2.1de
Bashier	0.2bcd	0.7b	1.4cd	1.8bc	2.3bcd	2.8bc	3.1b	3.4bc
Big rock	0.0de	0.3cd	0.6fg	1.0de	1.1fg	1.4e	1.5de	1.7ef
Cal j	0.5a	1.2a	1.8ab	2.3ab	2.8abc	3.4ab	4.1a	4.6a
Commando	0.1de	0.6bc	1.3cde	1.8bc	2.0cde	2.4cd	2.6bc	3.0cd
Crown	0.0de	0.2d	0.3g	0.5e	0.6g	0.6f	0.8e	0.8f
Nyati	0.1de	0.7b	1.3cd	1.8bc	2.1cde	2.6bc	3.0b	3.5bc
Nyota	0.1de	0.7b	1.1cdef	1.4cd	1.7def	2.0cde	2.4bcd	2.9cd
Onyx	0.2bcde	0.7b	1.1cdef	1.4cd	1.6def	2.0cde	2.6bc	2.8cde
President	0.2bcde	0.7b	1.4bc	1.8bc	2.3bcd	2.4cd	2.8bc	3.1cd
Raja	0.1de	0.5bcd	1.0cdef	1.5cd	1.6def	1.9cde	2.2bcd	2.7cde
Rambo	0.1de	0.6bcd	1.0cdef	1.3cd	1.7def	2.0cde	2.3bcd	2.6cde
Ranger	0.0de	0.6bc	1.2cde	1.3cd	1.8def	2.1cde	2.5bc	2.8cde
Rio grande	0.3bc	1.2a	2.0a	2.5a	3.1a	3.7a	4.1a	4.4ab
Sandokan	0.3b	1.3a	1.9a	2.4ab	3.0ab	3.4ab	4.0a	4.4ab
TO 135	0.0e	0.5bcd	0.8ef	1.1de	1.2fg	1.6de	1.9cd	2.2de
Tylka	0.1de	0.6bcd	1.0cdef	1.3cd	2.1cde	1.9cde	2.2bcd	2.5cde
Zara	0.1cde	0.6bc	1.1cdef	1.4cd	1.7def	1.9cde	2.3bcd	2.6de
Grand means	0.1	0.7	1.2	1.5	1.9	2.2	2.6	2.9
LSD	0.2	0.3	0.4	0.6	0.7	0.8	0.9	1.0
CV %	237.1	86.2	74.7	71.4	68.9	68.8	66.8	66.8
P Value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

The severity scores of bacterial wilt of tomato in Oloitoktok were observed when controlled with bio control agent. The eight affected varieties depicted varying results, with President having the highest score of more than 0.4. Only Sandokan had the severity score in the first week while the rest became affected in the second week. The results also show that the majority of the varieties

had scores of less than 1. However, there was no significant difference observed among the varieties across the weeks (Table 4.19).

Table 4. 19: Severity score means of bacterial wilt of tomato management in Oloitoktok

	Weeks after transplanting							
Varieties	1	2	3	4	5	6	7	8
Assila	0.0a	0.0ab	0.0a	0.1ab	0.1ab	0.1abc	0.1abc	0.1ab
Bashier	0.0a	0.0b	0.0a	0.0b	0.0b	0.0c	0.0c	0.0b
Big rock	0.0a	0.0b	0.0a	0.0b	0.0b	0.0c	0.0ac	0.0b
Cal j	0.0a	0.0ab	0.1a	0.1ab	0.1ab	0.1abc	0.2abc	0.2ab
Commando	0.0a	0.0ab	0.1a	0.1ab	0.1ab	0.1abc	0.1abc	0.2ab
Crown	0.0a	0.0b	0.0a	0.0b	0.0b	0.0c	0.0abc	0.0b
Nyati	0.0a	0.0ab	0.1a	0.1ab	0.1ab	0.1abc	0.1abc	0.2ab
Nyota	0.0a	0.0b	0.0a	0.0b	0.0b	0.0c	0.0abc	0.0b
Onyx	0.0a	0.0b	0.0a	0.0b	0.0b	0.0c	0.0abc	0.0b
President	0.0a	0.1ab	0.2a	0.2ab	0.3a	0.3a	0.4ab	0.5a
Raja	0.0a	0.1ab	0.1a	0.2ab	0.2ab	0.2abc	0.3abc	0.3ab
Rambo	0.0a	0.0b	0.0a	0.0b	0.0b	0.0c	0.0abc	0.0b
Ranger	0.0a	0.0b	0.0a	0.0b	0.0b	0.0c	0.0abc	0.0b
Rio grande	0.0a	0.0b	0.0a	0.0b	0.0b	0.0c	0.0abc	0.0b
Sandokan	0.1a	0.1ab	0.1a	0.2ab	0.2ab	0.2abc	0.3abc	0.3ab
TO 135	0.0a	0.0b	0.0a	0.0b	0.0b	0.0c	0.0abc	0.0ab
Tylka	0.0a	0.0b	0.0a	0.0b	0.0b	0.0c	0.0abc	0.0ab
Zara	0.0a	0.1a	0.2a	0.2a	0.3ab	0.3ab	0.4a	0.4ab
Grand means	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1
LSD	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.4
CV %	771.1	642.8	624.2	617.8	621.1	613.7	612.9	610.1
P Value	0.7	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

Severity scores of bacterial wilt of tomato were reported across most of the tomato varieties in Mwea with eleven out of the eighteen being affected. Cal j and Tylka were highly susceptible, with scores over 0.7, while the least affected varieties included Commando, Crown, Rambo, Ranger, and Rio grande (Table 4.20). The bacterial wilt of tomato was observed to progress over

time in all the affected varieties. However, there was no significant difference observed in the severity scores of bacterial wilt of tomato management between the first and last weeks.

Table 4. 20: Severity score means of bacterial wilt of tomato management in Mwea region

	Weeks after transplanting							
Varieties	1	2	3	4	5	6	7	8
Assila	0.0b	0.1bc	0.1ab	0.1c	0.1cd	0.1b	0.1bc	0.2bc
Bashier	0.0b	0.1abc	0.1ab	0.1ac	0.2abcd	0.2ab	0.2bc	0.3abc
Big rock	0.0b	0.0bc	0.0b	0.1c	0.1cd	0.1b	0.1bc	0.1bc
Cal j	0.1a	0.2a	0.3a	0.4a	0.5a	0.6a	0.7a	0.8a
Commando	0.0b	0.0c	0.0b	0.0c	0.0d	0.0b	0.0c	0.0c
Crown	0.0b	0.0c	0.0b	0.0c	0.0cd	0.0b	0.0c	0.0c
Nyati	0.0b	0.0bc	0.1ab	0.1c	0.1cd	0.1b	0.1bc	0.2bc
Nyota	0.0b	0.0bc	0.1ab	0.1c	0.2abcd	0.2ab	0.3abc	0.3abc
Onyx	0.0b	0.0bc	0.0b	0.0c	0.1cd	0.1b	0.1bc	0.1bc
President	0.0b	0.1abc	0.2ab	0.2abc	0.4abc	0.4ab	0.5ab	0.6abc
Raja	0.0b	0.0bc	0.1ab	0.1c	0.1cd	0.1b	0.1bc	0.1bc
Rambo	0.0b	0.0c	0.0b	0.0c	0.0cd	0.0b	0.0c	0.0c
Ranger	0.0b	0.0c	0.0b	0.0c	0.0cd	0.0b	0.0c	0.0c
Rio grande	0.0b	0.0c	0.0b	0.0c	0.0cd	0.0b	0.0c	0.0c
Sandokan	0.0b	0.1abc	0.1ab	0.1c	0.1cd	0.1b	0.1bc	0.2bc
TO 135	0.0b	0.0bc	0.1ab	0.1abc	0.1bcd	0.2ab	0.3bc	0.3abc
Tylka	0.0b	0.2ab	0.3a	0.4ab	0.5ab	0.6a	0.6ab	0.7ab
Zara	0.0b	0.0bc	0.1ab	0.1c	0.2abcd	0.2ab	0.3abc	0.3abc
Grand means	0	0.1	0.1	0.1	0.1	0.2	0.2	0.2
LSD	0.1	0.1	0.2	0.2	0.3	0.4	0.4	0.5
CV %	657.5	477.2	452	437.4	429.7	425	422.6	423.7
P Value	0.1	0	0.1	0.01	0.01	0.01	0.01	0.02

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

Stem discoloration scores was observed in all the tomato varieties in the green house in Kabete, five tomato varieties in Oloitoktok and a total of ten varieties in Mwea region. Cal J, Rio grande and Sandokan varieties had the highest scores more than 1 cm across all the sites (Table 4.21). In the field sites, President and Zara varieties had the largest scores in Oloitoktok while in Mwea Cal J and Tylka was highly affected. Additionally, Crown variety had the least score in both the field and under greenhouse conditions.

Table 4. 21: Mean scores of stem discoloration of bacterial wilt of tomato

Varieties	Stem discoloration (cm)		
	Kabete (Greenhouse)	Oloitoktok	Mwea
Assila	0.6def	0.0b	0.0b
Bashier	0.9bcd	0.0b	0.1ab
Big rock	0.5efg	0.0b	0.1b
Cal j	1.3a	0.0b	0.3a
Commando	0.8bcde	0.0b	0.0b
Crown	0.3g	0.0b	0.0b
Nyati	0.9bcd	0.1ab	0.1b
Nyota	0.8bcdef	0.0b	0.1ab
Onyx	0.7cdef	0.0b	0.0b
President	0.8cdef	0.2a	0.2ab
Raja	0.9bcd	0.1ab	0.0b
Rambo	0.7def	0.0b	0.0b
Ranger	0.7cdef	0.0b	0.0b
Rio grande	1.1ab	0.0b	0.0b
Sandokan	1.0abc	0.1ab	0.1b
TO 135	0.5fg	0.0b	0.1ab
Tylka	0.6def	0.0b	0.3a
Zara	0.9bcd	0.2a	0.1ab
Grand means	0.8	0.0	0.1
LSD	0.3	0.1	0.2
CV %	73.8	656.4	432.4
P Value	<.001	0	0.01

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

4.3.3 Growth parameter scores of bacterial wilt of tomato management

Considerable variations were noticed in the growth parameters of the bacterial wilt of tomato when utilizing a bio-control agent in the Kabete greenhouse. Plant height, root length, biomass and yield were some of the parameters where notable variations were observed (Table 4.22). The crown variety had the tallest height among all varieties, while Rio grande was the shortest. The crown variety also had the longest roots compared to other varieties, with Zara having the shortest. Most varieties had root lengths ranging between 22 to 28 centimeters. Conversely, the biomass output

showed significant differences, with Crown having the highest weight in fresh weight measured in kg, while the majority of the dry weight was between 20 to 22 kg (Table 4.22). The yield output was also significantly different, with Big rock recording the highest yield score of over 10.0, while Sandokan had the lowest yield score.

Table 4. 22: Growth parameters scores of bacterial wilt of tomato management in the greenhouse experiment in Kabete

Varieties	Plant height(cm)	Root Length(cm)	Fresh weight(kg)	Dry weight(kg)	Yield (kg)
Assila	46.7efgh	27.2bcd	53.1cdefg	22.0cdef	6.2ef
Bashier	45.1fghi	24.6ef	51.3fgh	22.4bcde	6.3def
Big rock	54.4ab	28.0abc	55.4bcde	22.9bcde	11.1a
Cal j	40.6jk	22.9fgh	45.1i	20.6fg	4.0g
Commando	42.3ij	22.9fgh	51.3fgh	21.9cdef	6.7def
Crown	55.4a	30.1a	60.5a	25.7a	7.8c
Nyati	42.5ij	24.2efg	51.5fgh	21.7cdefg	6.6def
Nyota	47.9defg	27.4bcd	52.2defg	23.3bcd	6.5def
Onyx	48.8def	28.0abc	55.8bcd	23.5bcd	6.2ef
President	47.0efgh	25.5de	53.0cdefg	21.7cdefg	6.8de
Raja	43.2hij	23.1fgh	54.3cdef	23.6bc	6.5def
Rambo	47.1efgh	26.1cde	52.0efg	21.7cdefg	6.5def
Ranger	49.3cde	28.5abc	56.2bc	22.6bcde	6.0f
Rio grande	37.6k	22.0gh	48.1hi	20.0g	3.8g
Sandokan	40.2k	22.0gh	49.4gh	21.4efg	3.6g
TO 135	52.7abc	28.8ab	58.2ab	23.9b	8.6b
Tylka	51.3bcd	28.8ab	53.1cdefg	21.7defg	6.9d
Zara	44.1ghij	21.5h	50.5gh	23.0bcde	6.4def
Grand means	46.5	25.6	52.8	22.4	6.5
LSD	3.5	2.1	3.2	1.6	0.5
CV %	14.9	16.4	12	13.7	16.6
P Value	<.001	<.001	<.001	<.001	<.001

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

There were significant differences observed in the growth parameter scores across the varieties. Notably, there were significant differences in dry weight and yield among the varieties. Plant heights had no significant difference among the eighteen varieties with the Tylka variety at more than 57 cm and President at the least (Table 4.23). In root length, there was no significant

difference among all the varieties with Assila and Crown having more than 26 centimeters. Big rock had the highest yield output while Tylka scored more than 31kg in biomass. However, there were no significant differences in plant heights and root length observed among the varieties.

Table 4. 23: Growth parameters scores of bacterial wilt of tomato management in Oloitoktok

Varieties	Plant height(cm)	Root Length(cm)	Fresh weight(kg)	Dry weight(kg)	Yield (kg)
Assila	56.8a	26.2a	66.7abcd	29.8abc	6.6d
Bashier	55.1ab	24.6a	65.3abcd	28.7bcde	6.8d
Big rock	55.9ab	24.7a	65.5abcd	29.3abcd	12.1a
Cal j	55.4ab	25.9a	66.3abcd	28.5cdef	4.3e
Commando	56.9a	25.1a	68.7a	30.5ab	7.1d
Crown	55.5ab	26.6a	67.5abc	28.9bcde	8.1c
Nyati	55.3ab	24.3a	65.2bcd	28.9bcde	6.8d
Nyota	56.2ab	25.3a	64.8bcd	27.9cdef	6.9d
Onyx	54.8ab	24.8a	66.5abcd	27.2def	6.8d
President	53.2b	25.2a	66.2abcd	28.0cdef	6.8d
Raja	54.2ab	24.6a	65.9abcd	26.5f	6.9d
Rambo	55.4ab	24.4aa	65.8abcd	28.0cdef	6.8d
Ranger	55.0ab	24.3a	64.5cd	28.0cdef	6.5d
Rio grande	56.9a	24.7a	66.3abcd	29.4abc	3.9e
Sandokan	55.5ab	25.0a	66.5abcd	28.5bcdef	3.9e
TO 135	54.7ab	24.6a	65.4abcd	28.2cdef	9.5b
Tylka	57.1a	25.5a	68.2ab	31.1a	6.9d
Zara	54.6ab	24.2a	63.8d	26.9ef	6.7d
Grand means	55.5	25	66.1	28.6	6.8
LSD	2.7	2	2.8	1.7	0.6
CV %	9.5	15.7	8.3	12	17.9
P Value	0.3	0.7	0.1	<.001	<.001

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

Significant differences were observed in the growth parameters of various tomato varieties days after transplanting. The results showed significant variations in plant height among the varieties, with Rambo being the tallest at over 70 cm, while Cal j was the shortest. There were also significant differences in root lengths, with Rambo having longer roots compared to the other varieties (Table

4.24). Furthermore, yield output was also significant, with the varieties reporting different scores. Big rock for instance had the highest score of over 9 kg, while Rio grande had the lowest of less than 3.6. Finally, Rambo had the highest weight both in fresh and dried form against other varieties that were tested in the Mwea region.

Table 4. 24: Growth parameters scores of bacterial wilt of tomato management in the Mwea region

Varieties	Plant height(cm)	Root Length(cm)	Fresh weight(kg)	Dry weight(kg)	Yield (kg)
Assila	69.1ab	29.8abcd	78.7ab	29.6abc	6.7cd
Bashier	63.9cde	28.0bcd	72.9cde	26.2def	6.8cd
Big rock	66.6abcd	29.6abcd	77.4abcd	27.4cdef	9.9a
Cal j	57.8f	23.5f	67.9f	25.0f	4.1e
Commando	68.3abc	30.0abc	78.3ab	30.9ab	7.3c
Crown	65.7abcd	28.6abcd	74.6bcd	27.7cdef	8.3b
Nyati	67.9abc	29.6abcd	77.5abcd	27.8cdef	6.4d
Nyota	65.4bcd	28.4bcd	75.9bcd	27.9cde	6.2d
Onyx	65.3bcd	28.1bcd	75.5bcd	27.4cdef	6.0d
President	59.6ef	24.9ef	67.9f	25.5ef	6.6cd
Raja	67.9abc	29.2abcd	78.9ab	29.2abc	6.5d
Rambo	70.5a	31.4a	81.3a	31.5a	6.7cd
Ranger	68.2abc	30.4ab	77.8abc	28.7bcd	6.1d
Rio grande	67.3abc	29.3abcd	77.3abcd	29.0abcd	3.6e
Sandokan	67.5abc	29.7abcd	77.8abc	28.6bcd	3.8e
TO 135	64.0bcde	27.6bcd	74.1bcd	28.1bcde	8.9b
Tylka	62.3de	27.0de	72.5def	27.1cdef	6.4d
Zara	63.4cde	27.2cde	74.8bcd	28.5bcd	6.3d
Grand means	65.6	28.5	75.7	28.1	6.5
LSD	4.2	2.4	4.3	2.4	0.7
CV %	12.7	16.8	11.3	17	21.3
P Value	<.001	<.001	<.001	<.001	<.001

Values with similar letter (s) are not significantly different in each column at ($p \leq 0.05$); LSD=Least Significant Difference at ($p \leq 0.05$); CV%=Coefficient of Variation

CHAPTER FIVE: DISCUSSION

5.1 Status of bacterial wilt in Kajiado and Kirinyaga counties

5.1.1 Knowledge of tomato bacterial wilt among farmers in Oloitoktok and Mwea regions

The findings of the current study indicates that the majority of the farmers in the Oloitoktok and Mwea regions were able to identify bacterial wilt of tomatoes in their farms. This could be due to the disease being common and destructive affecting tomato plants worldwide. The disease is caused by a bacterium called R. *solanacearum*, which attacks the vascular system of tomato plants and causes yellowing, wilting, and untimely death of the plant (Manda *et al.,* 2020). Since tomato plants are widely grown by farmers, many of them have encountered this disease and learned to recognize its symptoms. Additionally, the disease is known to spread quickly, so farmers are often motivated to learn how to identify and manage it in order to protect their crops. This is consistent with the findings of (Nuwamanya *et al*., 2023). At least less than ten percent of the famers in both regions were unable to identify the disease and this lack of knowledge is due to the farmer's ignorance about crop diseases and their importance (Khurana and Kumar, 2021; Mamphogoro *et al*., 2020). Most farmers engage in tomato farming for commercial purposes and do not want to incur costs and losses due to the disease, which has a prevalence of over 90% a finding that corresponds to Kariuki *et al*., 2020.

According to the study conducted by Kones *et al.,* (2020), the bacterial wilt of tomatoes was found to be significant in both regions and this suggests that the disease occurred in both areas. For example, in Mwea, the disease was of great importance as tomato farming has been practiced there for a long time, making it a persistent problem unlike in Oloitoktok where it was not considered a significant issue. These importance of the disease depended on several factors, such as the prevalence of the disease in the region, the economic importance of tomato production and the availability of management strategies. For instance, in Mwea region, tomato production is a vital source of income for farmers and since bacterial wilt is prevalent and causing significant yield losses, then it would be of high importance as compared to Oloitoktok region.

Studies indicate that bacterial wilt of tomatoes has been observed for over 50 years in Kenya (Kariuki *et al*., 2020). For instance, in both Oloitoktok and Mwea, farmers have observed the disease at equal measures of production for a period of six years. This is a result of tomato farming

for the last 5 years and being a mono-crop on the farm (Nuwamanya *et al*., 2023). Additionally, the results show that tomato farming has been practiced in Mwea for a long time as compared to Oloitoktok. The area community in Oloitoktok has been attributed as pastoralists and not involved in tomato farming, as revealed by the study. Furthermore, the findings suggest that farmers in Mwea have been engaging in crop rotation with other crops such as rice, bananas, and onions, which has reduced the inoculum levels.

According to studies carried out by Montemayor and Frischmann (2014), the source of seeds used to start a tomato crop is important and should be pathogen-free. Chemical and biological techniques were used to achieve this. The fact that bacterial wilt is not as severe in Oloitoktok is due to the fact that the majority of tomato producers utilize certified seeds from agro-shops. Farmers in Mwea, however, had access to a variety of seed sources, some of which they chose to use which increased the severity of bacterial wilt. The usage of various seed sources according to some farmers in Mwea is due to inadequate capital and therefore, they save seeds from their previous harvest. Furthermore, farmers in Mwea believe that saved seeds produce better yields than market seeds, which they find to be less productive. Farmers in both regions opted to use seedlings although there was a significant difference. The majority of farmers in Oloitoktok did not prefer seedlings because they wanted to avoid diseased seedlings from the nurseries which were not certified. The results are in tandem with those of Fredrick *et al.,* (2022). Farmers in Mwea decided to use seedlings because of the time and money saved unlike using seeds.

The study examined different management practices that have been observed in both regions, where rouging has proven to be an effective method for controlling the disease. Similarly, Kago *et al,* (2017) found that rouging was an effective method for managing bacterial wilt in tomato fields. This approach is preferred by the majority of farmers, as once a plant is affected and wilts, the chances of survival are minimal. The study also found that most farmers opted to leave the affected plant in place, as removing it provided no significant benefit. Chemical application, on the other hand, was an expensive practice that most farmers in Mwea could not afford as indicated by Muthoni *et al.,* (2012), only a few farmers in Oloitoktok were able to use chemicals to manage the disease.

Crop rotation comes with several benefits among them reducing disease inoculum in tomato fields. Research carried out by Ayana and Fininsa, (2017), found that practicing crop rotation in tomato

fields led to a declining trend in the bacterial inoculum with time. Crop rotation was a common practice among farmers, particularly in Mwea. This was due to the favorable climatic changes that occurred in the region, allowing for the planting of various food crops. The practice helped improve soil fertility and reduce inoculum levels in the soil. The farmers in Mwea observed the benefits of crop rotation when they planted multiple crops, including tomatoes, chilies, and capsicum in their greenhouses. Mono cropping was heavily practiced in Oloitoktok as most of the farmers' fields were rented mainly for commercial purposes.

Tomato farming majorly takes place in two production systems, Open fields, and a greenhouse. Most of the farmers in Kirinyaga and Kajiado counties plant their tomatoes in the open field (Thomas *et al.*, 2020). Farmers in Mwea have adopted both production systems as the farmers have smaller sizes of land. In Oloitoktok, the open field system was entirely adopted due to the large sizes of unused lands, which favored the open field system.

5.1.2 Isolation and identification of *Ralstonia solanacearum* in Mwea and Oloitoktok regions

Ralstonia solanacearum was identified in two regions, Mwea and Oloitoktok (Rostand *et al.*, 2018). Both regions had significant levels of Rs, but the levels in Mwea were higher than those in Oloitoktok. This indicates that the farmers in Mwea had been involved in tomato farming for a considerable amount of time, causing their soils to accumulate a significant amount of inoculum compared to Oloitoktok, which had low levels of Rs. Additionally, most of the tomato farms in Oloitoktok had been unused for a long time, making them less susceptible to soil disease.

Furthermore, despite having large farm sizes, the farmers in Oloitoktok were meticulous about keeping their fields clean and kept records of their crop management from start to finish, something that was not widely practiced in Mwea. It was also observed that most Mwea farmers were ignorant about the disease, with many believing that crop rotation was a sufficient method of disease management, which was not the case as the results showed high levels of inoculum compared to Oloitoktok. Finally, it was noted that most Mwea farmers were borrowing farm equipment without sanitizing them before use, which could contribute to the spread of *Ralstonia solanacearum*.

5.1.3 Pathogenicity of *Ralstonia solanacearum* on five tomato varieties

The pathogenicity test conducted in a greenhouse revealed a positive in incidence and severity of five tomato varieties. Even though both hybrid and non-hybrid varieties were tested, all the varieties showed the presence of Rs. This suggests that the inoculum taken from the fields in Mwea and Oloitoktok was effective, and wilt symptoms were observed in all five varieties, indicating the presence of the disease.

5.1.4 Susceptibility of newly introduced tomato cultivars to bacterial wilt

Screening for bacterial wilt was conducted on newly introduced tomato cultivars, and the results revealed that the majority of the cultivars displayed significant levels of tolerance and resistance to the disease. Specifically, when these varieties, which the company had referred to as tolerant and resistant to bacterial wilt of tomato, were inoculated with Rs, all varieties showed some level of susceptibility, although the degree of infection varied (Wang *et al.*, 2019). The study demonstrated that certain cultivars exhibited varying degrees of tolerance to bacterial wilt, while others were susceptible. This was measured by the time it took for a plant to wilt and die, as well as the appearance of infected leaves that turned yellow due to the presence of the Rs inoculum (Yan and Khan, 2021). Growth parameters were also evaluated, and the results indicated relative effects that were attributed to the inoculum. For example, the majority of the plants showed stunted growth and produced low yields, which was unexpected for high-yielding tomato varieties.

5.1.5 Efficacy of *Bacillus subtilis* in managing bacterial wilt in tomatoes

After undergoing screening, the eighteen varieties planted in the green house, Oloitoktok, and Mwea were treated with a bio control agent to manage bacterial wilt in tomatoes. The results indicated that the bio control agent was effective in all locations, as the majority of the tomato varieties displayed significant differences. Findings that conferred with the studies of (Peng *et al.*, 2016). These were attributed to the colonization of *Bacillus subtilis* on the roots of the tomato, particularly the ones inoculated in the greenhouse and those with natural inoculum in the field. The incidence and severity of the disease also showed significant differences, indicating that the bio control agent is an effective method of managing the disease. The growth parameters, such as yield, were also influenced, resulting in increased produce.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, the study shows that tomato bacterial wilt is a significant challenge for farmers in Kenya, particularly those in Oloitoktok and Mwea regions. However, many farmers lack knowledge about the disease and its management. The study suggests that this may be due to farmers' focus on commercial tomato farming and ignorance of crop diseases.

Bacterial wilt incidence and severity varied among the tested cultivars in the fields and under greenhouse management. Disease incidence and severity was higher under greenhouse conditions than in farmers' fields. Big rock, TO 135, Rambo, Ranger and Crown were tolerant to bacterial wilt in farmers' fields at Kirinyaga and Kajiado regions, and under greenhouse management.

The study findings indicate that the utilization of a bio control agent has proven effective in controlling bacterial wilt. The crops treated with *Bacillus subtilis* showed a remarkable reduction in disease incidence and a substantial increase in yield. Additionally the bio control agent displayed remarkable abilities in promoting plant growth and accumulating biomass. *Bacillus subtilis* demonstrated promising potential as a component of an integrated approach to manage bacterial wilt in the two regions where the disease poses as a significant obstacle to production.

6.2 Recommendations

i. Big rock, TO 135, Rambo, Ranger and Crown had the least severity of bacterial wilt and therefore more research can be done on the productivity so that they can be adopted by farmers as a way to reduce severity of bacterial wilt of tomatoes.
ii. Tomatoes treated with *Bacillus subtilis* recorded bacterial wilt less severely, farmers should adopt the treatment of tomato seedlings with Bacillus so that their crop is protected.
iii. Further research is needed to determine the most effective and practical strategies for using *Bacillus subtilis* in tomato production systems.

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APPENDIX 1. QUESTIONNAIRE

BACTERIAL WILT ON TOMATOES SURVEY QUESTIONNAIRE IN KAJIADO AND KIRINYAGA COUNTIES

A) Personal details

Name of Farmer/Respondent: .

Date of interview: . Gender: Male ☐ Female ☐

Relation to farm: owner ☐

Manager ☐

Employee ☐

Other ☐

B) Information on area

County . Sub County .

Location _____ Village _____

Agro ecological zone . Latitude: _____

Longitude _____ Elevation _____

C) General Production information

1. What is the total size of farm (Acre)

0 –2 acres ☐

3- 6 acres ☐

7 – 9acres ☐

> 10 acres ☐

2. What size of farm is used for tomato production?

0 – 2 acres ☐

3- 6 acres ☐

7 – 9acres ☐

> 10 acres ☐

3. How long have you been producing tomatoes? (months/years) _____

4. Do you grow tomatoes for household use or for selling?

Household ☐ Selling ☐

5. Do you use seedlings or seeds?

Seedlings ☐ Seeds ☐

6. What is your source of seeds?

Own saved seeds ☐ Local Market ☐

Neighbors ☐ Agro-shops ☐

Any other ☐ Specify .

7. What is your source of seedlings?

Own seedlings Local Market ☐

Neighbours Commercial Nursery

Any other Specify.

8. Give reasons for your sources of seeds/seedlings?

9. Which tomato variety (s) do you grow and reason?

Tomato variety	Reasons

10. Which systems of tomato production do you use?

(i) Open Field ☐

(ii) Green house ☐

(iii) Both ☐

11. What type of medium do you use?

(i) Soil

(ii) Cocoa peat

(iii) Vermiculite

(iv) Any other Specify .

12. Do you sterilize the medium used?

(i) Yes (ii) No

13. Do you practice rotation of the greenhouse?

(i) Yes (ii) No

14. Where do you plant your seedlings?

(i) Potting pots

(ii) Direct in the ground

(iii) Other Specify.

15. Which Implements do you use in land preparation?

(i) Tractor (ii) Animal drawn (iii) Hoe (iv) Any other Specify .

16. What is the source of your water?

(i) Harvested rainfall ☐

(ii) Borehole ☐

(iii) Dam ☐

(iv) River ☐

17. What method of irrigation do you use?

(i) Sprinkler ☐

(ii) Drip ☐

(iii) Furrow ☐

(iv) Basin ☐

Any other Specify .

18. What kind of fertilizers do you use?

(i) Compost manure ☐ (ii) Farmyard Manure ☐ (iii) Green manure ☐

(iv) Inorganic fertilizer ☐

19. What are the main sources of information on tomato production?

(i)Extension officer ☐ (ii) Magazines ☐ (iii) Radio/Tv ☐

(iv)Any other ☐ Specify .

D. <u>Production Challenges</u>

20. What are the main challenges of tomato cultivation beginning with the most challenging?

Challenges	Importance (Major/ Minor)

21. Which diseases do you know that affect tomatoes and how do you manage them?

Diseases	Rank of importance	Management methods

22. Which pests do you know that affect tomatoes and how do you manage them?

Pests	Rank of importance	Management methods

E. <u>Bacterial wilt</u>

23. Have you ever seen Bacterial wilt (pictogram) on your crop? (Show farmers pictures of diseased plants). Yes No

24. If yes for how long has it been a challenge?

0 – 2 years ☐
3- 6 years ☐
7 – 9 years ☐
> 10 years ☐

25. How serious is the disease affecting the tomato?

(i) Important (ii) Moderately important (iii) Not important

26. How does the bacterial wilt affect tomatoes?

Effect on the crop	

27. Which tomato varieties are more susceptible to the disease?

28. How do you manage Bacterial wilt?

Management methods	Reasons

29. Which is your most preferred management method and why?

30. Do you practice crop rotation?

Yes No

31. If yes which crops do you rotate tomatoes with?

32. Are you willing to use certified seeds from the agro-shops if available? Yes No

If YES, Why?

F. FIELD OBSERVATION

Assessment of the management of the farm and the agronomic practices on tomato production.

Practice	**Tick (done)**	**Score**
Presence of diseases and pests	**Tick if present**	**Score (incidence/ severity)**

Rank the Following Constraints in Order of Importance in Tomato Production

Constraint	**Rank**	**Propose a solution**

Thank you

APPENDIX I1. : PHOTO card

Bacterial wilt in Tomato

Early blight on tomato leaves

Late blight on leaves

Blossom end rot on tomato fruit

Blossom end rot on unripe tomatoes

Damping-off on tomato seedling

Verticillium wilt on tomato plant

Tomato yellow leaf curl virus

Some of Tomato pests

Tuta absoluta effect on tomato

Tuta absoluta on tomato fruit

White flies