POTENTIAL OF HOST RESISTANCE AND EFFICIENT WATER USE IN MANAGEMENT OF BACTERIAL WILT OF TOMATOES IN KAJIADO COUNTY

GRACE DAMA CHENGO

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

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This Thesis is submitted with our approval as University Supervisors

Prof. William M. Muiru

11/07/2023

Signature………………. Date……………………

Department of Plant Science and Crop Protection

University of Nairobi

Prof. W. E. Mutitu

11//07/2023

Signature………………. Date……………………

Department of Plant Science and Crop Protection

University of Nairobi

UNIVERSITY OF NAIROBI

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Name of Student GRACE DAMA CHENGO

Registration Number A56|36126|2019

College COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES

Faculty/School/Institute FACULTY OF AGRICULTURE

Department PLANT SCIENCE AND CROP PROTECTION

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This work is dedicated to my beloved son Williams Chengo, my brothers Osca Charo and Ngao Yeri for their moral support in my academic pursuit.

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ABBREVIATIONS AND ACRONYMS

CABI	Centre for Agriculture and Bioscience International
AVRDC	Asian Vegetable Research and Development Centre
AEZ	Agro-ecological zone
KEPHIS	Kenya Plant Health Inspectorate Services
ANOVA	Analysis of Variance
CFU	Colony forming units
Cm	Centimeter
DI	Disease Index
F1	First filial generation
FAO	Food and Agriculture Organization of the United Nations
FC	Field capacity
GMC	Gravimetric moisture content
HCD	Horticultural Crops Directorate
KALRO	Kenya Agricultural and Livestock Research Organization
Ml	Milliliter
Mm	Millimeter
NPSWS	Number of plants showing wilt symptoms
RCBD	Randomized Complete Block Design
TNPPT	Total number of plants per treatment
VMC	Volumetric moisture content
WHO	World Health Organization

GENERAL ABSTRACT

Tomato (*Solanum lycopersicum*) is an important vegetable consumed for its nutritional value in Kenya and around the world. Bacterial wilt disease caused by *Ralstonia solanacearum* is a major production constraint in tomato that cause an estimated yield loss of 35% to 90% in the field and greenhouse. Current management methods used to manage bacterial wilt are inadequate and they pose safety risks to the environment and human health. The objectives of this study were (i) To determine the reaction of common tomato varieties to infection by bacterial wilt disease, (ii) To assess the effect of watering regimes on bacterial wilt on selected tomato varieties in the greenhouse and (iii) To determine the effect of different irrigation systems on incidence and severity of selected tomato varieties infected with bacterial wilt disease. Greenhouse and field experiments were carried out at Kabete Field Station University of Nairobi and at Isinya in Kajiado County-Kenya respectively in the year 2021 and 2022. For the first objective, eighteen tomato varieties were screened against *R. solanacearum* and the experiment was laid out in a randomized complete block design (RCBD) with 3 replications. In the second objective, the experiment was laid out in a completely randomized design (CRD) with three varieties namely Big rock F1, Assila F1 and Riogrande subjected to three moisture levels: 50% field capacity(FC), 100%FC and 120%FC. For the third objective, field experiments on drip and furrow irrigation systems were laid out in a RCBD with 4 replications. For the greenhouse experiments plants were inoculated with *Ralstonia solanacearum* at a concentration of 1×10^8 cfu/ml two weeks after transplanting while disease assessment was done eight days after inoculation. Bacterial wilt incidence, severity, stem browning, growth and yield parameters were assessed. Data collected was subjected to analysis of variance and means separated by Protected Fishers Least significance difference at 5% probability. Incidence and severity of bacterial wilt were highly significantly different ($P<0.001$) among the varieties screened. Non-hybrid varieties Riogrande, Isisementi and Rionix were highly susceptible to bacterial wilt with high incidence and severity scores of disease index (DI) 0.61-0.9. Kilele F1 variety had the lowest incidence andseverity score of bacterial wilt and was found to be highly resistant to bacterial wilt with DI of
0.18. Terminator F1, Big rock F1 and Bravo F1 were resistant with a DI of 0.21 - 0.3. Inoculation of *R. solanacearum* to the eighteen varieties had significant effect on stem browning and growth parameters compared to the non-inoculated varieties. Big rock F1, Assila F1and Riogrande F1 had lowest severity scores of 0.83, 1.77, and 2.26 and lowest incidences of 35.4%,

58.3% and 77.1% at 50% field capacity (FC) respectively. At 100%FC, severity score and incidence were lowest in Big rock F1 with 1.04 and 41.7% and highest in Riogrande with 2.94 and 85.4% respectively. Highest incidence was recorded at 120% FC from Riogrande variety with 91.7%. On field experiments, lowest incidences were recorded in Big rock F1 and Big rock F1 with Chemical treatment (Brono pol) with 17.3% and 17.2% under drip and furrow irrigation systems respectively. Highest incidence was recorded on Riogrande variety with 44.5% in furrow irrigation and Riogrande with Chemical treatment with 38.8% under drip irrigation. Highest yields were obtained in drip irrigation on Big rock with chemical treatment with 78.2t/haon drip irrigation and 39.6t/ha on furrow irrigation while lowest yields were obtained from Riogrande variety with 44.3t/ha under drip irrigation and 16.2t/ha under furrow irrigation. The results showed that bacterial wilt disease is highly influenced by moisture levels in the soil. The type of irrigation system also influences the multiplication and spread of *R. solanacearum* in the soil. The findings showed that resistant cultivars had great positive impact in management of *R. solanacearum*. Therefore, the combination of resistant varieties with appropriate moisture undera suitable watering regime like drip can be incorporated in integrated disease management programs to manage bacterial wilt disease on infected fields.

CHAPTER 1: INTRODUCTION

1.1 Background information

Tomato (*Solanum lycopersicum L.,* syn. *Lycopersicon esculentum* Mill.*)* is one of the most important vegetables grown in many parts of Kenya and other areas of Eastern Africa (Sigei *et al.,* 2014). The major production areas of tomatoes in Kenya include Kirinyaga, Kajiado, Taita Taveta, Meru, Bungoma Counties and many other areas between 1150 and 1800 meters abovesea level (Avedi *et al*., 2022). Tomato is used for food and nutrition and most importantly as an income generating crop in peri-urban and high potential areas (HCD, 2015; Mbaka *et al.,* 2013; Waiganjo *et al.,* 2010). Globally, tomato production averages 38.2t/Ha while in Africa it is 16.1 t/Ha. China and Egypt remain the chief producers of tomato in the world and Africa respectively (FAOSAT, 2018). Diseases including late blight cause by *Phytopthora infestans*, bacterial wilt cause by *Ralstonia solanacearum,* early blight cause by *Alterneria solani*, fusarium wilt cause by*Fusarium oxysporum* f.sp. *lycopersici*, bacterial canker cause by *Clavibacter michiganensis*, and yellow leaf curl infection cause by *Tomato leaf curl virus* are the major tomato production constraints with losses of between 10-95% (Singh *et al*., 2014; Tahat and Sijam 2010; Jones, 2008).

Bacterial wilt caused by *Ralstonia solanacearum* is a damaging soil borne bacterium that causes death of tomatoes resulting to low yields and income earnings to farmers (Taylor *et. al*., 2011). During periods of high moisture conditions and high temperature, the pathogen causes yield loss of up to 35% to 90% (Singh *et al*., 2015). Once bacterial wilt infects the plant, it moves in to xylem vessels and multiplies to cause brown discoloration and rapid wilting and death of tomato plants (Vasse *et al*., 1995). Various strategies of managing bacterial wilt have been documented but still the crop succumbs to this pathogen posing a threat to tomato farming. The disease has a broad host range of up to 200 plant species (Grimault *et al*.,1994) and it can survive in soil, especially in deeper layers (Hsu, 1991). Wilting symptoms of tomato plants occur two to five days post-infection, and this depends on susceptibility level of the host, temperature and pathogen virulence (Jones *et al*., 1991). A study conducted showed that *R. solanacearum* relies on water to rapidly multiply and infect (Álvarez *et al*., 2010).

Kajiado is in the semi-arid region and bacterial wilt is a serious problem. Management of bacterial wilt by use of resistant varieties in infected fields and efficient water management options will be addressed in this study.

1.2 Statement of the problem

Bacterial wilt caused by *Ralstonia solanacearum* is the most devastating and widespread disease that destroys solanaceous crops including tomato, potato, chilli and eggplant (Pousier *et al*., 1999; Yabuuch *et al*., 1995). The pathogen has been grouped into five races based on variationof host range and five biovars based on metabolic properties (Alguthaymi *et al*., 2016). The disease causes serious losses on crops due to the pathogens' wide host range of more than 450 hosts and long survival period in the soil (Wicker *et al*., 2007). Spread of the pathogen has been effective through planting infected tomato seedlings in fields, contaminated irrigation water, recycling of irrigation water and seeds in the farm practiced by farmers to reduce productioncosts (Kanyua, 2018).

Soil fumigation, soil solarization and heating have been used to reduce inoculum concentration but lasts for a short term and is not adequate due to re-introduction of the pathogen through planting materials, irrigation water and crop handling by farmworkers in the field (Chellemi *et al*., 1997). Methods of irrigation such as furrow, surface, sprinkler and hose pipe have been reported to spread and increase build-up of the disease inoculum (Cabral *et al*., 2011).

Management strategies like field sanitation, use of pesticides, disease-free planting materials and crop rotation have been used but they are associated with chemical residual effects, susceptibility challenges and inadequate land for rotation. The sole application of each approach has been proven inefficient (Aslam *et. al*., 2017).

Due to the inability to manage the disease, tomato growers have continued to report brown discoloration inside the stem with rapid wilting and death of the crop resulting in serious losses**.** Many farmers have deserted their fields due to losses incurred of up to 90% and have ceased tomato farming because of this disease (Mallkirjun *et al*., 2008). This study will be carried out to evaluate tolerant varieties, effects of different water regimes and different methods of irrigation in the management of bacterial wilt on tomatoes.

1.3 Justification of the study

Tomato production is negatively affected by bacterial wilt of tomato. For instance, there are few studies that extensively established the level of tolerance of bacterial wilt in most preferred tomato varieties. Owing to the intense tomato production, the potential increase of losses due to severity and incidence of bacterial wilt of tomato is of great concern (Aslam *et al.,* 2017; Liu *et al.*, 2015). Alternative management approaches against bacterial wilt such as cultural, chemical and biological have been associated with challenges of inefficiency, human health complications and increasing the production cost (Latifah *et al.*, 2018; Perry and Wright, 2013; McManus *et al*.,2002). Adoption of tolerant tomato varieties would potentially address the challenge of bacterial wilt with minimal use of synthetic fungicides (Scott *et al.*, 2004), however, this requires identification of these varieties through screening. Given that bacterial wilt is a soil-bornepathogen, inoculum levels in the soil enough to induce symptoms are elusive despite studies indicating that inoculum levels of 10^7 -10^9cfu/ml has the potential of causing symptoms (Singh *etal.*, 2018; Aslam *et al.,* 2017). On the other hand, tomato production in Kajiado County heavily relies on furrow irrigation which is partly attributed to the spread of bacterial wilt. Similarly, there is minimal evidence relating water regimes in the soil with the spread of possible inoculum of bacterial wilt in tomato fields. Furthermore, association of water regimes in the soil and the bacterial wilt establishment and spread among the preferred tomato varieties is key to optimizingproduction resources (water) as well as yields. Therefore, this study seeks to develop a comprehensive soil water regime approach with the identified tolerant tomato varieties that would mitigate the spread and damages of bacterial wilt.

1.4 Objectives

1.4.1 Broad objective

To increase tomato production through effective and sustainable management of bacterial wilt.

1.4.2 Specific objectives

i. To determine the reaction of common tomato varieties to infection by bacterial wilt disease

ii. To assess the effect of watering regimes on bacterial wilt on selected tomato varieties in the greenhouse
iii. To determine the effect of different irrigation systems on incidence and severity of selected tomato varieties infected with bacterial wilt disease

1.5 Hypotheses

i. There is no significant difference on reaction of common tomato varieties to infection by bacterial wilt disease
ii. Different watering regimes in the soil have no significant effect on development and expression of *Ralstonia solanacearum* in the greenhouse
iii. Different irrigation systems have no significant effect on bacterial wilt development in the field

CHAPTER 2: LITERATURE REVIEW

2.1 Tomato origin and botany

Tomato plant, *Solanum lycopersicum L*. belongs to the family *Solanacea* together with other crops that include eggplant, pepper, potato, black night shade, chill, bell, tomatillo, tobacco and aubergine (CABI 2005). The origin of tomato plant is said to be in South America, the Andean region which presently covers parts of Colombia, Chile, Ecuador and Peru (CABI, 2005). Tomatoes were introduced in Kenya in the 16th Century during European arrivals at the coastal shores (Atherton and Rudich, 2012).

Tomato plant is classified as an annual plant that grows to a height of between one and three meters tall. It is a dicotyledonous plant exhibiting a tap root system and a branching stem style ofgrowth, with terminal buds at the tip that does the actual growth (Ricky, 1995).
When the terminal buds cease from growing due to flowering or pruning, the lateral buds overgrow into fully functional vines. Tomato plant has a weak stem that sprawls over the ground and vines on other plants. The vines have short hairs that contribute to the vining process. When the hair comes into contact with the ground and in the presence of moisture, it turns to roots (Ricky, 1995).

Tomato plant exhibits compound leaves that range from 10 to 25 cm long, odd-pinnate with 5 to 9 leaflets on petioles, each leaflet up to 8 cm long with serrated margins. The flowers have the anthers fused along the edges, forming a column around the eight pistil's style. The flowers are yellow in colour, 1-2 cm across with fine pointed lobes on the corolla. Tomato is a true fruit and classified as a berry. It emerges from the ovary of the plant after fertilization, the pericarp wall serves as the flesh. The berry contains hollow spaces full of seeds and moisture known as locularcavities (Ricky, 1995; Acquaah, 2008).

Tomato plant has two types of growth habit, it can be determinate or indeterminate. Side shoots are produced on the vines that end in a flower cluster in determinate tomato enabling the plant to grow to a height of between 90 to 120cm and yields are concentrated within 4 to 6 weeks during harvesting season. The indeterminate tomato grows and produces more vines and flower clusters during the growing season and may reach a height of 150 to 200cm (Ricky, 1995). The flowers are self-pollinating which is achieved through wind, insects and physical vibration of the plant. The plant is a warm season crop that grows in low medium area with supplemental irrigation during the off season. The plant is sensitive to frost and freezing temperature and this can result to death of the crop. Tomato can be cultivated in many types of soils ranging from sand to heavy clay. It prefers a soil that has a pH range of 6.0 to 7.0 and be well-drained, fairly good moisture holding capacity is ideal for growing tomato crop (Bawa, 2016).

2.2 Tomato production in Kenya

Tomato crop is grown in many parts across the Country, it is ranked second after potato in production. Tomato have gained their growth popularity especially after technology innovations of plastic houses and irrigation which has attracted hundreds of youths to venture into farming (Mbaka *et al*., 2013). The top growing Counties in Kenya are Kirinyaga, Bungoma, Kajiado, Makueni and Kiambu (HCD, 2015). The most practiced methods of watering regime during tomato growing are rain-fed agriculture and supplemental water application. Tomato in Mwea (Kirinyaga), Namelock (Loitoktok), and Kabaa in Machakos is grown under irrigation. Tomato farming has tremendously increased in recent years due to the use of high tunnels and greenhouses in production. However bacterial wilt has invaded these systems of production causing major yield loss of up to 50% making farmers to withdraw from tomato farming(KALRO, 2016).

In the past decade, tomato production has shown a gradual increase in both the area under cultivation and the harvested yield (Table 2.1). Most of the tomato produce is consumed locally (90%) while 10% is exported (Weirsinga *et al*., 2007). In 2018, tomato production was 21.2 t/ha, with the area harvested being 28,263 hectares yielding 599,458 tons (FAOSTAT 2018). In Kenya tomato growing is an attractive business that provides income generation to smallholder farmers, source of employment for many individuals and alleviating poverty in rural and peri urban areas (Mbaka *et al*., 2013).

Table 2.1: Tomato production in Kenya (2010-2018)

Year	Area harvested (ha)	Production (tons)	Tons/ha
2018	28263	599458	21.21
2017	27636	507142	18.35
2016	21921	410033	18.71
2015	19027	402513	21.15
2014	24531	443271	18.07
2013	23866	494037	20.70
2012	21874	444862	20.34
2011	20584	396544	19.26
2010	18477	539151	29.18

Source FAOSTAT, 2018

2.3 Tomato varieties

The source of seeds and seedlings of tomato is seed companies that include Simlaw seeds limited, East African limited, Kenya seed company limited, Greenlife Crop Protection, Amiran Kenya, Syngenta, Royal seed and Monsanto among others (Farmlink, 2017). These companies provide a wide range of tomato varieties to farmers and have met the demand of growers and consumer needs. Among the popular tomato varieties, Cal-J, Moneymaker and Riogrande are susceptible to bacterial wilt (Manani *et al*., 2020; Kathimba *et al*., 2018). The tomato varieties can be classified into open field varieties and greenhouse varieties. The open-pollinated field varieties include Riogrande, Cal-J, Moneymaker, Roma and Marglobe. Some of the greenhouse varieties include Kilele F1, Chonto, Anna F1- seminis, Tylka F1, Prostar F1, Eva, and Corrazon among others (KALRO, 2016). The popular tomato varieties that are grown by farmers are Riogrande, Cal-J, Anna F1 and these have substituted previous varieties such as Moneymaker, Fortune, Kentom, Neema, mansetRotade and Caltana that are not in favour with farmers (Weirsinga *et al*., 2007). Due to the surge of pests and disease, companies have come up with hybrid varieties that include Kilele F1, Anna F1, Rambo F1, Shanty, Nouvelle, Tropicana, and Nuru F1 that are high yielding and resistant to pests and diseases (KALRO, 2016; Monsato, 2013).

2.4 Nutritional and economic importance of tomato

Tomato as a fruit and vegetable is mostly consumed worldwide and its consumption can be raw or in several processed forms. The nutritional composition of tomato includes vitamins A and C, potassium, fiber and lycopene. Vitamin A helps in the division of cells, growth of bones and differentiation, respiration and maintaining surface lining of the eyes and helping in regulation of urinary and intestinal tracts. On the other hand, vitamin C helps in collagen formation which in turn provides structures to bones, cartilage, muscle and blood vessels (https://www.agrisupportonline.com/Articles/importance_of_the_tomato.htm). Tomato produces carotenoids such as lycopene in considerably high amounts. The lycopene which is a natural antioxidant is known to reduce chronic diseases and cardiovascular diseases (Arab and Steck, 2000). As an antioxidant, it helps to prevent the development of various forms of cancer (Freedman and Reimers, 2011). Tomatoes contain nicotinic acids hence used as antiseptic agent which is useful in fighting viruses stimulating blood flow and regulating cholesterol levels (Basu and Imrhan, 2007).

Tomato is a crucial dietary component, contributing to improved livelihoods of urban and rural people. Tomato fruit is sold in its fresh state but also can be processed into tomato paste, source and juice and can as well be dried. Tomato growing is an attractive business for smallholder farmers and a potential source of employment (Waiganjo *et al*., 2010).

2.5 Tomato production constraints in Kenya

Production constraints in tomato are both biotic and abiotic in nature. According to a study by Ochilo *et al.,* (2019), the major production limitations are insects 34%, fungi 23%, bacteria 13%, nutrient deficiencies 12%, mites 8%, viruses 3% and nematodes 2%. Major insects that cause damage include leaf miner, whiteflies, mites, thrips and aphids (Mwangi *et al.,* 2020; KALRO, 2016; Monsanto 2013). *Tuta absoluta* commonly known as leaf miner is the most devastating invasive insect that cause yield loss of between 50-100% in tomatoes. The caterpillar is known to mine inside the leaves, stems and green fruits and marketing of the commodity becomes a challenge when the insect is detected inside the fruit (KALRO, 2016).

Nutrient deficiencies, irregular watering and water logging are among the physiological disorders that cause remarkable loss on yield and quality of tomato. Lack of calcium in the plants causes blossom end rot (KALRO, 2016).

Tomato diseases of importance include late and early blight (*Alternaria solani*), fusarium wilt (*Fusarium oxysporum* f.sp *lycopersici*), bacterial wilt (*Ralstonia solanacearumdf)*, bacterial canker (*Clavibacter michiganensis* subsp. *Michiganensis)* and tomato spotted wilt (*Tomato spotted wilt virus*). Reports have shown that wilt disease caused by *R. solanacearum* can causeup to 100% yield loss in greenhouse tomato farming and 64% yield loss in open field tomato production (Mbaka *et al*., 2013). Management of the disease has greatly relied upon the excessive chemical application that results to disease resistance, health problems andenvironmental pollution.

2.6 Bacterial wilt disease

The causal agent of bacterial wilt is *Ralsonia solanacearum* an important pathogen of many solanaceaous crops (Tahat *et al*., 2012). The pathogen was initially reported for the first time on potato, tomato, tobacco and groundnuts in Asia, Southern USA and South America (EPPO, 2004). The disease exhibits a wide host range that include major crops such as potato, tobacco, pepper and eggplant (Champoiseau *et al*., 2009) The disease is known to be soilborne and is widely distributed in tropical and humid subtropical countries (Deberdt *et al*., 1999). In Kenya, bacterial wilt was initially reported in Embu County in 1945 in a potato farm. It is known to havebeen introduced in the area with contaminated seeds from Europe (Muthoni *et al*., 2012).Bacterial wilt has been reported in both highlands and lowlands regions in Kenya (Kago *et al.,* 2016).

Ralstonia solanacearum (Smith 1896), Yabuuch *et al*. (1995), is a gram negative, rod shaped and an aerobic bacterium that is 0.5-0.7 x 1.5-2.0 µm. It can survive for long periods in the soil, in plant debris, in asymptomatic weeds and in rhizospheres of non- host plants (Wenneker *et al*., 1999). The pathogen is disseminated through infected planting materials, infected farm tools and equipment, contaminated run off and irrigation water. It gains entry through the roots, on natural open wound or wounds made by root knot nematodes (Swansom *et al*., 2005). The bacteria move and colonizes the xylem, multiplies and forms tyloses that blocks the movement of water inducing wilting of the crop, yellowing and necrosis of the leaves and browning of the stem (Yabuuch *et al*.,1995; Swansom *et al*., 2005).

2.7 Bacterial wilt management in tomato

2.7.1 Phytosanitary and cultural practices

Cultural control methods include intercropping, intercropping green manure such as mung bean and crotalaria earlier followed by planting susceptible cultivars was found to effectively control bacterial wilt (Hartman *et al.,* 1993). Addition of poultry and farmyard manure in the soil increases the activity of microorganism which consequently inhibits the development of bacterialwilt (Islam and Toyota, 2004). Crop rotation for 5 to 7 years using non-host plants has given promising results in controlling bacterial wilt (Smith *et al*., 1995; Adebayo *et al*., 2009). Moreover, crop rotation is limited because the pathogen is known to survive for an extended period in the soil and the existence of a wide host range, alternate weed hosts such as black night shade (*Solanum nigrum*) and jimson (*Datura* spp) among other solanaceous volunteer plants (Fajinmi and Fajinmi 2010). Field and greenhouse practices have been put in place to reduce build- up of inoculum. They include rogueing, removal of plant debris and volunteer plants (Salamanca, 2015). Disinfection of tools and equipments and hand wash are important practices that can be used to manage the spread of the pathogen. Breeding resistant germplasm of tomato has proved to effectively control bacterial wilt (Persley, 1992).

2.7.2 Chemical control

Since 1960, chemical control methods have been used to control bacterial wilt to reduce yield loss of tomato (Yuliar and Toyota 2015). However, Hartman *et al.*, (1994) demonstrated that, controlling the pathogen is difficult due to the disease location inside the vascular tissues and pathogen ability to survive at deeper layers of the soil. Fumigation with chemicals and antibiotics such as tetracycline, penicillin, streptomycin and ampicilin have been reported to cause minimum suppression of the pathogen, however they are not widely used (Murakoshi andTakashi, 1984). Reports indicate that management of bacterial wilt by use of chemical has detrimental effects on human health, cause environmental pollution, are labor intensive and costly which highly discourages their use (Aslam *et al*., 2017; Hartman *et al.,* 1993).

2.7.3 Biological methods

Use of biological control agents to manage bacterial wilt has gained popularity over the years. A recent study by Yuliar and Toyota (2015) from the year 2005 to 2014 shows that 54% of research studies published had interest in management of bacterial wilt using biocontrol agents. Bacteria and fungi are the most dominant microbes making up biocontrol agents with 90% being bacteria and 10 % fungi (Yuliar and Toyota, 2015). Use of bacteriophages has been shown to inhibit *Ralstonia solanacearum* as demonstrated by Wall and Sanchez, (1992). The use of *Ralstonia picketii* QL-A6 a non-pathogenic organism in controlling bacterial wilt disease had a biological control efficacy of 73% in tomato as documented by Wei *et al*. (2013). Wei *et al*. (2011) found out that *Bacillus amyloliquefaciens* strains QL-5, QL-18 and inorganic fertilizerhad a biological control efficacy of 17% to 87% in controlling bacterial wilt disease in tomato. Bacillus isolate (CB64) was observed to reduce bacterial wilt incidence in the field by 53% and reduced *Ralstonia solanacearum* inoculation density in the soil by 93.17% (Kariuki *et al*., 2020).Fungi have been used to manage bacterial wilt disease, Masunaka *et al*., (2009) demonstratedthat *Pythium oligandrum* suppresses bacterial wilt by activating structural defense responses in the host plant. A study by Kariuki *et al*. (2020) demonstrated that Trichoderma *sp* isolate (T1) significantly reduce *Ralstonia solanacearum* (by 53%) in contaminated fields that were grown with tomatoes. However, biocontrol methods are faced with colonization inconsistencies and suppressing a narrow range of plant hosts or limited to one disease or pathogen. Therefore, they are mostly used as preventive because on their own they are insufficient to control diseases (Whipps, 2007).

2.8 Effects of soil moisture in the development of bacterial wilt

Water is an essential component in tomato farming and sufficient moisture is necessary during seedling, flowering and fruit enlargement stage. A full-grown tomato crop requires an approximate 550mm of water for optimum production (Ozbahce & Tari, 2010 and FAO, 1986). Bacterial wilt disease of tomato can cause yield loss of 35% to 90% under high moisture and high temperature conditions (Singh *et. al*., 2015). *Ralstonia solanacearum* inoculum is known to reduce at low moisture level (Weller *et al*., 2000), the size of the xylem vessels, epidermis and pits membrane are also reduced which leads to compaction of the xylem vessels therefore stopping the movement of *R. solanacearum* (Gupta *et al.,* 2018). Survival and multiplication of *Ralstonia solanacearum* is dependent on presence of moisture and temperature. Low moisture content in the soil reduces bacterial inoculum in the rhizosphere but it causes development of more lateral roots thereby increasing avenues for the pathogen to penetrate (Choudhary *et al.,* 2018). This makes the tomato plants to be potentially at risk of bacterial wilt infection but thelow soil moisture condition alters the composition, concentration and diffusion of plants root exudates that are associated in chemotaxis (Vasse *et al*., 1995). High temperature range of 30 to 35 ^{O}C increases the manifestation of the disease while temperatures lower than 20 ^{O}C is unsuitable for the manifestation of the disease (Singh *et al*., 2015).

2.9 Irrigation systems used in tomato farming

Arid and semi-arid areas of Kenya encounter water shortages for domestic and livestock consumption. Most of the smallholder farmers depend on rainfall to grow their crops, and with the pronounced climate change, which is being experienced all over the world, rainfall has become unpredictable, and seasons have changed. The demand for water has been on the rise and the available water sources should be used appropriately in these areas. Effective water utilization in the required amount at the appropriate time is important (Ozbahce and Tari 2010). Drip irrigation system offers numerous advantages over other irrigation system such as surface, furrow, localized and sprinkler irrigation (Ozbahce and Tari 2010). The drip system has uniform and precise emission of water and chemicals, improve yields and reduce evapotranspiration. It is also known to curb aerial diseases and phytophthora root rots that are commonly associated with furrow and sprinkler irrigation systems (Sanogo and Ji, 2013; Ristaino *et al.*, 1991). However, drip irrigation creates conducive environment for multiplication of soil borne diseases due to its localization method of application and elevates the severity of bacterial wilt disease while on the other hand furrow irrigation is associated with its spread in tomato plants as reported by (Cabral *et al.,* 2011; Marouelli *et al.,* 2005).

CHAPTER 3: REACTION OF COMMON TOMATO VARIETIES TO INFECTION BY BACTERIAL WILT DISEASE

3.1 Abstract

Climate change impacts food production through altering the climatic suitability of agricultural areas for crops, pests and associated natural enemies. Bacterial wilt caused by *Ralstonia solanacearum* is a major constraint in tomato production that is responsible for losses of up to 90% both in the open field and greenhouse conditions. The methods used to manage the disease are inadequate and pose health and environmental risks. Host resistance has potential to be effective in management of bacterial wilt. The current study evaluated eighteen tomato varieties for resistance to *Ralstonia solanacearum* in the greenhouse. The experiment was laid out in a randomized complete block design with three replications and varieties were inoculated with 1×10^8 cfu/ml of *R. solanacearum*. Severity of bacterial wilt was assessed 8 days after inoculation using a disease rating score of 0-4 to determine disease index (DI) of each cultivar, while the number of wilted plants was recorded and used to determine disease incidence. The severity and incidence of bacterial wilt was highly significantly different ($p < 0.001$) among the screened varieties. All non-hybrid varieties (Riogrande, Isisementi and Rionex) were highly susceptible to bacterial wilt with high severity and incidence scores of DI 0.61-0.9 compared to F1 tomato varieties. Among the F1's, Kilele had the lowest severity score and incidence of bacterial wilt and was found to be highly resistant to the disease with DI of 0.18. Terminator F1, Big rock F1 and Bravo F1 were resistant with a DI of 0.21-0.3. Eight F1's varieties were moderately susceptible with a DI of 0.41-0.5 while Onix F1 and Sifa F1 were susceptible to bacterial wilt with DI of 0.51-0.6. Inoculation of *R. solanacearum* to the eighteen tomato varieties had significant effect on growth and yield parameters and stem browning. Plant height significantly varied among the varieties while number of inflorescence and number of fruits per plant from theinoculated varieties was lower compared to their respective varieties not inoculated. Flowering initiation was delayed in cultivars that were inoculated with *R. solanacearum* than cultivars not inoculated. There was an increase in length and width of stem browning between the first and second month for all varieties, while the length of adventitious roots declined from the first and second month. Despite the climatic risks and impacts on tomato production, resistant cultivars can be recommended in the management of bacterial wilt for increased production and farmer incomes.

Key words: Bacterial wilt, cultivars, disease index, screening of tomato varieties, severity

3.2 Introduction

Tomato (*Solanum lycopersicum L.,* syn. *Lycopersicon esculentum* Mill.*)* is an important dietary component that is grown in many areas of Kenya (Sigei *et al*., 2014). The crop is used for food and most importantly as an income generating crop in peri-urban and high potential areas (HCD, 2015; Mbaka *et al*., 2013; Waiganjo *et al*., 2010). The nutritional composition of tomato includes vitamins A and C, potassium, fiber and lycopene and its regular usage is known to reduce the risk of cancer (Willcox *et al*., 2003). Tomato farming is constrained by pests and diseases,among them bacterial wilt disease caused by *Ralstonia solanacearum*. The soil-borne bacterium causes serious losses on crops because of its widespread host range of more than 450 hosts and long survival period in the soil (Taylor *et al*., 2011; Wicker *et al*., 2007). Bacterial wilt was reported to cause up to 64% losses in open fields and 100% in greenhouses (KALRO, 2016; Mbaka *et al*., 2013). Spread of the pathogen has been effective through planting tomato in infected soil, using infected tomato seedlings in fields, use of contaminated irrigation water, recycling of irrigation water and use of uncertified seeds in the farm often practiced by farmersto reduce production costs (Kanyua, 2018).

Management approaches against bacterial wilt such as cultural, chemical such as metam sodium, chloropicrin and biological have been associated with challenges of inefficiency, health complications and increasing the production cost (Latifah *et al.*, 2018; Aslam *et al*., 2017 ; Perry and Wright, 2013; McManus *et al*., 2002). According to Yuliar and Toyota (2015) introduction of tolerant varieties is the most effective and eco-friendly method to control bacterial wiltdisease. *Ralstonia solanacearum* resistant variety Hawii 7996 was developed by Wang *et al*. (2000) at the World Vegetable Centre. Kim *et al*. (2016) screened 285 tomato cultivars obtained from different parts of the world for resistance against bacterial wilt and found out that four genotypes were resistant. In Pakistan, evaluation of 30 varieties for resistance to the disease by Aslam *et al*. (2017) found out that only two varieties Lerica and Early king were resistant.

In Kenya, tomato hybrid varieties are majorly imported by private seed merchants and less attention is given to tomato improvement in the country (Kathimba *et al*., 2018). Manani *et al*.

(2020) evaluated six tomato varieties in the western region of Kenya and identified Heirloom Tall vine and Goliath pear varieties as being tolerant while the rest were susceptible. Adoption of tolerant tomato varieties would potentially address the challenge of bacterial wilt with minimum application of synthetic chemicals (Scott *et al.*, 2004). The present study aimed to identify tolerant tomato varieties through screening of hybrid and non-hybrid varieties commonly grown in Kajiado County in order to mitigate the spread, damages and yield losses caused by bacterial wilt disease.

3.3 Materials and Methods

3.3.1 Experimental site

This study was conducted in a greenhouse at Kabete site, University of Nairobi in Kenya. The area is located at an agro-ecological zone of upper midland zone three (UM3), on latitude 1º 15'South and longitude 36º 44' East at an altitude of about 1800m above sea level (Jaetzold, 2006). The mean daily temperature in the greenhouse was 18ºC between April and July 2021.

3.3.2 Isolation of *Ralstonia solanacaerum*

The bacterium, *R. solanacearum* was isolated from diseased plants that showed typical symptoms of bacterial wilt in the laboratory using Kelman's Agar medium (2,3,5 Triphenyl Tetrazolium Chloride) consisting of 10g bacto-peptone (Difco), 1g casamino acid, 5ml glycerol, 15g bacto agar (Difco),1000ml distilled water sterilized at 121ºC for 15 minutes (Kelman, 1954).After isolation, a single colony was purified and pathogenicity was conducted on a susceptible tomato variety Riogrande to confirm its virulence.

3.3.3 Tomato varieties collection

Eighteen tomato cultivars comprising 15 hybrids and 3 locals were obtained from seed companies and registered seed distributers. They included Stallion F1, Rambo F1, Kilele F1, Commando F1, Star F1, Danny F1, Bravo F1, Big Rock F1, Assila F1, Terminator F1, Gem F1, Sifa F1, Shanty F1, Onyx F1, Ranger F1, Isisementi, Rionex and Riogrande varieties. These varieties were selected because they are popularly grown by the farmers in Kajiado County.

3.3.4 Screening tomato varieties for resistance to *Ralstonia solanacearum*

Tomato seedlings were raised in a greenhouse in germination trays containing hygro-mix obtained from the local agrovets suppliers. The seedlings were watered as needed. The assessment of eighteen tomato varieties was carried out in the greenhouse. Polythene pots that measure 20.3cm by 35.6cm by 35.6cm were filled with pasteurized media composed of sand and soil in the ratio of 1:3 and each bag contained 6 kg of the media. Four weeks old seedlings of each variety were transplanted 3 per pot in 4 pots and then replicated three times. The experiment was laid out in a Randomized Compete Block Design Aslam *et al*. (2017), the designwas adopted because of shading effect caused by nearby greenhouses and trees during the day. *Ralstonia solanacearum* inoculum was prepared using nutrient agar media autoclaved at 121^{o}C for 15 minutes to prepare plates of pure cultures followed by incubation for 2 days at a temperature of 28^{o}C. The resultant growth of the pathogen was harvested by flooding the plate with sterile distilled water. Sterile L shaped glass rod was used to dislodge *Ralstonia solanacearum* from the plates and transferred to a conical flask to make a stock solution. One milliliter of stock solution was drawn and added to 9ml sterile distilled water in a universalbottle. The solution was mixed by shaking thoroughly and 1ml was drawn and transferred to a 9ml of sterile distilled water to make a dilution of 10^{-1}. This dilution process was repeated up to 10^{-9}. The last three dilutions were plated on the isolation media in triplicate. The concentration was obtained by multiplying the average number of colonies of each plated dilution with the reciprocal of the power (Singh *et al.,* 2018).

The inoculum concentration was adjusted to 1×10^{8}cfu/ml using pour plate method (Singh *et al*., 2018). One week after transplanting, seedlings of each variety were inoculated with 30mls of 1×10^{8}cfu/ml bacterial suspension through soil drenching. Pricking was done with a sharp scalpel blade on the roots before drenching to increase chances of penetration of the pathogen.

3.3.5 Agronomic practices

During transplanting 5 g of Diammonium Phosphate (DAP) was mixed with planting media in each pot. Seedlings were watered as per crop need and top dressed with Calcium ammonium nitrate (CAN) four weeks after transplanting followed by NPK (17:17:17) during flowering. Scouting of diseases and pests was done regularly and insect pests like *Tuta absoluta* and whiteflies were controlled using Coragen active ingredient: Chlorantraniliprole (Rynaxypyr)200g/l. Diseases such as early and late blight were controlled by spraying Milraz 76 WP with active ingredients: Propinep 700g/kg and Cymoxanil 60g/kg and Ridomil Gold MZ 68WG with active ingredients: Metalaxyl-M 40g/kg and Mancozeb 640g/kg.

3.3.6 Diseases assessment and data collection

3.3.6.1 Disease severity

Data collection started when the first wilting symptom was observed, this was three weeks after inoculation. Data on severity was collected based on a modified key described by Uwamahoro *et al.* (2018). Severity rating score: 0 – no symptoms, 1— one or two young leaves wilted, 2 – half of all the leaves wilted, 3 – almost all the leaves wilted, 4 – dead plant. Disease index was estimated by the formula described by Aslam *et al*. (2017). Data was collected every week for nine weeks.

$$\text{Disease Index} = \frac{\begin{array}{c}((\text{No.of plants at disease rating score"0"} \times 0) + \\ (\text{No.of plants at disease rating score"1"} \times 1) + \\ (\text{No.of plants at disease rating score "2"} \times 2) + \\ (\text{No.of plants at disease rating score "3"} \times 3)) + \\ (\text{No.of plants at disease rating score "4"} \times 4))\end{array}}{\text{Total number of plants observed} * 4}$$

3.3.6.2 Disease index reaction

The disease index reaction was used as described by *(Aslam et. al., 2017)*

0.00–0.2 Highly resistant

0.21–0.3 Resistant

0.31–0.4 Moderately resistant

0.41–0.5 Moderately susceptible

0.51–0.6 Susceptible

0.61–0.9 Highly susceptible

3.3.6.3 Area under disease progress curve (AUDPC)

Area under disease progress curve was calculated from the formula described by Simko *et al*. (2012)

$$\text{AUDPC} = \sum_{i=1}^{k} [(\text{SCBW}_i + \text{SCWB}_{i+1})(\text{t}_{i+1} - \text{t}_i)]/2$$

Where SCBW is severity score of bacterial wilts, t is time and *i* is the *i*th observation

3.3.6.4 Bacterial wilt incidence

Disease incidence was determined by counting the number of wilted plants per pot after every week for nine weeks. Percentage of bacterial wilt incidence was calculated per treatment by the formulae described by (Ayana *et al*., 2011).

$$WI = NPSWS/TNPT * 100$$

Where WI-wilt incidence, NPSWS-number of plants showing wilt symptoms and TNPT- total number of plants per treatment

3.3.7 Data collection on growth and yield parameters

Data on height was measured using a string and a meter ruler from the base of the plant to the apex of the plant after every two weeks. Stem browning data was collected by selecting three plants per treatment, the stems were dissected using a sharp scalpel. Browning of stem lengthand width were measured using a meter ruler twice during the growing season at thirty and sixty days after inoculation. Number of inflorescences, flowering initiation and number of fruits per plant were recorded three times at intervals of two weeks. Flowering data was done by counting the number of plants that had flowered per pot while number of inflorescences and number of fruits was determined by counting for each individual plant per pot (Traore *et al*., 2020; Manani *et al*., 2020).

3.3.8 Data analysis

Data on severity and incidence was subjected to analysis of variance using R statistical software version 4.1.2 and means were separated by Tukey's Honest Significant test (HSD). Data on growth and yield parameters namely, length of browning, flowering initiation, stem browning and length of adventitious roots were subjected to analysis of variance using GenStat software version 15th edition and means were separated by Fishers' Protected Least Significant Difference. For plant height, number of inflorescences and number of fruits the means were separated using t-test with ($P<0.05$).

3.4 Results

3.4.1 Response of tomato cultivars to *Ralstonia solanacearum*

3.4.1.1 Severity of bacterial wilt

The response of tomato varieties after inoculation with *R. solanacearum* showed significant differences in severity rating scores. Five varieties namely Kilele F1, Terminator F1, Bravo F1, Big Rock F1 and Ranger F1 were categorized into the following groups depending on their severity reactions; i) Kilele F1 was highly resistant (HR), ii) Terminator F1, Bravo F1 and Big Rock F1 were resistant (R) and iii) Ranger F1 was moderately resistant (MR) to *R. solanacearum.* Wilting was expressed in KileleF1, Terminator F1 and Bravo F1 at week 6 while in Big rock F1 and Ranger F1 at week 5 (Table 3.1). These five varieties had significantly low area under disease progress curve AUDPC with a (DI) of less than 0.32 (Table 3.2).

Out of the 18 varieties screened, 3 varieties namely, Riogrande, Isisementi and Rionex began wilting as early as week 2 (Table 3.1)**.** These varieties were also noted to have significantly high AUDPC with a (DI) ranging from 0.61 to 0.9 and were grouped as highly susceptible to *R. solanacearum*. Majority of the screened varieties (55%) were observed to be moderately susceptible and susceptible with disease index (DI) of 0.41-0.5 and 0.51-0.6 respectively. Riogrande, Rionex and Isisementi emerged to be highly susceptible with a disease index ranging from 0.61-0.7 (Table 3.2).

Table 3.1: Severity rating scores of bacterial wilt of eighteen tomato varieties evaluated under greenhouse conditions

	Weeks after inoculation								
Variety	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Assila F1	0.15a	0.65abc	0.79abcd	1.20bcdefg	1.79bcdefg	2.61abcde	2.85abc	3.01abcd	3.06abc
Big rock F1	0.04a	0.13bc	0.21cd	0.44efg	1.34defg	1.57cde	2.05bc	2.29cd	2.48bc
Bravo F1	0.00a	0.00c	0.00d	0.12g	0.74efg	1.96bcde	2.46abc	2.68abcd	2.72abc
Commando F1	0.11a	0.55abc	0.96abcd	1.54abcdefg	1.88bcdefg	2.68abcde	3.16ab	3.22abc	3.21abc
Danny F1	0.04a	0.85abc	1.80ab	2.33abc	2.83abc	3.38ab	3.52ab	3.52abc	3.67ab
Gem F1	0.08a	0.25bc	0.72abcd	1.29bcdefg	2.00bcde	3.07abc	3.32ab	3.42abc	3.42ab
Isisementi	0.42a	1.83a	2.25a	2.88a	3.58a	3.74a	3.86a	3.86ab	3.86ab
Kilele F1	0.00a	0.07bc	0.08cd	0.17g	0.45g	1.21e	1.45c	1.66d	1.80c
Onyx F1	0.11a	0.48abc	1.17abcd	1.76abcdef	2.38abcd	2.93abcd	3.55ab	3.48abc	3.67ab
Rambo F1	0.00a	0.23bc	0.32bcd	0.68defg	2.29abcd	2.97abcd	3.32ab	3.47abc	3.53ab
Ranger F1	0.04a	0.17bc	0.32bcd	0.50efg	1.19defg	2.27abcde	2.53abc	2.76abcd	2.86abc
Riogrande	0.31a	1.20abc	1.39abcd	1.93abcde	3.04abc	3.48ab	3.70a	3.79ab	3.87ab
Rionex	0.56a	1.42ab	1.63abc	2.54ab	3.25ab	3.63a	3.90a	3.95a	4.00a
Shanty F1	0.11a	0.35bc	0.64bcd	0.96cdefg	2.08bcde	2.89abcd	3.37ab	3.56abc	3.62ab
Sifa F1	0.38a	1.34ab	1.80ab	2.07abcd	2.10abcde	3.17ab	3.28ab	3.36abc	3.28abc
Stallion F1	0.00a	0.25bc	0.42bcd	0.79defg	1.58cdefg	2.67abcde	3.14ab	3.43abc	3.48ab
Star F1	0.04a	0.51abc	0.55bcd	1.08bcdefg	1.95bcdef	2.81abcde	2.86abc	3.29abc	3.29abc
Terminator F1	0.00a	0.19bc	0.19cd	0.27fg	0.48fg	1.50de	2.07bc	2.44bcd	2.50abc
Mean	0.13	0.58	0.84	1.25	1.94	2.69	3.02	3.18	3.23
C.V (%)	139.5	75.6	59.8	38.9	24.9	18.7	16.9	15.2	15.2
LSD	0.58	1.36	1.55	1.50	1.49	1.54	1.57	1.49	1.51
P-value(p<0.05)	0.0103	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Means in the same column followed by the same letter are not significantly different. Means were separated using Tukey's Honest significant Test (P<0.05).

Table 3.2: Disease index reactions to bacterial wilt and area under disease progress curve of eighteen tomato varieties.

Tomato Varieties	Type of Variety	Disease Index	Reaction	AUDPC
Kilele	F1	0.18	Highly resistant	225.67h
Terminator	F1	0.26	Resistant	330.00gh
Big Rock	F1	0.27	Resistant	365.50fgh
Bravo	F1	0.29	Resistant	364.00fgh
Ranger	F1	0.32	Moderately resistant	395.33efgh
Stallion	F1	0.41	Moderately susceptible	557.17defgh
Assila	F1	0.42	Moderately susceptible	573.17defg
Star	F1	0.43	Moderately susceptible	604.00cdefg
Rambo	F1	0.44	Moderately susceptible	600.50cdefg
Shanty	F1	0.46	Moderately susceptible	643.50cdefg
Gem	F1	0.47	Moderately susceptible	679.50cdef
Commando	F1	0.47	Moderately susceptible	709.50bcde
Danny	F1	0.48	Moderately susceptible	847.67abcd
Onyx	F1	0.52	Susceptible	796.33abcde
Sifa	F1	0.55	Susceptible	678.17cdef
Riogrande	Non hybrid	0.61	Highly susceptible	927.50abc
Rionex	Non hybrid	0.68	Highly susceptible	1058.33a
Isisementi	Non hybrid	0.72	Highly susceptible	1019.67ab
Mean				631.97
C.V (%)				17.4
LSD				338.03
P value($p<0.05$)				<.001

Disease index was calculated as a cumulative of the severity scores for all nine weeks for each variety. **AUDPC:** Area under disease progress curve

3.4.1.2 Incidence of bacterial wilt

Bacterial wilt incidence varied significantly among the 18 tomato varieties ($p<0.05$). More than 50% of the plant population in Isisementi, Rionex, Riogrande and Sifa F1 varieties showed bacterial wilt symptoms by week 3 whereas Big Rock F1, Kilele F1, Bravo F1 and Terminator F1 by week 6. By week 9, in Kilele F1 only 60% of the plant population wilted while Big rock F1 and Terminator F1 had 76% and 80% wilted plants. Riogrande and Rionex had 100%bacterial wilt incidences by week 7 (Table 3.3).

Table 3.3: Percentage incidence of bacterial wilt on the eighteen tomato varieties

	Weeks after inoculation							
Variety	**2**	**3**	**4**	**5**	**6**	**7**	**8**	9
Kilele F1	4.17cd	4.17cd	4.17f	18.45e	51.19b	55.36b	55.36b	60.11b
Big Rock F1	4.17cd	8.33bcd	12.50ef	37.50cde	61.90ab	66.67ab	71.43ab	76.19ab
Terminator F1	4.77cd	4.76cd	8.93ef	25.60de	56.94ab	70.83ab	75.00ab	80.56b
Bravo F1	0.00d	0.00d	12.50ef	44.05bcde	68.45ab	77.38ab	82.14ab	82.14ab
Shanty F1	12.50abcd	24.07abcd	37.50bcdef	62.50abcd	82.14ab	86.31ab	86.31ab	86.31ab
Commando F1	20.83abcd	25.00abcd	45.83abcde	54.17abcde	77.98ab	82.61ab	87.37ab	87.37ab
Gem F1	16.67abcd	29.17abcd	41.67bcde	54.17abcde	91.07ab	91.07a	91.07a	91.07a
Rambo F1	4.70cd	13.10bcd	26.79def	69.05abcd	91.67ab	91.67a	91.67a	91.67a
Ranger F1	4.76cd	9.52bcd	14.29ef	52.39abcde	82.22ab	82.22ab	94.44a	94.44a
Sifa F1	51.43a	51.43ab	56.19abcd	74.29abc	94.44a	94.44a	94.44a	94.44a
Assila F1	23.81abcd	27.98abcd	37.43bcdef	66.67abcd	84.92ab	89.68ab	95.24a	95.24a
Star F1	13.10abcd	21.43abcd	35.71cdef	61.31abcde	90.48ab	90.48a	95.24a	95.24a
Onyx F1	19.91abcd	43.98abc	54.17abcd	72.69abc	85.71ab	90.48a	95.24a	95.24a
Danny F1	45.83ab	64.29a	62.50abcd	77.98abc	90.48ab	90.48a	90.48a	95.24a
Isisementi	52.98a	61.90a	79.17a	91.67a	95.83a	95.83a	95.83a	95.83a
Stallion F1	8.33bcd	16.67bcd	30.36def	51.79abcde	90.48ab	90.48a	100.00a	100.00a
Rionex	43.98abc	50.00ab	72.69abc	84.26ab	91.53ab	100.00a	100.00a	100.00a
Riogrande	50.00a	50.00ab	73.21ab	87.96a	95.83a	100.00a	100.00a	100.00a
Mean	21.22	28.1	39.2	60.36	82.4	85.89	88.96	90.06
CV (%)	62.1	50.7	31	23.5	16.5	13.2	12.7	11.5
LSD	40.6	43.8	37.4	43.7	41.7	34.8	34.8	31.8
P-value<0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means in the same column followed by the same letter are not significant differently. Means were separated using Tukey's Honest significant Test (P<0.05).

3.4.2 Effects of *R. solanacearum* on growth parameters of selected tomato varieties

3.4.2.1 Plant height

Inoculated tomato varieties and non-inoculated tomato cultivars had significant difference ($P \leq 0.05$) on plant height. Inoculated tomato varieties namely Gem F1, Rambo F1, Sifa F1, Stallion F1 Assila F1 and Star F1 significantly differed from the respective non-inoculated varieties while the rest 12 inoculated varieties had no significant difference with their respective non- inoculated ones. The non-inoculated varieties recorded a mean range of 85.14 cm to154.61cm for Onyx F1 and Bravo F1 on plant heights respectively while the inoculated varieties recorded a range of 76.83cm to 136.28cm for Sifa F1 and Bravo F1 on plant heights respectively (Table 3.4).

Table 3.4: Mean plant height (cm) of inoculated and non-inoculated tomato varieties

Variety	Inoculated	Non-inoculated	P value
Bravo F1	136.28a	154.61a	0.113
Gem F1	110.10a	150.30b	0.030
Big rock F1	106.88a	126.55a	0.073
Ranger F1	107.40a	121.30a	0.206
Shanty F1	116.60a	121.10a	0.637
Rambo F1	90.30a	117.60b	0.009
Stallion F1	96.40a	112.40b	0.009
Kilele F1	103.80a	109.30a	0.562
Terminator F1	94.00a	109.10a	0.093
Commando F1	100.40a	102.20a	0.814
Riogrande	89.93a	100.73a	0.145
Star F1	93.69a	98.39b	0.331
Sifa F1	76.83a	93.61b	0.008
Assila F1	104.72a	92.5b	0.004
Isisementi	89.29a	89.56a	0.952
Rionex	82.53a	89.07a	0.095
Danny F1	81.74a	85.15a	0.448
Onyx F1	77.55a	85.14a	0.065

Means in the same row followed by the same letter are not significantly different.
Means were separated using a t-test with ($P<0.05$).

3.4.2.2 Effects of *R. solanacearum* on number of inflorescences

Inoculated tomato varieties recorded the lowest number of inflorescences compared to the Non-inoculated. Among the inoculated varieties Assila F1, Commando F1, and Danny F1 had the highest number of inflorescences of 3.34, 3.28 and 3.11 respectively while Bravo F1 recorded the lowest number of inflorescences of 2.07. In varieties not inoculated with *R. solanacearum*, Big rock F1, Commando F1 and Stallion F1 recorded the highest number of inflorescences of 3.83, 4.20 and 3.98 respectively while Issisementi and Star F1 recorded the lowest number of inflorescences of 2.241 and 2.24 respectively. Big rock F1, Rambo F1, Shanty F1 and Stallion F1 showed significant difference on the number of inflorescences due to the inoculation with *R. solanacearum* (Table 3.5).

Table 3.5: Mean number of inflorescences comparing inoculated and non-inoculated tomato varieties

Variety	Inoculated	Non-inoculated	P value
Assila F1	3.34a	3.54a	0.613
Big rock F1	2.52a	3.83b	0.036
Bravo F1	2.07a	2.52a	0.234
Commando F1	3.28a	4.20a	0.124
Danny F1	3.11a	3.62a	0.387
Gem F1	2.13a	2.65a	0.119
Isissementi	2.22a	2.24a	0.940
Kilele F1	2.88a	3.41a	0.307
Onyx F1	2.56a	2.98a	0.400
Rambo F1	2.47a	3.70b	0.037
Ranger F1	2.60a	3.26a	0.241
Riogrande	2.59a	2.81a	0.658
Rionex	2.20a	3.15a	0.151
Shanty F1	2.26a	3.13b	0.053
Sifa F1	2.11a	2.43a	0.470
Stallion F1	2.92a	3.98b	0.035
Star F1	2.16a	2.24a	0.796
Terminator F1	2.98a	3.06a	0.802

Means in the same row followed by the same letter are not significant different.
Means were separated a t-test with ($P<0.05$).

3.4.2.3 Effects of *R. solanacearum* on flowering initiation

Varieties not inoculated recorded the highest percentage of flowering on week 5 and 7 compared to the inoculated varieties. Bravo F1, Commando F1, Shanty F1, Terminator F1 varieties showed significant difference by week 5 while in week 7, only Shanty F1 and Stallion F1 showed significant difference in flower initiation. Big rock F1 was the earliest flowering variety (100%) for both the inoculated and none inoculated plants while Riogrande was the latest to flower (83%) by week 9 for the non-inoculated plants (Table 3.6).

Table 3.6: Mean number of flowering plants from inoculated and non-inoculated tomato varieties

	Weeks after Transplanting					
	Week 5		Week 7		Week 9	
Variety	Inoculated	Non-inoculated	Inoculated	Non-inoculated	Inoculated	Non-inoculated
Assila F1	73.00a	100.0a	91.70a	100.00a	100.00a	100.00a
Big rock F1	29.60a	88.90a	100.00a	100.00a	100.00a	100.00a
Bravo F1	59.30a	100.00b	91.70a	100.00a	100.00a	100.00a
Commando F1	37.00a	88.90b	87.50a	100.00a	100.00a	100.00a
Danny F1	88.90a	100.00a	87.50a	100.00a	100.00a	100.00a
Gem F1	70.40a	77.80a	70.80a	100.00a	100.00a	100.00a
Isisementi	66.70a	88.90a	78.60a	83.30a	100.00a	100.00a
Kilele F1	44.40a	77.80b	8.00a	88.90a	100.00a	100.00a
Onyx F1	59.30a	88.90a	70.80a	100.00a	100.00a	100.00a
Rambo F1	47.20a	66.70a	90.50a	100.00a	100.00a	100.00a
Ranger F1	81.50a	100.00a	90.50a	100.00a	100.00a	100.00a
Riogrande	25.90a	44.90a	66.70a	72.20a	100.00a	83.30a
Rionex	44.40a	55.60a	83.00a	83.00a	100.00a	100.00a
Shanty F1	59.30a	100.00b	82.70a	100.00b	100.00a	100.00a
Sifa F1	29.40a	44.40a	69.80a	88.90a	100.00a	100.00a
Stallion F1	55.60a	88.90a	85.90a	100.00b	100.00a	100.00a
Star F1	53.70a	55.60a	70.00a	83.00a	100.00a	100.00a
Terminator F1	76.90a	100.00b	100.00a	83.30a	100.00a	100.00a

Means in the same row followed by the same letter are not significantly different.
Means were separated using Fishers' Protected Least Significant Difference (P<0.05).

3.4.2.4 Effects of *R. solanacearum* on number of fruits

Inoculated tomato varieties had lower number of fruits per plant compared to tomato varieties not inoculated. Stallion F1, Shanty F1, Gem F1 and Onyx F1 showed significant difference whilethe other 12 varieties were not significantly different. Kilele F1 and Terminator F1 recorded the highest mean number of fruits for the inoculated varieties with 5.36 and 5.0 respectively while Onyx F1 and Riogrande recorded the lowest mean number of fruits with 2.04 and 2.21 respectively. For the Non-inoculated varieties Rambo F1and Commando F1 recorded the highest mean number of fruits at 7.47 and 7.17 respectively while Bravo F1 and Star F1 recorded the lowest mean number of fruits with 3.28 and 3.44 respectively (Table 3.7).

Table 3.7: Mean number of fruits from inoculated and non-inoculated tomato varieties

Variety	Inoculated	Non-inoculated	P-value
Assila F1	4.60a	5.17a	0.491
Big rock F1	2.93a	3.94a	0.241
Bravo F1	3.68a	3.28a	0.633
Commando F1	4.70a	7.17a	0.130
Danny F1	4.77a	6.78a	0.184
Gem F1	3.15a	4.89b	0.048
Isissementi F1	2.55a	4.13a	0.129
Kilele F1	5.36a	6.04a	0.717
Onyx F1	2.04a	5.17b	0.011
Rambo F1	3.86a	7.47a	0.080
Ranger F1	3.90a	6.89a	0.112
Riogrande	2.24a	3.83a	0.119
Rionex	2.37a	4.56a	0.194
Shanty F1	3.01a	5.68b	0.013
Sifa F1	2.66a	4.11a	0.154
Stallion F1	3.37a	6.89b	0.020
Star F1	3.51a	3.44a	0.927
Terminator F1	5.00a	6.22a	0.166

Means in the same row followed by the same letter are not significantly different.
Means were separated using a t-test with ($P<0.05$).

3.4.3 Effect of *R. solanacearum* on stem browning and adventitious root system of tomato varieties

The highest length and width of browning was two months after inoculation for all varieties except for Assila F1. One month after inoculation, Terminator F1 had the longest mean length of browning (5.37cm) while Commando F1 had the shortest length of browning 0.8cm. During the second month, Onyx F1 had the longest length of browning (15.07cm) while Bravo had the shortest length of browning (4.17cm). Width of browning was longest in Rionex and Sifa F1 (0.23cm) while Kilele F1 and onyx F1 had the shortest width (0.067cm) in the first month.

During the second month, width of browning was longest in Rambo F1 (1.1cm) and shortest in Riogrande (0.1cm). The length and width of browning had no significant difference among the varieties for both first and second month.

Length of adventitious roots was higher during the first month compared to the second month for all the eighteen varieties except for Big rock F1. Ranger F1 (7.17cm) and Big rock F1 (0.57cm) had the longest adventitious roots in the first and second month respectively while Onyx F1 and Kilele F1 had the shortest length of adventitious roots during the first and second month respectively. The length of adventitious roots did not significantly vary among the 18 varieties for both the first and second month (Table 3.8).

Table 3.8: Mean length and width (cm) of browning and adventitious roots from inoculated tomato varieties.

	L. of browning (cm)		W. of browning (cm)		L. of adventitious roots (cm)	
Variety	30 DPI	60 DPI	30 DPI	60 DPI	30 DPI	60 DPI
Assila F1	4.73	4.47	0.17	0.30	4.93	2.93
Big rock F1	1.37	9.60	0.10	0.33	4.10	4.67
Bravo F1	3.93	4.17	0.17	0.27	3.20	3.20
Commando F1	0.80	8.60	0.17	0.20	4.83	1.60
Danny F1	4.47	7.83	0.13	0.47	5.73	2.37
Gem F1	4.80	7.27	0.17	0.33	4.70	2.43
Isisementi	4.90	9.17	0.13	0.30	2.47	1.20
Kilele F1	0.90	6.10	0.07	0.20	3.93	0.57
Onyx F1	2.33	15.07	0.07	0.47	2.60	2.13
Rambo F1	2.97	9.93	0.10	1.10	2.13	2.03
Ranger F1	4.33	7.00	0.13	0.33	7.17	1.95
Riogrande	3.87	7.37	0.10	0.10	2.70	3.83
Rionex	2.00	6.07	0.23	0.17	4.80	2.40
Shanty F1	4.70	5.77	0.17	0.37	5.37	2.30
Sifa F1	2.23	4.60	0.23	0.33	4.43	3.23
Stallion F1	5.00	6.70	0.13	0.13	3.03	3.30
Star F1	2.77	7.30	0.27	0.33	5.83	3.17
Terminator F1	5.37	7.47	0.17	0.30	4.50	3.13
CV%	58.10	49.50	73.40	118.90	41.70	52.20
LSD	3.29	6.14	0.18	0.66	2.94	2.24
P-value	0.083	0.197	0.73	0.602	0.602	0.137

Means were separated using Fishers' Protected Least Significant Difference ($P<0.05$).
Key: DPA- Days Post Inoculation; L-Length; W-Width

3.4.4 Correlation analysis for disease incidence, severity and growth parameters of tomato

There was positive correlation between incidence and severity of bacterial wilt ($r=0.97$, $P<0.001$). Both incidence and severity had a negative correlation with plant height ($r=-0.62$, -0.60, $P<0.01$). There was a positive correlation between number of inflorescence and number of fruits ($r=0.67$, $P<0.01$) while number of fruits had negative correlation both incidence and severity ($r=-0.57$, -0.54, $P=0.05$). Also, a positive correlation was observed in number of fruits and percentage flowers ($r=0.55$, $P<0.05$), (Table 3.9).

Table 3.9: Correlation table for disease incidence, severity and growth parameters of tomato

	Percent flowered	Length A. roots	Incidence	L. Browning	Fruits No.	Inflor. no	Severity	W. browning	P. height
Percent flowered	-								
Length of A. roots	0.26	-							
Incidence	-0.29	-0.11	-						
L. browning	0.28	-0.27	0.24	-					
Fruits no.	0.55*	0.02	-0.57*	-0.22	-				
Inflor. no	0.30	-0.07	-0.29	0.06	0.67**	-			
Severity	-0.29	-0.21	0.97***	0.24	-0.54*	-0.29	-		
W.browning	0.17	-0.13	0.03	0.26	0.02	-0.32	0.05	-	
P. height	0.28	0.19	-0.62**	-0.32	0.28	0.02	-0.60**	-0.17	-

Key: Length of A. roots= Length of Adventitious roots, L. Browning= Length browning, W. browning = Width browning, Inflor. No = Inflorescence number, P. height =Plant height. Values abbreviated with '***' $P<0.001$, '**' $P<0.01$, '*' $P<0.05$

3.5 Discussion

Using resistant varieties is a simple, safe and economical strategy for managing bacterial wilt. This study identified hybrid varieties Kilele F1, Terminator F1, Bravo F1, Big rock F1 and Ranger F1 as resistant to bacterial wilt disease. Non-hybrid varieties Riogrande, Rionex and Isisementi were found to be highly susceptible to *Ralstonia solanacearum*. A similar study in Kenya by Manani *et al.* (2020) screened six varieties against *Ralstonia solanacearum* and found out that Heirloom Tall vine and Goliath pear hybrid were resistant to bacterial wilt. Riogrande variety was noted to be susceptible to *R. solanacearum* in studies conducted by Aslam *et al*. (2017) and Kathimba *et al*. (2018). Dossoumou *et al*. (2021) evaluated 21 tomato genotypes against *R. solanacearum* and found out that only one genotype Cobra 26 was resistant to bacterial wilt disease. In the current study, expression of bacterial wilt incidence and severity of resistant varieties was lower than susceptible varieties during the entire period of the experiment.Comparable findings were reported by Abebe *et al*. (2020) who evaluated 27 varieties and concluded that the varying levels of severity and incidence of bacterial wilt could be ascribed to different genetic make-up of the varieties. Manani *et al*. (2020) and Vanitha *et al*. (2009)concluded that low mean severity and incidence in the resistant tomato genotypes could be dueto the production and activities of phenylalamine ammonia lyase (PAL) and Polyphenol oxidase (PPO). PAL and PPO were found to be responsible for production and oxidation of phenolic compounds that enhance plant defense against *R. solanacearum* (Vanitha *et al*., 2009). In this study, five cultivars showed tolerance to bacterial wilt disease based on the scale defined by Aslam *et al*. (2017). Grimault *et al*. (1995) and Singh (1961) reported that resistance was due to certain single dominant genes and recessive genes that are present in the plant host genome. Based on Oliveria *et al*. (1999) additive effects of the genes can be attributed to resistance against bacterial wilt disease. The susceptible varieties expressed wilting early and succumbed easily due to the pathogen colonization in the vascular tissues that block water passage.

In this current study, the latent period was related to susceptibility and tolerance of tomato varieties to *R. solanacearum*. In tolerant varieties, wilts developed at week four while susceptibleones showed wilts at week two after inoculation. Similar findings were reported by Dossoumou*et al*. (2021). Since the experiment was conducted during the cold season from April to July withmean temperatures of 19ºC, 17ºC and 16ºC respectively, general bacterial wilt symptoms expression was lower. These observations agrees with those of Hanson et al. (1996) who reported that bacterial wilt resistance is influenced by climatic conditions and Singh *et al*.(2018); Bittner *et al*. (2016) reported that lower temperatures between 15ºC to 20ºC delays bacterial wilt disease expression symptoms.

Breeding for resistance to bacterial wilt is faced with difficulties. The resistant variety Hawii7996 was found to exhibit polygenic resistance (Gremault, 1995) and the resistance depends on the ecological conditions (Hayward 1991). Wang *et al*. (2000) described the resistance in Hawii 7996 as strain specific and a recent study by Carmeille (2006) suggested that the QTLs in variety Hawii 7996 may lead to the deployment of a phylotype-specific resistance. These findings illustrate the difficulties in breeding for a long-lasting resistance to *R. solanacearum* worldwide.

Significant variation on flower initiation among the inoculated and non- inoculated varieties can be associated with the infection of *R. solanacearum* that is known to cause stunted growth hence prolonging the days to flower initiation. These findings agree with those reported by Manani *et al.* (2020). Inoculated plants produced fewer number of fruits compared to the non-inoculated plants. Winstead and Kelman (1952) confirmed that *R. solanacearum* greatly influences fruit production in tomato plants and once the bacterium gains entry, it multiplies and colonizes vascular tissues. Some varieties like Rionex, and Riogrande succumbed during the fruiting stage.In this study, length and width of browning was observed to increase overtime after pathogen inoculation. This can be attributed to the effect of *R. solanacearum* colonization in the vascular bundles which causes brown discoloration of the stem. Pradhanang *et al*. (2005) reported similar results.

In conclusion, out of the 18 varieties popularly grown in Kajiado County, five varieties were found to be resistant while majority were moderately susceptible to *Ralstonia salanacearum*. Non-hybrid varieties Riogrande, Rionex and Isisementi performed poorly and emerged to be highly susceptible. Therefore, Kilele F1, Terminator F1, Bravo F1, Big rock F1 and Ranger F1 varieties can be recommended in the integrated diseases management to farmers in areas that are prone to bacterial wilt for up-scaling tomato production.

CHAPTER 4: EFFECT OF WATER REGIMES ON BACTERIAL WILT CAUSED BY *RALSTONIA SOLANACEARUM* ON SELECTED TOMATO VARIETIES IN THE GREENHOUSE

4.1 ABSTRACT

Soil moisture regimes have an important role in determining tomato productivity and also influencing bacterial wilt disease development in the field. The objective of this study was to assess the development of bacterial wilt disease on selected tomato varieties under different moisture regimes. An experiment was conducted during the period of November 2021 to January2022 in a greenhouse at Kabete site, University of Nairobi. The experiment was laid out in a completely randomized design in a 3 by 3 factorial arrangement. The treatments were three moisture levels of 50% field capacity (FC), 100%FC and 120%FC and three tomato varieties namely Big rock F1, Assila F1 and Riogrande. The varieties were inoculated with 10^8CFU/ml of *Ralstonia solanacearum* two weeks after transplanting. Severity and incidence of bacterial wilt was assessed one week after inoculation using a disease rating score of 0-4 and incidence was recorded by counting the number of wilted plants. Length of browning was measured with a meter ruler. Watering level and tomato variety had significant effect on incidence and severity ofbacterial wilt. Incidence and severity of bacterial wilt was observed to increase with increase in moisture level from 50% FC to 120% FC in all tomato varieties. Big rock F1, Assila F1 and Riogrande variety recorded the lowest severity scores at 50% FC while highest severity scores were recorded at 120% FC in all the varieties tested. At 100% field capacity, the lowest severity score was recorded in Big rock F1 followed by Assila F1 and the highest score was in Riogrande variety. Big rock F1consistently recorded the lowest severity and incidences of bacterial wilt while Riogrande variety recorded the highest severity and incidences under all watering levels. Lowest incidence of bacterial wilt was recorded from Big rock F1 while Riogrande recorded the highest bacterial wilt incidence at all watering levels. Assila F1 and Riogrande variety recorded 100% incidence at week four and five respectively at 100% FC and 120% FC. The length of browning significantly varied among the three tomato varieties. The shortest length was recordedin Big rock F1 with 6.3cm while the longest length of browning was recorded in Riogrande variety with 10.9cm. There was no significant variation observed in the width of browning in all the tomato varieties under all the watering levels. These finding shows that high moistureconditions favors the development of bacterial wilt disease. Big rock F1 variety showed some

resistance to the disease while Riogrande emerged to be highly susceptible. Since the disease expression in a tolerant variety like Big rock F1 is enhanced by optimal water level, there is need for an integrated approach where other management practices such as biological products can be combined to further suppress bacterial wilt disease in tomato production.

Key words: Moisture regimes, *Ralstonia solanacearum*, Incidence, Tomato varieties, severity.

4.2 Introduction

Bacterial wilt disease caused by *R. solanacearum* is an important soil borne bacterium thatcauses wilt disease in solanaceous crops. The survival of the pathogen in contaminated fields canpersist for quite some time and in potato fields it was observed to be present for a period of one year (Vas Elsas *et al*., 2000). The pathogen is known to infect over two hundred plant species in fifty different families (Grimault *et al*., 1994). The main inoculum sources are infected planting materials and soils, contaminated irrigation water, surface water, farm equipment and tools and weeds (Hayward, 1991). The disease causes high yield losses of up to thirty-five to ninety percent under high temperatures and high moisture conditions (Singh *et al*., 2015). The bacterium gains entry in to the tomato plant through tiny openings present in the root hairs and moves into the xylem vessels (Vasse *et al*., 1995). An extracellular polysaccharide (EPS), a virulent factor is produced by the pathogen which increases the fluid viscosity of the xylem. The viscous fluid blocks the passage of water in the xylem vessels that results into wilting of theplant (McGarvey *et al*., 1999).

Various mechanisms are involved in the interaction between soil moisture and bacterial disease. Soil moisture influences multiplication of *R. solanacearum* hence increasing inoculum amount and survival of the pathogen in the soil (Gupta *et al.,* 2018). Van Elsas *et al*. (2000) found out that drought stress causes a negative impact on the density of *R. solanacearum* in fields grown with potatoes. Low soil moisture condition contributes to small sized diameter of vessels and pitsthat leads to compaction of the xylem. The compact xylem vessels in tomato plants prevents the free movement of *R. solanacearum* (Nakaho *et al*., 2000). The host resistance of a crop is also very important in the management of *R. solanacearum*. Kim *et al*. (2016) and Nakaho *et al*. (2000) found out that resistant varieties have thickened pit membranes that inhibited the movement of *R. solanacearum* in the xylem vessels, an observation that was not made in the susceptible varieties. The findings also showed that high concentration of *R. solanacearum* was observed in the primary and secondary xylems of susceptible varieties. Production of tyloses was observed in *R. solanacearum* resistant cultivars that limited the movement of the pathogen to adjacent cells in the vessels. In susceptible varieties there was no production of tyloses, therefore free movement and spread of the pathogen occurred that led to death of the plants (Beattie, 2011; Fradin and Thomma 2006).

Studies by Mondal *et al*. (2014) and Van Elsas *et al*. (2000) have shown that bacterial wilt incidences were significantly reduced under low moisture conditions in the field. Marouelli *et al.* (2005) observed high wilt incidence in drip irrigation compared to sprinkler irrigation. This shows that plant and water regimes in the soil plays a significant role in determining tomato productivity and the severity of soil-borne *R. solanacearum* and spread. The study aimed to evaluate the development of *R. solanacearum* under different moisture regimes on selected tomato varieties in the greenhouse.

4.3 Materials and Methods

4.3.1 Description of experimental site

This trial was carried out in a greenhouse at Kabete site, University of Nairobi in Kenya. The area is located at an Agro-ecological zone of upper midland zone three (UM3), at latitude 1º 15'South and longitude 36º 44' East and at an altitude of about 1800m above sea level (Jaetzold, 2006). The mean daily temperature in the greenhouse was 21ºC between the period of November2021 and January 2022.

4.3.2 Planting materials

Three tomato varieties (Big rock F1, Assila F1 and Riogrande) were evaluated at three moisture levels namely 50%, 100% and 120% field capacity. Big rock F1, Assila F1 and Riogrande varieties were previously determined to be resistant, moderately susceptible and highly susceptible respectively in a greenhouse experiment as shown in (Section 3.4.1 Table 2). They were also the most frequently grown tomato varieties in Kajiado County. The seeds and potting medium (hygromix) were purchased from certified local agrovet suppliers and seedlings were raised in germination trays containing hygro-mix in a greenhouse. The seedlings were watered asper the plant requirements.

4.3.3 Experimental design and layout

The experiment was laid out in a completely randomized design in a 3 by 3 factorial arrangement. The treatments were three moisture levels (50%, 100% and 120% field capacity) and three tomato varieties (Big rock F1, Assila F1 and Riogrande variety). Red soil was collectedin a non-cultivated land at the field station. Polythene pots measuring 20.3cm by 35.6cm by 35.6cm were filled with heated media composed of soil and sand in the ratio of 3:1 and each bag contained 10 kg of the planting media. Thirty-day old seedlings were transplanted, one seedling per pot. One week after transplanting, the plants in the pots were moistened with water and pricking was done using a sharp scalpel blade on the seedlings' roots before drenching with 30ml of *Ralstonia solanacearum* inoculum at 1×10^8cfu/ml except for the control check. Each treatment combination (Variety +Moisture level) was assigned twelve pots where nine pots were inoculated with *R. solanacearum* and the other three pots served as a control.

Gravimetric moisture analysis method was used to calculate the different moisture levels (Reynolds, 1970). Gravimetric moisture content (GMC) of soil in the pot was obtained from the formula: (wet soil core weight - dry soil core weight)/dry soil core weight - core can weight) × 100. The GMC was used to determine the volumetric moisture content (VMC) of pot soil using the formula: (%GMC × bulk density of pot soil × Density of water) × volume of pot soil (cm^3). The results estimated 1.9litres of water per pot in 100%FC volume of moisture and the other treatments were obtained by multiplying 1.9litres by 50%FC and 120%FC to give 0.95litres and 2.28litres respectively (Maina, 2020; Reynold, 1970).

The lapsed period of time before successive watering was determined by water potential reading in a soil moisture meter inserted in pots at a depth of 10 cm. This indicated when to water the plants depending on the prevailing weather throughout the season. A spacing of one meter distance between the pots was maintained to avoid water getting in to the unintended pots. The moisture levels were imposed two weeks after transplanting to allow establishment of root system.

4.3.4 Agronomic practices

During transplanting, 5 g of Di-ammonium Phosphate (DAP) was mixed with planting media in each pot. Seedlings were watered in alternate days during the first two weeks before different moisture levels were effected. Calcium ammonium nitrate (CAN) was used to top dress 3 weeks after transplanting followed by NPK (17:17:17) during flowering.

Scouting of diseases and pests was conducted regularly and management of insect pests was done to prevent damage of the crop in the green house, the chemicals used were Coragen (Chlorantraniliprole and Rynaxypyr) 200g/l for tomato leaf miner (*Tuta absoluta*) and Nimbecidine on whiteflies. Early and late blight diseases were controlled by spraying Milraz 76 WP with an active ingredient of Propinep 700g/kg and Cymoxanil 60g/kg alternated with Ridomil Gold MZ 68WG with an active ingredient of Metalaxyl-M 40g/kg and Mancozeb 640g/kg.

4.3.5 Assessment of disease severity

Disease severity was scored every week after the first symptom was observed and continued for six weeks based on a modified key described by Aslam *et al.* (2017) and Uwamahoro *et al.* (2018). Severity rating score: 0 – no symptoms, 1— one or two young leaves wilted, 2 – half of all the leaves wilted, 3 – almost all the leaves wilted, 4 – dead plant.

4.3.6 Determination of disease incidence

Disease incidence was assessed by counting the number of wilted plants per pot after every seven days for six weeks. Percentage of bacterial wilt incidence was calculated per treatment by the formulae described by Ayana *et al*. (2011).

WI= NPSWS/TNPT *100

Where WI-wilt incidence, NPSWS-number of plants showing wilt symptoms and TNPT-total number of plants per treatment.

4.3.7 Scoring for stem browning

The length and width of stem browning was assessed once at the end of the growing season at 65 days after transplanting. The length and width were measured by use of a meter ruler.

4.3.8 Statistical analysis

Data on severity, incidence and stem browning were subjected to analysis of variance using

GenStat software version 15th edition and means were separated by Fishers' Protected Least Significant Difference at 5%.

4.4 Results

4.4.1 Effect of moisture levels on the severity of bacterial wilt on selected tomato varieties

Moisture level and tomato varieties had significant differences on severity of bacterial wilt. Severity scores were noted to increase gradually with increase in moisture levels for all the varieties tested. Severity was lowest at 50% field capacity (FC) (Figure 4.1) and highest at 120%FC (Figure 4.3) in all the three tomato varieties tested. Big rock F1 was noted to have the lowest severity scores in all the moisture levels compared to Assila F1 and Riogrande varieties. Riogrande recorded the highest severity scores in all the moisture levels while Assila F1 variety performed moderately. Disease symptoms started as early as week one for Riogrande variety and at week four for Big rock F1variety in all moisture levels (Figure 4.1, 4.2 and 4.3). In Assila F1 variety symptoms started by week two at 100%FC and 120%FC while at 50%FC symptom expression started by week three. Big rock F1 consistently recorded the lowest severity scores throughout the weeks and by week 6 it recorded 1.5 severity score at 50%FC while at 100% and 120%FC it had 2.5 severity score. In Riogrande variety all the plants were dead at 120% FC by week six (Figure 4.3).

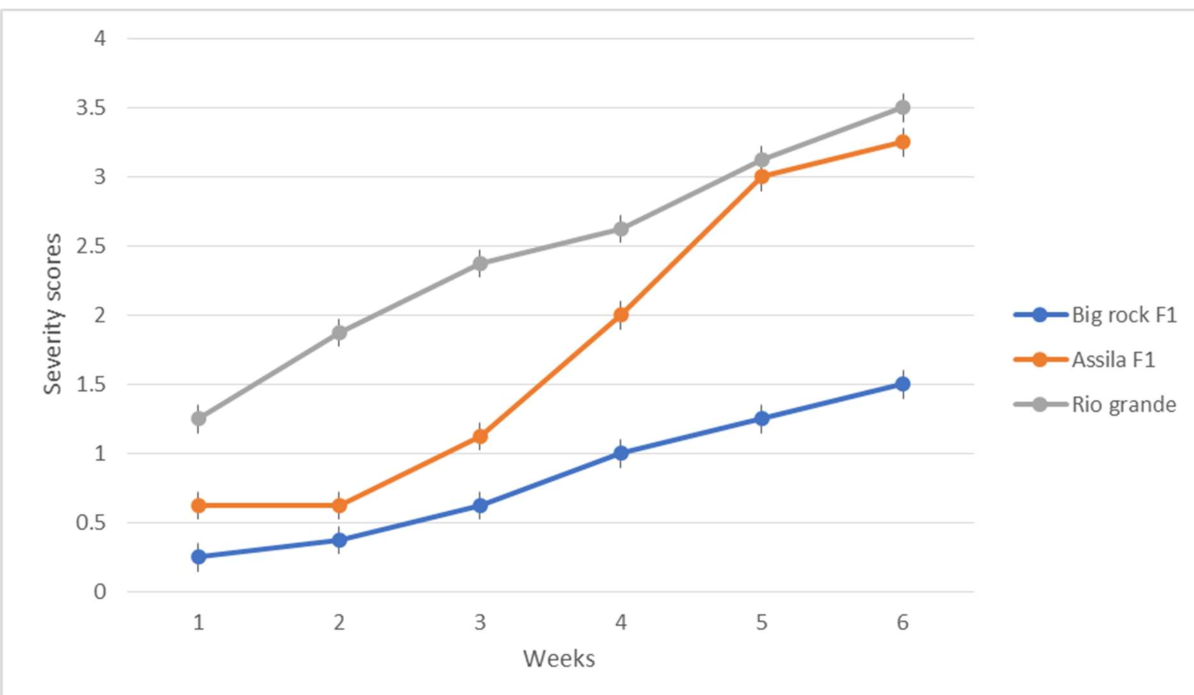

Figure 4.1: Response of tomato varieties on severity of bacterial wilt at 50% Field Capacity

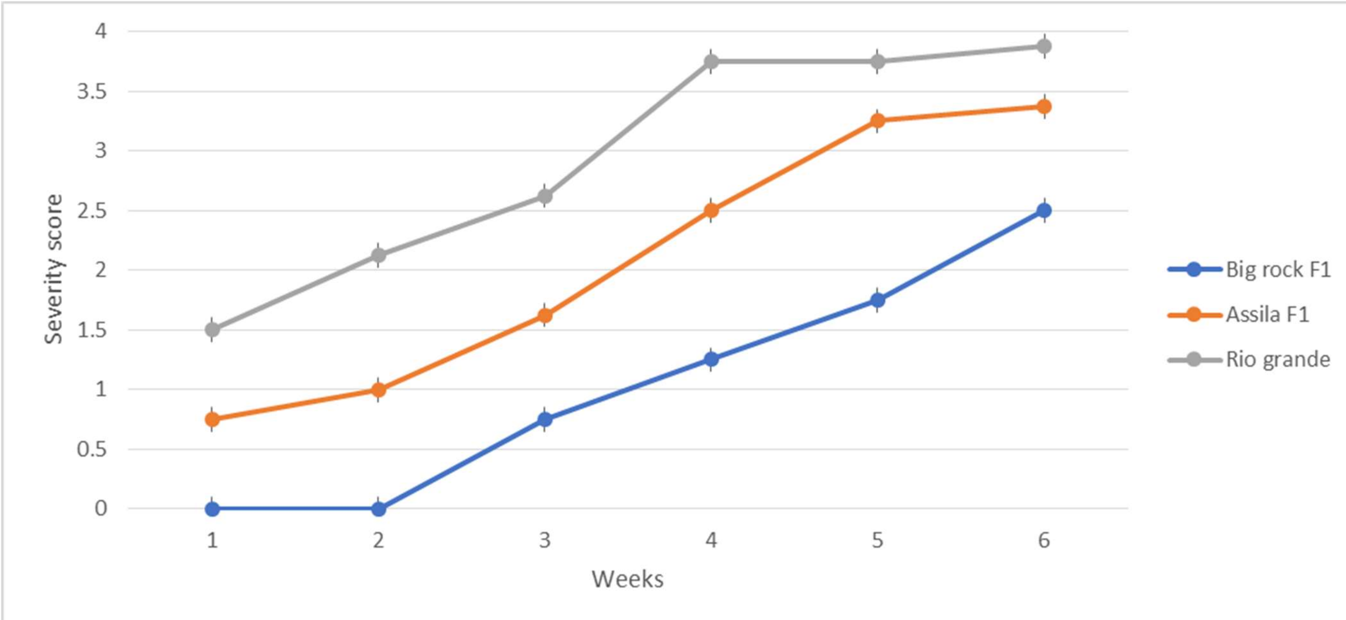

Figure 4.2*:* Response of tomato varieties on severity of bacterial wilt at 100% Field Capacity

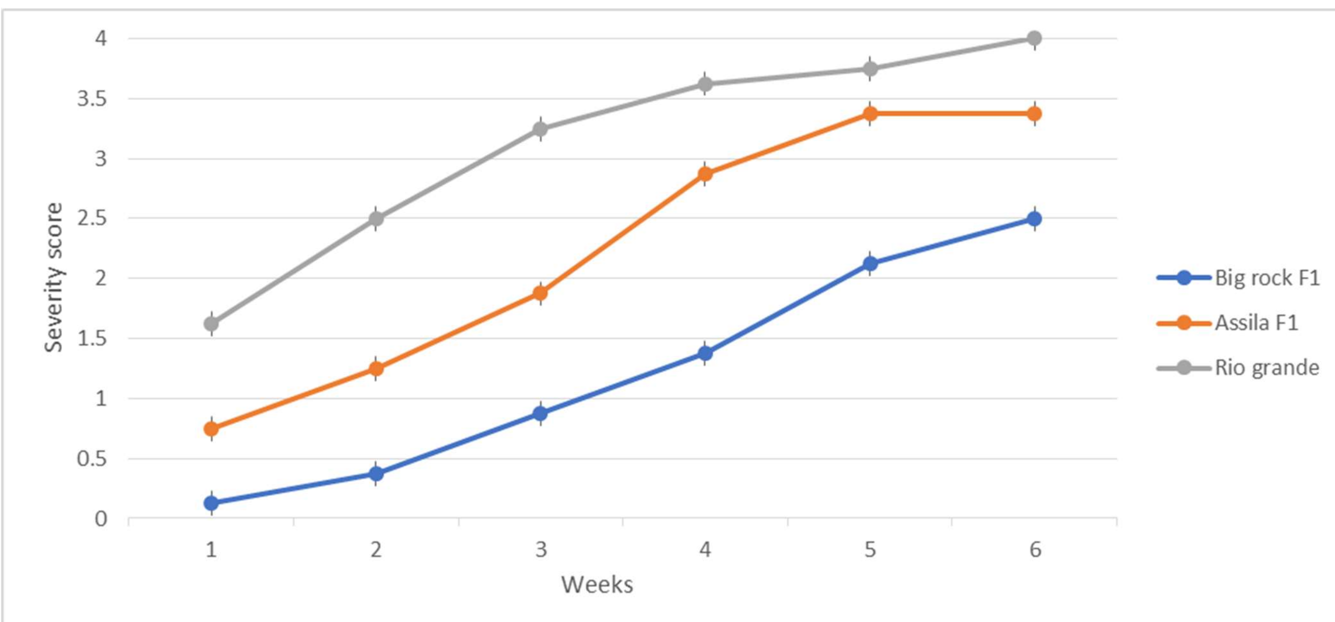

Figure 4.3*:* Response of tomato varieties on severity of bacterial wilt at 120% Field Capacity

4.4.2 Effect of moisture levels on incidence of bacterial wilt on selected tomato varieties

Moisture level and tomato varieties had significant differences on bacterial wilt incidence. Increase in moisture level from 50%FC to 120% FC increased the incidence of bacterial wilt in

Big rock F1, Assila F1 and Riogrande varieties (Figure 4.4, 4.5 and 4.6). Big rock variety recorded the lowest incidence compared to Assila F1 and Riogrande varieties in all moisture levels (4.4, 4.5 and 4.6). At 50% FC, lowest incidence was recorded in all the varieties while at 120%FC, highest incidence was recorded in all the varieties tested (Figure 4.4 and 4.6). By weekfour, Riogrande variety recorded 100% bacterial wilt incidence at 100%FC and 120%FC while atweek 5, Assila variety recorded 100% bacterial wilt incidence at 50%FC, 100%FC and 120%FC (Figure 4.4, 4.5 and 4.6).

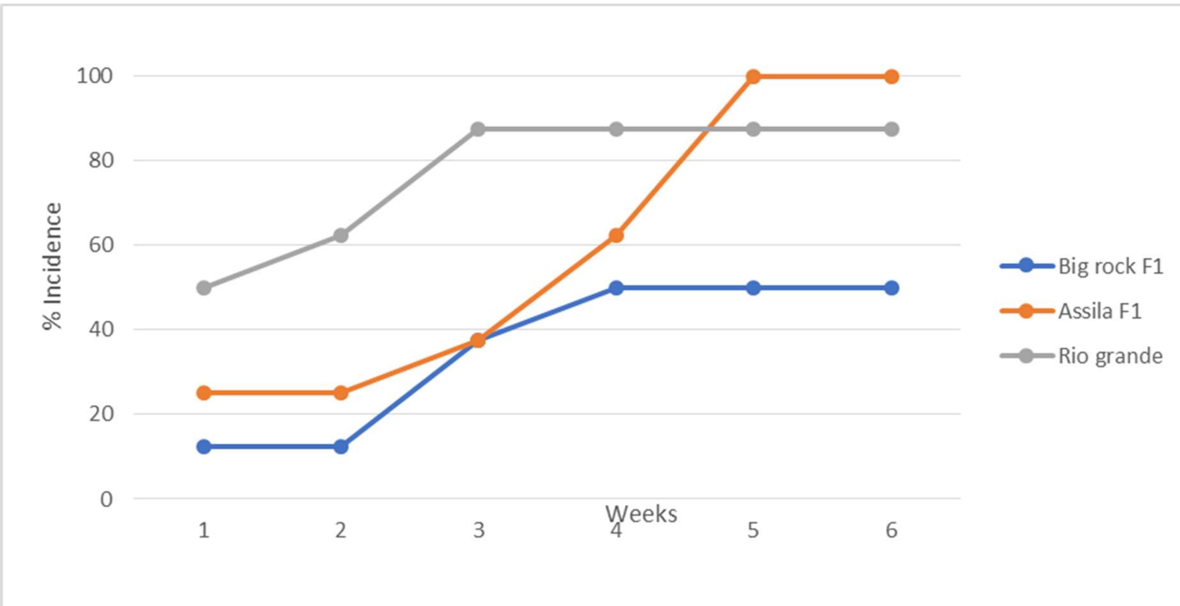

Figure 4.4: Response of tomato varieties on bacterial wilt incidence at 50% Field Capacity

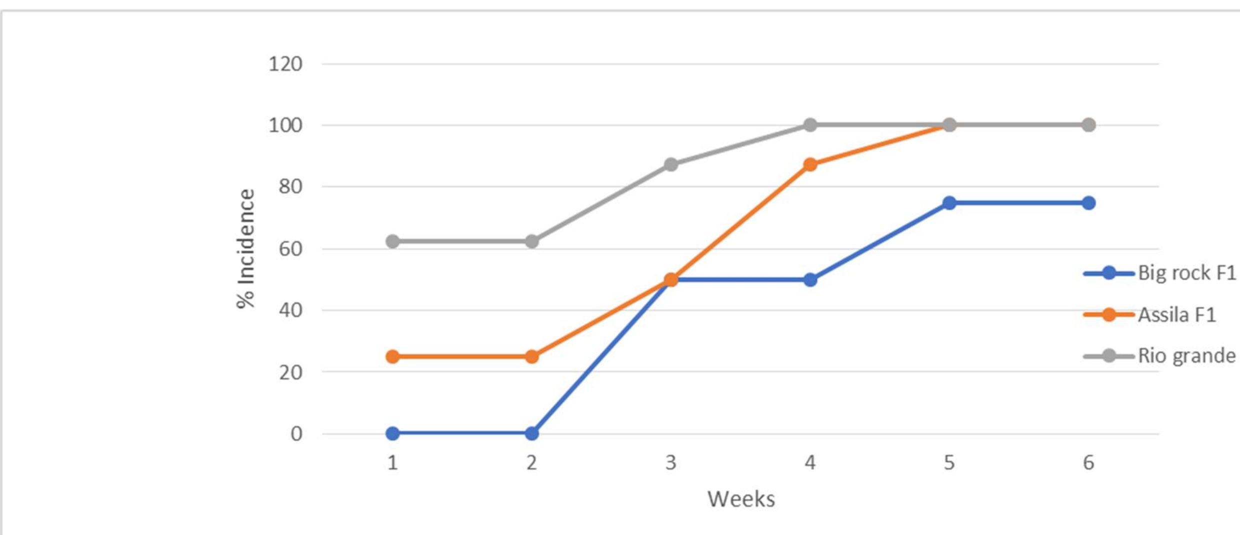

Figure 4. 5: Response of tomato varieties on bacterial wilt incidence at 100% Field Capacity

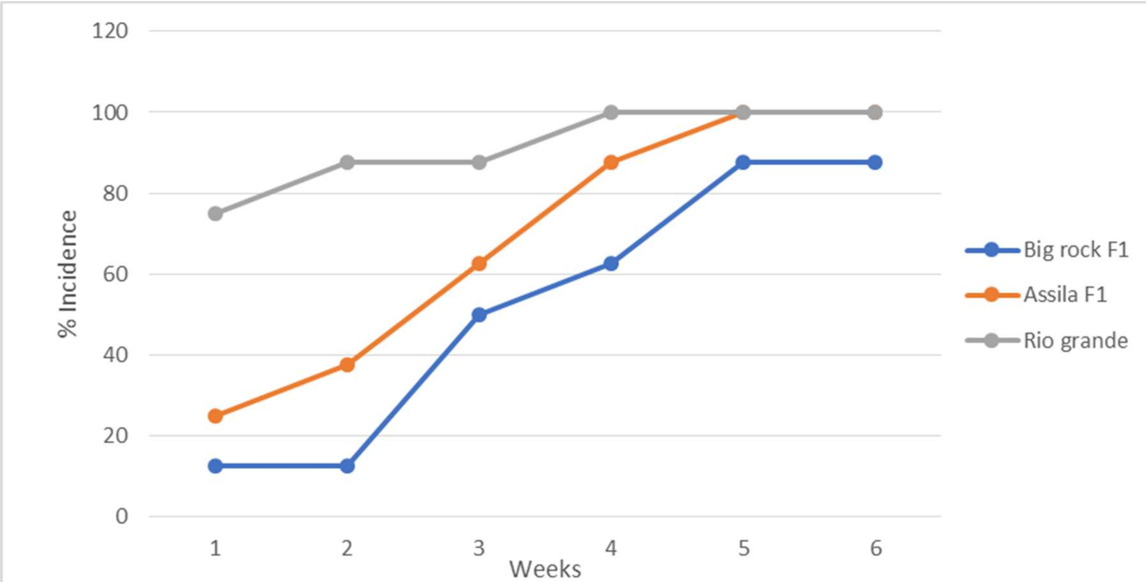

Figure 4. 6: Response of tomato varieties on bacterial wilt incidence at 120% Field Capacity

4.4.3 Effect of moisture levels on stem browning of selected tomato varieties

There was a significant difference on length of browning for all the tomato varieties tested. The highest length of browning (10.90cm) was recorded at 50%FC on Riogrande variety while the lowest (6.30cm) was observed at 50%FC on Big rock F1 (Figure 4.7). Lowest length of browning was recorded in Big rock F1 at all moisture levels while Riogrande variety had the highest length of browning (Figure 4.7, 4.8 and 4.9). There were no significant differences observed on tomato varieties and moisture level on the width of browning for the tomatovarieties tested. The width of browning was less than 1cm in Big rock F1, Assila F1 and Riogrande variety at all moisture levels (Figure 4.7, 4.8 and 4.9).

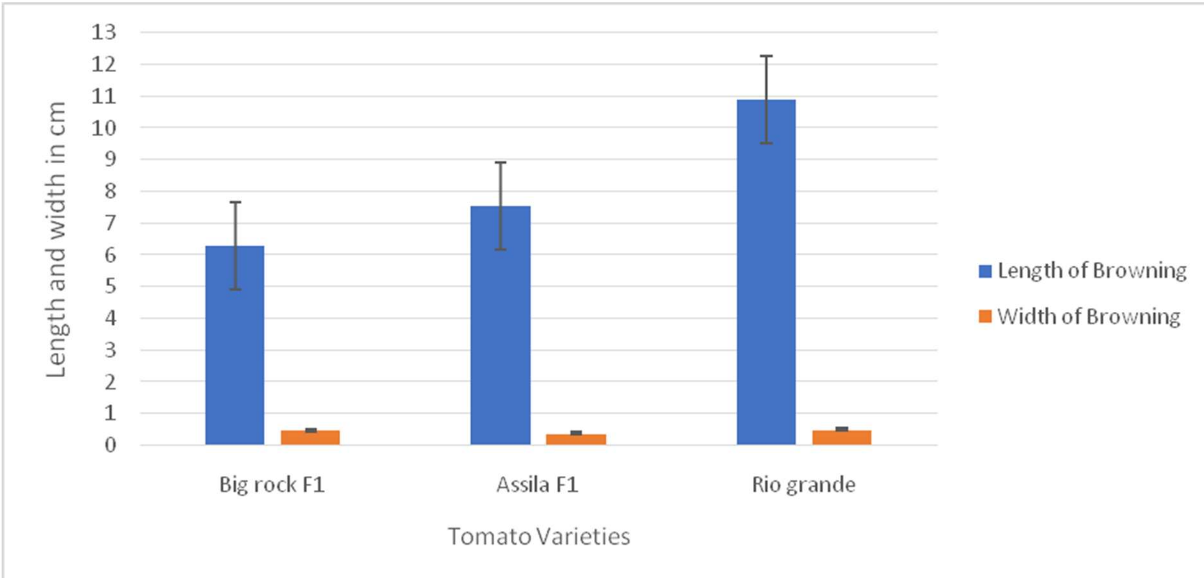

Figure 4.7: Response of tomato varieties on length and width of browning at 50% Field Capacity

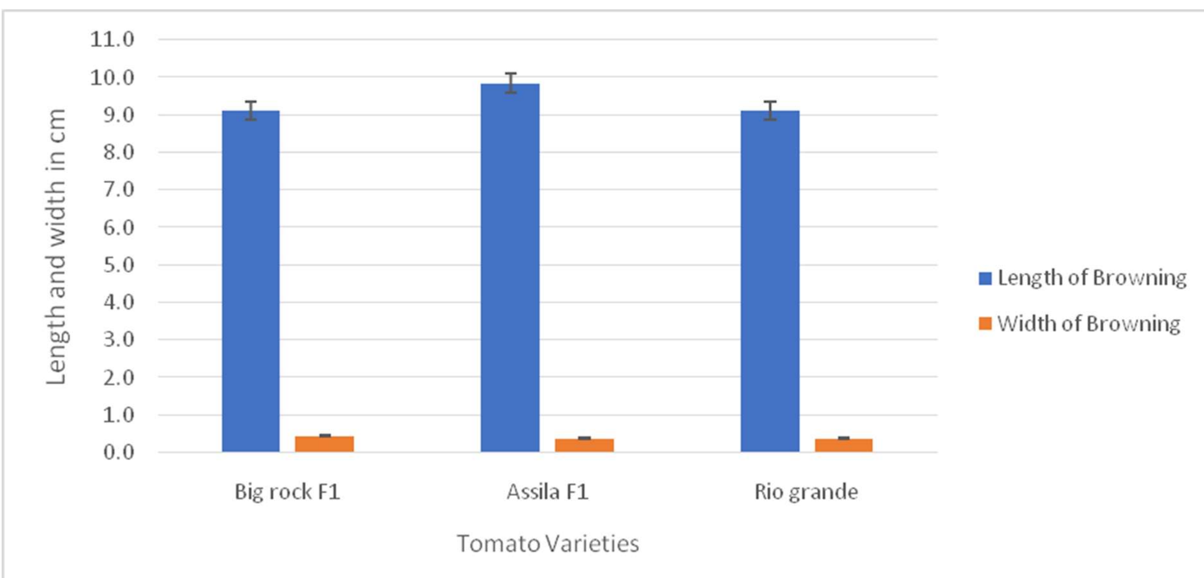

Figure 4.8: Response of tomato varieties on length and width of browning at 100% Field Capacity

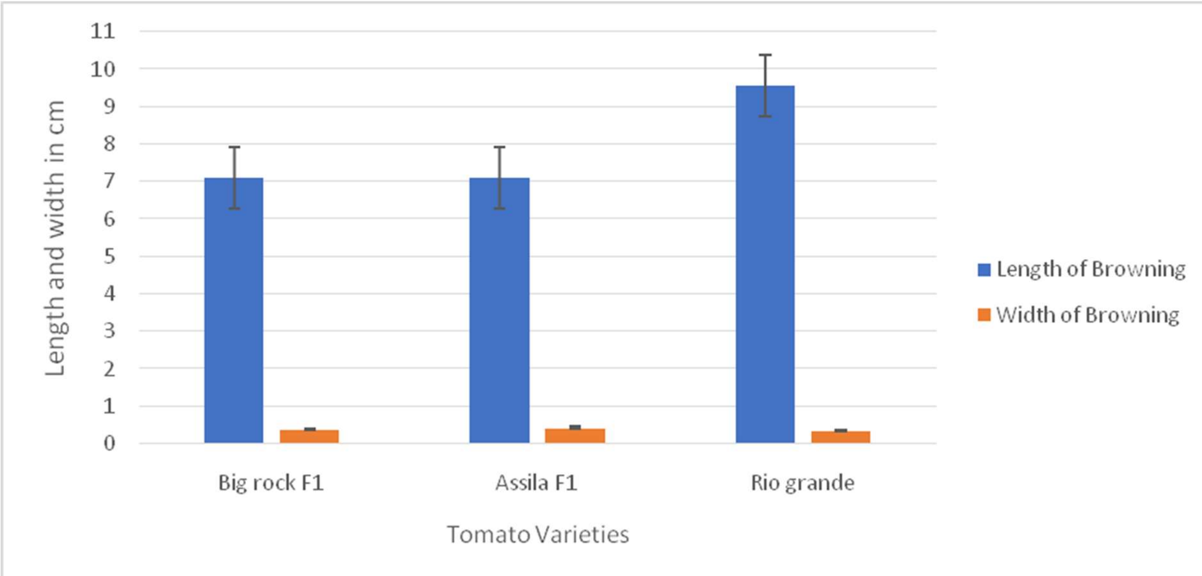

Figure 4.9: Response of tomato varieties on length and width of browning at 120% Field Capacity

4.5 Discussion

Soil moisture regime greatly influences the severity of *Ralstonia solanacearum* in tomato plants. In this study, bacterial wilt incidence and severity was noted to increase with increase inmoisture level. Wilt incidence and severity was positively influenced by high moisture conditions (100%FC and 120%FC). These results were similar to the results obtained by Mondal *et al*. (2014) who found out that during high rainfall (high moisture conditions) there was increased bacterial wilt incidence. Van Elsas *et al*. (2000) also reported that severe wilts were observed at the onset of potato growing season when moisture availability was high. A study by Gupta *et al*. (2018) showed that the high infection rate of bacterial wilt disease during high moisture conditions was due to the rapid multiplication of *R. solanacearum* once it gains entry through the tiny openings in the root hairs. The increased cell size, cortex and epidermis provides a suitable environment for the pathogen multiplication that leads to clogging of the xylem vesselshence death of the plant (Gupta *et al.,* 2018).

As described by Nakaho *et al*. (2000), at low moisture level, tomato plant tends to increase the growth of lateral roots for maximum absorption of available moisture and therefore potentially increasing entry of pathogen. Gupta *et al*. (2018) reported that low moisture conditions alter the concentration, content and diffusion of plant exudates involved in chemotaxis thereby reducing the attraction of *R. solanacearum* to the root hairs. In addition, movement of *R. solanacearum* from the roots to aerial parts of the plant is reduced by closure of stomata which inhibits the transpiration pull under low moisture levels (Nakaho *et al*., 2000).

In this study, resistant variety Big rock F1 had the lowest incidence and severity of bacterial wilt at all the moisture levels that it was exposed to compared to susceptible variety Riogrande that had the highest wilt incidence and severity at all the moisture levels. Studies conducted by Kim*et al.* (2016) and Nakaho *et al*. (2000) reported that the resistance could be due to thickened membrane that stops the movement of bacterial wilt pathogen in the xylems of resistant varieties which was not the case in susceptible varieties where there was observed high concentration of *Ralstonia solanacearum* in the primary and secondary xylem (Kim *et al*., 2016; Nakaho *et al*., 2000). Based on Gremault and Prior (1993) the resistance feature was linked to thickened pits of the plant that halted the establishment of *Ralstonia solanacearum* in the vascular bundles. A study by Hacisalihoglu *et al*. (2008) reported that, wilt susceptible variety exhibited high mineralcontent of nitrates as compared to wilt resistant variety. This shows that *Ralstonia solanacearum* a pathogen is dependent on inorganic compounds for its pathogenicity.

The incidence of bacterial wilt was significantly reduced under low moisture levels while significantly increased under high moisture levels. Big rock F1 variety showed a consistent lower disease infection compared to Assila F1 and Riogrande variety. Therefore, *Ralstonia solanacearum*-resistant tomato varieties can be used to manage bacterial wilt disease under varying degrees of moisture levels to improve tomato production

CHAPTER 5: EFFECT OF DIFFERENT IRRIGATION SYSTEMS ON INCIDENCE AND SEVERITY OF SELECTED TOMATO VARIETIES INFECTED WITH BACTERIAL WILT DISEASE

5.1 Abstract

Irrigation systems enhance sustainable tomato production through provision of adequate water for maximum yields. Presence of moisture in soil provides a breeding ground for *Ralstonia solanacearum* that causes bacterial wilt of tomato causing huge losses in tomato production. The aim of this study was to evaluate the effect of different irrigation systems on incidence and severity of bacterial wilt. Two experiments, one on drip and the other on furrow irrigation were conducted from the month of September 2021 to January 2022 in Isinya-Kajiado County on soilsinfected with *R. solanacearum*. Six treatments were laid out in a randomized complete block design (RCBD) with four replicates in each experiment. Severity of bacterial wilt was assessed four weeks after transplanting using a disease rating score of 0-4, while the number of wilted plants was recorded and used to determine percentage disease incidence. Severity and incidence were assessed every week for eight weeks. Tomato fruits were harvested once a week, weighed and yields converted to tons/hectare. The results showed high disease incidences in both drip andfurrow irrigation systems. The highest incidence (44.5%) was recorded in Riogrande variety under furrow irrigation while Riogrande variety with chemical treatment (Brono pol) had the highest incidence of 38.8% under drip system. Lowest incidence of 17.3% and 17.2% was observed in Big rock and Big rock with chemical treatment under drip and furrow systems respectively. Lowest severity was recorded in Big rock treatment with 1.75 under drip and Big rock with chemical treatment with 1.05 under furrow irrigation. Big rock with chemical treatment recorded the highest yields in both systems with 78.2t/ha under drip and 39.6t/ha underfurrow irrigation while Riogrande treatment had the lowest with 44.3t/ha and 16.2t/ha under drip and furrow systems respectively. Furrow irrigation demonstrated delayed disease symptoms but had the highest disease incidences towards the end of the growing season. Drip irrigation had expressed early bacterial wilt symptoms at the beginning of the season but had low disease incidences as compared to furrow. These results demonstrated that resistant cultivars such as Big rock F1 significantly gave higher yields with drip irrigation which is more efficient than furrow irrigation. This combination can be incorporated in disease management strategy for bacterialwilt resulting in increased tomato production.

Key words: Irrigation systems, *Ralstonia solanacearum*, incidence, tomato varieties, yields**.**

5.2 Introduction

Tomato (*Solanum lycopersicum*) is an important vegetable widely consumed around the world, and in Kenya it is ranked second after potato in terms of production and economic value (Sigei *et al.,* 2014). In 2018, tomato production was 21.2t/ha with the area harvested being 28,263hectares yielding 599,458 tons (FAOSTAT, 2018). Over the years, area under production has gradually increased but yields remain constant (Ochilo *et al.,* 2019) and this can be attributed to challenges that face tomato farming among them being pests and diseases and limited water supply. Tomato is a delicate plant and to obtain maximum yields, it requires management of insects, bacteria, fungal and viral diseases, adequate water and fertilizer application (Café-Filho*et al.,* 2019; Gupta *et al.,* 2018).

Ralstonia solanacearum, a soil born bacterium has been found to cause yield loses of up to 80% in tomatoes (Manani *et al.,* 2020). The bacteria survive well in moist conditions and multiplies rapidly when it gains entry into the host. As a result, infected plants show wilting symptoms and eventually die within a short period of time (Hayward, 1991). Management of *R. solanacearum* is difficult once the pathogen invades growing fields. Being a bacterial disease, there is no single strategy that can be used to effectively manage the pathogen. Farmers rely on cultural practices such as rogueing, crop rotation and use of tolerant varieties. The accumulation of loses andspread of the disease has led farmers to abandon tomato farming (Aslam *et al*., 2017).

The major tomato growing counties in Kenya include Kirinyaga, Kajiado, Bungoma, Meru, Kwale and Taita Taveta (Avedi *et al*., 2022). Rain fed agriculture and irrigation systems are the most practiced method of water application in tomato farming. Furrow irrigation system requires large amounts of water and has been reported to spread soil borne diseases including bacterial wilt. Drip irrigation is the most preferred method due to its effective water management and reduction of foliar diseases. However, studies have shown that drip system creates a favorable rhizosphere environment for multiplication of *R. solanacearum* (Marouelli *et al.,* 2005). Since *R.solanacearum* thrives well in moist soils, there is need for evaluation of suitable method of irrigation that will reduce the spread of *R. solanacearum* and obtain optimum yields. Therefore, the aim of this study was to evaluate the development of bacterial wilt on selected tomato varieties with varying resistance in different irrigation systems.

5.3 Materials and methods

5.3.1 Description of experimental site

The study was conducted in Kajiado County which is located at latitude 1^{0} 53' South and within longitude 36^{0} 47' East (GoK, 2009). The county is semi-arid and receives an annual rainfall of 500mm-800mm with an average annual temperature of 18.9 ^{O}C. It lies at 1733masl and within agro-ecological zone IV and an extension of zone V and is majorly covered with black cotton soils. Kajiado County has two distinct rainy seasons, the long rainy season which begins inMarch to May and the short rainy season from October to December (Bobadoye *et al.,* 2014). Table 5.0 shows the minimum and maximum temperatures during the experimental season in Isinya.

Table 5.0 Average temperature (^{O}C) readings from the month of September 2021 to January 2022 in Isinya

Month	Maximum	Minimum
September	25.00	14.47
October	27.16	15.42
November	26.83	16.53
December	24.13	19.32
January	26.61	15.65

Source: http//www.accuweather.com accessed on 8th July 2022

5.3.2 Selection of experimental site and detection of *R. solanacearum*

Visits were made in the tomato growing areas of Isinya and a focus group discussion was held with eight farmers with the help of an agricultural extension officer from the Ministry of Agriculture. Information on tomato varieties grown by the farmers, diseases of economic importance, cultural practices and water management systems used was recorded using a questionnaire. Diseased plant parts (leaves and stems) that showed bacterial wilt symptoms and soil samples were collected from the farms and carried in appropriate bags. Isolation of *Ralstonia solanacearum* from the diseased plants was carried out as described in section 3.3.2.

For soil samples, *R. solanacearum* was detected by taking 10 grams of soil sample mixed in 90ml sterile distilled water in 500ml conical flask. The samples were mixed thoroughly by putting them on a rotary shaker at 120 rpm for 10 minutes. One milliliter was drawn with a micropipette and transferred to a universal bottle containing 9ml of sterile distilled water to make a dilution factor of 10^{-1}. This dilution process was repeated up to 10^{-5} for each sample. The dilutions of each sample were plated in triplicate through pour plate method in petri-dishes containing 2,3,5 triphenyltetrazolium chloride (TZC) -casein-peptone-glucose agar and sterilized at 12 °C for 20 minutes. The molten media was amended with 0.5% percent aqueous tetrazolium at 50 °C (Popoola *et al.,* 2015). Observations were made after 36 and 48 hours and colonies that had a white and pinkish center were counted and recorded. Farms with the highest inoculum density of *R. solanacearum* were selected for field experiments.

5.3.3 Planting materials

Big rock F1, Assila F1 and Riogrande tomato varieties were selected for the studies under drip and furrow irrigation systems in open field. These varieties were observed to be resistant,moderately susceptible and highly susceptible respectively in the greenhouse experiment as shown in Section 3.4.1 (table 2) and were also the most frequently grown tomato varieties in Kajiado County. The seeds and potting medium (hygromix) were purchased from certified local agrovets suppliers and seedlings were raised in germination trays containing hygromix and placed in a greenhouse. The seedlings were watered as per the need.

5.3.4 Experimental layout and treatments

Two irrigation systems drip and furrow were laid out in fields that had been found to have high concentrations of *R. solanacearum.* The design used was randomized complete block design (RCBD) with four replications. In drip irrigation system, a plot size of 3.6m by 1.8m with four drip lines was used. Plant spacing along the drip line was 0.9m while spacing between the drip lines was 0.6m. Plots were separated from each other by 0.5m. The number of plants along each drip line per plot was 5 making a total of 20 plants per plot. For furrow irrigation a basin plot measuring 3m by 2m was used. Plant spacing along the length was 0.6m while along the width was 0.9m. Plots were separated from each other by a distance of 0.5m. Each plot contained a total number of 14 plants. Treatment blocks in drip and furrow irrigation system were separated from each other by a spacing of 1m.

The treatments in each irrigation system were Big Rock F1 variety, Assila F1 variety, Riogrande variety, Big Rock F1 variety+ Standard chemical (Enrich BronoPol**)**, Assila F1 variety +

Standard chemical (Enrich BronoPol), Riogrande variety + Standard chemical (EnrichBronoPol). One gram of Enrich BronoPol (Immunodulation-2-bromo-2 Nitropropane 1,3 Diol) was mixed with 3 litres of water as per the recommended rate of 20g/60L and applied as a foliar spray two weeks after transplanting seedlings and repeated after every 14 days. A maximum of four sprays were done during the entire growing season. Drip irrigation experiment was conducted between the months of September 2021 to January 2022 while furrow irrigation was conducted between the months of October 2021 to February 2022.

5.3.5 Crop husbandry

Land was harrowed, leveled and beds raised appropriately to support the drip lines in the drip irrigation system. The basins for furrow irrigation were prepared by digging trenches between crop rows in the field to make basins where water was directed and infiltrated in to the soil. Di-ammonium Phosphate (DAP) fertilizer was applied during transplanting at the rate of 5 grams per hole. Thirty-day old seedlings were transplanted and after three weeks top dressed with Calcium ammonium nitrate (CAN) followed by NPK (17:17:17) during flowering. Watering for each irrigation system was done as per the crop needs and also based on farmer's practice. Weeding, staking and pruning were done when required.

Scouting for diseases and pests was conducted regularly. Management of insect pests was done using Coragen (Chlorantraniliprole and Rynaxypyr) at 200g/l alternated with Tracer (Spinosad) for tomato leaf miner (*Tuta absoluta*) and Nimbecidine for whiteflies. Early and late blight diseases were controlled by spraying Milraz 76 WP with an active ingredient of Propinep 700g/kg and Cymoxanil 60g/kg alternated with Ridomil Gold MZ 68WG with an active ingredient of Metalaxyl-M 40g/kg and Mancozeb 640g/kg.

5.3.6 Assessment of disease severity

Disease severity was scored every week after the first symptom was observed and continued for eight weeks based on a modified key described by Uwamahoro *et al.* (2018). Severity rating score: 0 – no symptoms, 1— one or two young leaves wilted, 2 – half of all the leaves wilted, 3 –almost all the leaves wilted,4 – dead plant.

5.3.7 Determination of disease incidence

Disease incidence was assessed by counting the number of wilted plants per plot after every seven days for eight weeks. Percentage bacterial wilt incidence was calculated per treatment by the formula described by Ayana *et al*. (2011):

$$WI = NPSWS/TNPT * 100$$

Where WI-wilt incidence, NPSWS-number of plants showing wilt symptoms and TNPT- total number of plants per treatment.

5.3.8 Assessment of yield parameters

Yields of tomato were recorded after every five days from the first harvest of mature fruits at 83 and 78 days for drip and furrow irrigation systems respectively. A digital weighing balance was used to measure the weight of fruits per plot and then converted to tons per hectare (Diago and Wydra, 2007). Fruit quality was assessed by measuring the diameter of three fruits, the largest, medium and small fruits of each plot by use of Vernier caliper.

5.3.9 Assessment of stem browning

The length and width of stem browning was assessed once at the end of the growing season at 108 and 115 days after transplanting for drip and furrow irrigation systems respectively. The length and width were measured by use of a meter ruler.

5.3.10 Statistical analysis

Data on severity, incidence, yield parameters and stem browning were subjected to analysis of variance Using GenStat software version 15th edition and means were separated by Fishers' Protected Least Significant Difference at 5%. For the fruit size, means were separated by t- test with ($P<0.05$).

5.4 Results

5.4.1 Expression of bacterial wilt incidence on selected tomato varieties grown under drip and furrow irrigation

Bacterial wilt infection started earlier for all treatments under drip system. In furrow irrigation, infection delayed and was observed to rise in the last three weeks and surpassed the wiltincidence observed under drip irrigation. Bacterial wilt incidence varied significantly ($P<0.05$) inall the treatments throughout the experiment for drip irrigation system. There was no significant difference among treatments under furrow irrigation system at week 3, 4, 5 and 6 while at week 7 and 8 the treatment showed significant variation. Two treatments, Big rock and Big rock with chemical showed delayed expression of wilt incidence compared to other treatments. Wilt was observed from week 4 and 5 for Big rock with chemical and Big rock treatments respectively on drip irrigation system and at week 6 under furrow irrigation system. Big rock with chemical and Big rock treatments recorded consistently low disease incidence from the onset of disease compared to Riogrande and Riogrande with chemical in both drip and furrow irrigation systems. At week 8, Big rock recorded the lowest incidence of 17.3% followed by Big rock with chemical with 20.7% on drip while on furrow irrigation, Big rock with chemical recorded the lowest incidence of 17.2% followed by Big rock 20.5%. Riogrande and Riogrande with chemicalrecorded the highest percentage wilt incidence of 34.3% and 38.8% on drip and 44.5% and 41.1% on furrow respectively. Assila and Assila with chemical performed moderately well in both irrigation systems, however Assila with chemical outperformed Assila treatment only at week 7 on furrow irrigation systems (Table 5.1).

Table 5.1: Mean percentage disease incidence of selected tomato varieties evaluated on drip and furrow irrigation systems

	Weeks after transplanting					
Treatments	3	4	5	6	7	8
Drip						
Assila F1	9.00bc	9.04bc	16.64a	17.90ab	17.90bc	28.00ab
Assila+Chemical	10.90b	18.75ab	20.31ab	20.30ab	25.9abc	27.40ab
Big rock F1	0.00c	0.00c	1.47c	8.70b	10.20c	17.30b
Big rock+chemical	0.00c	1.32c	1.32c	6.60b	11.60c	20.70b
Riogrande	14.50ab	18.39b	27.55ab	30.20a	30.20a	34.30a
Riogrande+Chemical	22.40a	30.08a	34.105a	35.40a	38.83a	38.8a
Mean	9.31	12.9	16.9	19.9	22.4	27.8
LSD	9.5	11.53	15.34	18.22	16.58	11.99
CV%	65.1	59.2	60.3	60.9	49.1	28.6
P-value	<.001	<.001	0.002	0.025	0.014	0.015

	3	4	5	6	7	8
Furrow						
Assila F1	9.10a	14.10a	16.40a	22.00a	39.90a	39.90ab
Assila+Chemical	6.10a	6.10a	6.10a	18.30a	26.90b	31.90b
Big rock F1	0.00a	0.00a	0.00a	11.20a	20.50b	20.50c
Big rock+chemical	0.00a	0.00a	0.00a	8.90a	17.20b	17.20c
Riogrande	8.50a	8.50a	15.80a	28.30a	41.40a	44.50a
Riogrande+Chemical	9.60a	11.1a	15.70a	20.70a	41.10a	41.10ab
Mean	5.50	6.60	9.00	18.20	31.20	32.50
LSD	15.22	15.95	16.81	20.11	11.15	11.01
CV%	182.2	159.5	124.1	73.2	23.7	22.5
P -value	0.579	0.356	0.132	0.374	<.001	<.001

Means in the same column followed by the same letter are not significantly different. Means were separated using Fishers' Protected Least Significant Difference ($P<0.05$)

5.4.2 Expression of bacterial wilt severity under drip and furrow irrigation system

There was significant variation observed at $P<0.05$ among treatments for both drip and furrow irrigation system. All the treatments under drip system recorded higher severity scores in all the weeks than treatments under furrow irrigation. Big rock treatment recorded the lowest severity score of 1.75 while Big rock with chemical had severity score of under on drip and furrow irrigation respectively. High disease severity scores were noted in Riogrande with chemical treatment with 3.75 followed by Assila treatment with 3.6 on drip system while under furrow system, Riogrande recorded the highest severity score of 3.05 followed by Assila treatment with 2.6. Treatments Assila, Assila with chemical, Riogrande, Riogrande with chemical showed no significant difference for both irrigation systems in all the weeks except at week 7 under drip irrigation system. Big rock and Big rock with chemical treatments had no significant difference from each other but they highly differed significantly from the rest of the treatments in all the weeks in both drip and furrow irrigation systems (Table 5.2).

Table 5.2: Mean disease severity rating scores of selected tomato varieties evaluated on drip and furrow irrigation systems

	Weeks after transplanting					
Treatments	3	4	5	6	7	8
Drip						
Assila F1	0.65b	0.70c	1.25b	2.00b	2.20b	3.60a
Assila+Chemical	0.75b	1.65b	1.90b	2.30b	2.45b	3.25a
Big rock F1	0.00c	0.00d	0.05c	0.35c	0.45c	1.75b
Big rock+chemical	0.00c	0.05d	0.05c	0.60c	0.70c	2.35b
Riogrande	0.9ab	1.20bc	1.90b	2.50ab	2.50b	3.30a
Riogrande+Chemical	1.45a	2.50a	3.15a	3.10a	3.40a	3.75a
Mean	0.63	1.02	1.38	1.81	1.95	3.00
LSD	0.58	0.65	0.68	0.76	0.74	0.69
CV%	147.7	101.6	78.7	66.8	60.5	36.6
P-value	<.001	<.001	<.001	<.001	<.001	<.001

	3	4	5	6	7	8
Furrow						
Assila F1	0.35a	0.60a	0.70a	1.25a	1.80a	2.60a
Assila+Chemical	0.25a	0.35ab	0.35ab	0.85ab	1.65a	2.40a
Big rock F1	0.00a	0.00b	0.00b	0.30b	0.70b	1.45b
Big rock+chemical	0.00a	0.00b	0.00b	0.25b	0.70b	1.05b
Riogrande	0.35a	0.40ab	0.60a	1.15a	1.95a	3.05a
Riogrande+Chemical	0.35a	0.40ab	0.45ab	0.75ab	1.65a	2.45a
Mean	0.22	0.29	0.35	0.76	1.41	2.17
LSD	0.36	0.43	0.46	0.64	0.70	0.88
CV%	264.4	237.6	208.9	134.3	79.2	64.6
P-value	0.117	0.039	0.008	0.007	<.001	<.001

Means in the same column followed by the same letter are not significantly different. Means were separated using Fishers' Protected Least Significant Difference (P<0.05)

5.4.3 Effects of bacterial wilt on yields of tomato varieties on drip and furrow irrigation system

There was no significant variation in yields among treatments from week one to week four on drip irrigation system. In furrow irrigation system, treatments in week one and two showed significant differences ($P<0.05$) while treatments in week three and four had no significant differences ($P>0.05$). All treatments in drip irrigation recorded higher yields in all the four weekscompared to treatments in furrow irrigation system. The highest yields of tomato were harvested in week two for the treatments Assila, Assila with chemical, Big rock, and Big rock with chemical and Riogrande and Riogrande with chemical treatments at week three in drip system. All treatments recorded highest yields at week three on furrow irrigation except Big rock with chemical at week 2 **(**Table 5.3**).** Yields of each treatment on drip system significantly differed from those yields harvested in furrow irrigation system **(**Table 5.4). Big rock with chemical treatment recorded the highest yields at78.2t/ha and 39.6t/ha in both drip and furrow irrigation followed by big rock with yields of 73.0t/ha and 36.8t/ha respectively. Riogrande recordedlowest yields of 44.3t/ha under drip irrigation system while AssilaF1 treatment recorded the lowest yields of 16.2t/ha under furrow irrigation. Yields of each treatment on drip system were higher than those yields obtained in furrow irrigation system which were consistently lower **(**Table 5.4**).**

Table 5.3: Mean yields harvested in four weeks from selected tomato varieties under drip and furrow irrigation systems

	Yield in drip system (t/ha)					Yields in Furrow system (t/ha)				
		weeks				weeks				
Treatments	1	2	3	4	Total yields	1	2	3	4	Total yields
Assila F1	12.00a	25.50a	19.50a	6.26bc	63.3a	3.00a	4.02bc	7.92b	1.23b	16.2b
Assila+Chemical	9.00a	18.80a	15.80a	5.78c	49.3a	1.33b	8.28b	10.61ab	2.18ab	22.4b
Big rock F1	14.4a	27.90a	21.00a	9.75ab	73.0a	2.68ab	12.76a	15.15a	6.25a	36.8a
Big rock+chemical	14.2a	27.70a	25.80a	10.56a	78.2a	4.61a	16.96a	15.18a	2.83ab	39.6a
Riogrande	6.00a	13.50a	18.10a	6.71abc	44.3a	1.9b	3.78c	7.52b	5.03ab	18.2b
Riogrande+Chemical	9.00a	14.9a	19.4a	7.92abc	51.3a	1.73b	4.78bc	12.02ab	4.49ab	23.0b
Mean	10.80	21.40	19.90	7.83	59.9	2.54	8.43	11.40	3.67	26.0
LSD	10.95	20.72	14.93	3.92	34.69	2.07	4.26	6.01	4.09	10.46
CV%	67.4	64.3	49.7	33.2	0.270	54	33.6	35.0	73.9	26.7
P-value	0.541	0.514	0.799	0.102	38.4	0.045	<.001	0.053	0.143	<.001

Means in the same column followed by the same letter are not significantly different. Means were separated using Fishers' LSD (P<0.05)

Table 5.4: Mean yields (t/ha) obtained from selected tomato varieties under drip and furrow irrigation systems

	Total yields per treatment (t/ha)		
Treatments	Drip irrigation	Furrow irrigation	P-value
Assila F1	63.30a	16.20b	<.001
Assila+Chemical	49.30a	22.40a	0.199
Big rock F1	73.00a	36.80a	0.131
Big rock+chemical	78.20a	39.60b	<.001
Riogrande	44.30a	18.20b	0.007
Riogrande+Chemical	51.30a	23.00a	0.071

Means in the same row followed by the same letter are not significantly different.
Means were separated using a t-test with (P<0.05).

5.4.4 Effects of bacterial wilt on fruit size of selected tomato varieties under drip and furrow irrigation

Mean fruit length of large, medium and small fruits varied significantly among treatments under drip irrigation. There was no significant variation (P>0.05) among treatments under furrow irrigation for large, medium and small fruits. Average fruit length ranged from 78.25mm for Assila to 53.99mm for Riogrande with chemical under drip while under furrow average length was 67.0mm for Assila to 44.04mm for Riogrande with chemical (Table 5.5).

Significant variation was also observed on mean width in all treatments under both drip and furrow irrigation except in small fruits under furrow irrigation. The average width under drip irrigation ranged from 44.98mm for Big rock to 71.32mm in Riogrande treatment while under furrow irrigation the range was between 38.2mm to 61.77mm in Riogrande with chemical (Table5.6).

Table 5.5: Average fruit length of selected tomato varieties under drip and furrow irrigation system

	L. of large fruits		L. of medium fruits		L. of small fruits	
Treatments	Drip	Furrow	Drip	Furrow	Drip	Furrow
Assila F1	78.25a	67.04a	68.60a	60.50a	60.33a	49.20a
Assila+Chemical	77.76a	64.89ab	70.77a	53.70a	59.13a	48.51a
Big rock F1	72.81b	62.99a	63.99b	58.20a	56.88b	48.99a
Big rock+chemical	71.02b	63.88a	64.45b	57.80a	56.42b	51.44a
Riogrande	69.26b	60.51a	60.87c	55.50a	54.21c	48.64a
Riogrande+Chemical	69.23b	56.97b	61.68bc	51.20a	53.99c	44.04a
Mean	73.05	62.71	65.06	56.1	56.83	48.47
LSD	3.72	7.02	3.01	7.58	1.84	7.21
CV%	3.4	7.4	3.1	9.0	2.2	9.9
P-value	<.001	0.096	<.001	0.177	<.001	0.438

Means in the same column followed by the same letter are not significantly different.
Means were separated using Fishers' Protected Least Significant Difference (P<0.05).

Table 5.6: Average fruit width of selected tomato varieties under drip and furrow irrigation system

	W. large fruits (mm)		W. of medium fruits (mm)		W. of small fruits (mm)	
Treatments	Drip	Furrow	Drip	Furrow	Drip	Furrow
Assila F1	63.48b	52.76bc	55.52b	47.79abc	46.72bc	40.90a
Assila+Chemical	61.99bc	49.68c	54.86b	43.29c	47.88b	39.50a
Big rock F1	71.32a	61.77a	60.68a	53.39a	51.90a	55.20a
Big rock+chemical	71.28a	59.9ab	60.19a	52.47ab	50.93a	45.70a
Riogrande	57.93c	50.98c	54.19b	46.79bc	44.98c	41.10a
Riogrande+Chemical	59.22c	49.91c	53.08b	42.65c	46.05bc	38.20a
Mean	64.20	54.17	56.42	47.73	48.08	43.40
LSD	4.09	7.37	3.00	6.01	2.76	13.87
CV%	4.2	9.0	3.5	8.4	3.8	21.2
P-value	<.001	0.009	<.001	0.006	<.001	0.159

Means in the same column followed by the same letter are not significantly different. W-width, mm-millimeter. Means were separated using Fishers' Protected Least Significant Difference (P<0.05).

The length and width of large, medium and small tomato fruits were significantly higher in drip system compared to furrow system. Tomato fruits harvested from all treatments in drip irrigation system were significantly longer and wider than in furrow irrigation system (Tables 5.7. 5.8 and 5.9).

Table 5.7: Mean length and width (mm) of large tomato fruits in drip and furrow irrigation system

Treatments	Drip	Furrow	P-value
Length(mm)			
Assila	78.30a	67.00b	0.016
Assila+Chemical	77.80a	64.90b	0.021
Bigrock	72.80a	63.00b	0.020
Big rock+chemical	71.02a	63.88b	0.003
Riogrande	69.26a	60.51b	<.001
Riogrande+Chemical	69.20a	57.00b	0.009
	Drip	Furrow	P-value
Width(mm)			
Assila	63.50a	52.80a	0.064
Assila+Chemical	62.00a	49.70b	0.004
Big rock	71.30a	61.80b	0.021
Big rock+chemical	71.28a	59.90b	<.001
Riogrande	57.93a	50.98b	0.002
Riogrande+Chemical	59.20a	49.90a	0.065

Means in the same row followed by the same letter are not significantly different. Means were separated using a t-test with ($P<0.05$).

Table 5.8: Mean length and width (mm) of medium tomato fruits in drip and furrow irrigation system

Treatments	Drip	Furrow	P-value
Length(mm)			
Assila	68.60a	60.50a	0.065
Assila+Chemical	70.80a	53.70b	<.001
Big rock	63.99a	58.22b	0.016
Big rock+chemical	64.45a	57.75b	<.001
Riogrande	60.87a	55.54b	0.022
Riogrande+Chemical	61.70a	51.20a	0.074
	Drip	Furrow	P-value
Width(mm)			
Assila F1	55.50a	47.80b	0.011
Assila+Chemical	54.90a	43.30b	0.001
Big rock F1	60.68a	53.39b	<.001
Big rock+chemical	60.19a	52.47b	0.003
Riogrande	54.19a	46.79b	<.001
Riogrande+Chemical	53.10a	42.65a	0.089

Means in the same row followed by the same letter are not significantly different. Means were separated using a t-test with (P<0.05).

Table 5.9: Mean length and width (mm) of small tomato fruits in drip and furrow irrigation system

Treatments	Drip	Furrow	P-value
Length(mm)			
Assila F1	60.30a	49.20b	0.008
Assila+Chemical	59.10a	48.50b	0.009
Big rock F1	56.90a	49.00b	0.009
Big rock+chemical	56.42a	51.44b	0.013
Riogrande	54.21a	48.64b	0.003
Riogrande+Chemical	54.00a	44.00a	0.051
	Drip	Furrow	P-value
Width(mm)			
Assila F1	46.70a	40.90a	0.075
Assila+Chemical	47.90a	39.50b	0.012
Big rock F1	51.90a	55.20a	0.785
Big rock+chemical	50.93a	45.66b	0.007
Riogrande	44.98a	41.09b	0.012
Riogrande+Chemical	46.00a	38.20a	0.078

Means in the same row followed by the same letter are not significantly different.
Means were separated using a t-test with (P<0.05).

5.4.5 Effects of bacterial wilt on stem browning on selected tomato varieties grown under drip and furrow irrigation system.

Destructive sampling was done at the end of the experiment and length and width of stem browning recorded. There was no significant difference among treatments on average length and width of browning among treatments under both drip and furrow irrigation. However, Riogrande recorded the longest length and width of browning of 10.7cm and 1.59cm respectively under dripsystem while under furrow, Assila recorded the longest length and width of browning of 3.46cm and 0.383cm. In both irrigation systems, Big rock had the shortest length of browning 5.13cm in drip and 1.75cm under furrow. All treatments under drip system recorded higher values of both length and width of browning as compared to their respective treatments under furrow irrigation system (Table 5.10).

Table 5.10: Mean value of length (cm) and width (cm) of browning

	Drip system		Furrow system	
Treatments	L. of Browning	W. of browning	L. of browning	W of browning
AssilaF1	9.38a	1.07a	3.46a	0.38a
Assila+Chemical	6.48a	1.34a	2.63a	0.23a
Big rock F1	5.13a	1.07a	1.75a	0.23a
Big rock+chemical	6.58a	1.25a	2.20a	0.21a
Riogrande	10.7a	1.59a	2.21a	0.22a
Riogrande+Chemical	5.50a	0.90a	2.65a	0.30a
Mean	7.29	1.20	2.48	0.26
LSD	5.53	0.72	3.39	0.32
CV%	92.90	73.10	167.40	152.30
P-value	0.271	0.47	0.946	0.87

Means in the same column followed by the same letter are not significantly different. L-Length, W-width, cm-centimeter. Means were separated using Fishers' Protected Least Significant Difference ($P<0.05$).

5.4.6 Correlation of disease incidence, severity and yield parameters of tomato under drip irrigation system

Positive correlation was observed between disease incidence and severity of bacterial wilt ($r=0.98$, $P<0.001$). Both incidence and severity had a negative correlation with tomato yields ($r= -0.89$, $r= -0.87$, $P<0.01$). Large fruit length had a positive correlation with medium fruit and small fruit lengths (r= 0.96, r= 0.98, P<0.01 and P<0.001). There was also a positive correlation between medium fruit length and small fruit length (r=0.93, P<0.01), (Table 5.11).

There was a positive correlation between bacterial wilt incidence and severity (r=0.96, P<0.001). Yields of tomato had a negative correlation with incidence and severity of bacterial wilt (r= - 0.93, r= -0.99, P<0.01 and P<0.001). Length of browning had positive correlation with width of browning (r= 0.87, P<0.01), (Table 5.12).

Table 5.11: Correlation table for disease incidence, severity and yield parameters of tomato under drip irrigation system

	%Incidence	L. browning	Severity	W. browning	Yields	LF Length	MF Length	SF Length
%Incidence	-							
L. browning	0.24	-						
Severity	0.98***	0.23	-					
W. browning	0.01	0.63	-0.05	-				
Yields	-0.89**	-0.41	-0.87**	-0.36	-			
LF_Length	-0.23	0.06	-0.08	-0.08	0.06	-		
MF_Length	-0.23	-0.06	-0.07	-0.04	0.07	0.96**	-	
SF_Length	-0.40	0.06	-0.25	-0.11	0.26	0.98***	0.93**	-

Key: L. Browning= Length browning, W. browning = Width browning, LF Length=Large Fruit Length, MF Length=Medium Fruit Length, SF Length=Small Fruit Length.

Values abbreviated with '***' $P<0.001$, '**' $P<0.01$, '*' $P<0.05$

Table 5.12: Correlation table for disease incidence, severity and yield parameters of tomato under furrow irrigation system

	%Incidence	L. browning	LF Length	MF Length	severity	SF Length	Yields	W. browning
%Incidence	-							
L. browning	0.64	-						
LF Length	-0.32	0.35	-					
MF Length	-0.31	0.13	0.77	-				
Severity	0.96***	0.68	-0.10	-0.23	-			
SF Length	-0.62	-0.16	0.28	0.43	-0.66	-		
Yields	-0.93**	-0.71	0.01	0.18	-0.99***	0.67	-	
W. browning	0.61	0.87**	0.21	0.25	0.56	-0.22	-0.58	-

Key: L. Browning= Length browning, W. browning = Width browning, LF Length=Large Fruit Length, MF Length=Medium Fruit Length, SF Length=Small Fruit Length.

Values abbreviated with '***' P<0.001, '**' P<0.01, '*' P<0.05

5.5 Discussion

Irrigation methods used in tomato farming highly influence the expression of bacterial wilt. The study identified that both drip and furrow systems had high incidence of bacterial wilt of 38.8% and 44.5% respectively by the end of the experiments. A similar study conducted by Marouelli *et al.* (2005) reported that drip irrigation recorded 42.5% bacterial wilt incidence against 5% in sprinkler irrigation.

In drip system, incidence and severity of bacterial wilt started earlier for all the treatments as compared to furrow irrigation where bacterial wilt incidence was low at the beginning of the experiment and noted to rise and surpassed the wilt incidence observed in drip. These results are comparable with those of Cabral *et al*. (2011) who found out that water application made locally available in the rhizosphere of the plant root in the drip system had the soil layer saturated for longer hours. This created favourable conditions for the multiplication and infection by *Ralstonia solanacearum* around the root zone.

In furrow irrigation system, disease incidence delayed at the beginning of the experiment. However, in the last three weeks towards the end of the experiment, wilt incidences increased rapidly. Studies by Singh *et al*. (2015) have shown that water applied on the soil surface experiences high evaporation creating wet and dry condition. Mc Cater *et al*. (1969), conducted an experiment on vertical distribution of *R. solanacearum* on different types of soil and observedthat population of bacteria on the upper surface of the soil (0-15cm) was lower than in deeplayers of (15-30cm) and this was attributed to the greater variability of soil moisture in surface layers. Graham and Lloyd (1979) observed that *R. solanacearum* survives for a long period in infected pockets of soil at a greater depth than on the superficial layers of the soil. There was rapid increase of wilt incidence in the last three weeks in furrow irrigation. Studies have shown that free flow of water on contaminated soil contributed to the spread of *R. solanacearum* from one plant to another (Cabral *et al.,* 2011).

Tomato Big rock variety alone and Big rock combined with brono pol had low incidences and severity in both drip and furrow irrigation system. In this study Big rock variety was noted to be a resistant variety to bacterial wilt in the greenhouse and open field. Studies by Grimault *et al*. (1995) and Sigh (1961) found out that resistance is linked to certain single dominant genes and recessive genes while studies done by Oliveria *et al*. (1999) found out that, additive effects of the genes contributed to resistance against bacterial wilt.

However, bacterial wilt incidence and severity where tomato varieties were combined with Brono pol was not significantly different to tomato varieties planted alone. Brono pol (Immunodulation-2-bromo-2 Nitropropane 1, 3 Diol) is a biological product that acts as an immunomodulator that activates the plant host natural defense system against bacterial wilt diseases of potato, tomato, cabbage and beans (https://www.oshochem.com/pdf/crop/ENRICH.pdf).
This observation confirms the study of Aslam *et al* (2017) who reported that it is difficult to control bacterial wilt and no single method can be used to control the disease. However, breedingfor resistance against bacterial wilt as described by Kathimba *et al*. (2018) and Lebeau *et al*. (2011) is an effective method hence the lower percentage incidence recorded by Big rockF1variety.

Higher yields were obtained in drip than in furrow irrigation system and this can be explained by the target application of water on the root region of the plants. The effect of high evaporation was experienced more in furrow irrigation system because soil did not maintain the moisture for long after water application (Singh *et al*. 2015; Cabral *et al.,* 2011). High yields were also obtained in Big rock with chemical followed by Big rock F1 treatment and this was because theywere infected less by bacterial wilt disease. Big rock maintained more plant stand than Riogrande treatment which had low yields due to high bacterial wilt infection reducing the number of plant stand.

Fruits harvested in drip system had the longest length and width in all treatments while fruits harvested in furrow were smaller, which was influenced by the period of water availability for uptake by the plant roots in both systems. In drip system soil moisture persisted longer on the root zone creating adequate time for water uptake. Similar findings were reported by Medyouni*et al*., (2021) who found out that water deficit in tomato plants reduces the size of fruits and leaves. Stem browning of sampled plants was observed to be shortest in Big rock and Big rock with chemical treatments as compared to the rest of the treatments. Similar findings by Pragthanang *et al*. (2005) who reported brown discoloration in vascular bundles due to *R.solanacearum* colonization, however resistant varieties tend to fight against the mechanisms of the pathogen in the plants thus reducing the browning effect.

High yields were obtained in drip irrigation system in all the tomato varieties tested. Big rock variety was identified to be resistant and also high yielding in both systems. Based on the results of this study, Big rock F1 variety can be recommended to farmers in areas infected with bacterial wilt disease under drip irrigation systems.

CHAPTER 6. GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

6.1 General discussion

Host plant resistance of tomato crop against bacterial wilt disease remains the most effective method to manage *Ralstonia solanacearum*. The eighteen varieties that were evaluated in this study demonstrated varying degrees of resistance against bacterial wilt disease. The five hybrids Kilele F1, Terminator F1, Bravo F1, Big rock F1 and Ranger F1 showed resistance against the disease while the local cultivars Riogrande, Rionex and Isisementi were susceptible. In Kenya, a similar study was done by Manani *et al*. (2020) and found out that Tall vine and Goliath pear hybrids were resistant to *Ralstonia solanacearum* among six varieties tested while the other four varieties were susceptible. Findings by Kathimba *et al*. (2018) and Aslam *et al*. (2017) showed that Riogrande variety was susceptible to *R. solanacearum*.

In the present study, resistant varieties expressed delayed symptoms of bacterial wilt compared to the susceptible varieties. Resistant varieties have been bred against bacterial wilt and studies have shown that delayed expression of disease could be due to enhanced defense system. Manani*et al*. (2020) and Vanitha (2009) found out that production and activity of phenylalamineammonia liase (PAL) and Polyphenol oxidase (PPO) led to the secretion of phenolic compounds that enhances the plants' defense system against bacterial wilt. Grimault *et al*. (1995) concluded that the resistance of tomato cultivars to bacterial wilt was due to certain single dominant and recessive genes and in addition additive effects of the genes contributed to resistance against the pathogen (Oliveria *et al*., 1999). Early symptoms of the bacterial wilt disease were observed on susceptible varieties and the plants easily wilted and died. Studies have shown that *Ralstonia*- susceptible variety results to a heavy and rapid colonization of the pathogen that blocked water movement in the xylem vessels leading to death of the crop (Hacisalihoglu *et al*., 2008).

Soil moisture played a significant role in the development and expression of bacterial wilt disease in the greenhouse experiment. At high moisture levels of 100% field capacity (FC) and 120%FC, bacterial wilt incidence and severity were noted to be high. These findings agree with those of Mondal *et al*. (2014) who found out that high incidences of bacterial wilt occurredduring high rainfall season than during low rainfall season. Gupta *et al*. (2018) found out that theincreased bacterial wilt incidences during high moisture conditions can be ascribed to rapid multiplication of *R. solanacearum* once it gains entry in the xylem vessels through the tiny pores in the root hairs. High moisture conditions make the tomato plants to exhibit enlarged cell size, cortex and epidermis that provides a favorable condition for *R. solanacearum* multiplication that leads to clogging of xylem vessels hence death of the plants (Gupta *et al*., 2018; Vasse *et al*., 1995).

At low moisture level 50%FC, there was reduced incidence and severity of bacterial wilt. These results are comparable to those of Gupta *et al*. (2018) who reported that during low moisture conditions, tomato plants tend to increase the growth of lateral roots for maximum water absorption from the soil. The increased growth of lateral roots potentially creates a risk by increasing the surface area of hair roots for *R. solanacearum* entry into the plant to cause infection (Vasse *et al*., 1995). However, the low level of moisture in the soil, concentration, composition and diffusion of plants exudates involved in chemotaxis are altered reducing the attraction of the pathogen to the root hairs (Gupta *et al*., 2018). Additionally, *R. solanacearum* movement from the roots to the shoots of the plants is hindered by closing of stomata whichalters the transpiration pull under low moisture condition (Nakaho *et al*., 2000).

Irrigation methods practiced in the field during tomato production influences the development of bacterial wilt disease. The present experiments conducted in the field on drip and furrow irrigation systems recorded bacterial wilt incidences of 38.8% and 44.5% on drip and furrow irrigation systems respectively. Similar findings were reported by Maroueli *et al.* (2005) with 42.5% bacterial wilt incidence on drip irrigation system and 5% wilt incidence on sprinkler irrigation system. The findings of the current study also showed that there was early onset of bacterial wilt disease on drip irrigation while on furrow irrigation system, there was a delay in disease expression but towards the end of the growing season, bacterial wilt incidence and severity surpassed those recorded in drip irrigation. Cabral *et al.* (2011) and Marouelli *et al.* (2005) conducted a similar study and found out that the high incidence in drip irrigation was due to application of water in the root zone of the drip irrigated plants which ensured long hours of moisture to the plant roots. Singh *et al*. (2015) and Cabral *et al.* (2011) demonstrated that high evaporation of water applied on the surface of the soil created a dry soil condition for the plants which led to delayed disease incidence and severity of bacterial wilt. There was increased wiltingof plants towards the end of the season in furrow irrigation. Studies have shown that free flow of water on contaminated soil contributed to the spread of *R. solanacearum* from one plant to another (Cabral *et al.,* 2011).

Higher yields were achieved in drip irrigation system compared to furrow irrigation system. The high yields obtained in drip irrigation can be associated with target of water application which ensured constant available moisture to the plants during the growing season. In the field experiments, Big Rock F1 had the highest yields in both drip and furrow irrigation systems compared to Assila F1 and Riogrande variety. Big rock F1 variety was among the resistant varieties in the screening experiment and therefore continued to express significantly reduced incidence and severity of bacterial wilt in the subsequent experiments. Findings by Kim *et al*. (2016) and Nakaho *et al*. (2014) showed that resistant varieties have thickened pit wall membranes that hinder the flow of *R. solanacearum* in xylem vessels unlike susceptible varieties that had high concentration of the pathogen in the primary and secondary xylem. Based on Hacisalihoglu *et al*. (2008), high content of nitrates in susceptible varieties is associated with increased wilts because *R. solanacearum* depends on inorganic minerals for its pathogenicity.

6.2 Conclusion

The eighteen screened tomato varieties showed varying degrees of susceptibility to *R. solanacearum*. Five varieties demonstrated resistance to bacterial wilt disease and they include Kilele F1, Terminator F1, Bravo F1, Big rock F1 and Ranger F1. Non hybrid varieties including Riogrande, Rionex and Isisementi showed susceptibility to the disease. When Big rock F1, Assila F1 and Riogrande variety were planted on different moisture regimes the expression of thedisease was lower in Big rock F1 and high in Riogrande variety. When the three varieties were grown under drip and furrow irrigation systems, Big rock F1 had low disease incidence while Riogrande variety had high bacterial wilt incidences. The application of Brono pol product to tomato varieties had no effect on suppressing bacterial wilt disease in the field. The results showed that bacterial wilt is greatly influenced by moisture levels where high moisture conditionincreases the bacterial wilt disease. The use of drip irrigation system increases the yields of tomato. The findings also showed that resistant varieties were able to reduce bacterial wiltdisease on both drip and furrow irrigation and also resulted in increased tomato produce.

6.3 Recommendations and further studies

6.3.1 Recommendations

i. The resistant cultivars, Kilele F1, Terminator F1, Bravo F1, Big rock F1 and Ranger F1 which can be recommended for use by farmers in an integrated diseases management system in areas that are prone to bacterial wilt in Kajiado County. This will significantly increase tomato productivity where used.

ii. Under varying degrees of moisture levels and in soils infected with *Ralstonia solanacearum*, Big rock F1 consistently gave a lower incidence of bacterial wilt compared to Assila F1 and Riogrande. The variety can therefore be recommended for enhanced tomato production under similar environments as above.

iii. High yields were obtained under drip irrigation system in all the tomato varieties tested. Big rock F1 was identified to be resistant and also high yielding in both systems. Based on the results of this study, Big rock F1 variety can be recommended to farmers in areas infected with bacterial wilt disease under drip irrigation systems.

6.3.2 Further studies

i. Periodically, screening of tomato varieties grown in Kenya is required to determine the level of resistance to bacterial wilt.

ii. Since bacterial wilt is a difficult disease to manage and one management strategy is not adequate, further studies should be done on integration with other control measures like use of biological control agents that suppress the disease.

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