EFFECTS OF INORGANIC FERTILIZER ON TOMATO – BACTERIAL WILT INTERACTIONS IN HIGH TUNNEL TOMATO VARIETIES

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Effects of inorganic fertilizer on tomato – bacterial wilt interactions in high tunnel tomato varieties

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DECLARATION

This is my original work and has not been presented for a degree in any other university

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DEDICATION

To my beloved parents; Salesius Ireri & Rose Wangari Ireri, and sister Christine Kagendo Ireri, without whose dedication, sacrifice and prayers to educate me, I would not have managed to come this far. To my late brother, Kelvin Macharia Ireri, who in his lifetime challenged me to go beyond my limit.

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LIST OF ABBREVIATIONS

BW	Bacterial wilt
CAN	Calcium Ammonium Nitrate
CaClO	Calcium chloro-oxide
CFU	Colony Forming Units
DAP	Di-Ammonium Phosphate
EXPs	Extracellular proteins
EPS	Extracellular Polysaccharide
HPLC	High performance liquid chromatography
ICIPE	International Centre of Insect Physiology and Ecology
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KALRO	Kenya Agricultural and Livestock Research Organization
LC/MS	Liquid Chromatography/Mass Spectrometry
NaOCl	Sodium hypochlorite
SMSA	Semi-selective medium, South Africa
VOCs	Volatile Organic Compounds

ABSTRACT

Tomato (*Solanum lycopersicon L*), is one of the most common and important vegetable crops in the world with over 18,477 hectares under tomato production in Kenya. Tomato production is however, greatly threatened by bacterial wilt (BW). Bacterial wilt, caused by *Ralstonia solanacearum* is a serious disease in the tropics and subtropics, causing severe losses in many agricultural crops. To control its infection, several measures have been deployed but with minimum success. Alternative integrated strategies are therefore needed for the management of this pathogen. This study sought to test the hypothesis that particular rates of inorganic fertilizers influence certain secondary metabolites in the tomato that affect the plant's response to bacterial wilt. The hypothesis was tested by: (i) Investigating the farmers' knowledge, attitude and practices (KAP) towards *R. solanacearum* in high tunnel tomato production in Kiambu County, central Kenya. This was done by conducting a focused group discussion (FGD) for 32 farmer groups which was followed by soil sampling from the high tunnels in six sub counties in the period July – September 2016 using a checklist with open ended questions. (ii) establishing site specific soil chemical properties influencing population density of *R. solanacearum* in the high tunnels in Kiambu County, which was done by analyzing the chemical properties of the soil samples collected and relating that to the BW populations from the same soil samples as quantified; (iii) evaluating the pathogenicity of *R. solanacearum* under different inorganic fertilizer rates in high tomato varieties grown under high tunnels by conducting potted trials with different levels of NPK fertilizer treatments, namely, 0 g, 2.5 g, 5 g, 10 g and 20 g per 2 kg of sterilized 2:1 red soil and on two tomato varieties; and (iv) identifying the chemical components released by tomato plants grown under different inorganic fertilizer rates done by extracting and quantifying phenolics from the various treatments at the International Centre of Insect Physiology and Ecology. Results from the KAP survey showed that about 78.1% of high tunnels were in use for 1-2 years and 62.5% of farmers taking part in the study could identify symptoms caused by BW, which resulted in crop loss of 50%–100%. About 82.6% had the soil in which they produced tomato in the tunnels analyzed for nutrition and presence of pathogens, but the majority (71.7%) never followed recommendations on how to amend their soils. These findings indicate that more research and information are required so that farmers can optimize high tunnel production of tomato under tropical conditions. Analyses of soil samples collected showed that BW was present in all the Sub counties, with Gatundu North sub county testing up to an average concentration of 12.551×10^9 cfu/ml of *R. solanacearum*, which was 3.8 times higher than Juja sub county. Results showed that there was a strong positive correlation between *R. solanacearum* with soil nitrogen (78%) and phosphorous (87%), and a weak negative correlation with soil pH (42%), electrical conductivity (8%), zinc (23%) and copper (4%). When pathogenicity of *R. Solanacearum* was examined on Anna F1 and Tylka F1, results showed that no significance difference ($P = < 0.05$) existed between the two varieties in terms of BW attack and yield. When treatments were separated for treatments, 5 and 10 g yielded best (no significance difference at $P=0.05$). Wilting was highest (87.13%) in the control and least (30%) at 10 g. Qualitative tests results revealed that alkaloids, flavonoids, tannins, saponins and terpenoids were all present in all the treatments. In

addition, when quantified using the Folin Ciocalteu method, total phenolics were found to be highest in 10 and 20 g respectively with no significance difference (P=<0.05) between them, with concentrations of 126.46 and 132.75 milligram gallic acid equivalent (mg GAE)/g dry weight of extract (mg/gGAE) respectively. Least concentration was found in the control with a concentration of 45.71 mg/gGAE. A strong negative correlation (r = 0.7002) was found between wilt incidence and the total phenolics present in the treatments meaning that wilt incidence decreased with increase in total phenolics. This indicates that plant defense against the bacterial wilt pathogen is influenced by phenolic content in the plant. In conclusion, proper application of inorganic fertilizers at affordable and non-toxic rates (10g/hole) for tomato production offers a promising bacterial wilt management alternative because of the ability of enhancing the plant defense system. As such, identification and subsequent understanding of the functions of the specific chemical exuded by tomato plants due to fertilizers applied would not only address the existing knowledge gap, but also offer hope for the development of a new approach or the improvement of existing bacterial wilt control strategies.

CHAPTER ONE

INTRODUCTION.

1.1 Background Information

Tomato (*Solanum lycopersicum L*), is one of the most common and important vegetable crops in the world (Brickell *et al*., 2002). The top five tomato producing countries in the world that include China, India, Turkey, United States of America and Egypt, account for 170 million tons of the crop, with China producing over a quarter of the total produce (FAO, 2013). In Africa, Egypt is the leading producer in the continent, producing and estimated 7 million tons with Kenya producing 283,000 tons (FAO, 2013). In Kenya, it is one of the mostly widely grown and consumed vegetable. Tomato is a source of vitamins A and C, potassium and calcium. It has antioxidant properties and has been named as a functional food because of its nutritional and health benefits (Naika *et al*., 2005). It can be consumed fresh in salads, cooked in other dishes or processed into other food products such as jam and puree (Hortinews, 2016). In Kenya, tomato is grown in both open field and in high tunnels (commonly referred to as 'greenhouses'), whereby in the latter production creates an ideal production environment that results in high yields, efficient water utilization, high fruit quality, prolonged production, shortened maturity period, low pest and disease incidences, reduced use of land to achieve the same results i.e. ratio of about 1:10, low labor input and timing of market (Hortinews, 2016).

To meet the demands of an ever-expanding human population, global crop production needs to double by 2050; however, current estimates of vegetable crops are far below what is needed (Ray *et al*., 2013). Plant pests decrease the production of crops worldwide by 36%, and diseases alone have been shown to reduce crop yields by 14% (Agrios, 2005). Yield losses of tomatoes caused by soil-borne and foliar pathogens give problems wherever tomato is grown (Schäfer *et. al*, 2006). Diseases caused by different fungal and bacterial pathogens (Neher, 2010) are among the major constraints of tomato production.

One of the most devastating soil pathogens in tomato production is the bacterial wilt caused by the soil-borne bacterium *Ralstonia solanacearum*. It is a serious disease in the tropical, sub-tropical and temperate regions in the world, causing severe losses in many agricultural crops (Hayward, 1991). The bacterium, *R. solanacearum,* has five races (Race 1-5) per its host range and five biovars (biovar 1-5) according to its utilization of disaccharides and alcohols. Race 1 and biovar 1 of *R. solanacearum* is associated with bacterial wilt in tomato, potato and other solanaceous crops (Hayward, 1991). *Ralstonia solanacearum* enters plant roots either through natural openings or from wounds caused by tilling activities or nematode injury in the soil. It invades xylem vessels and spreads quickly to aerial parts of the plant through the vascular system. The high level of multiplication in the vascular system leads to wilting symptoms and, ultimately, plant death (Genin, 2010). Its lethality is enhanced by the ability of *R. solanacearum* to survive in soils for many years since it forms latent infections within alternate hosts such as indigenous weeds which makes its eradication difficult. Simple test to identify the bacterial pathogen includes observation of a slimy ooze from the infected stem when placed in clear/distilled water (Hayward, 1991).

Plants use many mechanisms to defend themselves against the invasion of herbivores and pathogens, including constitutive and inducible defenses (Freeman and Beattie, 2008). Defense against herbivores requires measures different from defense against pathogens (Freeman and Beattie, 2008). In general, phyto-pathogens are less mobile than herbivores, and migration across or through their host plant is often passive and occurs over relatively short distances. For example, plant-infecting viruses usually need plant proteins for tissue-to-tissue transport, plant-infecting fungi can only relocate by growing longer hyphae and plant-infecting flagellar bacteria can only travel short distances by taxis in fluid media. Hence, the infected host can attack such relatively immobile pathogens on the spot; at the site of infection, plants will often respond by producing structural reinforcements (e.g. cell wall thickening and callose deposition) or toxins (e.g. phytoalexins or alkaloids), or by initiating programmed local cell death (apoptosis) to isolate and possibly kill the pathogen (Dangl and Jones, 2001).

Information on plant defense mechanism to the infection of the soil-borne bacterium *Ralstonia solanacearum* is limited (Chen, 2008). Recently, the strategies used to manage plant diseases depend upon induced systemic resistance in the plant such as generation of phenolic compounds. *Saccharomyces cerevisiae* yeast extract was used for improving the health of butter lettuce (*Lactuca sativa* L.) Where it increased the total phenolic compounds and chlorophyll content (Zlotek and Swieca, 2016). Such of these compounds, e.g., indole and auxin are produced by numerous plant-associated bacteria and fungi. These phytohormones have biological effects on plant growth and several physiological processes (Bari and Jones, 2009). These compounds possess two mechanisms, direct promotion of plant growth and indirect inhibition of plant pathogens (Davies, 2010). The efficacy of simple organic compounds, including amino acids, sugars, and organic acids, on bacterial wilt in the tomato has been evaluated in pot experiments. The production of the active compounds by plants is influenced by many factors that are either agronomic and environmental including fertilizer application (Geneva *et al*., 2008).

The use of fertilizers and amino acids has been showed to cause increased phenolic contents in plant tissues (Osuagwu, 2008). The application of lysine, an amino acid used in synthesis of proteins, to a pumice culture medium (0.25 mg g^{-1}) and soil (2.5 mg g^{-1}) reduced bacterial wilt in the tomato by 85-100% (Igawa, *et. al.*, 2008). These authors found that the suppression mechanism was not attributed to the induction of systemic resistance, but to shifts in the soil microbial community structure that led to the more rapid death of the pathogen (Posas and Toyota, 2010). In contrast, riboflavin (1mM) induced a series of defense responses and secondary metabolism in cell suspensions and, thus, has been reported to protect tobacco against *R. solanacearum* (Liu, *et al*., 2010). Another DL, -3-aminobutyric acid (BABA), when drenched, was reported to increase polyphenol oxidase activity and lower the catalase in tomato plants, suggesting the role of induced of resistance to bacterial wilt in the tomato (Hassan and Abo-Elyousr, 2013). Another study showed that methyl gallate (20 μg/l) exhibited strong bactericidal effects on *R. solanacearum* (Fan *et. al*., 2014).

The current study sought to establish the effects of fertilizer on the bacterial wilt and tomato complex in the soil and understand the possible ways in which the inorganic fertilizers help in disease reduction. Initial studies to understand the effects of this disease in the farmers' high tunnels was conducted using focused group discussions and also by sampling soils in the visited farms. High tunnel pot experiments were conducted to determine the effects of different rates of applications of inorganic fertilizer NPK on the bacterial wilt disease and to provide a baseline on the phytochemicals which may be playing a role in the defense against the disease.

1.2 Problem Statement

Bacterial wilt of tomato caused by *R. solanacearum*, causes a considerable amount of damage to tomatoes and many other crops in tropical, subtropical and warm temperature regions of the world and limits the production of many crops e.g. potato, tomato, eggplant and pepper (Ji *et al*., 2005). It can infect over 200 plant species representing over 50 botanical families (Aslam *et al.*, 2017).

Its harmfulness, wide host range, persistence and huge genome plasticity have made it one of the world's most important phytopathogenic bacteria and one of the most intensively studied. Bacterial wilt caused by *R. solanacearum* is one of the major diseases of tomato and the disease causes concern for tomato production because it can drastically reduce tomato up to 90%. Kim *et al.* (2016) reported that, bacterial wilt of tomato caused by *R. solanacearum* is a devastating disease that limits the production of tomato in Korea and other parts of the world (Wei *et al*., 2018). In Kenya, bacterial wilt is the most limitation factor to tomato production in the counties with highest incidence recorded in Kiambu and lowest in Kajiado (Onduso, 2014). It was first reported in Kenya in 1945 around the Embu, upper eastern, Kenya, from where it spread to other parts of the country (Otipa *et al*., 2003). Of late, the disease has spread to all potato and tomato growing areas of the country (Jane *et al*., 2013), affecting over 70% of crops of the nightshade family and causing yield losses of 50 to 100%; followed by late blight (67%), and viral diseases (12%) (Kaguongo *et al.*, 2010). Currently, bacterial wilt has spread countrywide (The Organic Farmer, 2013).

Bacterial wilt lethality is also enhanced by presence of other pathogens. The bacterial wilt pathogen commonly coexists with polyspecific nematode populations in tropical and subtropical areas. The wounding of roots by nematodes is usually invoked to explain the correlation between nematode infection and bacterial wilt, since this wounding increases the number of sites for bacterial entry (Deberdt, 1991).

Control of bacterial wilt is difficult for tomato growers, particularly for growers with limited capacity to rotate out of solanaceous crops. The wide host range of *R. solanacearum* (Kelman, 1997) further restricts rotational options, and effective crop rotation programs in severely infested soils may require multiple years out of tomato production (Lemaga, *et al.*, 2001). Even soil fumigants have little success against this pathogen (Driver and Louws, 2002), and vertical movement of *R. solanacearum* in soils (Satou, *et al.*, 2006) may allow the bacterium to recolonize fumigated beds quickly. Soil pre-treatment has recently been widely used to control soil-borne pathogens, such as soil fumigation with methyl bromide, methyl iodide, and propargyl bromide, and these methods have achieved good outcomes (Luo *et al*., 2010). However, the use of these fumigant pesticides has been restricted in many countries due to their environmental risks and hence their subsequent ban (Montzka *et al.*, 1996). Some physical methods, such as solarization, steaming and heating, have also been employed to control soil-borne disease including bacterial wilt. However, the efficiency of physical methods is limited by climatic conditions, soil type (Liu *et al.*, 2016).

1.3 Justification

A sustainable, affordable and effective control method needs to be introduced to prevent further crop loss. The management strategy must guarantee continuous and increased production of tomato. Tomato production being a key income earner to some households, ensures increased incomes for the farmer and fair prices to the consumers (Taylor *et al*., 2011). Farmer practices play a role in prevention of soil borne pathogens. Studies done have shown that Farmers have poor practices toward the prevention of BW and RKNs. For example, farmers do not obtain clean planting

material, do not sterilize the soil, and dispose-off diseased plants outside the high tunnels in which bacterial wilt problems are experienced (Ireri *et al*., 2018). Bacterial wilt is a soil borne pathogen and only good farming practices are predictors of prevention (Yuliar and Toyota, 2015). The relationship between plant pathogens and their hosts is often affected by such factors as nutrition, weather and soil conditions (Davis *et al*., 2003). Soil fertility and fertilizer practice has the potential to influence tomato in numerous ways (Davis *et al.,* 2003). Soil amendments that enhance host plant resistance to pathogens have been given due consideration as options of bacterial wilt control (Datnoff *et al*., 2001). Amendment of soil with organic and/or inorganic fertilizers under field conditions have been reported to reduce incidence of bacterial wilt on *Solanum tuberosum* L. (Lemaga *et al*., 2005) and increased yield. These authors also reported that the application of nitrogen (N) + phosphorus (P) + K and N + P (application rate of each fertilizer = 100 kg ha^{-1}) reduced bacterial wilt by 29% and 50%, respectively, and increased the yield of potatoes to 18.8 t ha^{-1} and 16.6 t ha^{-1}, respectively, which was 40% higher than that in untreated controls. Also, it has been found out that the use of both organic i.e. farm yard manure (except compost) and inorganic fertilizer (NPK) reduced the survival period of the bacteria by 50% in Egyptian and Dutch soils (Messiha, 2006). The author further found out that Potassium and Calcium rich soils had less incidences of bacterial wilt which were attributed to the ability of the nutrients to increase plant resistance. Li and Dong (2013) showed that the combined amendment of rock dust and commercial organic fertilizer reduced the incidence of bacterial wilt in tomato. A single amendment with rock dust also effectively reduced the incidence of bacterial wilt in tomato and higher soil pH and calcium content were key factors in the control of bacterial wilt by the rock dust amendment. Many elements in the cell walls influence the susceptibility or resistance of plants to infections by pathogens and silicon is considered to be a beneficial element for plants and higher animals (Epstein, 1991). Kiirika *et al.* (2013) reported that the combined application of silicon and chitosan reduced the incidence of bacterial wilt in the tomato by inducing resistance. Silicon and chitosan exhibited synergistic effects against the disease. Silicon has also been reported to have a significant effect in

reducing bacterial wilt incidence in tomato in a hydroponics culture system, and peat substrate (Dannon and Wydra, 2004).

Therefore, soil chemical ecology may act as a crucial factor for soil community organization which requires innovative, simple and affordable eco-friendly technologies in order to effectively manage soil-borne pathogens for improved production of vegetable crops. This chemical ecology is influenced by day to day farmer practices which include fertilizer application, which, if they are showed to offer an integrated approach to prevent the bacterium, would be cheaper options of control. The research conducted was to provide information on an alternative management practice for Bacterial Wilt which has been identified as very harmful to the tomato crop. The study explored the use of fertilizers in the control of the bacteria through modification of soil ecosystem in which it exists, which has been deduced in previous studies by identification and subsequent understanding of the functions of the chemical exuded by tomato plants due to fertilizers applied. This would not only address the existing knowledge gap, but also offer hope for the development of a new approach or the improvement of existing BW control strategies.

1.4 Objectives

1.4.1. General objective

The general objective of this study was to determine the effects of different rates of inorganic fertilizer, NPK, in bacterial wilt tomato interactions for improved management of the disease, in Kiambu County, Kenya.

1.4.2. Specific Objectives

i. To evaluate the knowledge, attitude and practices of high tunnel tomato farmers towards *R. solanacearum* in Kiambu
ii. To establish site specific soil chemical properties in respective to high tunnels influencing population density of *R. solanacearum*

iii. To determine the pathogenicity of *R. solanacearum* under different inorganic fertilizer rates on different tomato varieties
iv. To identify the chemical components released by tomato roots under different treatments in an inorganic fertilizer-pathogen interaction

1.5 Hypothesis

i. Farmer knowledge, attitudes and practices do not impact on *R. solanacearum* in high tunnel tomato production
ii. The soil chemical properties do not influence *R. solanacearum* population in high tunnels in Kiambu County
iii. The pathogenicity of *R. solanacearum* is not influenced by inorganic fertilizer in tomato varieties grown in high tunnels
iv. There is no variation in chemical components released by tomato plants infected by *R. solanacearum* under different inorganic fertilizer regimes

CHAPTER TWO

REVIEW OF LITERATURE

2.1. Bacterial wilt origin and existence

Bacterial wilt caused by the pathogen *R. solanacearum*, formerly called *Pseudomonas solanacearum* (Yabuuchi, *et al*., 1995*)*, is an important disease of many crops. The genus *Ralstonia* belongs to the β-proteobacteria (Palleroni *et al*., 1973) and it is a gram-negative aerobic bacterium, which is rod-shaped and has polar flagella (Holt *et al*., 1994). The pathogen is considered one of the most important plant pathogenic bacteria due to the economic losses that occur globally resulting from bacterial wilt disease (Hayward, 1991). Historically, *R. solanacearum* strains have been classified into five races based loosely on host range and into five biovars based on ability to produce acid from a panel of carbohydrates (Denny, 2006). In biological taxonomy, race is an informal rank in the taxonomic hierarchy, below the level of subspecies. It has been used as a higher rank than strain, with several strains making up one race (Rithchie and Dittapongpitch, 1991). A biovar is a variant prokaryotic strain that differs physiologically and/or biochemically from other strains in a particular species. The five races of *R. solanacearum* have different host ranges and geographic distributions. Race 1 is a poorly-defined group with a very wide host range and is endemic to the southern United States as well as Africa, Asia and South America. Race 2 principally attacks bananas and is found mainly in Central America and Southeast Asia. Race 3 is distributed worldwide and has primarily been associated with potato. Race 4 affects ginger in much of Asia and Hawaii and race 5 affects mulberries in China (Kelman, 1997; Denny, 2006). While the origin of *R. solanacearum* is not clear, previous reports suggests that it existed before the geological separation of the continents as the bacterium has been found in virgin jungle in South America and Indonesia (Hayward, 1991). However, race 3 biovar 2 strains are believed to originate in the Andean highlands and this near-clonal subgroup is widely distributed in tropical ones throughout the world and some temperate regions such as Europe and northern Asia (Hayward, 1991).

2.2 Host range and epidemiology

Bacterial wilt has been described on a wide range of hosts in many tropical and subtropical regions (Agrios, 2005). In the absence of susceptible crops, alternative weed hosts and non-host plants play important roles for the survival of *R. solanacearum* strains (Granada and Sequeria, 1983)

The bacterium infects over 200 plant species representing more than 50 plant families including: solanaceous crops such as tobacco, tomato, potato and eggplant; leguminous plants such as groundnuts and French beans (Genin and Boucher, 2002); and in monocotyledonous plants, such as banana, the pathogen causes Moko disease. *Ralstonia solanacearum* also causes bacterial wilt disease on several shrub and tree species such as cashew, mulberry and olive (Shiomi *et al*., 1989).

High soil moisture in well-drained soils is conducive for the survival of *R. solanacearum* which depends on temperature. For example, a constant high day temperature of 40 $^{\circ}$C of more than four hours has been shown to reduce bacterial populations (van Elsas *et al*., 2000). Also an increase in ambient temperature between 30-35 $^{\circ}$C has been correlated with an increase in disease incidence and rate of onset of bacterial wilt on hosts such as tomato (Hayward, 1991). In addition, nematode infection especially *Meloidogyne species* contributes to spread of *R. solanacearum*. This is a result of the increase in wounding of plants by the nematodes, which promotes bacterial infection; however, the nematode may also modify plant tissue making it suitable for bacterial invasion (Hayward, 1991).

The pathogen, *R. solanacearum* is also able to survive in aquatic habitats and contaminated irrigation water. Wastewater used in the processing of diseased plant tissue, has been recognized as sources of inoculum (Elphinstone *et al.,* 1998). A host may often be regarded as healthy since disease symptoms are not visible however the pathogen can be present in the plant at high inoculum levels. The pathogen over-winters in diseased plants or plant debris, in vegetative propagative organs such as potato tubers or banana rhizomes, on the seeds of some crops like capsicum and

tomato, and in the rhizosphere of weed hosts e.g. *Solanum dulcamara, Solanum carolinense* and *Solanum cinereum* (Hayward, 1991). This results in latent infection as the host is sometimes further cultivated (Denny *et al.*, 2001).

2.3 Disease symptoms

The major and most common characteristic of *R. solanacearum* is sudden wilting of foliage and the young plant is affected more. The symptoms occur as discoloration of the vascular system from pale yellow to dark (Gota, 1992). Older plant leaves first show wilting before the youngest leaves or one sided wilting and stunting and finally the plant wilts permanently and dies (Agrios, 2005). In other instances, the infected plant may express all or none of these symptoms, even under typical environmental conditions that are ideal for the pathogen and typically this is a commonly observed condition known as latency. The pathogen enters roots through wounds caused by transplanting, cultivation, nematode, insects and through natural wounds. Then it starts to multiply rapidly in the vascular system, finally the xylem elements are filled with bacterial cell and slime (Kelman and Sequeira, 1995).

Successful colonization requires production of molecular mass Extracellular Polysaccharide (EPS) in high amounts and multiple extracellular proteins (EXPs) (Denny, 2006). Extracellular Polysaccharides physically obstructs plant water transport, or that EPS cloaks the bacterium from host plant recognition and subsequent defense (Milling, *et al*., 2011). Disease incidence may range from a few scattered plants or loci of infection in fields where low or erratic natural infestations occur to the rapid death of the plants (Kelman and Sequeira, 1965). The bacterium rapidly spreads upward in the vascular system from secondary roots to larger roots and then to the stem. After that, the plant starts to suffer from wilting irreversibly (Kelman and Sequeira, 1965).

2.4 Control methods

The management of bacterial wilt with chemical, physical, biological and cultural methods has been investigated for decades. Elphinstone (2005) extensively reviewed bacterial wilt in 2005, and many studies have since been conducted on this topic.

2.4.1 Chemical control

Pesticides such as algicide (3-[3-indolyl] butanoic acid), fumigants (metam sodium, 1,3-dichloropropene, and chloropicrin), and plant activators generating systemic resistance on tomato (validamycin A and validoxylamine) have been used to control bacterial wilt. Fortnum and Martin, (1998) reported that a combination of methyl bromide, 1,3-dichloropropene, or metam sodium with chloropicrin reduced bacterial wilt by 18% and increased the yield of tobacco and the tomato yields. Edwards-Jones (2008) reported that pesticides offered greater net benefits than other control methods, but this has not always been the case. For example, if farmers use pesticides carelessly or without proper knowledge, a percentage of the pesticide may remain in the environment for many years (Gadeva and Dimitrov, 2008), become a contaminant in soil and/or groundwater and be poisonous to farmers and animals (Dasgupta *et al*., 2007).

Bactericides (triazolothiadiazine [0.5 to 12 mM, in solution] (Khanum *et al*., 2005), streptomycin sulfate (Lin *et al*., 2004), other chemicals such as bleaching powders as sterilizers (Sharma and Kumar, 2009), or weak acidic electrolyzed water (40 ppm of available chlorine, in pH 5.6 solution) have also been shown to effectively destroy microorganisms. Acibenzolar-S-methyl (ASM) has been proposed to induce systemic resistance (Pradhanang *et al*., 2005). The combination of ASM and thymol significantly reduced the incidence of disease and increased the yield of the tomato, whereas ASM or thymol alone did not (Hong *et al*., 2011). Silicon (Kurabachew and Wydra, 2014) or Si and chitosan (Kirkegaard *et al*., 1996) reduced the incidence of bacterial wilt through induced resistance. Wang *et al.* (2013) reported that Si-mediated resistance was associated with increases in the amount of microorganisms in the soil

as well as soil enzyme activity (urease and acid phosphatase). The soaking of seeds in a low sodium chloride solution was previously found to increase seedling vigor and tolerance to *R. solanacearum* in the tomato (Nakaune *et al*., 2012). The mechanism of action of non-pesticide chemicals that suppress bacterial wilt is considered to involve either induced systemic resistance or antibacterial activity (Kawaba *et al.,* 2005). Some new methods have been reported to suppress bacterial wilt. Live microbial cells of the pathogen were captured with 10 g kg−1 of coated sawdust with 1% of an equimolar polymer of N-benzyl-4-vinylpyridinium chloride with styrene (PBVPco- ST) or coagulated in the soil with 10 mg kg−1 of a co-polymer of methyl methacrylate with N-benzyl-4- vinylpyridinium chloride at a molar ratio 3:1 (PMMA-co- BVP) (Kawaba *et al.,* 2005). Infection by the bacterial wilt pathogen was prevented through bacteriostatic actions with a phosphoric acid solution (Norman *et al*., 2006). Various non-pesticide chemicals have the potential to be applied in the field in order to control bacterial wilt disease because they have less damaging effects on the environment; however, economic considerations often influence the chemicals selected. Expensive chemicals and repeated applications are only possible for valuable crops that may incur substantial economic losses in the absence of treatments. Since crop yield and quality are not damaged when disease severity is low or in the absence of pathogens, a diagnosis based on economic thresholds is essential for determining whether chemical treatments are needed (Yuliar and Toyota, 2015).

2.4.2 Physical Methods

Several physical methods have been tried and have proved to be effective against bacterial wilt. Solarization using transparent plastic mulches for 60 d prior to the planting of tomatoes reduced the incidence of bacterial wilt (Vinh *et al*., 2005). Baptista *et al*. (2007) advanced the mechanisms of soil solarization that reduced bacterial wilt in tomato. It was shown to reduce soil pH, potassium, sodium, boron and zinc contents, microbial biomass and microbial respiration in the soil but did not affect other soil chemical properties. A heat treatment at either 45 ^{o}C for two days or a minimum temperature of 60 ^{o}C for two hours of the infected soils before tomato

planting reduced the total bacterial wilt population by 60-97% and the incidence of bacterial wilt by 50-75% (Kongkiattikajorn and Thepa 2007). Cold temperatures are also sometimes effective in control of the pathogen. It has been reported that bacterial wilt rarely occurs in tobacco crops planted in winter whereas the disease developed when crops are planted in spring, particularly where the disease had previously occurred and crop rotation not practiced (Akiew and Trevorrow, 1994). Lower moisture conditions (20-30% maximum water holding capacity) and pre-incubation at lower temperatures (4°C) reduced bacterial wilt and had a negative impact on the survival of *R. solanacearum (*Islam and Toyota, 2004).

Biofumigation, which refers to the agronomic practice of using volatile chemicals released from plant residues to suppress soil-borne plant pathogens, has recently been attracting attention (Kirkegaard *et. al*., 1996). Biofumigation is called biological soil disinfection (BSD) and the production of organic acids or heavy metal ions is involved in the suppression of pathogens (Momma, 2008).

2.4.3 Biological control

2.4.3.1 Biological control agents (BCAs)

Biocontrol can be simply defined as the application of one living organism to control another. This process is also referred to a biological control. The biological application is mainly introduced to reduce the population of a pest and to produce pest-free yields. It is a self-sustaining and long-term treatment method, for managing pests (Flint and Dreistadt, 1998). The interest in BCAs has increased due to concerns over the general us of chemicals in pest control (Whipps, 2001).

Surveys have shown that BCAs have been dominated by bacteria (90%) and fungi (10%) (Montesinos, 2003). Previous studies showed the potential value of some promising BCAs, which are dominantly avirulent strains of *R. solanacearum* followed by *Bacillus* spp., *Streptomyces* spp., and other species, in controlling bacterial wilt. A total of 109 strains of endophytic or rhizobacteria were recently screened for their

antibacterial activities against *R. solanacearum,* and effective isolates (a total of 22) consisted of *Pseudomonas* spp. (18 isolates) and *Bacillus* sp. (2 isolates) (Ramesh and Phadke, 2012). Several new or uncommon BCAs have been reported to control bacterial wilt such as *Acinetobacter* sp. (Xue *et al*., 2009), *Burkholderia nodosa*, *B. sacchari*, *B. tericola*, *B. pyrrocinia* (Nion and Toyota, 2008) and bacteriophages (Yamada *et al*., 2007). The possible suppression mechanisms of these species are competition, induced systemic resistance, antibiosis, and the production of enzymes that degrade the cell wall and siderophores. In the inoculation methods of BCAs, pouring or drenching soil was more prevalent than other methods, whereas the biocontrol efficacy range appeared to be lower than that of the dipping of roots or seed coating method.

2.4.3.2 Organic matter/ amendments

Organic amendments to soil have direct impacts on plant health and crop productivity. They are advantageous because they improve the physical, chemical, and biological properties of soil, which can have positive effects on plant growth (Bailey and Lazarovits, 2003). Organic matter originates from recently living organisms and decays or is the product of decay. It is categorized into plant or animal origins, and simple organic carbons. In the previous references to an *R. solanacearum* study, different organic matter, such as plant residue (80%), animal waste (10%), and simple organic matter (10%), were shown to control bacterial wilt disease. Larkin (2008) found that biological amendments were generally effective for delivering microorganisms to natural soil, resulting in a wide variety of effects on soil microbial communities depending on the particular types, numbers, and formulations of organisms added. A new approach is the suppression of bacterial wilt in an organic hydroponic system through a rhizosphere biofilm that only forms on roots in the organic system (Fujiwara, 2012).

2.4.4 Cultural Practices

2.4.4.1 Crop rotation

The benefits of crop rotation are maintenance of the soil structure and organic matter, and a reduction in soil erosion that is often associated with continuous row crops (Janvier *et al*., 2007). While continuous cropping with the same susceptible host plant will lead to the establishment of specific plant pathogenic populations, crop rotation avoids this detrimental effect and is often associated with a reduction in plant diseases caused by soil-borne pathogens (Kurle *et al*., 2001). For example, the onset of bacterial wilt was delayed by 1 or 3 weeks and wilt severity was reduced by 20-26% when a susceptible tomato variety was grown after corn, lady's fingers, cowpea, or resistant tomato (Adhikari and Basnyat, 1998). Potato cultivation rotated with wheat, sweet potato, maize, millet, carrots, sorghum, or phaseolus beans reduced the incidence of wilt by 64 to 94% while the yield of potatoes was 1- to 3-fold higher than that of monocultured potatoes (Katafiire *et al.,* 2005).

2.4.4.2 Soil amendment

Studies done earlier have showed that application of fertilizers reduced incidence of bacterial wilt. Calcium is the most well-known fertilizer to suppress disease (Yamazaki *et al*., 2005). Increased concentration of the fertilizer reduces severity of bacterial wilt as well as the populations of the bacteria in the stem of tomato (Yamazaki *et al*., 2005). Yamazaki *et al*., (2005) further reported that an increase in calcium uptake by tomato correlates with lower levels of disease severity. Lemaga *et al*., (2005) also reported that the application of nitrogen (N) + phosphorus (P) + K and N + P (application rate of each fertilizer = 100 kg ha^{-1}) reduced bacterial wilt by 29% and 50%, respectively, and increased the yield of potatoes to 18.8 t ha^{-1} and 16.6 t ha^{-1}, respectively, which was higher than that in untreated controls (11.2 t ha^{-1}). It has also been reported that bacterial wilt induced changes in the distribution of nutrients, especially Ca, B, and P in tomato leaves. Li and Dong (2013) showed that the combined amendment of rock dust and commercial organic fertilizer reduced the incidence of bacterial wilt in the

tomato. A single amendment with rock dust also effectively reduced the incidence of bacterial wilt in the tomato and higher soil pH and Ca content were key factors in the control of bacterial wilt by the rock dust amendment. Many elements in the cell walls influence the susceptibility or resistance of plants to infections by pathogens and silicon is considered to be a beneficial element for plants and higher animals. Kiirika *et al.* (2013) reported that the combined application of silicon and chitosan reduced the incidence of bacterial wilt in the tomato by inducing resistance. Si and chitosan exhibited synergistic effects against the disease.

2.4.5 Resistant Cultivars

The growth of cultivars that are resistant to bacterial wilt is considered to be the most economical, environmentally friendly, and effective method of disease control (Elphinstone, 2005). Breeding for resistance to bacterial wilt has been concentrated on crops of wide economic importance such as the tomato, potato, tobacco, eggplant, pepper, and peanut, and has commonly been influenced by factors such as the availability of resistance sources, their diversity, genetic linkage between resistance, and other agronomic traits, differentiation and variability in pathogenic strains, the mechanism of plant-pathogen interactions, and breeding or selection methodology (Elphinstone, 2005). For example, the *Arabidopsis* NPR1 (non-expresser of *PR* genes) gene was introduced into a tomato cultivar, and enhanced resistance to bacterial wilt and reduced the incidence of wilt by approximately 70% 28 d after the inoculation (Lin *et al.*, 2004). Potato genotype BP9, which is a somatic hybrid between *Solanum tuberosum* and *S. phureja*, successfully reduced bacterial wilt by 90–100% (Dahal *et al*., 2010). Resistance to bacterial wilt in many crops has generally been negatively correlated with yield and quality. Thus, the release of resistant cultivars may be poor because of other agronomic traits and are not widely accepted by farmers or consumers. The breeding of a good resistant cultivar is expected in the future through stronger efforts in the genetic enhancement of bacterial wilt resistance through biotechnology approaches in order to improve yield crop.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Farmers' knowledge, attitude and practices towards *R. solanacearum* in Kiambu County

3.1.1 Study site

The study was conducted from January to September 2016 in high tunnels (18-25m length × 8 m wide × 2 m high) covered with translucent UV-treated plastic sheeting in the Sub-Counties Gatundu North, Gatundu South, Juja, Kiambu, Ruiru and Thika of Kiambu County, Central Kenya. The County is divided into the broad topographical zones of Upper Highland, Lower Highland, Upper Midland, and Lower Midland. Kiambu County is characterized by the presence of broad categories of soils: high-level upland soils, plateau soils, and volcanic footbridge soils. The high-level upland soils, which are from volcanic rocks, are fertile. Low fertility soils are mainly found in the middle zone and the eastern areas such as Juja, Thika, Ruiru, Gatundu North, and Gatundu South Sub-Counties. The soils are either sandy or clay. Mean temperature in the area is 26 °C with temperatures ranging from 7 °C in some parts of Gatundu North and Gatundu South Sub-Counties, to as high as 34 °C in Thika sub county (Anonymous, 2015a). This temperature range can support tomato production even under protected cultivation.

3.1.2 Sampling procedures

Purposive sampling was used to select the sample size (Mugenda and Mugenda, 2003). Based on information from County and sub county crop officers, 80 high tunnels, managed by a group of 8-30 farmers, were constructed by the Kiambu County Government and the National Irrigation Board for horticultural production in 2014. Of these, 32 high tunnels located in the Sub-Counties were selected based on previous challenges farmers experienced with pests and diseases in tomato production (Anonymous, 2015b). This comprised a sample size of 40% (of the 80 tunnels that

exist), which is a good representation of the target population (Mugenda and Mugenda, 2003). The selected high tunnels comprised 311 respondents (141 males and 170 females). Data were collected using focused group discussions using a checklist with open-ended questions. The checklist was constructed in consultation with a statistician to ensure that questions were written correctly and would allow for valid statistical analysis. The checklist was pretested among sub county crop officers and enumerators (10 respondents) before administration in its final form. The checklist consisted of sections that included farmer: (1) knowledge, (2) attitude, and (3) practices in high tunnel tomato production in relation to the key target pests. The knowledge section consisted of 12 questions used to compute a knowledge score. For each question, a correct response was assigned 1; a wrong response, or "don't know," 0. The number of correct responses for the 12 questions was summed to calculate the group knowledge and subsequently respective sub county scores. In the attitudes section, three "yes/no" questions on the impact of bacterial wilt toward high tunnel tomato production were asked. Those who said yes were considered to have a positive attitude; those that said no were considered to have a negative attitude. For the practices section, questions were asked using the "When, Where, What, Which, Who, and How method" to evaluate precautionary measures respondents took against bacterial wilt. One point was assigned for each measure and a 0 if a measure was not mentioned in subsequent responses. A practice score for each group was computed by summing the number of responses out of 10 questions.

Data on focused group discussions was entered into Microsoft Excel 2007, cleaned to detect any missing or invalid variable. The data were analyzed with SPSS (ver. 22, Released 2013, IBM SPSS Statistics for Windows, Armonk, NY). Means, frequencies, and percentages were calculated and separated using Tukey's HSD test. Locations of the high tunnels were marked using a Global Positioning system device (Magellan, Triton windows CE core 5.0 00039_272_446_822 X11_15302).

3.2 Site specific soil chemical properties influencing population density of *R. solanacearum* in high tunnels in Kiambu County

3.2.1 Soil sampling

Soil samples were collected from 32 high tunnels in the Kiambu County, in which tomato crops were grown. Seven tunnels were sampled in Thika, 5 in Juja, 2 in Ruiru, 10 in Kiambu, 5 in Gatundu South and 3 in Gatundu North. The sub-counties were identified for the study during focus group discussions. The location of each high tunnel was identified using a GPS device (Magellan, triton windows CE core 5.0 00039_272_446_822 X11_15302, Integritytech, IL) (Table 1). A systematic pattern was used to divide high tunnel into 4 quadrants to collect soil samples. Five sub-samples per quadrant were collected with a soil auger to a depth of 15 cm in a cross-diagonal pattern (Coyne *et al*., 2014). The 5 sub-samples were mixed in a plastic basin to make a composite sample of ~1 kg and placed in a labelled plastic bag. A similar procedure was repeated across quadrants with 4 samples (comprised 20 sub-samples) collected from each high tunnel and constituting a total of 128 samples for the 32 high tunnels. Soil samples were placed in cooler boxes and transported to the laboratory at JKUAT and stored at 10 °C for 1-2 weeks for *R. solanacearum* and soil chemical analyses.

3.2.2 Soil analyses

Soil samples from high tunnels for each Sub-County were air dried on the bench in the laboratory (25 ± 2°C) for 3 days and their chemical properties determined using validated protocols as follows: total nitrogen (N) by Kjedahl's method (Kjeldahl, 1883; McGeehan and Naylor, 1988); available phosphorus (P) using the Double Acid Extractable P ($HCl–H_2SO_4$) method (Mehlich, 1953; Olsen, 1954); potassium (K), calcium (Ca) and magnesium (Mg) using the ammonium acetate extraction method (Jones Jr, 1999); iron (Fe), copper (Cu) and zinc (Zn) using the EDTA extraction method (Lindsay and Norvell, 1978); electrical conductivity (EC) by the 4 electrode

method (Nadler and Frenkel, 1980) and soil pH using an electric pH meter (Conkling and Blanchar, 1988)

3.2.3 Isolation and identification of *R. solanacearum*

To isolate *R. solanacearum*, 1g of soil from each composite sample was put in a 28 ml universal glass bottle filled with 10 ml of double distilled water and mixed thoroughly using a laboratory shaker (Stuart Lab-Scale Linear Reciprocating Shaker, Model SSL2, Cole Parmer Ltd, Staffordshire, UK) to ensure optimal dissolving of the soil and yield a concentration of 100 $mg \cdot ml^{-1}$. The stock solution was then serial diluted up to ten-fold dilution. Of these, 50 µL were obtained from the 10^{-5} (1:100,000), 10^{-6} (1:1000,000) and 10^{-7} (1:10,000,000) dilutions and subsequently streaked using a wire loop on Kelman's Tetrazolium Chloride (TZC) medium (Kelman, 1954). For each concentration, two Petri dishes were used to grow *R. solanacearum* from soil obtained from respective high tunnels. Inoculated Petri dishes were incubated (Laboratory incubator PC-IB-150, Xi'an HEB Biotechnology Co., Ltd, Shaanxi, China) at 32 °C for 30-48 h in an upside-down position using existing protocols (Hayward, 1991; Schaad *et al*., 2001). Virulent colonies of *R. solanacearum* were identified by their large and elevated size, fluidal nature, and if they were either entirely white, or with a pale red center (Buddenhagen and Kelman, 1964). Mutant, or non-virulent, type colonies of *R. solanacearum* are uniformly round and dark red, smaller in size, and butyrous or dry on TZC. Bacteria colonies (virulent and non-virulent) were counted cumulatively on a daily basis for three consecutive days. The average colony count was used to determine colony forming units per ml ($cfu \cdot ml^{-1}$) which was calculated as (number of colonies × dilution factor)/volume of culture plate (Sieuwerts *et al*., 2008).

Several biochemical tests *viz*. Gram staining reaction, potassium hydroxide solubility test, Kovac's oxidase test, Levan test and Sugar fermentation test were performed for confirmation of *R. solanacearum* isolates as described previously by Rahman *et al.* (2010). Single isolates of *R. solanacearum* from each high tunnel and from the control sample of sterilized soil were randomly selected for biochemical tests.

3.3 Pathogenicity of *R. solanacearum* in tomato grown under different fertilizer rates

3.3.1 Plant establishment

Two hybrid and indeterminate tomato varieties Tylka F1 and Anna F1 were used for the study. These varieties were selected since they were commonly grown by high tunnel tomato farmers in Kiambu County. Seeds of Tylka F1 and Anna F1 were purchased from a local agrochemical store in Thika Town, Kiambu County. Seedlings were raised in trays (36 holes per tray) filled with sterilized cocopeat in a screen house at the Jomo Kenyatta University of Agriculture and Technology (JKUAT) (latitude 0°10′48″ S, longitude 37°7′12″ E; altitude 1525 m a.s.l.), Kenya. The Plants were watered after every two days with tap water and weeded regularly to ensure no were weeds present at any time. The tomato plants were pruned regularly to avoid development of any unwanted shoots.

3.3.2 Experimental design

The seedlings established in a greenhouse at JKUAT were transplanted after 4 weeks into pots with sterilized (by autoclaving at 1.5 kilo pascals for 20 minutes) red soil and sand mixed at 2:1 ratio, each pot containing 2kgs of the total. Three days after transplanting, plants were fertilized with 2.5, 5, 10 and 20 g pot^{-1} of NPK (17:17:17) as the treatments. Control plants did not receive any fertilizer treatments (0g). The rates were determined from those (5 g and 10 g) used by high tunnel farmers in Kiambu County. Each treatment had 20 plants replicated three times, constituting 60 plants per treatment and laid in a completely randomized design. Each tomato plant was inoculated with 5 x 10^7 colony forming units (CFU) ml^{-1} a week after transplanting.

3.3.3 Preparation of the *R. solanacearum* inoculum and application

Three days prior to inoculation, a Tripheny tetrazolium chloride (TZC) plate was streaked with *R. solanacearum* from frozen stock cultures stored at -80°C. One day prior to inoculation, a single colony was transferred from a fresh TZC plate to Casamino Acid-Peptone-Glucose (CPG) broth, grown to an optical density, (OD) 600

in a baffled flask at 28 °C with vigorous shaking (250 rpm) for 24 hours. The *R. solanacearum* cultures prepared above was centrifuged at room temperature in non-sterile centrifuge bottles at 6000 rpm for 20 minutes. The rotor was maintained at room temperature and the supernatant poured off. The pellets were re-suspended in a known amount of room temperature distilled water by gentle inversion. The initial spectrophotometric reading at OD (optical density) 600 was then taken. Using the equation, $C_1V_1 = C_2V_2$ whereby C_1 is the initial concentration, V_1 is the initial volume, C_2 is the final volume and V_2 is the final volume, the dilution required to obtain the desired inoculum concentration was calculated. A 50 ml of a cell suspension containing 5 x 10^7 cfu/ml (2.5 X 10^9 total cells or 3.1 X 10^7 cfu/gram soil) was used to inoculate each tomato plant. The 50 ml of inoculum was poured using a pipette at the base of the tomato plant after injuring the plant roots with a sharp lab tweezers (Kiirika *et al*., 2013).

3.3.4 Monitoring of disease incidence

The pathogen behavior which entails their population growth or suppression was monitored at weekly intervals for twelve weeks for respective treatment. This entailed checking the progress of the plant wilt for each of the treatment, which was by intensity of wilting/incidence on the plants in a treatment being monitored. The wilt progress/incidence was rated on a 1-4 scale with 0 representing no wilt, 1 representing 1-25% of plants wilted, 2 representing 26-50% of plants wilted, 3 = 51-75% of plants wilted and 4 = 76-100% of plants wilted (Swanson *et al.,* 2005). Quantitatively, the bacterial cell population growth was checked by plating soils from a selected consistent pot every week and quantifying the amount of bacterial growth by extracting and culturing the bacteria on Kelman's modified media.

For growth analysis, treated and control plants were checked and analyzed every week after inoculation for determination of plant height, shoot and root fresh weight progress. Yields from each treatment were also determined by harvesting mature tomatoes over a month and accumulating the various harvests weight/ yield per treatment to get the final yield.

Disease incidence was calculated as % diseased plants over the total number of plants. Wilt progress and populations were analyzed using Repeated Measures ANOVA. Bacterial wilt inoculum densities were subjected to analysis of variance procedures and means separated with the Tukey's multiple range test (P = 0.05) using R-statistical software (R-Development-Core-Team, 2013).

3.4 Chemical components released by tomato roots under different treatments in a inorganic fertilizer-pathogen interaction

3.4.1 Extraction of root phytochemical components from roots

After the 1st, 6th and 9th week of growth the whole root system of intact tomato plants was carefully removed from each treatment, washed with running tap water, to remove the sand and soil debris followed by rinsing twice with sterile distilled water. The roots were then freeze-dried and stored at -80℃ until use.

The freeze-dried plant roots were subsequently ground into a fine powder using a mortar and pestle. The powder was then passed through a 2 mm sieve to remove the large debris and stored at -80℃. To obtain an aqueous extract, 1 g of powder from respective treatments, was soaked in 10 ml of distilled water for 30 min and then sonicated for extraction of components (Frey & Mayers, 2010).

3.4.2 Phytochemical screening

Test for alkaloids:

About 1.5 ml of 1 % HCL was mixed with 2 ml of the plant aqueous extract and then heated in a water bath for 3-5a few minutes. Six drops of Mayers' reagent were added. Appearance of an orange precipitate indicated presence of alkaloids and vice versa (Rasool *et. al.,* 2010).

Test for terpenoids:

About 2 ml of plant aqueous extract were mixed with 2 ml of chloroform, the mixture evaporated, and the resulting fine powder mixed with concentrated sulphuric acid and the mixture heated for 2 minutes. Formation of a greyish color indicated presence of terpenoids (Vijay *et. al.,* 2013).

Test for flavonoids:

Two drops of lead acetate were mixed with 2 ml of plant aqueous extract. A yellow precipitate indicated presence of flavonoids (Salhan *et. al.,* 2011).

Test for tannins:

Two drops of ferric chloride were mixed 5 ml of aqueous extract. A blackish precipitate appearance indicated presence of tannins (Saidulu *et. al*., 2014).

Test for saponins:

2 ml of plant aqueous extract was added to 5 ml of distilled water and agitated for 3 minutes. A frothing appearance and persistence indicated presence of saponins.

3.4.3 Determination of total phenolics by Folin-reagent method

Total phenolic content of the plants at 9 weeks was determined by the Folin-Ciocalteu reagent method (Elizabeth and Kelly, 2007). A ground sample (100 mg) was transferred into a 50 ml centrifuge tube. Then, 20 ml of 70% acetone were added and vortexed for 10 seconds. The samples were centrifuged for 10 minutes at 2500 rpm at 4 ºC. The first supernatant was taken from the centrifuge tubes and its volume recorded. The extraction was repeated thrice. Supernatants from respective treatments were stored at 4 ºC. The first and second supernatants were combined for analysis.

Analysis of total phenolics

For analysis of total phenolics, 200 µl of 10% Folin Ciocalteu were added into 100 µl of sample in triplicates into 2 ml microtubes and vortexed thoroughly for one minute. An aliquot, 800 µl of 700 mM sodium carbonate was then added into the samples and incubated for two hours at room temperature. Samples were then transferred into cuvettes and the UV absorbances recorded at 765 nm in UV spectrophotometer (UV-VIS Spectrophotometer, basis model with 2nm bandwidth) for each sample.

A calibration curve based on Gallic acid (Sigma-Aldrich) was prepared using the Folin-Ciocalteu reagent method with a few modifications. Gallic acid (3 mg) was dissolved in 10 ml of methanol to achieve a concentration of 300 mg/L. It was diluted by adding methanol to prepare serial concentrations of 200, 100, 50 and 25 mg/L. The UV absorbance was then measured for all standard solutions prepared above by using a UV-spectrophotometer at a constant wavelength 750 nm.

A standard curve was derived from the blank-corrected A_{765} of the gallic acid standards. Total phenolics were calculated as gallic equivalents using regression equation between gallic acid standards and A_{765}. Results were expressed as milligram gallic acid equivalent (mg GAE)/g dry weight of extract.

Presence of various phytochemicals tested was marked; (+) present, and then the quantified amounts of phenols as per their absorbance compared statistically between the treatments by subjecting them to analysis of variance procedures and means separated with the Tukey's multiple range test (P = 0.05). Total phenolic contents were used to quantify the phytochemicals present in the tomato roots for the various treatments. Using the Folin-Ciocalteu reagent method, the total phenolics for each treatment in every variety were calculated and expressed as milligram gallic acid equivalent (mg GAE)/g dry weight of extract, mg/gGAE.

CHAPTER FOUR

RESULTS

4.1 Farmers knowledge, attitude and practices towards *R. Solanacearum* in Kiambu County, Kenya

Characteristics of farmers and high tunnels in Kiambu County varied (

Table 4. 1). More female than male farmers used high tunnels. Most high tunnels had been utilized for 1-2 years, a few exceeded 2 years. Half of the high-tunnels had been used for 2 cropping cycles within a year. The majority of farmers grew tomato in the high tunnels for commercial purposes (

Table 4. 1). Overall, bacterial wilt and root knot nematodes were ranked as the most problematic diseases in high tunnel tomato production with the former being more important than the latter (Table **4.** 2). All respondents in Gatundu South and Ruiru Sub-Counties considered bacterial wilt as the most problematic; fewer from other sub-counties were of the same opinion (Table 4. 2).

Table 4. 1: Characteristics of respondents and high tunnels used by farmer groups in Kiambu County, Kenya

	Frequency (n)	Proportion (%)	P value
Sub-County			
Thika	8	25.0	
Juja	5	15.6	
Gatundu South	6	18.6	
Kiambu	8	25.0	
Ruiru	2	6.3	
Gatundu North	3	9.4	
Respondent gender			
Male	141	45.3a[a]	0.271
Female	170	54.7a	
Age of greenhouse			
<1 year	5	15.6b	0.007
1-2 years	25	78.1a	
>2 years	2	6.3c	
Cropping cycles			
1	9	28.1b	
2	16	50.0a	

3	5	15.6c	
>4	2	6.3d	
Purpose of growing tomato			
Sale (commercial) (a)	25	78.1 a	<.001
Subsistence (b)	0	0	
Both (a and b)	7	21.9 b	

[a] values in columns followed by the same letter are not significantly different (P≤0.05; Tukey's HSD test).

Table 4. 2: Ranking disease challenges in tomato producing high tunnels in Kiambu County

		Sub County							
Rank	Challenge	Thika (%)	Juja (%)	Gatundu South (%)	Kiambu (%)	Ruiru (%)	Gatundu North (%)	P value	Kiambu County (Mean %)
1.	Bacterial wilt	75a[a]	80ab	100b	75a	100b	66.7a	0.042	82.8c
2.	RKNs[b]	50a	60ab	83.3c	75c	50a	66.7b	0.027	64.2c
3.	Blight	50b	60	100	37.5a	50b	33.3a	0.019	55.1b
4.	Fusarium wilt	62.5c	40b	66.7c	75c	0a	33.3b	0.002	46.3b
5.	Blossom end rot	37.5b	40b	83.3d	12.5a	50c	33.3b	0.001	42.8 b
6.	Powderly mildew	50d	80f	66.7e	25b	0b	33.3c	<.001	42.5b
7.	Tomato yellow leaf curl	25b	0a	50c	37.5bc	0a	0a	0.018	18.8a
8.	Root rot	12.5b	0a	50d	0a	0a	33.3c	0.006	16a
	P value								0.0073

[a] values in columns followed by the same letter are not significantly different (P≤0.05; Tukey's HSD test).

[b] Root-knot nematodes.

Knowledge on bacterial wilt varied between respondents in different Sub-Counties the Sub-Counties. The respondents had a knowledge score that indicated a high knowledge of bacterial wilt (Figure). Most of the farmers reported a crop loss of above 50% from BW (Figure 4.1a). A majority of farmer groups indicated that bacterial wilt was a serious threat to tomato production (Figure 4.1b). Of these 81.2% perceived bacterial wilt as a major disease contributing to decline in tomato production (Figure 4.1b). Farmers also reported other challenges (Figure 4.1c) leading to the decline of tomato production but diseases being the major. Despite the challenges, 71% retained an interest in tomato production (Figure 4.1c).

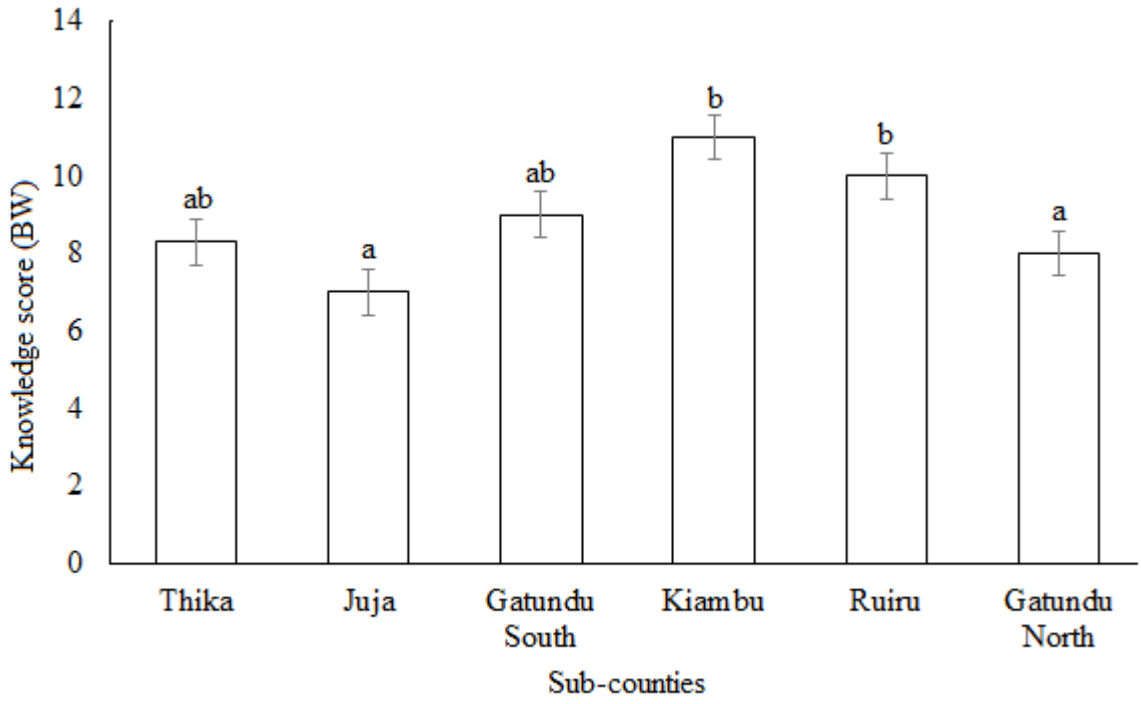


Figure 4.1: Knowledge score on bacterial wilt (BW) among greenhouse tomato farmers in Kiambu County (P≤0.05)

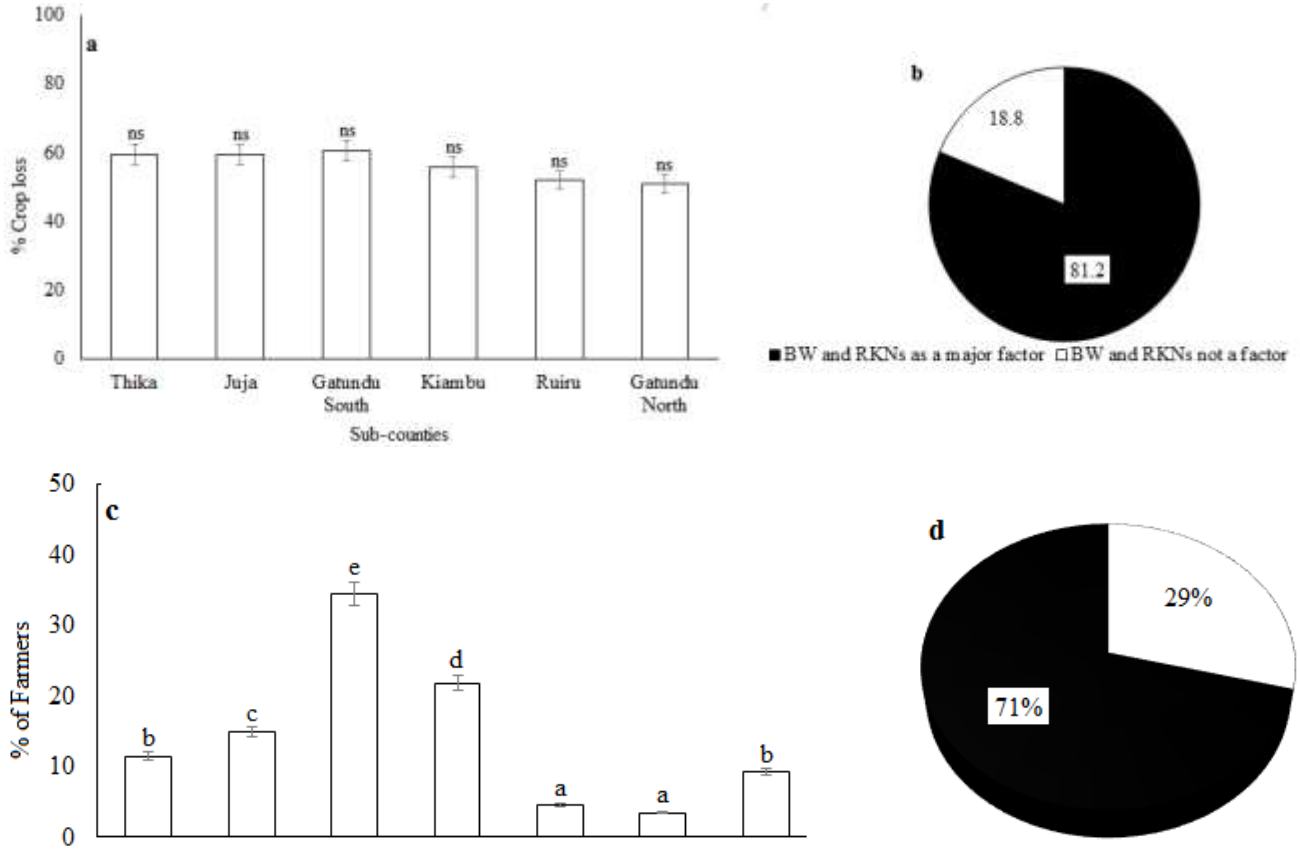


Figure 4.1: Perception of farmers on bacterial wilt (BW) (a) Crop loss due to bacterial wilt; (b) perceptions towards bacterial wilt as a factor leading to loss in tomato (c) contributors to tomato production decline and (d) If farmers have lost interest in tomato production

Of the tomato varieties grown in the high tunnels, ‘Tylka F1’ was the most preferred by nearly half of farmer groups (Table 3). The County government mainly provided tomato seedlings. Most farmers dug up soil in the high tunnels to obtain planting media and it was not treated before establishing the crop. Although most farmers took soil samples for analysis, they did not follow the recommendations. Most farmers used ≈10 g of fertilizer at planting or as top dressing (Table 3). Although a diverse number of methods were used by farmers to control bacterial wilt, uprooting and soil fumigation were the main practices used. Despite their numbers, these methods were considered not to be effective because most farmers disposed of diseased plants outside the high tunnels. Most farmers practiced crop rotation, a minority used other tomato varieties alleged to be resistant to bacterial wilt for rotation. Plants were watered using water from shallow wells and rivers being most common (Table 3).

Table 4.3: Farmer practices in tomato producing high tunnels in Kiambu County

	Sub-County							
Source	Thika (%)	Juja (%)	Gatundu South (%)	Kiambu (%)	Ruiru (%)	Gatundu North (%)	P value	Kiambu County [Overall mean (%)]
Tomato variety								
Tylka F1	40b[a]	71.4d	71.4d	45.5b	50c	20a	0.003	49.7c
Anna F1	10a	14.3a	28.6b	9.1a	0a	60c	<.001	20.3b
Chonto F1	10a	0a	0a	18.2a	25b	20ab	0.037	12.2a
Bravo F1	10a	0a	0a	18.2a	25b	0a	0.034	8.9a
Prostar F1	0a	14.3a	0a	0a	0a	0a	0.237	2.4a
Unknown	30b	0a	0a	9.1a	0a	0a	0.04	6.5a
P value								0.021
Planting material								
Seed	11.1a	28.6b	42.9c	25ab	0a	0a	0.007	17.9a

Seedlings	88.9b	71.4a	57.1a	75a	100b	100b	0.04	82.1b
P value								<.001
Source of planting materials								
County government	60a	71.4a	57.1a	66.7a	66.7a	66.7a	0.08	64.8b
Agrovets	20a	28.6b	28.6b	22.2a	0a	0a	0.039	16.6a
Seed company	0a	0a	14.3a	0a	33.3b	33.3b	0.006	13.5a
Greenhouse contractor	20a	0a	0a	11.1a	0a	0a	0.74	5.2a
P value								0.001
Source of planting media								
Greenhouse earth	100a	100a	83.3a	100a	100a	100a	0.431	97.2b
Forest	0a	0a	17.7a	0a	0a	0a	0.38	2.8a
P value								<.001
Media treatment								
No	87.5b	80b	66.7a	87.5b	100b	100b	0.041	86.9b
Yes	12.5a	20a	33.3b	12.5a	0a	0a	0.033	13.1a
P value								0.001
Soil test								
Yes	37.5a	100c	83.3c	75bc	100c	100c	0.07	82.6b

No	62.5b	0a	16.7a	25a	0a	0a	0.004	17.4a
P value								0.0018
Following recommendations								
No	66.7a	80b	50a	83.3b	50a	100b	0.03	71.7b
Yes	33.3b	20a	50b	16.7a	50b	0a	0.016	28.3a
P value								0.0022
Fertilizer quantities used								
10 g	50a	40a	50a	62.5b	100c	66.7b	0.01	61.5c
5 g	50bc	60c	33.3b	37.5b	0a	33.3b	0.017	35.7b
>10 g	0a	0a	16.7a	0a	0a	0a	0.09	2.8a
P value								0.001
BW and RKN control method								
Uprooting	38.1b	41.7b	50b	26.9b	18.2a	14.3a	0.013	31.5b
Soil fumigation	23.8a	41.7b	30b	23.1a	18.2a	21.4a	0.03	26.4b
Chemical	9.5a	8.3a	20a	15.4a	18.2a	21.4a	0.473	15.5a
Door management	9.5a	0a	0a	15.4a	18.2a	21.4a	0.05	10.8a
Ashes	4.8a	8.3a	0a	3.8a	18.2b	7.1a	0.041	7a
Side nets	4.7a	0a	0a	7.7a	9.1a	0a	0.039	3.6a

Footbath	9.5b	0a	0a	7.6b	0a	0a	0.043	2.9a
Traps	0a	0a	0a	0a	0a	14.3b	0.028	2.4 a
P value								0.014
Effectiveness of method								
No	62.5b	40a	33.3a	50a	100c	33.3a	0.014	53.2a
Yes	37.5a	60b	66.7b	50ab	0a	66.7b	0.02	46.8a
P value								0.051
Crop rotation								
Yes	87.5a	80a	100a	87.5a	100a	100a	0.09	92.5b
No	12.5b	20c	0a	12.5b	0a	0a	0.0281	7.5a
P value								<.001
Crops for rotation								
Other tomato varieties	40c	14.3a	27.3b	42.9c	50c	42.9c	0.028	36.2e
Onions	26.7d	28.6d	18.2c	14.3b	0a	28.6d	0.017	19.4d
Spinach	6.7b	28.6d	18.2c	14.3c	25d	0a	0.005	15.5d
Kale	13.3b	14.3b	9.1b	0a	25c	0a	0.0039	10.3c
Cucumber	6.7b	0a	0a	21.4c	0a	14.3b	0.02	7.1b
Capsicum	0a	0a	18.2c	7.1b	0a	14.3b	0.015	6.6b

Coriander	0a	14.3b	9.1b	0a	0a	0a	0.021	3.9 a
P value								0.019
Disposal of diseased plants								
Piling outside the greenhouse	25a	80d	57.1c	57.1c	50c	33.3b	0.03	50.4c
Burning	62.5d	0a	28.6b	28.6b	50c	33.3b	0.014	33.8b
Burying	12.5b	20c	14.3b	14.3b	0a	33.3d	0.008	15.7a
P value								<.001
Irrigation water source								
Shallow wells	22.2b	0a	37.5c	66.7d	0a	60d	0.01	31.2d
River	55.5d	33.3c	37.5c	22.2b	20b	0a	0.004	28.1c
Rain	11.1b	33.3e	25d	0a	20c	20c	0.019	18.2b
Water pans	0a	16.7b	0a	11.1b	40d	20c	0.019	14.6a
Water company	11.1b	16.7b	0a	0a	20c	0a	0.019	7.9a
P value								0.0036

[a] values in columns followed by the same letter are not significantly different (P≤0.05; Tukey's HSD test

4.2 Site specific soil chemical properties influencing population density of *R. Solanacearum* in the high tunnels

4.2.1 Chemical characteristics of soils from high tunnels in Kiambu County

The soil pH was slightly acidic to neutral (5.3-7.0), and differed significantly (P = <0.001) between the six sub-counties. However, the EC, which was within the normal range, and it was not significantly (P = 0.144) different across the sub-counties (Table 4). Except Zn, Fe and Mn, other elements i.e. N, P, K, Ca, Mg, Na and Cu differed significantly (P = <0.001) between the sub-counties (Table 4). Although high levels of N were recorded in soils from Gatundu North (0.6%), significantly low levels were found in Juja, Ruiru and Thika sub-counties. Moreover, P was significantly low across the sub-counties, except in Gatundu North that was within the normal range **(**Table 4). Notably, Mg was three times higher in Gatundu North relative to the other sub-counties, and above the normal range across all the sub-counties (Table 4).

Table 4.4: Chemical characteristics of soil collected in high tunnels in Kiambu County

Characteristic	Normal range	Sub-County						P value
		Gatundu North	Gatundu South	Juja	Kiambu	Ruiru	Thika	
Soil pH	5.5-6.8	5.3a[a]	5.6a	7.0b	6.6b	6.6b	6.7b	<.001
Soil EC[b] ($\mu S \cdot cm^{-1}$)	<0.8	0.4a	0.7a	0.5a	0.6a	0.4a	0.6a	0.144
N (%)	>0.25	0.6c	0.3a	0.1a	0.4b	0.2a	0.2a	<.001
P ($mg \cdot kg^{-1}$)	0.01-0.3	0.01e	0.004c	0.002a	0.005d	0.002a	0.003 b	<.001
K ($cmol(+) \cdot kg^{-1}$)	0-2	0.6d	0.4b	0.3a	0.6d	0.5c	0.5c	<.001
Ca ($mg \cdot kg^{-1}$)	2-200	254c	120a	338d	204b	293c	186b	<.001
Mg ($mg \cdot kg^{-1}$)	1-120	884b	192a	168a	315a	169a	187a	<.001

Na ($mg \cdot kg^{-1}$)	20-250	11.6b	10.1ab	8.3a	11.2b	10.9ab	8.3a	<.001
Zn ($mg \cdot kg^{-1}$)	2-1600	303.1a	444.6a	415.8a	383.0a	486.6a	320.3 a	0.561
Fe ($mg \cdot kg^{-1}$)	2000-100000	4291.0a	2828.0a	2540.0 a	3874.00 a	555.00a	4832.00a	0.336
Cu ($mg \cdot kg^{-1}$)	2-960	19.2a	15.8a	21.6a	20.5a	25.1b	19.2a	<.001
Mn ($mg \cdot kg^{-1}$)	37-4600	3122.0a	3039.0a	3029.0 a	3038.0a	547.0a	2807.0a	0.723

[a]*Means followed by the same letter within rows are not significantly different; P≤0.05; Tukey's HSD test.* [b]*EC = Electrical conductivity; N = Nitrogen; P = Phosphorus; K= Potassium; Ca = Calcium; Mg = Magnesium; Na = Sodium; Zn = Zinc; Fe = Iron; Cu = Copper; Mn = Manganese; µS = micro-Siemens; cmol(+)·kg^{-1} = centimoles of positive charge·kg^{-1} soil.*

4.2.2 Abundance of *R. solanacearum* in Kiambu County

All samples tested from the high tunnels tested positive for R. *solanacearum*, which was confirmed by the biochemical tests. The high tunnels had varying populations of the pathogen (Figure 3), with high of up to an average concentration of 12.551×10^9 cfu/ml in Gatundu North subCounty. Lest concentrations were in Juja subCounty, 33.102×10^8 cfu/ml, varying significantly within the sub counties at p=0.05 (Figure 3). The abundance of *R. solanacearum* in Gatundu North was two and four-fold higher than in Gatundu South and Juja sub-counties respectively, and ~1.7 times higher than in Kiambu, Ruiru and Thika Sub-Counties (Figure 3).

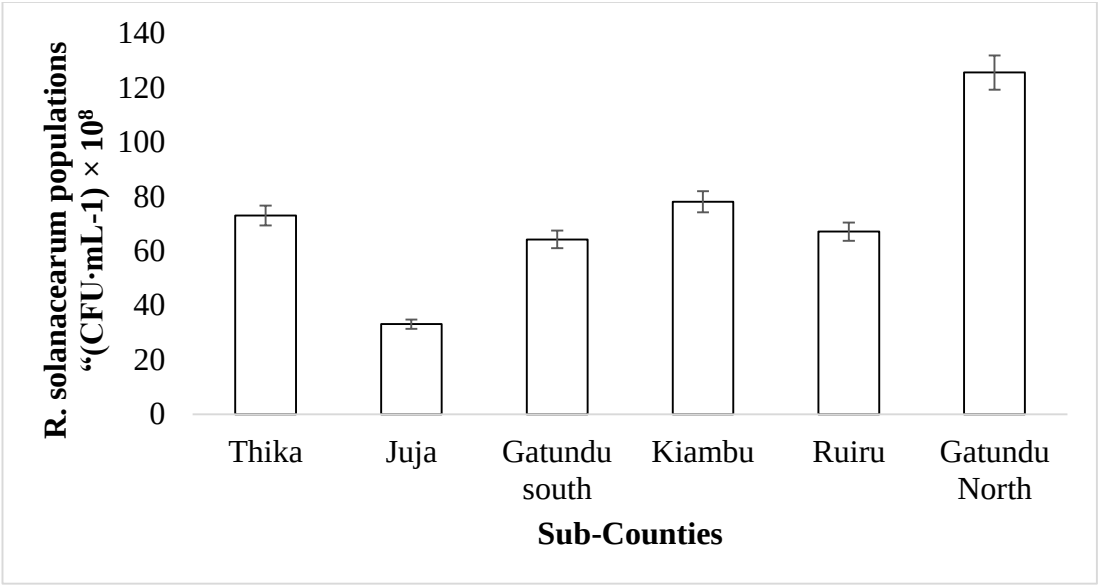


Figure 4.2: *R. solanacearum* populations within Kiambu County (P≤0.05; means separated using Tukey's HSD test)

4.2.3 Correlations between soil chemical characteristics and *R. solanacearum*

There were weak positive and negative correlations between *R. solanacearum* populations with the soil pH, EC, K, Na, Zn, Fe, Cu and Mn, we found a strong positive correlation with the soil N and P (Table 5). Notably, Calcium had strong negative correlations with *R. solanacearum* compared with other elements (Table 5). Notably, we found a strong positive correlation between *R. solanacearum* and *Meloidogyne* spp. Populations in Kiambu County (r = 0.7562).

Table 4.5: Correlation co-efficient (r) of *Ralstonia solanacearum* relative to soil chemical property

Soil chemical property	*Correlation co-efficient (r)*
Soil pH	-0.4203
Soil Ec	-0.0811
Nitrogen	0.7793
Phosphorus	0.8717
Potassium	0.3197
Calcium	-0.4752
Magnesium	0.3583
Sodium	0.1110
Zinc	-0.2318
Iron	0.1550
Copper	-0.0391
Manganese	0.3859

4.3 Monitoring of pathogenicity of *R. Solanacearum* under different fertilizer regimes

4.3.1 Effects of fertilizer treatments on *R. solanacearum*

The two tomato varieties used in the experiments had no significant differences with regards to wilting (P = 0.237) (**Error! Reference source not found.**a). However, the treatments affected the wilt incidence differently. Control (0 g) plants wilted most (87.32%) due to the BW disease which was significantly higher (P = 0.01) than all other treatments. At 10 g, plants had the least wilt incidence at 30%, which was three times lower than control (**Error! Reference source not found.**b). Wilting had a strong negative correlation (r = -0.54) with the yield of tomato (Figure 6) indicating that as the wilt severity increased, yield reduced.

4.3.2 Effects of fertilizer treatments on tomato yield per treatment

Two tomato varieties (Tylka F1 and Anna F1) were investigated. The varieties, Tylka F1 and Anna F1 respectively yielded 1.15 and 1.12 kg on a cumulative average (without considering the treatment effect on yield). This was not significantly different at P = 0.05 indicating no differences in the varieties used in terms of yielding (Figure 4.4a). However, the treatments affected the yield of the tomato varieties significantly (P = 0.05). Yield was least at 0 g for all tomato varieties used followed by 20 g. Most plants in the 20 g treatment never reached maturity due to fertilizer toxicity. The best yield was achieved at 10 g application rate, significantly better than all other treatments at P = 0.05 but not significantly better than 5 g treatment for Anna F1 tomatoes (Figure 4.4b).

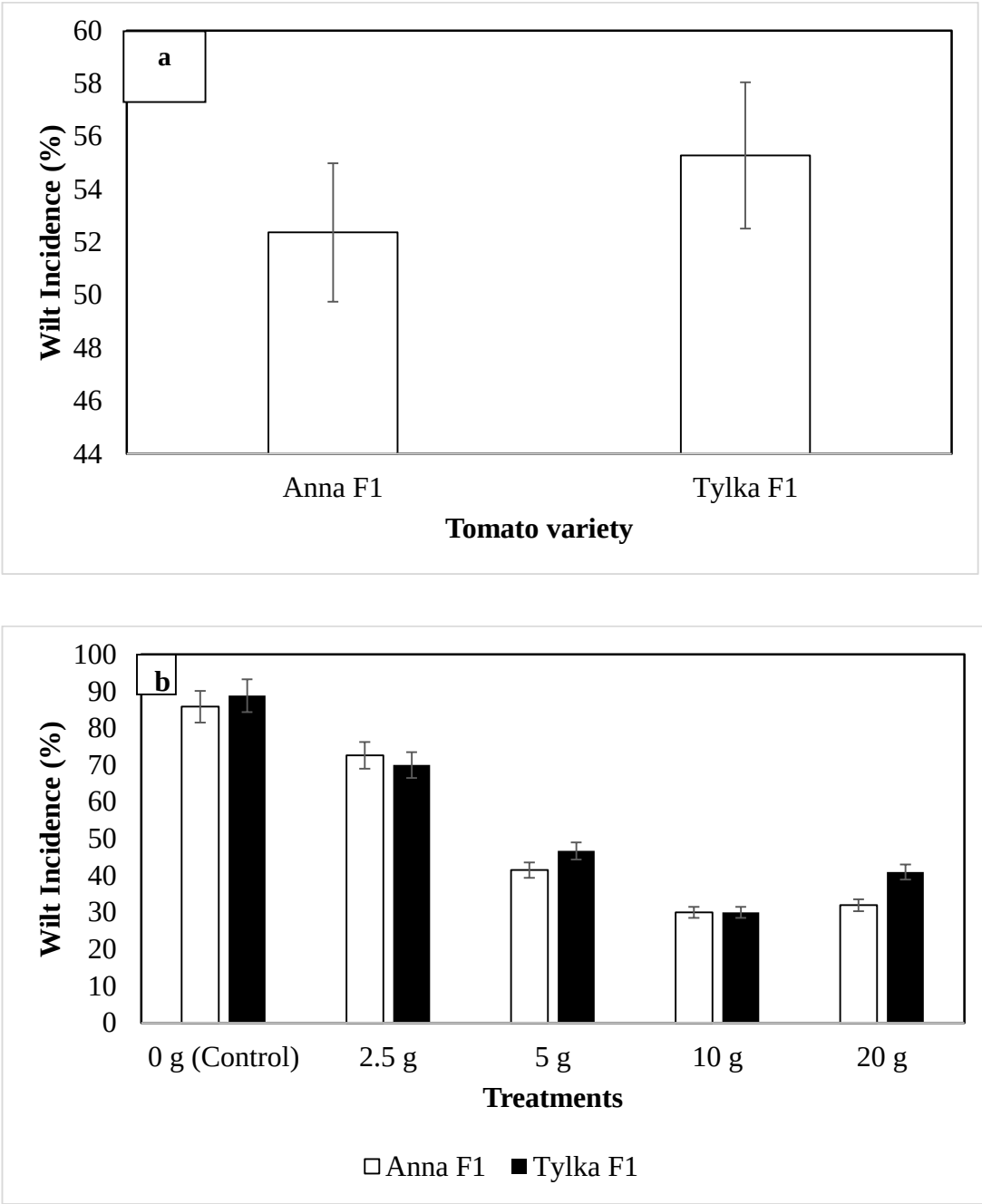


Figure 4.3: Tomato wilt incidence due to *R. solanacearum* as affected by (a) variety, and (b) fertilizer treatments (P≤0.05; Tukey's HSD test)

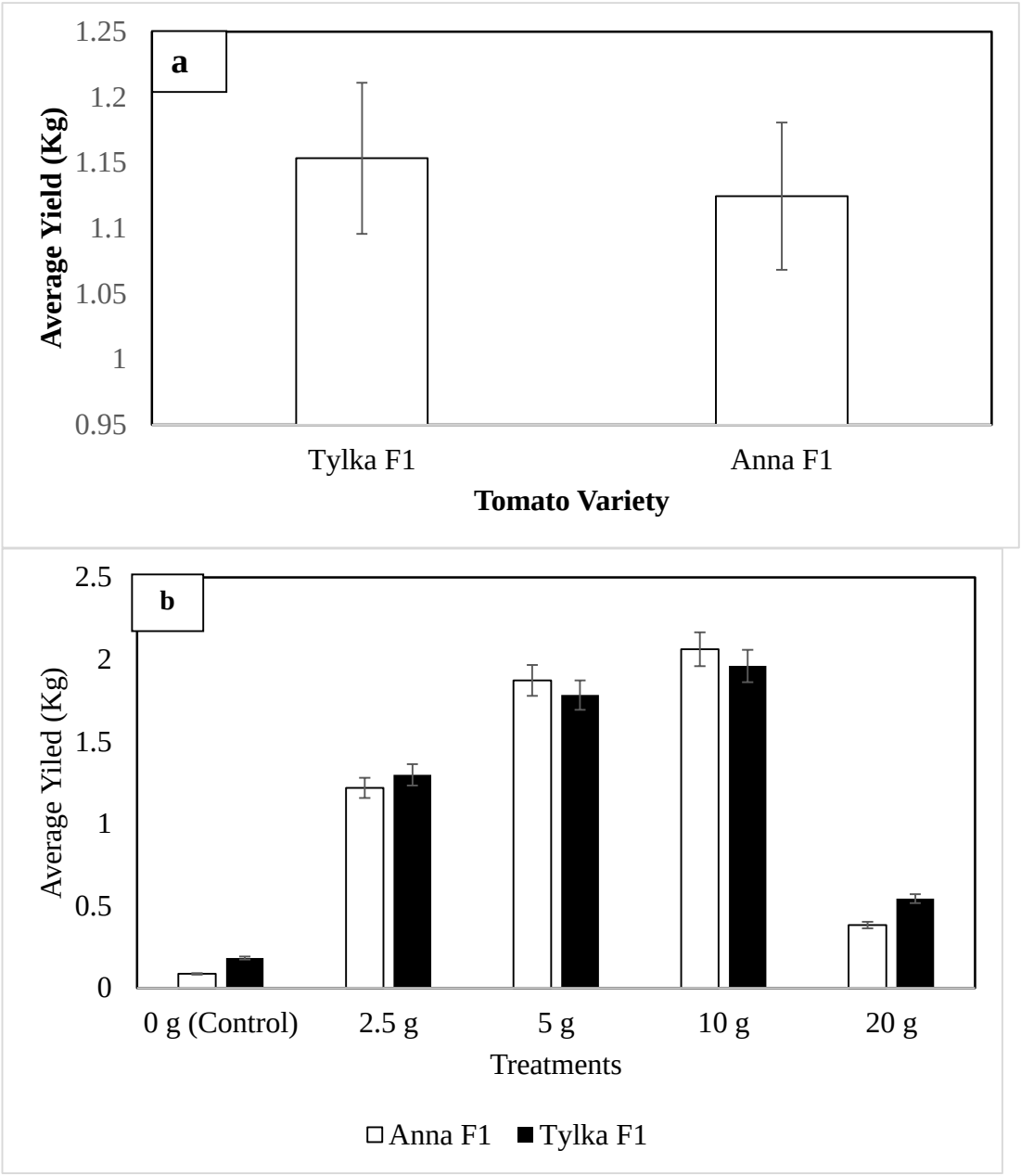


Figure 4.4: Average yield of tomato as affected by (a) variety, and (b) fertilizer treatments (P≤0.05; Tukey's HSD test)

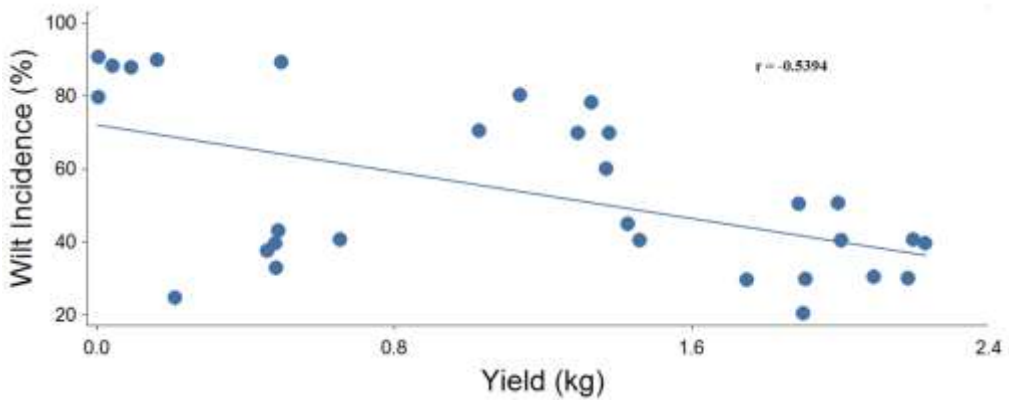


Figure 4.5: Correlation between wilt incidence and yield of tomato

4.4 Chemical components released by tomato roots infected with bacterial wilt treated with different fertilizer rates

4.4.1 Phytochemical components of tomato roots

Results of phytochemical screening showed that alkaloids, flavonoids, tannins, saponins, and terpenoids were present in all the root exudates under different fertilizer treatments for the tomato varieties used (Table 6).

Table 4.6: Phytochemical profiles of tomato roots treated using different fertilizer levels

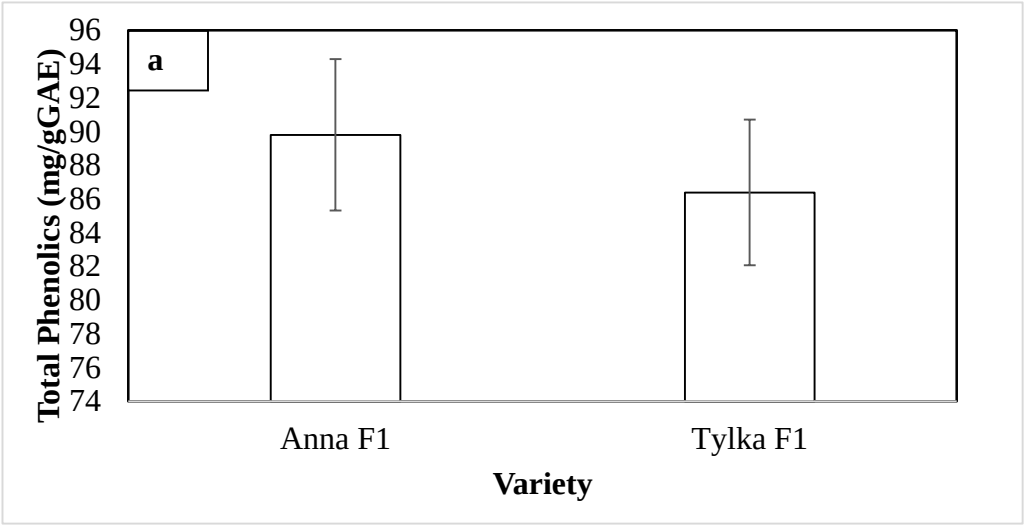
	Fertilizer rate (g/2 kg of soil)				
Phytochemical test	**Control**	**2.5**	**5**	**10**	**20**
Alkaloids ***(Meyer's Test)***	+	+	+	+	+
Flavonoids ***(Lead acetate Test)***	+	+	+	+	+
Tannins ***(Ferric Chloride Test)***	+	+	+	+	+
Saponins ***(Distilled water test)***	+	+	+	+	+
Terpenoids ***(Liebermain-Burchard Test)***	+	+	+	+	+

NB: (+) = Present

4.4.2 Phenolic content of tomato plant roots under different fertilizer rates

The tomato varieties used did not significantly differ (P = 0.083) in the total phenolic content in the roots (Figure 7a) irrespective of the fertilizer rates. When the effects of the different fertilizer rates were tested, the total phenolic content varied significantly (P = 0.0001) across the treatments. However, total phenolics content was found not to

be significantly different (P = 0.091) between 10 g and 20 g treatments which had the highest means of 126.5 and 132.8 mg/gGAE at 10 g and 20 g at respectively (Figure 7b). The control had low levels of total phenolics (45.7 mg/gGAE) relative to the other treatments. The total phenolic content was found to be fertilizer rate dependent (Figure 7b). Whereas a weak positive correlation (r = 0.29) was found between total phenolics and tomato yield (Figure 8a), a strong negative correlation (r = 0.70) existed between wilt incidence and the total phenolics (Figure 8b).



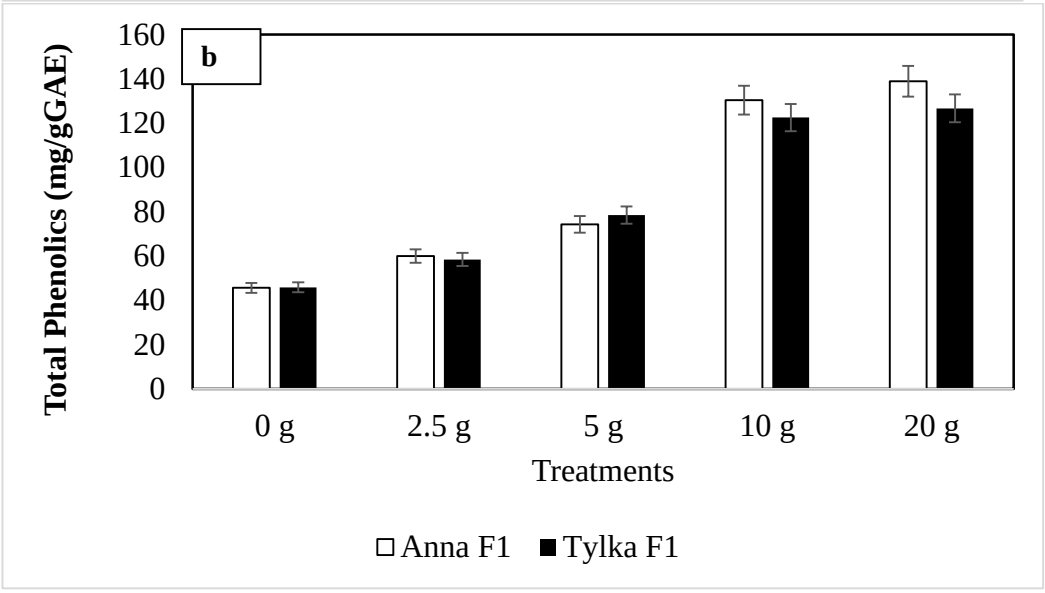


Figure 4.6: Total phenolics as affected by (a) variety, and (b) fertilizer treatments per variety (P≤0.05; Tukey's HSD test)

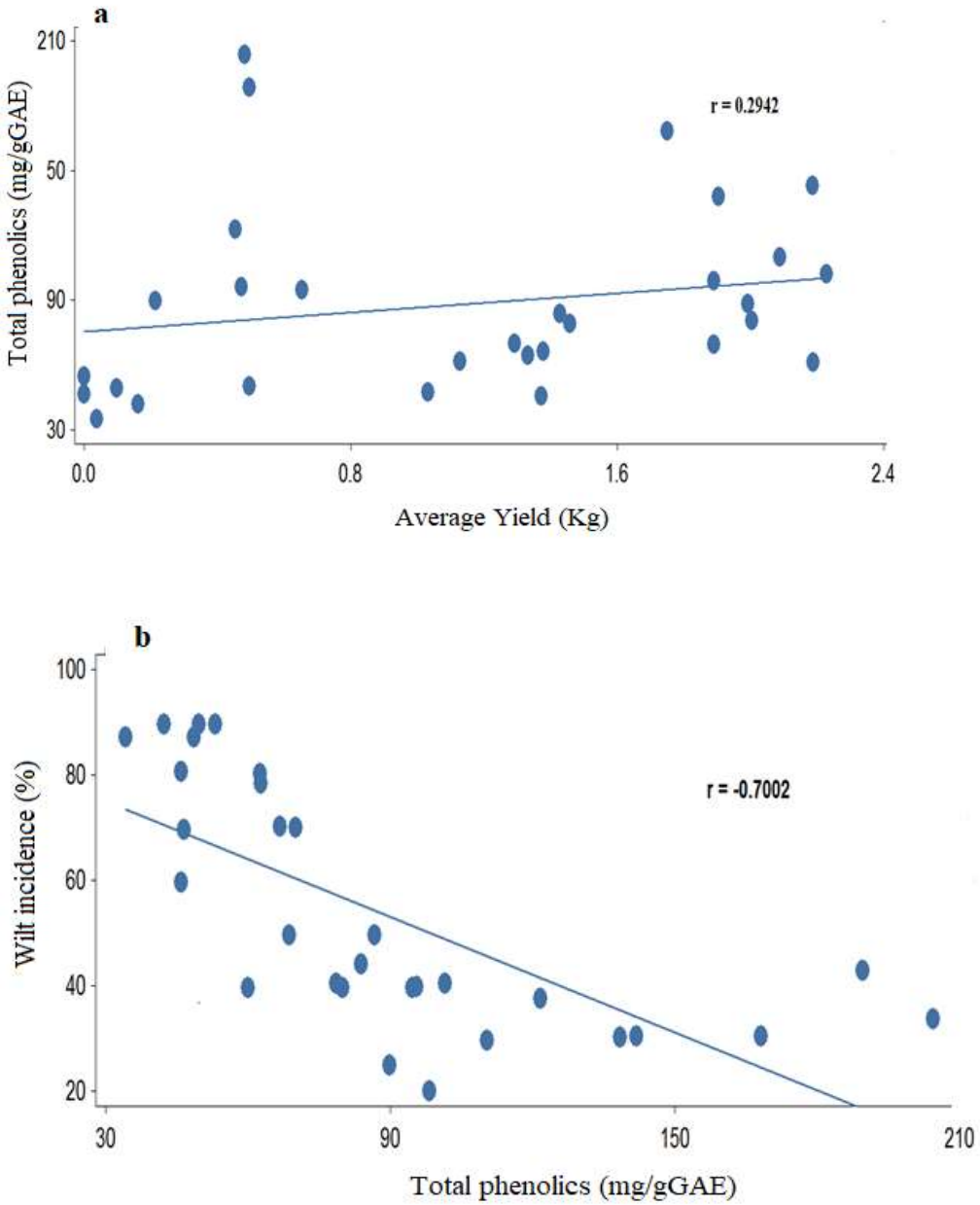


Figure 4.7: Correlations (a) Total phenolics against average yield, and (b) total phenolics against wilt incidence, for the fertilizer treatments

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

In Kenya, tomato is grown mainly under open field, until recently when high tunnels were introduced. Nearly all groups interviewed had/or were growing tomato in high tunnels. This was not unusual as the crop was ranked first in a prioritization of vegetable crop value chains in Kenya (KARI, 2011; Anonymous, 2012; 2016b). Most high tunnels had only been in existence for 1-2 years indicating farmers were yet to realize the full potential of producing tomato in these structures.

Most farmer groups reported bacterial wilt and RKNs as problematic in high tunnel tomato production. Despite this general perception, the farmers had a good knowledge about bacterial wilt which may be attributed to wilt symptoms being visible on aerial parts of the plants (Hayward, 1991). Despite individual farmer groups experiencing crop losses of between 50-100%, and average individual Sub-County losses between 45-65%, there was a high interest in producing tomato in high tunnels. Most farmers were interested to know more about the diseases and prevention methods.

Farmers have poor practices towards prevention of bacterial wilt. Farmers did not obtain clean planting material, did not sterilize the soil, and disposed of diseased plants outside the high tunnel. Bacterial wilt and root-knot nematodes are soil-borne pathogens (Hayward, 1991), and only good farming practices are predictors of prevention (Yuliar and Toyota, 2015). Previous reports indicated that improved cultural practices including planting clean healthy seed, crop and field sanitation, and disinfection of agricultural tools, reduced incidence of bacterial wilt disease and led to increased yield (Yu, 1999; Lemaga *et al.*, 2005). Amendment of soil with either organic and/or inorganic fertilizers under field conditions reduced bacterial wilt incidence on *S. tuberosum* L. (Lemaga *et al.*, 2005) and increased yield. Crop rotation has been shown to suppress nematode populations (Guerena, 2006). Studies using

these field-tested practices need to be conducted in high tunnels to establish their impact on bacterial wilt.

Despite their high interest in producing tomato in high tunnels, farmers have poor farming practices that intensify the severity of bacterial wilt, and experience large crop losses. Unsterilized media, unclean seedlings and poor disposal of infected plants are continuous sources of inoculum in high tunnels. Baseline information about the current knowledge of farmers about BW and practices to manage such pathogens in high tunnel tomato production indicates that more work is required to optimize this technology. With appropriate production practices, the use of high-tunnel tomato production may be optimized and applicable for smallholder farmers in the tropics. The mineral composition of nutrient fertilizers may alter the reaction of tomato plants to pathogenic agents. In this study, the effect of soil chemical properties on the population levels of *R. solanacearum* were investigated *in situ* in six sub-counties of Kiambu County. It was established that soil chemical properties, which differed across the sub-counties, variously influenced *R. solanacearum* populations. Whereas Gatundu North Sub-County reported high populations of *R. solanacearum*, the soil pathogen was significantly low in Gatundu South, Thika, Kiambu, Ruiru and Juja sub-counties. We found that the soil pH in Gatundu North was slightly acidic relative to the other sub-counties which consequently led to high *R. solanacearum* populations. These results agree with those of Li *et al*., (2017) who reported that acidified soils of a pH <5.5 increased the multiplication and infestation of *R. solanacearum* in solanaceous crops. Acidic soils have also been found to increase the population of soil microbial communities particularly *R. solanacearum* (Kesba and Al-Shalaby, 2008).

When the soil chemical characteristics were correlated with *R. solanacearum*, positive and negative relationships existed suggesting their role in pathogen-host interactions (Desaeger and Rao, 2000; Wang *et al*., 2004). Despite the fact that the soil pH had weak positive and negative correlations with the abundance of *R. solanacearum*our results suggest their role in influencing nematode diversity (Ingham *et al*., 1985; Zhong and Cai, 2007). Our results suggest that soil pH may alter the soil microbiota by

affecting the soil microbial activities (Rocha *et al*., 2006). Previous studies reported that continuous use of mineral fertilizers such as NPK and CAN decreased the soil pH (Adamtey *et al*., 2016), consequently affecting the soil microbes and diversity (Zhong *et al*., 2010). Differences in soil chemical properties and their subsequent effect on *R. solanacearum* populations could be attributed to changes in farmer practices and varying environmental conditions in the high tunnels which were, however, not measured in this study.

Tomato varities Anna F1 and Tylka F1 yielded the same. This is not unusual as F1 hybrids have been found to be more superior to inbred varieties with less variability amongst them (Crill and Burgis, 1970). Considering the treatments, it was observed that the average yield per plant varied with varying treatments. The highest yield was observed at 10 g application rate, with 0 g application rate giving the least yield per plant. Over fertilizing at 20 g/plant resulted into significantly lower yield. Application of NPK fertilizer at different rates has been shown to affect significantly, maximum plant height, stem thickness, leaf number, number of flower clusters and longevity of fruit picking period (Singh *et.al*., 2005). However, fertilizer at higher doses leads to excessive vegetative growth and delayed maturity, which may account for the poor performance for the 20 g application rate (Pandey et.al., 1996). Excess fertilizer application also alters the soil by creating too high of a salt concentration, and this can hurt beneficial soil microorganisms. Over-fertilization can lead to sudden plant growth with an insufficient root system to supply adequate water and nutrients to the plant. Use of a high-nitrogen fertilizer mixture increases the soil's mineral salts; excessive elemental nitrogen takes water away from the plant while leaving the salts behind. As a result, the plant leaves take on a burnt look from dehydration. Leaf edges become yellow or brown and wilt. Flushing the area with water to remove the excess nitrogen is the best course of action to revive the plant. Although addition of nitrogen produces desired large foliage, it can lead to leaf burn at high nitrogen levels (Rodriguez, 2018).

To test the effect of application of fertilizer on *R. solanacearum* incidence, results from the current study showed the presence of high levels of pathogen in the lower rates of

fertilizer application, decreasing to 10g fertilizer and a small spike at 20 g level. The application of fertilizers affects the incidence of diseases and pests induced by the plants' nutritional status and it indirectly produces dense stands and alterations in the interception of light and moisture within the crop (Agrios, 2005). Studies have reported that adequate mineral nutrition is central to crop production. However, it can also exert considerable negative influence on disease development. Fertilizer application can increase or decrease development of diseases caused by different pathogens, and the mechanisms responsible are complex, including effects of nutrients on plant growth, plant resistance mechanisms and direct effects on the pathogen (Parthasarathy, 2015). Two basic plant resistance mechanisms that mineral nutrition can affect in plants are: 1. Formation of mechanical barriers (cell wall strengthening) and 2. Synthesis of defense compounds that protect against pathogens (Spann and Schumann, 2010). The role of specific elements and their compounds is much more complicated. Certainly deficiencies of molecules such as Calcium and Potassium can interrupt either of these defense mechanisms.

Nitrogen fertilizer applied above the recommended rates can result in increased disease incidence and lesion area. This has been demonstrated for biotrophic fungal pathogens such as powdery mildews and rusts and necrotrophic pathogens such as *Magnaporthe grisea,* the cause of rice blast (Parthasarathy, 2015). Several studies have reported no effect of nitrogen on disease severity, also the effect of nitrogen depended on the type of pathogen. Thus, nitrogen increased susceptibility of tomato to the powdery mildew pathogen *Oidium lycopersicum* and the bacterial pathogen *Pseudomonas syringae* pv. *tomato,* while it had no effect on susceptibility to the vascular wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici*.

Wilt diseases have also been studied in relation to disease occurrence. Keim and Humphry (1984) showed that nitrogen source reduced the incidence of wilt cause by *Fusarium oxysporum* f.sp. *hebe* in Veronica. In their system, ammonium sulfate promoted disease and calcium nitrate prevented fusarium infections.

Increased disease level at 20 g application rate may have been due to weakening of the plant due to burning and salt concentration earlier discussed which leads to a weaker plant prone to attack by diseases. Results indicated presence qualitatively in all our fertilizer treatments and an increasing trend quantitatively from low fertilizer treated plants to higher treatment rates of our fertilizers. The availability of secondary metabolites in various plant parts can be influenced qualitatively and quantitatively by managing ecological factors or farming practices (Sereme *et. al.*, 2016). Studies have shown that fertilization organically and inorganically has an effect in increasing the amount of total phenolic contents in tomato plants (Sereme *et. al*., 2016). Studies by Ibrahim *et. al*., (2013) showed that application of N based fertilizers enhanced total phenolics and flavonoids at 12% and 22%, respectively. The work further reported that optimum fertilization occurred at 90 kg N/ha, where it was observed that total phenolics had the highest values (total phenolics = 1.32 mg/g gallic acid dry weight). Increased fertilization rates above 90 kg N/ha to 180 and 270 kg N/ha resulted in a decrease in the total phenolics contents. The results are in agreement with the curve of total phenolics observed in our studies. The strong negative correlation between the total phenolics and wilting of plants due to BW in our studies suggest that wilting decreases with increase in total accumulated phenolic compounds. The importance of phenolic compounds in a wide variety physiological processes is increasingly evident. There is hardly any plant disease which would not be associated with changes in metablosim of aromatic compounds. It is proposed that an important first line in plant defense against infection is provided by the very rapid synthesis of phenolics and their polymerization in the cell wall (Kneusel and Matern, 1988). Phenolic compounds with less complex structures, such as catechol and coumarin, have also shown to exhibit bactericidal and fungicidal activities (Cowan, 1999). Increased accumulation of phenolic phytoalexins in plants can promote host defense against pathogens. One outstanding example is the resistance of grape phenolic stilbenes to fungal colonization (Jeandet *et. al.*, 2012).

Flavonoids, phenolic compounds synthesized by plants in response to pathogen attack have the ability to make complexes with extracellular and soluble proteins. They

coagulate bacterial cell proteins and affect enzymes involved in synthesis of essential aminoacids (Al-Obaidi, 2015). Tannins have anti-microbial properties due to their basic character. They react with prolin-rich proteins and form stable water soluble compounds. They also kill bacteria directly by damaging their cell membrane (Mainasara *et. al.*, 2012). They also have ability to bind to adhesins so that the bacteria cannot attach to the surface of the host cell and in this way they remain unable to cause infection (Tanaka *et. al*., 2006). Terpenoids inhibit bacterial growth by denaturation of proteins or acting as dehydrating agents. They act upon the phospholipid bi-layers of the cell due to which, different processes like electron transport, protein translocation, phosphorylation steps and other enzyme dependent reactions are affected and finally membrane disruption occurs, resulting to inhibition of bacterial growth (Dorman and Deans, 2000). The same effect of prevention against *R. solanacearum* by increased accumulation of total phenolic compounds was exhibited but should be studied further.

5.2 Conclusion

From the studies conducted, the following conclusions were deduced;

1. Bacterial wilt is the greatest constraint to high tunnel tomato production in Kiambu County. Farmers were fully aware of the disease symptoms and effects.
2. Despite the high level of awareness of the pathogen, farmers have poor farming practices that intensify the severity of the pathogen.
3. Soil chemical characteristics influence occurrence of bacterial wilt pathogen in high tunnels. From the studies done in the farmers' high tunnels, it was noted that Soil pH, Calcium copper and Ec have a negative correlation with the pathogen, whereas Nitrogen, Phosphorous potassium magnesium and manganese had a positive correlation with nitrogen and phosphorous being a strong positive correlation. Farmers and stake holders can use this information to develop bacterial wilt control strategies which are based on nutrients available in their soils and use the information to make nutrients amendments

based on their soil analysis reports. This would in turn impact positively on the livelihoods of small holder tomato high tunnel farmers.

4. Application of fertilizer at a lower rate (2.5 g/2kg soil) did not offer benefits in terms of disease occurrence/expression in the tomato plants and resultant yields were low.
5. Application of fertilizer at 20 g/2kg soil was toxic to the plants despite the high level of phenolics tested at that rate. Wilting at this rate also was observed to go higher than in 10 g which had least wilt.
6. Application of fertilizer at a rate of 10 g per planting pot/hole offered potential to reduce the severity of bacterial wilt and had a yield advantage with no toxicity observed.
7. Application of NPK fertilizer influenced the expression of phenolic contents in the tomato roots. Phenolics expression was optimal at 10 g and 20 g fertilizer rates and there was no significant difference at the rates. A strong positive correlation was statistically identified to exist between the total phenolic contents and the bacterial wilt disease incidence.

5.3 Recommendations

From this study, the following should be up taken and researched further in order to cement the establishment of a proper bacterial wilt control strategy and in turn increase the productivity of tomatoes in high tunnels;

- There is need for continuous farmer education and intensified extension services by well-trained personnel to increase adoption of good farming practices. This will impact positively on tomato production and proper utilization of the high investment high-tunnels.
- Identification of the specific races and biovars affecting tomato production
- Onsite trials be conducted with 5 g and 10 g fertilizer/plant rates in farmer high tunnels

- Testing individual elements N, P & K to establish which actually influences the established pathogen behaviour
- Identification of the specific phenolic compounds and their mode of action

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APPENDICES

Appendix I: Checklist of focus group discussion with open-ended questions

Checklist for a focused group discussion on farmer knowledge attitude and practices on management of pests and diseases in high tunnel 'greenhouse 'tomato production in Kiambu County

A) Introduction

i. How many members do you have? Total: ……. Male ……. Female ……

ii. When did the group start practicing greenhouse farming?

B) Knowledge of the problem

i. How long have you been using the greenhouse

ii. During the time you have been using the greenhouse, which vegetables have you grown?

iii. How many seasons have you grown each crop?

iv. Are the vegetables for sale or for home consumption?

v. If for sale, are they profitable?

vi. How profitable are they compared to open field production?

vii. What are the main pest and disease challenges in growing the vegetables?

viii. Have you observed the following problems (*show pictures of Bacterial wilt symptoms & RKN damage on roots and patches in the field*) in tomato?

ix. What do you associate these (above) symptoms with?

x. At which stage do you mostly observe the above symptoms in a crop's life?

xi. Approximately, what proportion of the crop population is affected?

xii. Do you have a local name for these problems? Which one?

xiii. Has anyone of you participated in a group discussion or survey before to discuss RKN and bacterial wilt problems in your farms?

xiv. What did you discuss?

C) Attitude towards the problem

i. Which factors do you consider as the main contributors to the decline in greenhouse tomato production?

ii. Do you consider the above issues (*RKN and Bacterial wilt*) among major factors leading to decline in crop production?

iii. How?

iv. Do you think any greenhouse practices affect the level of any of the problems above? Which one and how?

v. Is there any method that you can suggest, as a control of the problem(s) (RKN and bacterial wilt)? Which one and how?

vi. How much is the estimated yield loss in tomato and other crops due to RKN and bacterial wilt?

vii. Has this affected your interest in growing tomato?

How?

D) Practices

i. How do you grow tomato for maximum production?

ii. Which variety of tomato do you plant?

iii. Where do you acquire your seeds/seedlings from?

iv. How long does tomato take from planting to the first harvest?

v. Do you uproot tomato and plant another crop (tomato or other crop) immediately? If not how long? And if yes, which other crop after tomato?

vi. Which type of planting media do you use?

vii. Where do you obtain it from?

viii. How do you prepare the media before planting?

ix.	Do you test your soil before planting?
x.	Where and what test?
xi.	If you don't, why?
xii.	How often do you test after the first test?
xiii.	Do you follow the test recommendations?
xiv.	Which fertilizer type do you apply?
xv.	How do you apply these fertilizers?
xvi.	At what stage of plant growth do you apply the fertilizer?
xvii.	How many times do you apply each fertilizer?
xviii.	How much fertilizer do you apply; a) per stem/hole b) the whole greenhouse
xix.	Which methods do use to you control the pests and diseases in the greenhouse?
xx.	How do you control the above problems (*show pictures of Bacterial wilt symptoms & RKN damage on roots and patches in the field)*?
xxi.	What is the cost implication of each of these methods?
xxii.	How do you apply these methods?
xxiii.	How effective are these methods in reducing the above problems?
xxiv.	Do you find any differences in terms of weather conditions in the severity of problems?
xxv.	Under which conditions is the bacterial wilt and RKN problems most severe?
xxvi.	Do you practice crop rotation in the greenhouse?
xvii.	If yes which crops?
xviii.	If no why?
xxix.	How dispose diseased plants?
xxx.	Where do you get your irrigation water from?
xxxi.	What type of irrigation do you use?

E) Wrap-up session

i.	Other than the ministry of Agriculture are there any Organizations or actors you are working with concerning the same pest and disease problems.
ii.	What kind of support have they given you?

iii. Which pest and/or disease would you prefer to have the greatest focus for intervention?

Why?

F) Participants gender (by observation)

i. Male
ii. Female

Appendix II: Journal article

Farmer Knowledge of bacterial wilt and root knot nematodes and practices to control the pathogens in high tunnel tomato production in the tropics, International Journal of Vegetable Science;doi: https://www.tandfonline.com/doi/full/10.1080/19315260.2018.1499690

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Farmer knowledge of bacterial wilt and root-knot nematodes and practices to control the pathogens in high tunnel tomato production in the tropics

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