INFLUENCE OF BUNCHING ONION (*Allium fistulosum*) CRUDE EXTRACT AND IRRIGATION LEVELS ON BACTERIAL WILT INCIDENCE AND TOMATO (*Solanum lycopersicum* L.) GROWTH, YIELD AND QUALITY

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EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted before in any institution for any other award.

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Recommendation

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

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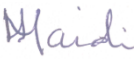
Signature …  Date 30/06/2022…

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DEDICATION

This thesis is dedicated to Almighty God, my beloved parents Mr Saul Peter Shikoli and Mrs Jackline Nasimiyu Shikoli, my siblings Consolata Shikoli, Peter Shikoli and Mercy Shikoli.

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ABSTRACT

Tomato is the second most important vegetable crop in Kenya after potato whose production is limited due to abiotic and biotic constraints among them water availability and bacterial wilt caused by *Ralstonia solanacearum.* The study was conducted at KALRO-Kakamega, Kenya. The objectives of the study were; to determine the effects of different concentrations of bunching onion (*Allium fistulosum*) crude extract and irrigation levels on (i) bacterial wilt inhibition in-vitro (ii) incidence and severity and (iii) growth, yield and quality of tomato. The study entailed a laboratory *in- vitro* antibacterial bioassay and a greenhouse experiment and employed a single factor treatment design with combinations of different levels of *Allium fistulosum* crude extract and different levels of irrigation treated as distinct treatments. Data was collected on diameter of zone of inhibition, disease incidence and severity, growth parameters, yield parameters and quality parameters. Results indicated that the highest inhibition mean diameter of 11.48 mm was obtained under 20% concentration of *Allium fistulosum* in the in-vitro antibacterial assay while lowest inhibition was under negative control treatment (distilled water). In the greenhouse experiment, use of *Allium fistulosum* crude extract combined with irrigation levels significantly($P<0.05$) reduced disease incidence and severity, improved growth, yield and quality parameters of tomato plant. During all data collection days, the lowest disease incidence and severity was recorded under 20% combined with one litre and half a litre of water while the highest disease incidence was recorded under positive control (Greencop) and negative control combined with two liters of water. Treatments with 20% combined with two liters and one and a half liters of water in both experiments significantly ($P<0.05$) had the tallest plants, highest number of branches and internodes. Highest length of internodes and thickest stems were obtained under 20 % combined with two litres and one and a half litres of water. Early flowering was achieved under 20% combined with one litre of water treatment. Significantly highest number of tomato fruits and fruit weight were produced under 20% combined with two litres of water. Shelf-life of fruits was highest under 20% combined with one and a half litres of water while total soluble solids, ascorbic acid content, lycopene content while beta-carotene content under 20% combined with one litres and half a litre of water. To conclude, 20% concentration of *Allium fistulosum* crude extract combined with one and a half litres of water can be considered for use by tomato growers in order to improve tomato growth and yields while 20% combined with one litres of water for improving quality of tomato fruits.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ASCU	Agricultural Sector Coordination Unit
ASM	Acibenzolar-s-methyl
CAN	Calcium Ammonium Nitrate
CFU	Colony Forming Unit
CPG	Casamino Peptone Glucose
CRD	Completely Randomized Design
GDP	Gross Domestic Product
GLM	General Linear Model
FAO	Food and Agriculture Organization
HCD	Horticultural Crop Directorate
HSD	Honest Significant Difference
KALRO	Kenya Agricultural and Livestock Research Organization
KCSAP	Kenya Climate Smart Agriculture Project
MOA	Ministry of Agriculture
Nm	Nanometer
NPK	Nitrogen Phosphorous Potassium
SAS	Statistical Analysis System
TSS	Total Soluble Solids
TZC	Triphenyl tetrazolium chloride
w/v	Weight per volume

WHC	Water Holding Capacity
WL	Water level

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Agriculture is an important sector in the Kenyan economy. In the year 2018, agriculture contributed 29.3% to the Gross Domestic Product (GDP) and accounted for 80% of national employment (HCD, 2019). According to Kenya Economic Survey (2019), the leading subsectors as per rank in 2018 were dairy, tea and horticulture. The horticulture industry in Kenya plays an important role in food security, employment creation and poverty alleviation (Agricultural Sector Coordination Unit, 2015). It also contributes enormously to food security and household income to majority of Kenyan producers who carry out one form of horticultural production or another. The industry has also employed over six million Kenyans both directly and indirectly thus improving their livelihoods (MOA, 2019). During the year 2018, the domestic value of the industry amounted to KES 236.45 Billion as compared to KES 213.11 Billion in 2017, representing an increase of 11%. Within the same period, the area under cultivation increased by 7% from 546,936 Ha to 584,597 Ha with a total production of 8.589 Million Tonnes in 2018 compared to 7.89 Million Tonnes in 2017 (HCD, 2019).

Vegetable production contributes significantly to the horticulture sector. For instance, in 2018, vegetables contributed KES 23.4 Billion which was an increase of 23% from the year 2015. Vegetable exports also increased from 68,942 Tonnes in 2017 to 78,790 Tonnes in 2018. The significant increase had a positive impact on food security enhancement, improved nutrition and generation of foreign exchange earnings for the country (HCD, 2019).

Tomato is the second most important vegetable in Kenya and is mostly grown for domestic market. It is grown both under irrigation and rain fed conditions. The vegetable demand is on the rise which has made farmers adopt high yielding varieties and modern technologies like greenhouse production to ensure all year round production (HCD, 2019). Production is mainly carried out by small scale growers with land sizes between 0.5 to 2.5Ha (Mbaka *et al*., 2013). The fruit contains ß-carotene, ascorbic acid and phenolic compounds which have nutritional benefits such as cancer prevention, growth, development and repair of all body tissues. According to HCD (2019), the area under production of tomato increased from 18,378 Ha in 2017 to 20,111 Ha in 2018 representing 11% increase while production increased from 330,679 Tonnes to 341,026 Tonnes representing a 6% increase. The increase was due to enhanced irrigation and expansion of greenhouse production. During the year

2018, yields realized under an area of production of 14,595 Ha were 283,000 Tonnes compared to 410,033 Tonnes in 2017. However, the increasing production is challenged by the prevalence of diseases such as bacterial wilt in many of the producing regions in the country (FAO, 2019).

Tomato bacterial wilt disease is caused by *Ralstonia solanacearum*. The pathogen also infects potato, eggplant, geranium, many weeds and wild plants and can survive in surface water, soil and plant debris and infected planting material (Mwanikemwa, 2015). It is a soil borne and waterborne pathogen that can survive in soils and water, thus facilitating further spread especially in irrigated fields (Fajinmi & Fajinmi, 2010). Bacterial wilt also infects plants through roots and vascular bundles that have wounds formed by lateral root emergence (Onduso, 2014). Mbaka *et al*. (2013) reported that the disease causes over 64% tomato crop loss for open field production and up to 100% loss in greenhouse production systems in Kenya. Bacterial wilt in tomato can be controlled by use of a number of different methods like crop rotation with non-host crops to suppress the soil borne populations of the pathogen. However, this method has a challenge because the pathogen can survive in soil in association with weed hosts, thus limiting the effect of crop rotation. In addition, the available land owned by small scale farmers is not enough for practicing rotational programmed (Fajinmi & Fajinmi, 2010). Another common method of controlling the disease is the use of chemicals. This method is quite expensive and unaffordable to small scale farmers who rely on tomato as a source of livelihood. The chemicals are not ecofriendly to humans, animals as well as the environment. Besides, the pathogen is also soil borne and systemic in nature and thus the use of copper based bactericides and antibiotics has not given satisfactory control (Fajinmi & Fajinmi, 2010). This has made farmers to look for alternative methods of controlling the disease.

According to Balestra *et al*. (2009), plant extracts of bunching onion (*Allium fistulosum*) have been reported to have potential of controlling a number of crop pests and diseases. *Allium fistulosum* produces sulfur volatiles when *Allium* tissues are degraded. It also contains allicin (diallyl- thiosulfinate), which has significant antibiosis effects against a wide range of plant-pathogenic bacteria and fungi. These properties of the plant extract therefore stand to offer a potential ecofriendly alternative for controlling tomato bacterial wilt (Buyela, 2017).

Irrigation is the backbone of greenhouse agriculture which enhances optimized water use under varying climatic conditions (Impron, 2011) and produces yields 5 to 10 times

higher than in the field (Vox *et al*., 2010). Irrigation water requirement in a greenhouse varies depending on the season and size of the crop cultivated. Transplanted tomato plants require about 0.05 liters per plant per day while at maturity and during sunny days, plant water requirement may rise to two point seven liters per plant per day (Georgios *et al*., 2018).

According to Agather *et al*. (2017), *Ralstonia solanacearum* depends on water for proliferation and infection and extent of disease development depends on moisture during the growing season. Soil moisture significantly affects reproduction and survival of the pathogen. High soil moisture and prolonged periods of wet weather or rainy seasons are associated with increased bacterial wilt incidence and severity. Besides, the pathogen can survive in extremely diverse environment by travelling along water ways. The aim of this study was therefore, to help establish a combination of plant extracts of bunching onion (*Allium fistulosum*) with optimum irrigation water level that can reduce bacterial wilt incidence and severity thus improving growth, yield and quality for greenhouse grown tomato plant.

1.2 Statement of the Problem

Production of tomato has intensified over the years. However, yield continue to remain low due to a myriad of constraints. Bacterial wilt caused by *Ralstonia solanacearum* is one of the major challenges that causes up to 100% loss in greenhouse production systems in potential tomato production areas in Kenya. This has predominantly lowered the farmers' choice of enterprises for their farms. The pathogen depends on water for proliferation and infection and the extent of disease development depends on moisture levels during the growing season. Farmers who grow tomatoes using irrigation where there is sufficient supply of water apply too much water not knowing the required quantity that can help reduce bacterial wilt incidences. This has contributed to high prevalence of bacterial wilt in their farms. Farmers have attempted different methods of controlling the disease including use of bactericides and antibiotics which are expensive for most smallholder farmers to afford apart from the problem of development of resistance following repeated applications of the chemicals. These chemicals are also hazardous to human, animals and the environment. Plant extracts obtained from *Allium fistulosum* have given promising results in the management of a wide range of plant diseases. However, research on the use of such plant extracts in combination with optimum irrigation level in the control of tomato bacterial wilt is still limited.

1.3 Objectives of the study

1.3.1 Broad Objective

To contribute to improved tomato yields and quality by developing an integrated, sustainable and environmental sound approach for management of bacterial wilt disease.

1.3.2 Specific Objectives

The specific objectives of the study were:

i. To determine the effects of different concentrations of *Allium fistulosum* crude extract on inhibition of *Ralstonia solanacearum In-vitro*.
ii. To determine the effects of different concentrations of *Allium fistulosum* crude extract and irrigation levels on bacterial wilt incidence and severity in greenhouse grown tomato.
iii. To determine the effects of different concentrations of *Allium fistulosum* crude extract and irrigation levels on growth and yield of greenhouse grown tomato.
iv. To determine the effects of different concentrations of *Allium fistulosum* crude extract and irrigation levels on quality of greenhouse grown tomato.

1.4 Hypotheses

The following hypotheses were tested:

i. Concentrations of *Allium fistulosum* crude extract have no significant effect on *Ralstonia solanacearum In-vitro*.
ii. Concentrations of *Allium fistulosum* crude extract and irrigation levels have no significant effect on bacterial wilt incidence and severity.
iii. Concentrations of *Allium fistulosum* crude extract and irrigation levels have no significant effect on growth and yield of tomato.
iv. Concentrations of *Allium fistulosum* crude extract and irrigation levels have no significant effect on quality of tomato.

1.5 Justification of the Study

Tomato is an important vegetable crop due to the fact that consumers appreciate its taste, nutritional value and its broad application in the human diet. The crop is also a source of income, more so to small scale farmers in Kenya. To youths, it is emerging as a source of employment and livelihood. Commercially, tomato can be used for fresh market and processing purposes. However, for full potential of this vegetable to be realized, there is need

for development of effective and sustainable methods of controlling diseases especially bacterial wilt disease which is one of its major production challenges.

Crop rotation with non-host crops as a method to control bacterial wilt disease by farmers is limited in that the pathogen can survive in the soil for a long period of time in association with alternative weed hosts. Also the available land owned by small scale farmers is not sufficient for practicing crop rotation. Use of chemicals on the other hand, is quite expensive and unaffordable to small scale farmers who rely on tomatoes as a source of livelihood. These chemicals are also not ecofriendly to humans, animals and the environment. Resistance of the pathogen to chemicals also builds up due to repeated application of chemicals through emergence of new races.

Furthermore, infection and spread of bacterial wilt is favoured by high soil moisture. High soil moisture and prolonged periods of wet weather result into increased severity of the disease. Soil moisture also affects reproduction and survival of the *Ralstonia solanacearum* pathogen. In addition, excess water enhances faster spread of the pathogen due to accumulation of high moisture levels in soil. Excess water also affects lycopene content in tomato fruit. Frequent irrigation at the time of fruit development has a negative impact on fruit total soluble solids. Therefore, determining the quantity of irrigation water to be applied will provide an eco-friendly alternative for production of tomato in order to reduce the incidence of bacterial wilt thereby improving tomato yields and quality.

For tomato to maintain its economic role in the country, affordable and environmental ecofriendly methods focusing on the use of plant extracts and irrigation levels therefore need to be put in practice. The use of plant extracts has been shown to be effective against many plant pathogens. Plant extracts have the capacity to reduce populations of soil and foliar pathogens, thereby controlling plant diseases. This is achieved due to the fact that plant extracts contain active ingredients which act on the pathogen directly or induce systemic resistance in host plants. The extracts also exert toxic effects by disrupting the normal metabolic activities of the pathogenic organism. Apart from the ability of the extracts in controlling plant diseases, they are also of natural origin, biodegradable and do not leave toxic residues to accumulate in the fruit and environment. The use of *Allium fistulosum* crude extract therefore poses great potential in addressing the problem of bacterial wilt due to above mentioned benefits. However, for this potential to fully benefit tomato growers, research needs to be done to document the effective concentrations and the quantity of irrigation that can be used to reduce the risks of development of tomato bacterial wilt while maximizing on

tomato yield and quality. It is also anticipated that the findings of this research will contribute to existing scientific knowledge on tomato disease management and improved tomato growth, yields and quality.

CHAPTER TWO
LITERATURE REVIEW

2.1 Origin and Botany of Tomato

Tomato (*Solanum lycopersicum* L.) is a fruit vegetable native to South America. It spread to Europe in the 16th century and then later to East Africa in the 1900 (Blanca *et al*., 2012). The crop belongs to the Solanaceae family and is second most important vegetable crop cultivated in the world (Wani, 2011). The family consists of a diverse group of vegetables possessing different characteristics. They include; tuber-bearing members such as potato and fruit bearing members such as tomato, pepper and eggplant. The family is also composed of 100 genera and 2500 species (Dias & Resende, 2013).

Tomato is an annual herb with an erect to prostrate stems with a strong taproot consisting of dense lateral and adventitious roots (Oduor, 2016). Stem is solid, coarsely hairy and glandular. Leaves are spirally arranged, imparipinnate with no stipules and the petiole length varies from 3 to 6 cm. Leaflets vary in size and are irregularly toothed and sometimes pinnatifid at the base. Inflorescence is a cyme but sometimes compound flowered. Flowers are bisexual and regular in shape and often with a yellow corolla. The fruit is a berry usually red but may sometime vary from pink, orange to yellow when ripe. The growth habit can be indeterminate, semi-determinate or determinate (bushy). The fruit size can be small round, medium-large round, beef steak and ribbed while the fruit shape is round, heart-shaped, pear-shaped, plum-shaped, elongated or flat (Dias & Resende, 2013).

2.2 Climatic Requirements and Uses of Tomato

Tomato thrives well under warm conditions with a temperature range of 15-25℃. Lower temperatures delays color formation and fruit ripening while temperatures above 30℃ inhibit fruit set, flavor and formation of lycopene due to failure of germination of pollen germ tube. It is fairly adaptable to a wide range of soil types rich in organic matter, well aerated with a pH range of 5 to 7.5. Higher or lower pH values can cause mineral deficiencies or toxicities (Oduor, 2016). The crop cannot tolerate frost but requires low to medium rainfall. Greenhouse technology has enabled farmers to utilize small pieces of land to produce high quality tomato for specialized markets (Mbaka *et al*., 2013).

Tomato fruit can be consumed fresh or used in preparation of a wide range of processed products such as tomato juice, soup, paste, puree, ketchup and sauce (Ray *et al*., 2011). According to Mbaka *et al*. (2013), the crop is economically important with high potentials of improving the livelihoods of small scale farmers in Kenya. It is also the more

cultivated vegetable crop with the highest consumption rate and economic value worldwide (Mbaka *et al*., 2013). It is valuable nutritionally and medicinally due to its high content of antioxidants, including carotenoids, lycopene, ascorbic acid and phenolics, which have health promoting potential for consumers (Fajinmi & Fajinmi, 2010; Wang *et al*., 2011).

2.3 Challenges to Tomato Production in Kenya

Tomato industry has reduced poverty through improvement of livelihood of most rural and peri-urban farmers (Anang *et al*., 2013). However, the full potential of the crop has been under-exploited because of a number of challenges. For instance, in Kenya, lack of effective irrigation systems renders tomato farming rain-fed (Adenuga *et al*., 2013). According to Robinson and Kolavali (2010), incidences of pests and diseases, low quality and insufficient quantity of tomato produced faces competition from foreign imports which hinders tomato production.

According to HCD (2019), diseases, pests, poor quality seeds, high cost of inputs and adverse weather conditions are additional challenges facing tomato farmers in Kenya. The main insect pests include; whiteflies, nematodes, spider mites, thrips, leaf minors, African bollworm and aphids. These insect pests cause damage by sucking plant sap and production of sooty mould which reduces plant productivity and fruit quality. They also spread viral diseases such as tomato yellowing leaf curl virus. These insect pests accounts for 56% tomato yield losses (Monsanto, 2013).

Diseases are also a major constraint to tomato production in Kenya. According to Tahat *et al*. (2010), diseases are mainly spread in lowlands, highlands, tropics and can cause 15-95% tomato crop loss. These diseases include; bacterial wilt, early and late blight, fusarium wilt, yellow leaf curl virus, tobacco mosaic virus, septoria leaf spot, powdery mildew and bacterial canker (Singh *et al*., 2014). According to Asante *et al*. (2013), farmers used pesticides excessively with over 40 applications per season to control these pests and diseases. However, these pesticides had negative effects by causing food poisoning to consumers and polluting the environment. Of these diseases including early and late blight, fusarium wilt, yellow leaf curl virus, tobacco mosaic virus, septoria leaf spot, powdery mildew and bacterial canker, bacterial wilt is the major challenge causing higher yield losses in tomato. This is because of the complex nature of the pathogen which has a wide host range and biodiversity. The disease is a threat due to its limited control strategies (Mbaka *et al*., 2013).

On the other hand, William and George (2014) reported that inadequate water supply during tomato growth leads to low yields. Water supply is limited due to increase in demand for agricultural, urban and industrial uses and also due to decrease in river flows. Excess amount of water on the other hand increases the incidences of bacterial wilt due to accumulation of high moisture levels in soil. Waterlogging is also another problem associated with excess water. Seepage from irrigation canals and irrigated fields causes water to accumulate in the upper soil levels. In absence of adequate drainage, water tables rise in the upper soil level including plant root zone thus inhibiting crop growth (Tan, 2013).

2.4 Methods of Controlling Bacterial Wilt and their Challenges

Use of pesticides has been the common method of crop disease control in Kenya (Asante *et al*., 2013). Schreinemachers and Tipraqsa (2012) reported that pesticide use Ha^{-1} including herbicides, insecticides, fungicides and bactericides increased with crop output Ha^{-1}. Schreinemachers and Tipraqsa (2012) also revealed that a 1% increase in crop output Ha^{-1} was associated with 1.8% increase in pesticide use Ha^{-1} as a result of excessive usage of the pesticides. Bacterial wilt has been mainly controlled by pesticides such as algicide (3-[3-indoly] butanoic acid) and fumigants such as metam sodium, 1, 3-dichloropropene and chloropictrin. Pesticide usage increased tomato yields by 1.7 to 2.5 fold higher. However, contrary to the positive effects of pesticides on crop yield, their usage also have negative impacts (Vinh *et al*., 2005). Careless usage of pesticides leads to accumulation of residues in the environment for many years, contamination of soil and ground water while usage without proper knowledge is poisonous to farmers as the pesticides accumulate in the environment leaving out residues which are detrimental (Lin *et al*., 2010). Usage of bactericides including (triazolothiadiazine zero 0.5 to 12mM) in solution and streptomycin sulfate (400 mg Kg^{-1} of soil have been shown to destroy soil living microorganisms while acibenzolar-s-methyl (ASM) has been proposed to induce systemic resistance (Hong *et al*., 2011).

Another approach used to control bacterial wilt is the use of physical methods such as solarization. In a study conducted by Yuliar *et al*. (2015), soil solarization using transparent plastic mulches for 60 days prior to planting of tomatoes reduced bacterial wilt incidence by 50-95%. In another study, it was reported that rhizome solarization in ginger seeds for two to four hours reduced bacterial wilt by 90 to 100% 120 days after planting. Results by Kago *et al*. (2019) showed that heat treatment at 45 ℃ for two days or 60 ℃ for two hours of infected soil prior to tomato planting reduced the total bacterial population by 60-97% and the incidence of bacterial wilt by 50-75%.

Crop rotation is another method which has been used to control bacterial wilt disease. According to Yuliar *et al*. (2015), the onset of bacterial wilt was delayed by one to three weeks and wilt severity was reduced by 20 to 26% when a susceptible tomato variety was grown after corn, ladys' finger, cowpea and resistant tomato. The incidence of wilt reduced by 64 to 94% when potato was rotated with wheat, sweet potato, maize, millet, carrot, sorghum and Phaseolus bean. This eventually increased the yield of potato by one to three-fold higher than monocultured potato. However, the main limitation of using crop rotation as a method of bacterial wilt disease control is that the pathogen has a wide host range. In addition, the available land owned by small scale farmers in the country is not sufficient for practicing crop rotation.

According to Lee *et al*. (2016), disease onset, pathogen dispersal and rate of disease progress is affected by choice of irrigation system. Furrow irrigation requires large volumes of water which can predispose tomato plant to many diseases. Soil borne pathogens are also easily spread in the irrigation furrows following water flow. Areas infested with *Ralstonia solanacearum* usually have an increased tomato wilt incidence and reduced yield when furrow irrigation systems are used. Overhead sprinkler irrigation when used has a lower disease level and results to higher yields. Drip irrigation is more efficient in terms of water use and its use leads to development of less foliar diseases. Lee *et al*. (2016), reported that bacteria require free water on the leaf surface to initiate infectious processes and leaf wetness duration is the most determinant microclimate variable for bacterial wilt disease establishment and progress.

2.5 Effect of Bacterial Wilt on Yield of Crops

Bacterial wilt disease caused by race one strain belonging to biovar three is a destructive and prevalent soil borne disease that limits tomato production in the tropics, sub-tropics and warm temperate regions of the world (Ramesh *et al*., 2014). The pathogen causing the disease has a wide host range consisting of solanaceous plants, leguminous plants, and a small number of monocotyledons, trees, shrubs and certain ecotypes of *Arabidopsis thaliana* (Van *et al*., 2000). It persists in soil or water for several years to form latent infections, within native weeds contributing to the difficult eradication of the bacterium (Avinash *et al*., 2016).

The direct yield losses caused by bacterial wilt vary widely according to host, cultivar, climate, soil type, cropping practice and pathogen strain. The level of damage can be expressed on a crop by crop basis and can range from minimal crop loss to a very high

economic damage. The yield losses due to tomato bacterial wilt ranges from 10% to 80% depending on crop seasons as reported by Wei *et al*. (2015). Liu *et al*. (2017) reported that tobacco bacterial wilt caused great economic losses with disease incidence of 15 to 35% which can go up to 75% or even higher in association with other root diseases like black shank caused by *Phytophthora nicotiana* var. *nicotianae.* Yield loss of 90% has been recorded in potato as a result of potato bacterial wilt. In areas where tobacco is planted as a monocrop, yield reduction range of 50 - 60% and up to 100% during extreme outbreaks has also been reported. Similar findings have been reported in chili, ginger and peanut with yield losses as a result of bacterial wilt ranging from 20 to 50% (Tan *et al*., 2014), 20 to 30% (Liu *et al*., 2017) and 10 to 20% and can go up to 50 to 100% in extreme cases (Yu *et al*., 2011), respectively.

According to Ayandiji *et al*. (2011), *Ralstonia solanacearum* causes tomato yield losses ranging from 10 to 100% worldwide. The pathogen infects tomato roots through wounds or natural openings, colonizes the xylem and produces extracellular polysaccharides that clog the vascular tissue. This prevents movement of water up through the stem and total collapse of the plant may occur in 2 to 5 days leading to severe yield losses (Wright *et al*., 2016). The presence of the pathogen often results in growers abandoning infested fields for tomato production. Results of the study by Wang *et al*. (2013) show that the yield losses in tomato vary from zero to 91% as a result of bacterial wilt. This is due to the fact that the pathogen grows endophytically, survives in soil and travels along water (Chen *et al*., 2005).

2.6 Effect of Plant Extracts and Irrigation Levels on Crop Diseases

The use of plant extracts in management of crop diseases is an ecofriendly alternative measure (Sales *et al*., 2016). The antifungal activities of both *Monsonia burkeana* and *Moringa oleifera* have been tested against *Penicilum digitatum* and *Sclerotium rolgsii*. Methanol extracts of the two plants significantly reduced pathogen growth *In-vitro*. The methanol extracts of *Monsonia burkeana* and *Moringa oleifera* reduced citrus grey mould, cowpea damping off and stem rot. Other studies by Mohammed (2015), revealed that leaf extracts of Moringa had a significant effect on soil born fungal pathogens including *Rhizoctonis* and *Pythium spp*. On the other hand, *Thymus vulgaris* extract was also found to be highly inhibitory towards mycelial growth of *Fusarium oxysporum* under laboratory conditions (Torre *et al*., 2016). Results obtained by Gayatri and Rajani (2015) showed that aqueous neem extract acted as a good antifungal agent by reducing early blight by 55.12% in

tomato plant. Similarly, garlic and neem oil have also been shown to be effective in reducing severity of early blight disease of tomato.

According to Hussain *et al*. (2013), *Azadirachta indica* plant extract reduced leaf spot incidence of ground nut. The crude extracts of neem and neem seed at concentrations ranging from five to 30% inhibited growth of *Fusarium oxysporum* (Ogechi *et al*., 2006). The bio-efficacy of neem extract over pathogens is attributed to the fact that neem has active compounds such as azadirachtin, nimbidin, nimbin, nimbinene and azadirone which are antifungal, antibacterial and anti-insecticidal in nature (Bohra *et al*., 2006).

On the other hand, irrigation has a significant effect on development of crop diseases. Findings of Oduor (2016) showed that lower soil moisture levels (20 to 30% of moisture water holding capacity and pre-incubation at low temperature (four to 20 ℃) reduced the incidence of wilt in tomato plants compared to soils with higher moisture content. The wilt incidence of tomato was high under high moisture conditions (> 35% Water Holding Capacity). Similarily, findings by Nesmith and Jenkins (1985) revealed that soil moisture significantly affected reproduction and survival of *Ralstonia solanacearum* in non-sterile soils. The culturable population in soil increased within 7 to 10 days of introduction at higher soil moisture levels (from flooded to -1 bar), but did not increase in drier soils (from negative five to -15 bars). Similarly, a decrease in water content from 70% of water holding capacity (WHC) to 50% WHC induced a 30% reduction of culturable population of *Ralstonia solanacearum* in soils. A 10% reduction in *Ralstonia solanacearum* population was observed when soils were further dried to 10 to 15% (Oduor, 2016).

2.7 Effects of Plant Extracts and Irrigation on Growth, Yield and Quality of Tomato

Extracts of some medicinal plants, trees and crop residues have been reported to influence growth, yield and quality of vegetable crops. The extracts of these medicinal plants, trees and crop residues have shown to have a promotive effect on length of shoots, number of branches, leaves, buds, flowers and fruit numbers. These growth parameters are also influenced positively by irrigation (Farooq *et al*., 2008).

(a) Effects on Growth and Yield

In a study by Gayatri and Rajani (2015), neem aqueous extracts showed a promotive effect on growth and yield of tomato. The shoot lengths of tomato increased by 58.12%, branches by 32.65%, leaf numbers by 11.85%, number of buds by 11.8%, number of flowers by 14.5% and number of fruits by 14.16%. This promotive effect could be due to the high

volume of the concentration used. Pattnaik *et al*. (2012) also observed the growth stimulating effect of ten medicinal plant extracts on tomato. These extracts included; *Psolarea pinatta*, *Aegle marmelos*, *Azadirachta indica*, *Brassica campestris*, *Solanum nigram*, *Euphobia tirucalli*, *Vitex negundu*, *Ageratum conyzoides*, *Tagetes patula* and *Ziziphus jujube.*

Similar experiment carried out by Okunola and Thomas (2013) demonstrated the effect of *Azadirachta indica* and *Piper guineense* on the growth and yield of jute under sole and mixed cropping. All the growth parameters increased in comparison to the control treatment where the plant extracts were used. However, a different finding was reported on ethanolic extracts of *Melia azedarch*, *Eucalyptus robusta* and *Sapium sebiferum* application of the extracts had no significant influence on growth and development of soybean seedlings (Wan *et al*., 2012). Different concentrations of tea seed extracts increased the growth, yield and biomass of beet, mustard, oat and barley (Thomas *et al*., 2015). The growth stimulating effect was not exclusively by its adverse effect on the pathogen or by an increase in nutrient uptake but substances with hormone like properties could have also stimulated the effect of biomass allocation in plants (Anderson, 2010). In addition to hormones, some medicinal plant extracts contain saponins and polyphenols which could be the active compounds causing the effect on growth (Singh *et al*., 2010).

Leaf extracts of *Moringa oleifera* have been reported to accelerate growth of young plants including tomato and potato, strengthen plants, increase leaf area, increase number of roots, produce more and larger fruits and generally increase yield by 20 to 35% (Lowell *et al*., 2000). In a study by Bashir *et al*. (2014), plant height and leaf number increased with constant application of Moringa leaf extract at a concentration of 80%. The average plant height was initially 16.3 cm, which increased to 23.0, 24.2, 30.0 and 33.3 cm. Leaf number also increased from 56.3 to 67.6, 115.3 and 182.3. This was attributed to the chemical components in the extracts which were responsible for development of tomato plants in comparison to the control ones. The highest plant height was 40.8cm while leaf numbers were 236.0 with constant application of moringa extract at a concentration of 80%. This was attributed to the fact that Moringa leaf extracts enhanced the germination of tomato plants by 20 to 80%. Foidl *et al*. (2001) reported that aqueous extract of *Moringa oleifera* at a ratio of 1:10 (w/v) prepared in a 30 g of plant leaf material with 300ml of distilled water influenced the duration of height and hypocotyl growth in tomato. In their study, Schon and Einhellig (1982) demonstrated that the incorporation of dried sunflower leaf material into the soil and their findings revealed that leaf leachates inhibited germination and growth of tomato. In

addition, application of *Moringa oleifera* root and leaf extract two to three weeks after planting significantly increased fresh fruit weight, number of stems, flowers and branches of tomato plant. However, recycling crop residues and leachates from plants to the soil have been reported to inhibit seed germination and early seedling growth in tomato (El-khalal, 2007). According to Croxton *et al*. (2011), application of moringa extract inhibited the growth rate of shoots and roots in tomato seedling.

Irrigation on the other hand is also reported to influence crop growth and yield. Results obtained by Wang and Xing (2016) revealed that tomato plant height was 40.8cm when 150 mm of irrigation water was applied. This value represented 6.9% and 10.3% higher than those in 262 mm and 206.5 mm irrigation water. The results also showed that at 23 days after transplanting, the highest value of stem diameter was eight point nine mm. However, the rate of stem diameter increase decreased 37 days after transplanting and the added value ranged from zero point nine to two point two mm at the blossoming and bearing fruit stage. The highest leaf expansion rate was four point five cm^2 $leaf^{-1}$ day^{-1} at 23 to 37 days after transplanting when 150 mm of irrigation water was applied. The highest fruit yield was 96.7 Tonnes Ha^{-1} when 262 mm of irrigation water was applied. This gave nine point six percent and 17.7% increase compared to when 206.5 mm and 150 mm of irrigation water was applied, respectively. The results showed that an increase in irrigation level increased the yield of tomatoes.

Tya and Othman (2014), studied the effect of irrigation water depth on tomato yield. Results showed that tomato yields increased with the depth of water applied up to an optimum value of 14.2 Tonnes Ha^{-1} after which the yield decreased with application of more water. The yields were realized from 125 mm depth of water. The amount of water that was applied was 1755 mm. The lowest average tomato crop yield of nine point three Tonnes Ha^{-1} was obtained when 3080 mm amount of water was applied. They also observed that the water use efficiency decreased with the increase in water depth. This led to a decrease in tomato yields per unit depth of water.

2.7.2 Effects on Quality

The use of herbal extracts can enhance the shelf life and bioactive compounds of tomatoes used as fresh-cuts thus maintaining or increasing the contents of lycopene, ascorbic acid and total phenolic compounds (Ayala- Zavala *et al.*, 2008). In a study undertaken by Surekha *et al*. (2010), garlic (*Allium sativum* Linn) and ginger (*Zingiber officinale* Rose) extracts were used at concentrations of one percent, five percent and 10% to enhance the

shelf life of tomato. Tomato keeping quality was improved when a concentration of 10 % garlic and ginger extracts was sprayed. The garlic and ginger extracts were able to reduce spoilage, physiological weight loss and growth of microorganisms on the surface of fruits. The efficacy of the extracts increased with their increasing concentration. Similar results were reported by Sharma and Bohra (2003) who used leaf extracts of *Barehaeria diffusa* and *Salvadora persica.* On the other hand, use of leaf extracts of *Moringa oleifera* resulted to higher, vigorous and good quality tomato seedlings (Bashir *et al*., 2014). The quality of tomato seedlings was facilitated by the increase in plant height, leaf number and number of flowers in the field as a result of an increase in the concentration used.

Another study by Mahmoud and Abdulrasoul (2012) revealed that irrigation of tomato plants with none saline water gave higher fruit weight and more vitamin C content than irrigation with saline water by 31.4% and 12.7% respectively. The results disagreed with those of Favati *et al*. (2009) whose findings revealed that the larger the size of tomato fruit, the lower the content of vitamin C content. Results also showed that irrigation with saline water gave significantly higher values of total soluble solids (TSS) and acidity (pH) than irrigation with non-saline water by 11.1% and six point nine percent (6.9%) respectively. This significant increase was due to a reduction in water intake by the fruits (Al-yahyai *et al*., 2010).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Site Description

The experiment was conducted at KALRO-Kakamega, Kenya. The field lies at a longitude of 34°35´ E and latitude of 0°35´ N in the Upper Midland Zone IV (UM_4) Agro Ecological Zone at an altitude of 1585 meters above sea level. The average daily temperatures are 22 ℃. The area receives an average annual rainfall of 1850 mm. The soils are predominantly well drained ferralsols and acrisols (Jaetzold & Schmidt, 2010).

3.2 Plant Materials

The variety used in the study was Sodagar F1 an indeterminate type that is suitable for greenhouse production as a trellised crop. The variety has a high vigour, but is highly susceptible to bacterial wilt. Tomato seedlings were started in a nursery until they attained the three to four true leaf stage before being transplanted. Prior to transplanting, pots of 22 cm diameter by 36 cm height were each filled with five kilograms of soil and NPK 14:28:14 was applied at a rate of 200 Kg Ha^{-1} (elemental N=28, elemental P=56, elemental K =28) (Oseko & Dienya, 2015). Bunching onion plant material was used in the study as it has antibacterial properties that have potential for controlling various plant pathogens.

3.3 Soil Preparation

Soil to be used in the study was obtained from KALRO Kakamega forest. Steam sterilization was done in batches. Each batch sterilizing 3.5 wheelbarrows of soil in three hours at 80°C. Water was heated to generate steam used to sterilize the soil. Sterilization for the total of soil required for the experimental trials took three weeks. Two tanks were used. The first tank contained soil and the other one water which was heated and the steam produced used to sterilize the soil in the first tank. This was to help in reducing soil borne pests such as weeds, plant pathogens, nematodes and insects (Gelsomino *et al*., 2010).

3.4 Preparation of Bunching Onion Extracts

Bunching onions were extracted using water according to Odey *et al*. (2012). Collected plant parts (roots) were washed using distilled water to remove mud. The roots and leaves were then chopped into small pieces. The plant parts were then placed in an oven to dry for 48 hours at a temperature of 65℃ thereafter ground into a powder. The different concentrations of water extracts were prepared by 0g, 5g, 10 g, 15 g and 20 g in 100 ml sterile distilled water to produce extract concentrations of zero percent, five percent, 10%,

15% and 20%, respectively. The extracts were then sieved through cheese cloth and stored in clean containers for experimental use.

3.5 Isolation Procedure for *Ralstonia solanacearum* Pathogen from Tomato Plants

A sample of 10 tomato plants showing symptoms of wilting and vascular discoloration were collected from the fields in Kakamega County. An ooze test was used to confirm presence of the *Ralstonia solanacearum* pathogen in the host (Kumar *et al*., 2017). Isolation was done on solidified triphenyl tetrazolium chloride (TZC) agar medium as shown in figure 1 below. Infected stems were cut into small pieces aseptically, and surface sterilized in 0.5% sodium hypochloride and then rinsed in three series of sterile water to remove traces of sodium hypochloride. The infected tissue pieces (1 g) were then suspended in a test tube containing sterilized water for 10 minutes in order to see proliferation of fine milky white strands. Bacterial suspension obtained through the proliferation of fine milky white strands was then streaked on the surface of TZC medium using a wire loop (Chaudhry & Rashid, 2011). The inoculated plates were then incubated at 33 ℃ for 48 hours. The plates were observed visually after 48 hours for the development of well-separated virulent colonies. Purification of bacterial colonies was by picking the highly virulent colonies which were pinkish in color and then streaked on the surface of TZC medium contained in petri dishes (Figure 1). The three to four loopfuls of well-separated virulent colonies were suspended in sterile distilled water taken in vials. The vials were then stored at 5℃ and served as stock culture for further studies. The bacterium isolate was identified on the basis of morphological, cultural and physical characteristics as described by Onduso (2014).

Figure 1: Positive samples from a tissue test of isolated *Ralstonia solanacearum* pathogen from infected tomato plants showing its red and fluidal shape

3.6 Preparation of Bacterial Inoculum to be used for Inoculating Healthy Plants and Inoculation Procedure

Bacterial inoculum was prepared by measuring with aid of a micro-pipette 25µl of *Ralstonia solanacearum* stock suspension which was then added to 10 ml Casamino acid peptone glucose (CPG) broth (1g casamino acid, 10g peptone and 5g glucose per liter), 30g agar, 1000 ml distilled water and 50 mg of 2,3,5-triphenyl tetrazolium chloride (TZC) agar medium suspended in nine petri dishes of 90 mm diameter and 15 mm height. The petri dishes were streaked with bacterial suspension from a specific isolate at concentrations of 1×10^1, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6 1×10^7 and 1×10^8 colony forming unit per ml by use of an inoculating loop (Pontes *et al*., 2017). The concentration of 1×10^8 colony forming unit per ml was adjusted with the aid of hemocytometer with 0.01 % Tween 20 a surfactant which amends the properties of the carrier to ensure it dissolves. The plates were then incubated upside down at 33 ℃ for 48hrs to avoid water condensation which causes colonies to flow into each other inhibiting their separation (Popoola *et al*., 2015). Two loopfuls of bacterial colonies were transferred to 5 ml of distilled water suspended in a bottle and stored at -20℃ for experimental use. Inoculation of tomato plants was carried out as described by Sangayomi *et al*. (2011). It was done during transplanting by pouring 30 ml of *Ralstonia solanacearum* suspension into the pots in a ring form around the root zone of the plants.

3.7 Pathogenicity Tests in Healthy Tomato Seedlings to Confirm the Presence of *Ralstonia solanacearum*

Pathogenicity was confirmed by the development of wilt symptoms on transplanted plants after seven days of inoculation. This was then followed by reisolation and identification of the causal organism from the diseased plants based on the method described by Singh *et al*. (2018).

3.8 Laboratory Study:

3.8.1 *In-vitro* Antibacterial Inhibition Assay

Paper disc diffusion method of Teng *et al*. (2010) on TZC agar media was used. A volume of 10 ml of prepared TZC agar was poured aseptically into petri dishes of 90 mm diameter and 15 mm height. A concentration of 25 µl of the bacterial suspension prepared was dispensed at the center of the petri dish and spread evenly on the surface of TZC agar media by the aid of an inoculating loop. Sterile five mm filter paper discs were prepared by

dipping each disc in 1 ml of each of the treatments used in the study. The discs were then air dried in the laminar flow hood for 3 hours after which they were aseptically placed on the surface of TZC agar media inoculated with the pathogen. The petri dishes were sealed with parafilm and incubated in dark conditions at 28 ± 2℃ for 48 hours. The diameter of zone of inhibition was measured in mm using a vernier caliper and recorded as a measure of the antibacterial activity of each treatment. The treatment that resulted in a zone of inhibition equal to or greater than 10 mm was considered effective and was used in greenhouse study (Teng *et al*., 2010).

3.8.2 Experimental Design and Treatments

The experiment was set up in a Completely Randomized Design (CRD) with three replications. The experiment was repeated once. There were six treatments namely 0% (negative control), Greencop 50g/20L (positive control), *Allium fistulosum* (5%), *Allium fistulosum* (10%), *Allium fistulosum* (15%), *Allium fistulosum* (20%) (Table 1). Each petri dish represented a treatment and the treatments were replicated three times giving a total 36 petri dishes in the first and second trial. The experiment was laid out as shown in figure 2 below.

Table 1. Treatment Design

Notation	Treatments
T_1	0% (negative control)
T_2	Greencop 50g/20L (positive control)
T_3	*Allium fistulosum* (5%)
T_4	*Allium fistulosum* (10%)
T_5	*Allium fistulosum* (15%)
T_6	*Allium fistulosum* (20%)

The experimental layout was as shown in figure 2 below;

T_2	T_6	T_6	T_1	T_4	T_5
T_3	T_6	T_2	T_1	T_5	T_3
T_4	T_1	T_3	T_5	T_2	T_4

Figure 2: Experimental Layout for *In-vitro* Laboratory Experiment

3.8.4 Data Collection

Data was collected on the diameter of zone of inhibition. The diameter of zone of inhibition was measured in millimeters (mm) using a vernier caliper and recorded as a measure of the antibacterial activity of each treatment. Treatments that resulted in a zone of inhibition equal to or greater than 10 mm was considered effective and was used in greenhouse study (Teng *et al*., 2010).

3.8.5 Data Analysis

Data collected was subjected to analysis of variance (ANOVA) at $P \leq 0.05$ level of significance. Mean separation was done using Tukey's Honestly Significant Difference (Tukey's HSD) test at $P \leq 0.05$. The general linear model procedure of the Statistical Analysis System (SAS) program, SAS version 9.1 (SAS institute Inc, 2010) was used for data analysis. The linear statistical model for the experiment was:

$$Y_{ij} = \mu + A_i + \varepsilon_{ij} \quad \text{.....Equation (1)}$$

where;

Y_{ij} = observation made in the i^{th} treatment and j^{th} replication

μ = is the overall mean which is an unknown constant

A_{i-} = is the effect due to treatments

ε_{ij} - Random error component

3.9 Greenhouse study:

3.9.1 Crop Establishment and Maintenance

Tomato seedlings were established in a nursery until they attained the stage of 3 to 4 true leaves. Seedlings were hardened off one week before transplanting by reducing watering frequency in the nursery bed. Prior to transplanting, the experimental greenhouse was maintained weed free to avoid buildup of feeding of nutrients competition and pest and disease transmission. Pots of 22 cm diameter by 36 cm height were filled with 5 Kg of sterilized soil. Soil to be used in the study was first sterilized by steam where two tanks were used. The first tank contained soil and the other one water which was heated and the steam produced used to sterilize the soil in the first tank. This was to help in reducing soil borne pests such as weeds, plant pathogens, nematodes and insects. Basal fertilizer (NPK 14:28:14) was applied at a rate of 200 Kg Ha^{-1} (elemental N =28, elemental P =56, elemental K =28) (Oseko and Dienya, 2015). Tomato seedlings were watered thoroughly in the nursery bed 5

hours before uprooting in order to minimize root damage. Inoculation of *Ralstonia solanacearum* pathogen was done during the time of transplanting by dipping of roots in the bacterial inoculum solution. Top dressing fertilizer (CAN 26:0:0) was applied at the rate of 200 Kg Ha^{-1} (elemental NH_3 and N = 52) in two splits (Oseko & Dienya, 2015). The first topdressing was done two weeks after transplanting and the second one four weeks later. Maintenance practices involved; gapping, weeding, watering, trellising, staking and flower pruning which were done uniformly in all experimental units. Tomato at different growth stages during the study are shown in Figure 3. On completion of the experiment, the pots containing infected soil and plants were sterilized and safely disposed off to avoid further spread of the pathogen. This was done by autoclaving at a temperature of 121℃ for 15 minutes in order to kill the pathogen.

a) Seedlings in nursery bed

b) Fruiting stage

Figure 3: Tomato seedlings at different growth stages (a) Tomato seedlings in nursery bed and (b) Tomato at fruiting stage

3.9.2 Experimental Design and Treatment for Greenhouse Experiment

The experiment was a single factor pot experiment in a Completely Randomized Design (CRD) with three replications. *Allium fistulosum* (15%) + Water Level of 0.5, *Allium fistulosum* (15%) + Water Level of 1, *Allium fistulosum* (15%) + Water Level of 1.5, *Allium fistulosum* (15%) + Water Level of 2, *Allium fistulosum* (20%) + Water Level of 0.5, *Allium fistulosum* (20%) + Water Level of 1, *Allium fistulosum* (20%) + Water Level of 1.5 and *Allium fistulosum* (20%) + Water Level of 2, *Allium fistulosum* (0%) + Water Level of 2L (negative control), Greencop 50g /20L + Water Level of 2L (positive control). *Allium fistulosum* crude extract of 20 % and 15 % concentrations were most effective in the laboratory experiment hence used in greenhouse experiment. The irrigation levels were

chosen based on the daily water requirement for greenhouse grown tomato plant which is 2.7 L/day at maturity and 0.05 L/day for new transplants. Treatment combinations used are as shown in Table 2. Each replicate therefore consisted of 10 experimental units with each represented by 5 pots.

Table 2: Treatments for Greenhouse Experiment

Notation	Treatments
T_1	Negative control WL2
T_2	Positive control (Greencop 50g/20L) WL2
T_3	A15%WL0.5
T_4	A15%WL1.0
T_5	A15%WL1.5
T_6	A15%WL2.0
T_7	A20%WL0.5
T_8	A20%WL1.0
T_9	A20%WL1.5
T_{10}	A20%WL2.0

KEY:

A- Concentrations of *Allium fistulosum* extract (%) **WL-** Irrigation Water level (L/pot/week)

A15%WL0.5– A15%+ 0.5L WL; **A15%WL1**- A15%+1L WL; **A15%WL1.5**- A15%+ 1.5L WL; **A15%WL2** - A15%+ 2L WL; **A20%WL0.5** - A20%+ 0.5L WL; **A20%WL1** - A20%+ 1L WL; **A20%WL1.5** - A20%+ 1.5L WL; **A20%WL2** - A20%+ 2L WL

3.9.3 Treatment Application and Randomization

Before treatment application, 0.05 litres of water level was applied to the young transplants for a period of one week since it is the amount recommended for young transplants. Different concentrations (15% and 20%) of the *Allium fistulosum* crude extract were applied by drenching on the soil contained in pots and inoculated with *Ralstonia*

solanacearum pathogen since the disease is soil borne. The *Allium fistulosum* crude extract was applied at an interval of one week (15g and 20g each dissolved in 100ml of distilled water) until the fruits became mature. Irrigation water was applied per weekly in every pot (L/week/pot). To take care of the existing inoculum, borehole water was used since pathogenicity tests that was done showed absence of bacterial wilt (Gelsomino *et al*., 2010). The experimental layout for the greenhouse study was as shown in figure 4 below;

T_1	T_8	T_2	T_9	T_1	T_8	T_{10}	T_6	T_3	T_6
T_9	T_7	T_4	T_2	T_8	T_1	T_4	T_{10}	T_4	T_3
T_6	T_7	T_5	T_3	T_9	T_7	T_5	T_2	T_{10}	T_5

Figure 4: Experimental Layout for Greenhouse Experiment

KEY:

AF- *Allium fistulosum* extracts (%) **WL-** Water level (L/pot/week)

T_1 - Negative control +2L WL ; **T_2**- Positive control (Greencop 50g/20L) + 2L WL ; **T_3**- A15%+ 0.5L WL **;T_4** - A15%+ 1L WL; **T_5** – A15%+ 1.5L WL ; **T_6** - A15%+ 2WL; **T_7** - A20%+ 0.5L WL; **T_8** - A20%+ 1L WL; **T_9** – A20%+ 1.5L WL;**T_{10}** -A20%+ 2WL

3.10 Data Collection

Data collection commenced two weeks after transplanting and continued on a two weekly interval until termination of the experiment. Four plants were tagged randomly in every experimental unit for the purpose of data collection. At each instance of data collection, the mean for all variables from each replicate were computed.

3.11 Disease Assessment

Symptom development from the four tagged plants in each experimental unit were evaluated at an interval of one week after transplanting until the completion of the experiment.

3.11.1 Disease Incidence

Disease incidence among the four tagged plants was assessed as percentage of wilted plants within each experimental unit. Disease incidence was then calculated according to Getachew *et al*. (2011).

$$DI = \frac{\text{NPSWS}}{NPPT} \times 100\% \quad \text{.....Equation (2)}$$

where;

DI = Disease Incidence

NPSWS = Number of plants showing wilt symptoms

NPPT = Number of plants per treatment

3.11.2 Disease Severity

A six point rating scale (0-5) as modified by Getachew *et al*. (2011) was used for wilt severity scoring, where;

0 = no wilt symptom

1 = one leaf wilted

2 = two or more leaves wilted

3 = all leaves except the tip wilted

4 = whole plant wilted

5 = death (collapse) of whole plant.

Percentage severity index (PSI) was calculated using the method described by Cooke (2006).

$$PSI = \frac{\sum scores \times 100}{NPR \times MSC} \text{.....Equation (3)}$$

where;

PSI = Percent severity index

NPR = Number of plants rated

MSC = Maximum scale of the scores

3.12 Growth Variables

3.12.1 Plant Height

Tomato plant height (cm) was measured from the ground level to the tip of each of the four tagged plants by means of a ruler and data obtained used to compute the average plant height.

3.12.2 Number of Branches

The number of branches of the four tagged plants was counted and results expressed as number of branches per plant (no./plant).

3.12.3 Number of Internodes

The number of internodes of the four tagged plants was counted and recorded. It was then expressed as average number of internodes per plant (no./plant).

3.12.4 Length of Internodes

The length of each internode of the four tagged plants was measured using a ruler and data obtained used to compute the average internode length per plant in cm.

3.12.5 Stem Collar Diameter

Stem collar diameter of the four tagged plants was measured using a digital vernier caliper (Model 599-577-1/ USA). Stem collar diameter was obtained from the middle part of the stem which was determined by counting the number of internodes divided by two. The data obtained was used to compute the average stem collar diameter in millimeters (mm).

3.12.6 Number of Days Taken by 50% of the Plants to Flower

The number of days from transplanting to when 50% of the 4 tagged plants in each experimental unit had at least one flower was monitored and recorded. Data obtained was used to compute the mean number of days to 50% flowering for each treatment.

3.13 Yield Variables

3.13.1 Number of Fruits per Plant

Harvesting was done once every week from when the first fruits attained breaker stage till termination of the experiment. The number of fruits from the four tagged plants from each experimental unit was counted after each harvest. The data obtained was used to compute total number of fruits per plant (no,/plant).

3.13.2 Total Fruit Weight

At every harvest, the weight of fruits picked from the 4 tagged plants was determined in Kilograms (Kg) using a weighing balance (Advanced Technocracy Inc. Ambala) and data recorded. Total yield was then recorded in Kg/plant.

3.14 Quality Variables

3.14.1 Total Soluble Solids (TSS)

Total soluble solids of fresh fruits was determined using a hand held refractometer (RHW Refractometer, Optoelectronic Technology Company Limited, UK) as per the procedure described by Majidi *et al*. (2011) and expressed in °Brix. The refractometer was cleaned with distilled water after each observation before moving to the next sample to avoid errors.

3.14.2 Ascorbic Acid (Vitamin C)

Ascorbic acid content was determined by titration with 2, 6-dichlorophenolindophenol dye using the method described by AOAC (2005) method number 218.02±1.14 as shown in Figure 5. Ten grams (10 g) of the fruit sample was extracted in 30ml of 5% oxalic acid using a mortar and pestle, and then filtered through Whatman No. 1 filter paper. Standard indolphenol solution was prepared by dissolving 0.05 g of 2, 6-dichlorophenolindophenol in 100 ml of distilled water and filtered. Ascorbic acid standard solution was prepared by dissolving 0.05 g of pure ascorbic acid in a small volume of 5% oxalic acid solution and then diluted to 250 ml with the same oxalic acid solution. 10 ml of the ascorbic acid standard solution was titrated with the indolphenol solution to a slight pink end point. Oxalic acid volume of 10 ml was titrated as a blank. The amount of ascorbic acid corresponding to 1ml of indophenol solution was then be calculated. 10 ml of the filtered sample extract was pipetted into a 50 ml flask and made to the mark with the 5% oxalic acid solution. It was then filtered through glass wool. The standard indophenol solution was used to titrate 10 ml of the filtrate. The amount of ascorbic acid was calculated using the following formula and expressed as mg/100 g sample.

Ascorbic acid = A x V x (DF/WT)……...…Equation (4)

where;

A = ascorbic acid (mg/100g fresh weight)

V = volume of dye to be used for titration of diluted samples

DF = dilution factor (100ml of distilled water)

WT = sample weight in g

a) Extraction of tomato juice

b) Prepared oxalic acid

Figure 5: Determination of ascorbic acid content in tomato fruits (a) extraction of tomato juice and (b) prepared oxalic acid.

3.14.3 Fruit Lycopene Content

Lycopene content of three fruits randomly picked from the pooled harvest of the four tagged plants in each experimental unit was extracted from tomato fruits using acetone and analyzed in a spectrophotometer at 503 nm as described by Babitha (2006) as shown in Figure 6 below. Lycopene content (mg/100g fresh weight) was then calculated by using the formula given by Ranganna (1986) where:

Lycopene Content (mg/100g fresh weight) = 3.1206×A×V×D×100/W×1000….Equation (5)

where

A=Absorbance

V=Volume made up (ml)

D= Dilution

W= Weight of Sample (g)

a) extraction of tomato fruits

b) samples in test-tubes

c) samples in test-tubes

Figure 6: Determination of lycopene content in tomato fruits (a) extraction of tomato fruits (b) samples in test-tubes and (c) samples in test-tubes

3.14.4 Fruit ß-Carotene Content

The carotene content of 3 fruits picked from the 4 tagged plants in each experimental unit was determined by the method described by Pritwani and Mathur (2017) as shown in Figure 7 below. The fruits were finely cut using a knife and ground in a mortar and pestle. 2.5g was weighed and extracted with 40ml acetone, 60ml petroleum ether and 0.1g magnesium carbonate and then blended for 5 minutes in a kitchen blender. The sample was filtered and residue washed with two 25 ml acetone, 25 ml petroleum ether and the extracts were combined. They were then evaporated to dryness and residue re-dissolved in acetone. The volume was made up to 5 ml using acetone. The carotene content was measured at a wavelength of 450 nm in a spectrophotometer and expressed in μg/100 g sample.

Figure 7: Determination of beta-carotene in tomato fruits (extraction of tomato juice)

3.14.5 Fruit Shelf Life

After harvesting, tomato fruits were kept at room temperature. The shelf life of the fruits was monitored daily by counting the days from harvest to decay.

3.15 Data Analysis

Data collected was subjected to analysis of variance (ANOVA) at $P \leq 0.05$ level of significance. Significant treatment means at F- Test were separated using Tukey's Honestly Significant Difference (Tukey's HSD) test at $P \leq 0.05$. The general linear model procedure of the Statistical Analysis System (SAS) program, SAS version 9.1 was used for data analysis.

The linear statistical model for the experiment was:

$Y_{ijk} = \mu + A_i + \varepsilon_{ijk}$ .....Equation (6)

where;

Y_{ij} = observation made in the i^{th} treatment and j^{th} repetition

μ = is the overall mean which is an unknown constant

A_{i-} = is the effect due to i^{th} treatment

ε_{ij} - Random error component which is assumed to be independent and normally distributed with zero mean and a common variance.

The results obtained from laboratory and greenhouse have been presented and discussed in the subsequent chapters of this document.

CHAPTER FOUR

RESULTS

This chapter presents the results of both the laboratory and greenhouse experiments. The order followed in the presentation is as follows; effects of *Allium fistulosum* crude extract concentrations on inhibition of bacterial wilt *In-vitro*, effects of *Allium fistulosum* crude extract concentrations and irrigation levels on; (i) disease variables, (ii) growth variables, (iii) yield variables and (iv) quality variables.

4.1 Effects of Concentration of *Allium fistulosum* Crude Extract on Inhibition of *Ralstonia solanacearum In-vitro*

Use of *Allium fistulosum* crude extract significantly influenced inhibition of *Ralstonia solanacearum* pathogen *in-vitro*. In the two trials conducted, crude extract concentration of 20% recorded the highest inhibition diameter throughout the study period while negative control (sterile distilled water) recorded the lowest inhibition diameter. Results also showed that treatment 20% crude extract concentration significantly inhibited growth of *Ralstonia solanacearum* pathogen compared to the other treatments (Table 3 and Figure 8). The results further showed that the second best treatments in inhibition of the growth of the pathogen were 15% and 10%, which did not significantly differ from each other (P=0.05). In the first trial, mean inhibition diameter was 11.467 mm for 20% *Allium fistulosum* crude extract, 10.833 mm for 15% *Allium fistulosum* crude extract, 10.533 mm for 10% *Allium fistulosum* crude extract, 10.10 mm for 5% *Allium fistulosum* crude extract, 9.5 mm for positive control (Greencop) and 5.8 mm for negative control respectively. A similar trend was also observed in the second trial. Figure 8 shows the inhibition zones by the different treatments.

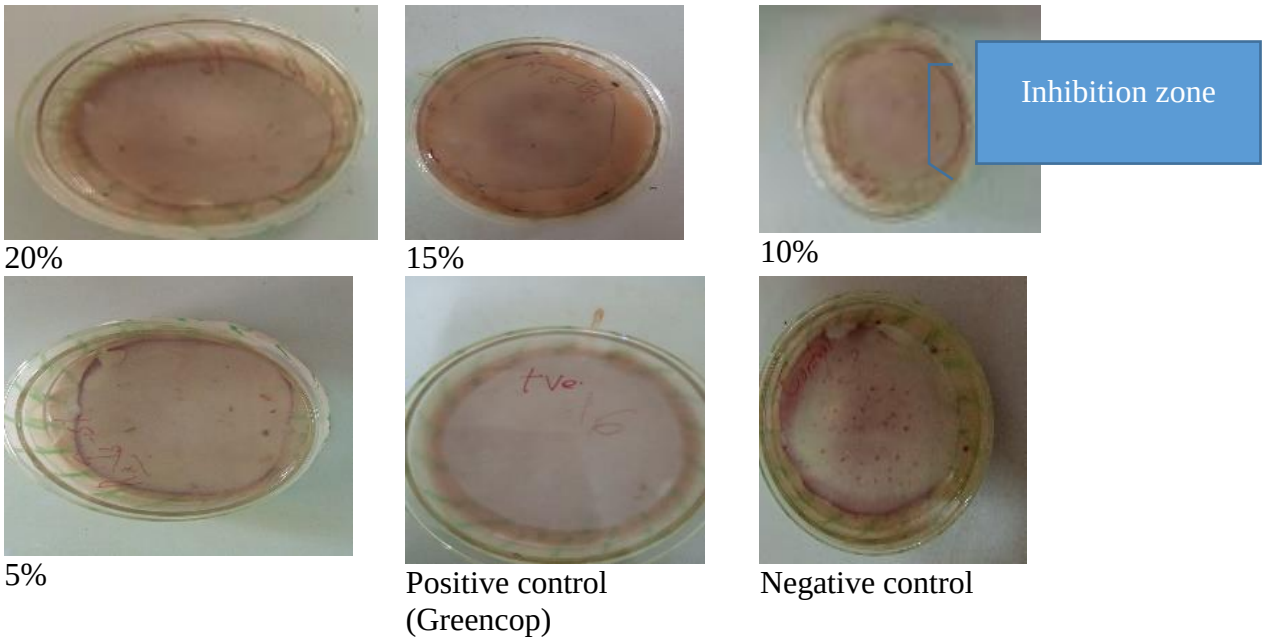

Figure 8: Effect of different concentrations of *Allium fistulosum* crude extract on diameter of zone of inhibition.

Table 3: Treatment means in millimeter (mm) for diameter of zone of inhibition of growth of *Ralstonia solanacearum* pathogen

Treatments	Trial one	Trial two
20%	11.467a*	11.500a*
15%	10.833b	10.700b
10%	10.533b	10.467b
5 %	10.100 c	9.867 c
Positive control (Greencop)	9.500c	9.367c
Negative control (sterile distilled water)	5.8000d	6.033d

*Means within a column followed by the same letter are not significantly different (P≤0.05, Tukey's HSD test)

4.1 Effects of Concentration of *Allium fistulosum* Crude Extract and Irrigation Levels on Bacterial Wilt Incidence and Severity in the Greenhouse

4.1.1 Effect on Bacterial Wilt Disease Incidence

Bacterial wilt disease incidence of tomato was significantly influenced by the use of *Allium fistulosum* crude extract with and irrigation at different levels (Table 4). The highest disease incidence was observed in the negative control treatment (distilled water combined with two litres of irrigation water (-ve+2L) followed by the positive control (Greencop at 50g/L spray) combined with two litres of irrigation water (+ve+2L). Disease incidence recorded under the negative control treatment was significantly higher than that recorded under all other treatments during all sampling dates. Although disease incidence was higher under the positive control treatment, the difference in disease score between this treatment and all the other treatments was not significant during most sampling dates. Among the other treatments, application of *Allium fistulosum* crude extract at 15% combined with either two litres (15%+2L) or one and a half litres of irrigation water (15%+1.5L) resulted in a higher disease incidence than the other treatments with the difference amongst them not significantly different (P=0.05) in both trials. Levels of disease score in these treatments were closely followed by those of *Allium fistulosum* crude extract at 15% combined with either one litre (15%+1L) and a half litre of irrigation water (15%+0.5L), followed by *Allium fistulosum* crude extract at 20% combined with two litres of irrigation water (20%+2L) and *Allium fistulosum* crude extract at 20% combined with one and a half litres of irrigation water (20%+1.5L) treatment recording lowest disease incidence. The plants exhibited characteristic symptoms of wilting such as wilting of youngest leaves, yellowing of leaves and browning of stems (Figure 9).

a) Yellowing symptoms on leaves b) Brownish discoloration of stem

Figure 9: Tomato plants in greenhouse showing different disease symptoms: (a) Yellowing

symptoms on leaves (b) Brownish discoloration of stem.

Table 4: Effect of concentration *Allium fistulosum* crude extract and irrigation levels on bacterial wilt disease incidence in Percentage (%), Disease severity (scale 0-5) and Disease severity index (%)

Treatments	Disease incidence (%)					Disease severity (scale 0-5)					Disease severity index (%)				
	14dat	28dat	42dat	56dat	70dat	14dat	28dat	42dat	56dat	70dat	14dat	28dat	42dat	56dat	70dat
-ve+2L	83.3a*	79.2a*	75.0a*	66.7a*	58.3a*	3.7a*	3.5a*	3.0a*	3.0a*	3.0a*	18.3a*	17.5a*	15.0a*	15.0a*	15.0a*
+ve+2L	50.0b	50.0b	41.7b	41.7b	33.3b	1.5b	1.3b	1.0b	1.0b	1.0b	7.5b	6.7b	5.0b	5.0b	5.0b
15%+2L	33.3bc	25.0c	25.0bc	16.7c	16.7bc	1.3b	1.0b	1.0b	0.85bc	0.85bc	6.7bc	5.0bc	5.0b	3.3b	3.3b
15%+1.5L	29.2cd	25.0c	16.7cd	16.7c	16.7bc	1.0bc	1.0b	0.85bc	0.65bc	0.65bc	5.0bcd	5.0bc	3.3bc	3.3b	3.3b
15%+1L	25.0cd	25.0c	16.7cd	12.5c	12.5c	1.0bc	1.0b	0.65bc	0.35c	0.35c	5.0bcd	5.0bc	3.3bc	2.5bc	2.5bc
15%+0.5L	25.0cd	20.8c	12.5cd	8.3c	8.3c	1.0bc	0.9bc	0.5bc	0.0c	0.0c	5.0bcd	4.2bc	2.5bcd	0.0c	0.0c
20%+2L	25.0cd	16.7cd	8.3cd	0.0c	0.0d	1.0bc	0.9bc	0.15c	0.0c	0.0c	5.0bcd	3.3bcd	1.7cd	0.0c	0.0c
20%+1.5L	20.8cd	12.5cde	0.0d	0.0c	0.0d	0.7bc	0.35c	0.0c	0.0c	0.0c	3.3cd	2.5cd	0.0d	0.0c	0.0c
20%+1L	12.5d	4.2de	0.0d	0.0c	0.0d	0.5bc	0.0d	0.0c	0.0c	0.0c	2.5d	0.0d	0.0d	0.0c	0.0c
20%+0.5L	12.5d	0.0e	0.0d	0.0c	0.0d	0.35c	0.0d	0.0c	0.0c	0.0c	2.5d	0.0d	0.0d	0.0c	0.0c

*Means within a column followed by the same letter are not significantly different (P≤0.05, Tukey's HSD test)

KEY:

-ve+2L– negative control + two litres; **+ve+2L**– positive control + two litres; **15%+2L**– 15% *Allium fistulosum* + two litres; **15%+1.5L**– 15% *Allium fistulosum* + one and a half litres; **15%+2L**– 15% *Allium fistulosum* + one litre; **15%+0.5L**– 15% *Allium fistulosum* + half a litre; **20%+2L**– 20% *Allium fistulosum* + two litres; **20%+1.5L**– 20% *Allium fistulosum* + one and a half litres; **20%+1L**– 20% *Allium fistulosum* + one litre; **20%+0.5L**– 20% *Allium fistulosum* + half a litre, **dat-** days after transplanting

4.1.2 Effect on Bacterial Wilt Severity and Percentage Severity Index

Severity of bacterial wilt on tomato plants was significantly reduced by combined use of *Allium fistulosum* crude extract and manipulation of the irrigation levels during both trials (Tables 4). In both trials, negative control (-ve+2L) treatment recorded the highest disease severity and percentage severity index followed by the positive control treatment (+ve+2L) in all days after transplanting. Treatments (15%+2L) and (15%+1.5L) followed in terms of disease severity and percentage severity index but the difference amongst them was not significant (P=0.05) in trials. Among the other treatments, use of the (15%+0.5L) treatment resulted in a high disease severity and percentage severity index, followed by (20%+2L), (20%+1L) and (20%+0.5L) recording lowest severity and percentage severity index. Higher disease severity could be attributed to the high temperatures experienced in the greenhouse that enhanced the thriving of the disease.

4.2 Effects of Concentration of *Allium fistulosum* crude extract and irrigation levels on growth and yield of tomato.

4.2.1 Effect on Growth

(a) Effect on Plant Height

Tomato plant height was significantly influenced by the use of *Allium fistulosum* crude extract combined with and irrigation at different levels (Table 5). The results indicated that, the tallest tomato plants were recorded in (20%+2L) treatment followed by the (20%+1.5L) treatment in all the sampling days. Treatments (15%+2L) and (15%+1.5L) followed in terms of tomato plant height and the difference amongst them was significant (P=0.05) in both trials. Among the other treatments, use of the (20%+1L) treatment resulted in taller tomato plants, followed by (15%+1L) and the difference amongst them was significant (P=0.05) in both trials. Shortest tomato plants were recorded in the negative control (-ve+2L) treatment followed by positive control (+ve+2L) treatment.

(b) Effect on Number of Branches

Branching of tomato plants was significantly influenced by the use of *Allium fistulosum* crude extract combined with and irrigation at different levels (Table 5). The highest number of branches of tomato plants were recorded in (20%+2L) treatment followed by the (20%+1.5L) treatment in all the sampling days. Treatments (15%+2L) and (15%+1.5L) followed in terms of number of branches of tomato plants and the difference amongst them was significant (P=0.05) 14dat, in both trials. Among the other treatments, use

of the (20%+1L) treatment resulted in higher number of branches of tomato plants, followed by (15%+1L) and the difference amongst them was significant (P=0.05) at 28dat, 56dat and 70dat in both trials. Lowest number of branches of tomato plants were recorded in the negative control (-ve+2L) treatment followed by positive control (+ve+2L) treatment but the difference amongst them was not significant (P=0.05).

(c) Effect on Number of Internodes

The number of internodes of tomato plants was significantly influenced by the use of *Allium fistulosum* crude extract combined with and irrigation at different levels (Table 5). The highest number of internodes of tomato plants were recorded in (20%+2L) treatment followed by the (20%+1.5L) treatment in all the sampling days. Treatments (15%+2L) and (15%+1.5L) followed in terms of number of internodes of tomato plants and the difference amongst them was significant (P=0.05) 14dat, in both trials. Among the other treatments, use of the (20%+1L) treatment resulted in higher number of internodes of tomato plants, followed by (15%+1L) and the difference amongst them was significant (P=0.05) at 28dat, 56dat and 70dat in both trials. Lowest number of internodes of tomato plants were recorded in the negative control (-ve+2L) treatment followed by positive control (+ve+2L) treatment but the difference amongst them was not significant (P=0.05).

Table 5: Effect of Concentration of *Allium fistulosum* Crude Extract and Irrigation Levels on Tomato Plant Height (cm), Number of Branches and Internodes

Treatments	Plant Height (cm)					Number of branches					Number of internodes				
	14dat	28dat	42dat	56dat	70dat	14dat	28dat	42dat	56dat	70dat	14dat	28dat	42dat	56dat	70dat
20%+2L	9.3a*	18.0a*	50.0a*	90.0a*	125.0a*	5.0a*	6.0a*	9.3a*	11.0a*	11.5a*	5.0a*	6.0a*	9.3a*	11.0a*	12.0a*
20%+1.5L	9.0ab	15.8b	48.7a	88.7a	123.3a	5.0a	6.0a	9.0a	10.3a	10.7a	5.0a	6.0a	9.3a	10.7a	11.7a
15%+2L	8.2bc	15.8b	47.7a	82.8b	118.8b	4.7ab	5.5ab	8.9a	10.3a	10.3ab	4.7ab	5.5ab	8.9a	10.3a	11.2ab
15%+1.5L	8.0bcd	14.5bc	43.2b	77.7c	115.0c	4.0bc	5.3abc	8.7a	10.0a	10.3ab	4.0bc	5.3abc	8.7a	10.0a	10.9ab
20%+1L	7.5cde	14.0cd	43.0b	75.5c	115.0c	4.0bc	5.3abc	8.1ab	9.7a	9.0b	4.0bc	5.3abc	8.2ab	9.7a	10.4b
15%+1L	7.0def	13.5cd	41.3bc	73.8cd	110.0d	4.0bc	4.7bcd	8.0ab	8.0b	8.0bc	4.0bc	4.7bcd	8.0ab	8.0b	9.0bc
20%+0.5L	7.0def	12.7de	40.0bc	73.3cd	105.0e	3.3cd	4.3cd	7.0bc	8.0b	9.0bc	3.3cd	4.3cd	7.0bc	8.0b	9.0bc
15%+0.5L	6.7ef	11.7ef	40.0bc	70.0d	105.0e	3.0d	4.0d	6.0c	8.0b	8.0bc	3.0d	4.0d	6.0c	8.0b	8.5bc
+ve +2L	6.0f	11.0fg	38.0cd	70.0d	100.0f	3.0d	4.0d	6.0c	7.1b	7.0c	3.0d	4.0d	6.0c	7.2b	7.5c
-ve +2L	4.0g	10.0g	36.0d	69.5d	95.0g	3.0d	4.0d	6.0c	7.0b	7.0c	3.0d	4.0d	6.0c	7.0b	7.5c

*Means within a column followed by the same letter are not significantly different (P≤0.05, Tukey's HSD test)

KEY:

-ve+2L– negative control + two litres; **+ve+2L**– positive control + two litres; **15%+2L**– 15% *Allium fistulosum* + two litres; **15%+1.5L**– 15% *Allium fistulosum* + one and a half litres; **15%+2L**– 15% *Allium fistulosum* + one litre; **15%+0.5L**– 15% *Allium fistulosum* + half a litre; **20%+2L**– 20% *Allium fistulosum* + two litres; **20%+1.5L**– 20% *Allium fistulosum* + one and a half litres; **20%+1L**– 20% *Allium fistulosum* + one litre; **20%+0.5L**– 20% *Allium fistulosum* + half a litre

(d) Effect on Length of Internodes

Internode length of tomato plants was significantly influenced by the use of *Allium fistulosum* crude extract combined with and irrigation at different levels (Table 6). The longest internode lengths were recorded in (20%+2L) treatment followed by the (20%+1.5L) treatment in all the sampling days. Treatments (15%+1.5L) and (15%+2L) followed in terms of lengths of internodes of tomato plants and the difference amongst them was significant (P=0.05) in both trials. Among the other treatments, use of the (20%+1L) treatment resulted in longer internode lengths of tomato plants, followed by (15%+1L) and the difference amongst them was significant (P=0.05). Shortest internode lengths of tomato plants were recorded in the negative control (-ve+2L) treatment followed by positive control (+ve+2L) treatment but the difference amongst them was not significant (P=0.05).

e) Effect on Stem Collar Diameter

Stem collar diameter of tomato plants was significantly influenced by the use of *Allium fistulosum* crude extract with and irrigation at different levels (Table 6). The largest stem collar diameter was recorded in the (20%+2L) and (20%+1.5L) treatment followed (15%+2L) and (15%+1.5L). The difference in number of days between these control treatments and all the other treatments was significant during most sampling dates. Among the other treatments, application of either *Allium fistulosum* crude extract at 20% combined with one litre (20%+1L) or *Allium fistulosum* crude extract at 15% combined with half a litre (15%+0.5L) of irrigation water produced larger stem collar diameter than the other treatments with the difference amongst them was not significantly different (P=0.05) in both trials. Smallest stem collar diameter of tomato fruits was recorded in the negative control (-ve+2L) treatment followed by positive control (+ve+2L) treatment but the difference amongst them was not significant (P=0.05).

Table 6: Effect of Concentration of *Allium fistulosum* Crude Extract and Irrigation Levels on Tomato Length of Internodes (cm) and Stem Collar Diameter (mm)

Treatments	Internode length (cm)					Stem collar diameter (mm)				
	14dat	28dat	42dat	56dat	70dat	14dat	28dat	42dat	56dat	70dat
20%+2L	4.0a*	5.0a*	7.0a*	8.0a*	8.7a*	2.0a*	3.7a*	5.5a*	5.9a*	6.7a*
20%+1.5L	3.7b	5.0a	7.0a	7.2b	8.7a	2.0a	3.4ab	5.0ab	5.9a	6.5a
15%+1.5L	3.0c	5.0a	7.0a	6.8b	8.2ab	1.9a	3.2ab	4.9b	5.3b	6.4ab
15%+2L	3.0c	4.5ab	6.0b	6.2c	7.2b	1.9a	3.2ab	4.0c	4.4c	5.9bc
20%+1L	3.0c	4.3ab	6.0b	6.0c	7.0b	1.7ab	2.5b	3.4d	4.2c	5.4bc
15%+1L	3.0c	4.0bc	5.0c	6.0c	6.3b	1.5ab	2.5b	3.2d	4.0c	5.0c
20%+0.5L	2.0d	3.3cd	4.3d	4.7d	4.7c	1.4ab	2.5b	3.2d	4.0c	5.0c
15%+0.5L	2.0d	3.3cd	4.0de	4.0e	4.7c	1.0b	2.5b	3.2d	3.9d	4.5d
+ve +2L	2.0d	3.0de	3.7e	4.0e	4.3c	1.0b	1.9c	2.7e	3.2de	4.0d
-ve +2L	2.0d	2.3e	3.5e	3.7e	4.0c	1.0b	1.5c	2.5e	3.0e	3.5e

*Means within a column followed by the same letter are not significantly different (P≤0.05, Tukey's HSD test)

KEY:

-ve+2L– negative control + two litres; **+ve+2L**– positive control + two litres; **15%+2L**– 15% *Allium fistulosum* + two litres; **15%+1.5L**– 15% *Allium fistulosum* + one and a half litres; **15%+2L**– 15% *Allium fistulosum* + one litre; **15%+0.5L**– 15% *Allium fistulosum* + half a litre; **20%+2L**– 20% *Allium fistulosum* + two litres; **20%+1.5L**– 20% *Allium fistulosum* + one and a half litres; **20%+1L**– 20% *Allium fistulosum* + one litre; **20%+0.5L**– 20% *Allium fistulosum* + half a litre.

(f) Effect on Days to 50% Flowering

Days to 50% flowering of tomato plants was significantly influenced by the use of *Allium fistulosum* crude extract with and irrigation at different levels (Table 7). The longest days to 50% flowering were observed in the negative control treatment (distilled water combined with two litres of irrigation water (-ve+2L) followed by the positive control (Greencop at 50g/20L spray) combined with two litres of irrigation water (+ve+2L). Days to 50% flowering recorded under the negative and positive control treatment were significantly higher than that recorded under all other treatments during all sampling dates. The difference in number of days between these control treatments and all the other treatments was significant during most sampling dates. Among the other treatments, application of either *Allium fistulosum* crude extract at 15% combined with one and a half litres (15%+1.5L) or *Allium fistulosum* crude extract at 20% combined with one and a half litres (20%+1.5L) of irrigation water resulted in longer days to achieve 50% flowering than the other treatments with the difference amongst them significantly different (P=0.05) in both trials. Number of days to 50% flowering in these treatments were closely followed by those of *Allium fistulosum* crude extract at 15% combined with either one litre (15%+1L) and two litres of irrigation water (15%+2L), followed by *Allium fistulosum* crude extract at 20% combined with two litres of irrigation water (20%+2L) and *Allium fistulosum* crude extract at 15% combined with one and a half litres of irrigation water (20%+1.5L) treatment recording lowest number of days to achieve 50% flowering.

Table 7: Effect of Concentration of *Allium fistulosum* Crude Extract and Irrigation Levels on Tomato Days to 50% Flowering

Treatments	Days to 50% Flowering
+ve +2L	45.0a*
-ve +2L	45.0a
15%+0.5L	44.7ab
20%+0.5L	44.0abc
15%+1L	43.7bc
15%+2L	43.7bc
20%+2L	43.0cd
15%+1.5L	43.0cd
20%+1L	42.3d
20%+1.5L	42.0d

*Means within a column followed by the same letter are not significantly different (P≤0.05, Tukey's HSD test)

KEY:

-ve+2L– negative control + two litres; **+ve+2L**– positive control + two litres; **15%+2L**– 15% *Allium fistulosum* + two litres; **15%+1.5L**– 15% *Allium fistulosum* + one and a half litres; **15%+2L**– 15% *Allium fistulosum* + one litre; **15%+0.5L**– 15% *Allium fistulosum* + half a litre; **20%+2L**– 20% *Allium fistulosum* + two litres; **20%+1.5L**– 20% *Allium fistulosum* + one and a half litres; **20%+1L**– 20% *Allium fistulosum* + one litre; **20%+0.5L**– 20% *Allium fistulosum* + half a litre

4.2.2 Effect on Yield

(a) Effect on Total Number of Fruits

The total number of tomato fruits were significantly influenced by the use of *Allium fistulosum* crude extract combined with and irrigation at different levels (Table 8). In both the trials, the highest total number of tomato fruits were recorded in (20%+2L) treatment followed by the (15%+1.5L) treatment and (20%+1.5L) treatment in all the sampling days. Treatments (15%+2L) and (20%+1L) followed in terms of total number of tomato fruits but the difference amongst them was not significant (P=0.05) in both trials. Among the other treatments, use of the (20%+0.5L) treatment resulted in higher total number of tomato fruits, followed by (15%+1L) but the difference amongst them was not significant (P=0.05). Lowest total number of tomato fruits were recorded in the negative control (-ve+2L) treatment followed by positive control (+ve+2L) treatment but the difference amongst them was not significant (P=0.05).

(b) Effect on Total Fruit Weight

The total fruit weight was significantly influenced by the use of *Allium fistulosum* crude extract combined with and irrigation at different levels (Table 8). In both the trials, the highest total fruit weight was recorded in (20%+2L) treatment followed by the (15%+1.5L) treatment in all the sampling days. Treatments (20%+1.5L) and (15%+2L) followed in terms of total fruit weight but the difference amongst them was not significant (P=0.05) in both trials. Among the other treatments, use of the (20%+1L) treatment resulted in higher total fruit weight, followed by (20%+0.5L) but the difference amongst them was not significant (P=0.05). Lowest total fruit weight was recorded in the negative control (-ve+2L) treatment followed by positive control (+ve+2L) treatment but the difference amongst them was not significant (P=0.05).

Table 8: Effect of Concentration of *Allium fistulosum* Crude Extract and Irrigation Levels on Number of Tomato Fruits and Total Tomato Fruit Weight

Treatments	Number of tomato fruits		Total tomato fruit weight	
	Trial 1	Trial 2	Trial 1	Trial 2
20%+2l	22.0a*	21.7a*	2.35a*	2.31a*
15%+1.5l	20.7ab	21.3ab	2.25ab	2.19ab
20%+1.5l	20.0ab	20.7ab	2.08ab	2.07ab
15%+2l	20.0ab	19.3ab	2.06ab	2.03ab
20%+1l	19.0ab	18.3ab	1.95abc	1.92abc
20%+0.5l	17.0abc	16.7abc	1.93ac	1.91abc
15%+1l	16.0abc	16.0abc	1.53bcd	1.50bcd
15%+0.5l	15.7abc	15.3abc	1.23cd	1.21cd
+ve+2l	13.0bc	12.7c	1.1d	1.10d
-ve+2l	10.0c	10.3c	0.93d	0.90d

*Means within a column followed by the same letter are not significantly different (P≤0.05, Tukey's HSD test)

KEY:

-ve+2L– negative control + two litres; **+ve+2L**– positive control + two litres; **15%+2L**– 15% *Allium fistulosum* + two litres; **15%+1.5L**– 15% *Allium fistulosum* + one and a half litres; **15%+2L**– 15% *Allium fistulosum* + one litre; **15%+0.5L**– 15% *Allium fistulosum* + half a litre; **20%+2L**– 20% *Allium fistulosum* + two litres; **20%+1.5L**– 20% *Allium fistulosum* + one and a half litres; **20%+1L**– 20% *Allium fistulosum* + one litre; **20%+0.5L**– 20% *Allium fistulosum* + half a litre

4.3 Effects of Concentration of *Allium fistulosum* Crude Extract and Irrigation Levels on Quality of Tomato

4.3.2 Effect on Tomato Shelf-life

The shelf-life of tomato fruits was significantly influenced by the use of *Allium fistulosum* crude extract combined with and irrigation at different levels (Table 9). In both the trials, the longest days on shelf-life of fruits was recorded in (20%+1.5L) treatment followed by the (15%+1.5L) treatment in all the sampling days. Treatments (20%+2L) and (15%+2L) followed in terms of days on shelf-life of fruits and the difference amongst them was significant (P=0.05) in both trials. Among the other treatments, use of the (20%+1L) treatment resulted in longer days on shelf-life of fruits, followed by (20%+0.5L) but the difference amongst them was not significant (P=0.05). Shortest days on shelf-life of fruits were recorded in the negative control (-ve+2L) treatment followed by positive control (+ve+2L) treatment and the difference amongst them was significant (P=0.05).

4.3.2 Effect on Total Soluble Solids

Total soluble solids (TSS) of fresh tomato fruits was significantly influenced by the use of *Allium fistulosum* crude extract combined with and irrigation at different levels (Table 9). In both the trials, the highest total soluble solids (TSS) in tomato fruits was recorded in (20%+0.5L) treatment followed by the (15%+1L) treatment in all the sampling days. Treatments (20%+2L) and (20%+1.5L) followed in terms of total soluble solids (TSS) in tomato fruits and the difference amongst them was significant (P=0.05) in second trial. Among the other treatments, use of the (15%+1.5L) treatment resulted in a high total soluble solids (TSS) in tomato fruits, followed by (15%+2L) and the difference amongst them was significant (P=0.05). Lowest values of total soluble solids (TSS) in fruits were recorded in the negative control (-ve+2L) treatment followed by positive control (+ve+2L) treatment and the difference amongst them was significant (P=0.05). The trend in the two trials showed that total soluble solids (TSS) increased with decrease in irrigation level.

4.3.3 Effect on Ascorbic Acid

Ascorbic acid expressed in (mg/100g) was significantly influenced by the use of *Allium fistulosum* crude extract combined with and irrigation at different levels (Table 9). In both the trials, the highest ascorbic acid content in tomato fruits was recorded in (20%+0.5L) treatment followed by the (15%+1L) treatment in all the sampling days. Treatments (20%+2L) and (20%+1.5L) followed in terms of ascorbic acid content in tomato fruits but the

difference amongst them was not significant (P=0.05) in both trials. Among the other treatments, use of the (15%+1.5L) treatment resulted in a high ascorbic acid content in tomato fruits, followed by (15%+2L) and the difference amongst them was significant (P=0.05). Lowest values of ascorbic acid content in fruits were recorded in the negative control (-ve+2L) treatment followed by positive control (+ve+2L) treatment and the difference amongst them was significant (P=0.05).

4.3.4 Effect on Lycopene

Lycopene expressed in (mg/100g) was significantly influenced by the use of *Allium fistulosum* crude extract combined with and irrigation at different levels (Table 9). In both the trials, the highest lycopene content in tomato fruits was recorded in (20%+0.5L) treatment followed by the (15%+1L) treatment in all the sampling days. Treatments (20%+2L) and (20%+1.5L) followed in terms of lycopene content in tomato fruits but the difference amongst them was not significant (P=0.05) in both trials. Among the other treatments, use of the (15%+1.5L) treatment resulted in a high lycopene content in tomato fruits, followed by (15%+2L) and the difference amongst them was significant (P=0.05). Lowest values of lycopene content in fruits were recorded in the negative control (-ve+2L) treatment followed by positive control (+ve+2L) treatment and the difference amongst them was significant (P=0.05). The trend showed that Lycopene content in tomato fruits increased with decrease in irrigation level.

4.3.5 Effect on ß-Carotene

ß-Carotene expressed in (µg/100g) was significantly influenced by the use of *Allium fistulosum* crude extract combined with and irrigation at different levels (Table 9). In both the trials, the highest ß-Carotene content in tomato fruits was recorded in (20%+0.5L) treatment followed by the (15%+1L) treatment in all the sampling days. Treatments (20%+2L) and (20%+1.5L) followed in terms of ß-Carotene content in fruits but the difference amongst them was not significant (P=0.05) in both trials. Among the other treatments, use of the (15%+1.5L) treatment resulted in a high ß-Carotene content in tomato fruits, followed by (15%+2L) but the difference amongst them was significant (P=0.05). Lowest values of ß-Carotene content in fruits were recorded in the negative control (-ve+2L) treatment followed by positive control (+ve+2L) treatment and the difference amongst them was significant (P=0.05). The trend showed that ß-Carotene content in tomato fruits increased with decrease in irrigation level.

Table 9: Effect of Concentration of *Allium fistulosum* Crude Extract and Irrigation Levels on Tomato Shelf life (days), Total Soluble Solids (°Brix), Ascorbic Acid Content (mg/100g), Lycopene Content (mg/100g) and b-carotene Content (µg/100g)

Treatments	Shelf life		Total Soluble Solids (°Brix)		Ascorbic Acid Content (mg/100g)		Lycopene Content (mg/100g)		b-carotene Content (µg/100g)	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
20%+2l	14.0a*	14.0a*	3.8a*	3.8a*	25.1a*	24.6a*	48.4a*	40.3a*	7.2a*	5.8a*
15%+1.5l	14.0a	13.3ab	3.6b	3.7a	22.8ab	21.8ab	47.1a	33.1b	6.1b	4.6b
20%+1.5l	13.0b	12.3b	3.5c	3.5b	22.0ab	20.4abc	39.7b	30.8bc	5.8b	4.2c
15%+2l	12.0c	11.0c	3.0d	3.0c	20.4ab	19.7abcd	37.1bc	28.0bcd	5.2c	4.0c
20%+1l	10.0d	9.3d	3.0d	3.0c	18.5ab	16.9bcde	33.4bcd	27.1cde	4.7d	4.0c
20%+0.5l	10.0d	9.0de	2.9d	2.9d	18.3ab	16.3cde	32.1cde	26.1cde	4.6d	2.8d
15%+1l	9.0e	9.0de	2.6e	2.6e	17.7ab	15.1cde	29.7def	24.9de	4.1e	2.5d
15%+0.5l	8.0f	8.0ef	2.5e	2.5e	14.5b	14.4de	26.9ef	23.2de	3.7e	2.2e
+ve+2l	7.0g	7.0f	2.1f	2.0f	14.2b	13.7e	24.9f	22.5e	2.5g	2.0e
-ve+2l	5.0h	5.0h	2.0f	2.0f	13.8b	12.0e	16.3g	12.6h	1.6h	1.0f

*Means within a column followed by the same letter are not significantly different (P≤0.05, Tukey's HSD test)

KEY: -ve+2L– negative control + two litres; **+ve+2L**– positive control + two litres; **15%+2L**– 15% *Allium fistulosum* + two litres; **15%+1.5L**– 15% *Allium fistulosum* + one and a half litres; **15%+2L**– 15% *Allium fistulosum* + one litre; **15%+0.5L**– 15% *Allium fistulosum* + half a litre; **20%+2L**– 20% *Allium fistulosum* + two litres; **20%+1.5L**– 20% *Allium fistulosum* + one and a half litres; **20%+1L**– 20% *Allium fistulosum* + one litre; **20%+0.5L**– 20% *Allium fistulosum* + half a litre

CHAPTER FIVE

DISCUSSION

5.1 Effects of Concentration of *Allium fistulosum* Crude Extract on Inhibition of Bacterial Wilt *In-vitro*

Use of plant extracts in management of crop diseases especially bacterial wilt of tomato can be regarded as a significant supply of anti-microbial and antibacterial compounds that have properties that suppress pathogens attacking the crop (Naz *et al*., 2015). These include active substances such as enzymes, sulphur- rich compounds, steroid alkaloids, glycol-alkaloids, saponins and antioxidants (Alemu *et al*., 2013). Presence of high content of these active compounds is known to offer an inhibitory effect of growth of pathogens *In-vitro* (Goncagul & Ayaz, 2010). This makes use of plant extracts as a cheap, environmentally friendly and readily available alternative source of sustainable pesticides for farmers (Dubey *et al*., 2010).

Bacterial wilt is a devastating disease that has no effective management strategy. The plant extract *Allium fistulosum* was used in this study as it offered an alternative eco-friendly method, readily available for disease management. In the current study, use of *Allium fistulosum* crude extract was found to be effective in reducing growth of bacterial wilt *in-vitro*. This reduction was attributed to presence of sulfur volatiles produced on degradation of *Allium fistulosum* during grinding. The extract also contains allicin which has antibacterial properties for controlling a number of bacterial diseases. This active ingredient acted on the pathogen directly by inhibiting its growth (Balestra *et al*., 2009).

The results indicated that the antibacterial properties of crude extracts of *Allium fistulosum* are highly effective against *Ralstonia solanacearum* at higher concentrations. On the other hand, treatments 10%, 5% and negative control had an inhibition diameter of 9.98mm, 9.43mm and 5.8 mm respectively which was <10mm an indication of less effectiveness in reducing growth of pathogen. However, these treatments showed significant differences amongst themselves at P=0.05. The presence of *in- vitro* growth in this extract may be explained by the fact that there was sufficient concentration of secondary metabolite in the onion plant material. Allicin is a sulphur volatile compound that possessed anti-microbial properties responsible for inhibition (Hussein *et al*., 2017).

These results were also in agreement with those obtained by Patrice *et al*. (2018) who used ethanolic extracts of *Allium fistulosum* to control tomato bacterial wilt. The highest

inhibition diameter of 16.5 mm was recorded when 15 mg/ml was used while the lowest inhibition of 11 mm was recorded. This inhibition was attributed to bioactive substances such as alkaloids, tannins, flavonoids and phenolic compounds present in *Allium fistulosum* crude extracts that inhibits growth of pathogen (Lee & Mitchell, 2011). In another study by Deberdt *et al*. (2012), *Allium fistulosum* extracts inhibited the growth of *Ralstonia solanacearum* Phylotype IIB/4NPB *In- vitro*. Similar findings were also obtained by Wagura *et al*. (2015) who used crude medicinal plant extract of *Ocimum gratisimum*, *Brassica oleracae* and *Ipomoea batatas* to manage *Ralstonia solanacearum* pathogen *In- vitro.* Contrary to the above, Sangoyomi *et al*. (2011) reported that aqueous extracts of medicinal plants did not inhibit *Ralstonia solanacearum* pathogen growth *In-vitro*.

Anton *et al*. (2021), also conducted a study on use of plant products such as *Lantana camara, Allium sativum, Azadirachta indica* and *Solanum incanum* to control *Ralstonia solanacearum* causing bacterial wilt in tomatoes (Solanum lycopersicum). Findings revealed that Water extracts at 10% and 20% (w/v) from Lantana camara, A. sativum, A. indica and S. incanum gave a significant inhibitory effect ($p > 0.05$) on the growth of R. solanacearum. Din *et al*. (2016) also conducted a study and results were similar to those of Anton *et al*. (2021). Findings revealed that in a disc diffusion experiment, aqueous extracts of dried leaves from *Lantana camara, Allium sativum, Azadirachta indica* and *Solanum incanum* and the mature fruits of *Solanum incanum* had a significant inhibitory effect on the rate of growth of R. solanacearum, compared to control treatments.

Findings of the current study were in concurrent with those of Jang *et al*. (2019) who investigated the inhibitory effect of extracts of allium plants on development of crop pathogens. Results revealed that use of flesh *Allium sativum* suppressed growth rates of *Pyricularia oryzae* and *Phytophthora cactorum* at a highest percentage. On the other hand, suppression of Colletotrichum coccodes was at a rate of 94% and 84% when 5% concentration of *Allium fistulosum* root and *Allium sativum* fresh water extracts were used respectively. Furthermore, these results were similar to those obtained by Mitali *et al*. (2012) who used plant extracts of *A. conyzoides* to control *Clavibacter michigenesis*. Antibacterial activity tested revealed that diameter of zone of inhibition of 10.67 mm was recorded when a concentration of 15% was used. In summary the results of these studies stated above are in agreement with the results of the current study where treatment 20% significantly inhibited growth of *Ralstonia solanacearum* pathogen by giving an inhibition mean diameter of 11.48 mm followed by 15 % which gave an inhibition mean diameter of 10.77 mm. These

treatments had an inhibition mean diameter of >10mm an indication that they had a positive impact in inhibiting growth of *Ralstonia solanacearum* pathogen *In-vitro.*

5.2 Effects of Concentration of *Allium fistulosum* Crude Extract and Irrigation Levels on Bacterial Wilt Incidence and Severity in Greenhouse

Combination of *Allium fistulosum* crude extract concentrations and irrigation levels in the current study enhanced a reduction in disease incidence and severity. Plants were examined for disease symptoms by counting number of plants wilted for disease incidence and scoring on a scale of 0-5 for disease severity. Disease incidence and severity remained lower under combination of 20% *Allium fistulosum* crude extract and half a litre of irrigation water compared to positive control (Greencop at a recommended rate of 50g/L) and negative control (water alone) combined with two litres of irrigation water. This may be explained by reduced amount of water applied and increased concentration of *Allium fistulosum* crude extract. According to Balestra *et al*. (2009), *Allium fistulosum* crude extract combined with optimum water level has been shown to reduce a number of crop diseases due to presence of sulfur volatiles produced upon degradation of allium tissues. *Allium fistulosum* also contains allicin with potential antibacterial properties for controlling a number of bacterial and fungal diseases. These active ingredients in this extract reduced bacterial wilt disease incidence and severity by acting on *Ralstonia solanacearum* pathogen directly or through stimulation or induction of systemic resistance in tomato plant. In addition, extract is also of natural origin, biodegradable and do not leave toxic residues to accumulate in the fruit and environment in comparison with use of chemicals. Results of present study are supported by findings of Baba *et al.* (2019) who reported that extracts of *Allium fistulosum* reduced incidence and severity of *Callosobruchus maculatus*.

Findings of the present study concur with those of Kamal *et al*. (2020) who carried out a research on induction of resistance to bacterial spot on tomato plants by plant extracts for a sustainable system. Results revealed that treatments composed of extracts of *Citrullus colocynthis* contributed to the greatest reduction of disease severity by 20% and 21.2% for ethanol and water extracts with no significance differences between them. The findings of the present study are also in agreement with those of Hassan *et al*. (2013) who stated that application of certain plant extracts reduced bacterial wilt disease of potato plants as compared to control plants. This reduction was due to lower number of pathogens recorded due to application of plant extracts in treated plants and induction of some antioxidant

enzymes that reduced number of pathogens in the tissues. In addition, findings of this study are in agreement with those of Draz *et al*. (2019) who used plant extracts of pomegranet (*Punica granatum*), acalypha (*Acalypha wilkesiana*), Lenna (*Lawsonia inhemis*), lantana (*Lantana camara*) and china berry (*Melia azedarach*) to induce resistance in wheat (*Triticum aestivum*) against rust disease. Results showed that all these plant extracts reduced disease severity and incidence.

On the other hand, bacterial wilt disease development depends on levels of water and moisture conditions during the growing period. High soil moisture conditions and prolonged periods of wet weather or rainy seasons are associated with increased disease incidence and severity (Agather *et al*., 2017). In the present study, highest disease incidence and severity was recorded under the control treatments that is positive control (Greencop at a recommended rate of 50g/L) and negative (distilled water) control combined with two litres of irrigation water level. This was attributed to the fact that there was absence of active ingredients (allicin and sulphur volatiles) contained in in extract responsible for combating thriving of the disease. In addition, moisture levels were high due to high amount of water applied, thus bringing about this differential effect. Lowest disease incidence was recorded when 20% concentration of *Allium fistulosum* crude extract was used combined with half a litre of irrigation water level.

Furthermore, the findings of the present study were in agreement with those reported by Oduor (2016) who studied on effects of soil moisture levels ranging from 20% to 35% of bacterial wilt incidence and severity of tomato. Results revealed that high bacterial wilt disease incidence and severity was recorded under high moisture conditions of 35%. Oduor (2016) also demonstrated that a decrease in water content from 70% of water holding capacity (WHC) to 50% water holding capacity induced a 30% reduction of population of *Ralstonia solanacearum* in soil thereby leading to a reduction in disease incidence and severity. In the present study application of two litres of irrigation with no combination of *Allium fistulosum* crude extract provided high soil moisture conditions and prolonged periods of wet weather which are associated with increased disease incidence and severity.

5.3 Effects of Concentration of *Allium fistulosum* Crude Extract and Irrigation Levels on Growth and Yield of Tomato

Combinations of *Allium fistulosum* crude extract concentrations and irrigation levels in the current study significantly influenced growth and yield parameters of tomato including plant height, number of branches, number of internodes, length of internodes, stem collar

diameter, days to 50% flowering, total number of fruits and total fruit weight.

5.3.1 Effect on Growth

Use of *Allium fistulosum* crude extract concentrations in combination with irrigation levels in the present study significantly increased the growth parameters of tomato crop. This could be explained by the fact that these extract promoted plant growth by decreasing negative impact of pathogen (stunting) which is a characteristic of bacterial wilt disease. These findings concurred with those of Alemu *et al*. (2014), who reported that tomato plants treated with different plant extracts at different time of application increased growth parameters of tomato crop including plant height, number of branches and internodes and stem collar diameter.

Plant height significantly increased due to combination of *Allium fistulosum* crude extract and different irrigation levels. Results of present study agrees with those of Tasisa and Fatih (2021) who found out that longest plants were obtained on use of full irrigation throughout the growing season while longer plants on giving optimum amount of water and shorter plants when plants were stressed by skipping irrigation during growing period. This increase in plant height in the current study could also be attributed to provision of the optimum amount of irrigation water which gave better availability of soil moisture that enhanced vegetative growth of plants by increasing cell division and elongation. Furthermore this could also be attributed to the favourable effect of water in maintaining turgor pressure of cell, which is a major prerequisite for growth (Ramada & Ramanathan, 2017). On the other hand, shorter plants obtained due to low water level maybe explained by the fact that tomato plants experienced partial stomatal closure and reduced $C0_2$ diffusion and nutrient uptake by the plants hence photosynthesis and other biochemical processes were hampered thus, adversely affecting plant growth negatively (El-Noemani *et al*., 2009). The result of the present are also in agreement with the findings of Ramada and Ramanathan (2017), who reported that highest and lowest plant height of shallot were obtained from irrigation levels ranging from 1 to 0.5 ETc respectively. Higher level of irrigation (1.2lw) and increased soil water supply resulted in highest plant height and increased plant growth was observed by David *et al*. (2016), and Enchalew *et al*. (2016).

Stem diameter is an important growth parameter as it serves as a reservoir for the amount of nutrients supplied per unit cross section to developing leaves and for the flow of photosynthates from mature leaves to the rest of plant parts. Tasisa and Fatih (2021) reported

that an increase in water level resulted in a significant increase in stem diameter across increasing depth of water application. Plants treated with sufficient water at any growth stage produced widest stem collar diameter while those subjected to deficit irrigation of 25% ETc produced narrow stem collar diameter. In the present study, increase in stem collar diameter with increasing level of irrigation water could be explained by a better supply of optimum water and less competition for other factors of growth among tomato plants (David *et al*., 2016).

In this study, the number of branches and number of internodes of tomato plants were significantly affected at (P=0.05) by different irrigation levels. These results are in agreement with those of David *et al*. (2016) who obtained high number of branches and internodes by interaction effects of irrigation levels and skipping irrigation at one stage of growth. The high number of branches and internodes in the current study could be explained by irrigation effect which facilitated nutrient availability and photosynthesis for continuous and undisrupted growth of tomato plants. On the other hand, reduced number of branches and internodes due to half a litre level of irrigation water (less optimum water) is attributed to the effects of water stress on cell expansion (Abbey & Joyce, 2014). This was an indication that when plants respond to water stress (lack of water) by closing their stomata to slow down water loss by transpiration, gas exchange within the leaf is limited, as a consequence, photosynthesis and growth slowed down in plants. Results of the present study are also in agreement with findings of Ramada and Ramanathan (2017) who reported that number of branches and internodes had a positive linear correlation with the availability of soil moisture.

Length of internodes of tomato plants was significantly influenced by varying different irrigation water levels. Their increase in the present study was due to better availability of soil moisture that promoted vegetative growth of plants by increasing cell division and elongation. On contrary to above, decrease in length of internodes could be attributed to stomatal closure and reduced CO_2 and nutrient uptake by plants thus altering photosynthesis and biochemical processes such as respiration affecting plant growth (Ramada and Ramanathan, 2017). This statement provides further explanation as to why treatment with less optimum water applied and no application of plant extract, had decreased length of internodes and poor vegetative growth.

Analysis of variance showed existence of significant differences at (P=0.05) on days to 50% flowering of tomato plants due to different levels *Allium fistulosum* crude extract and irrigation water applied. Results indicated a significant influence of irrigation levels on days

to 50% flowering amongst different treatments. These differences were due to different levels of water applied. Longer days taken to 50% of plants to flower during growing period could be due to high amount of water applied. This in turn promoted more vegetative growth and delayed transition to reproductive period. Results of the current study also concurs with those of Guluma (2009), who reported that length of days to enlargement of onion bulb was longer as frequency and amount of water application increased due to frequent water application, which promoted vegetative growth and delayed development period.

5.3.2 Effect on Yield

In the current study, tomato yields were highly influenced following combination of *Allium fistulosum* crude extract concentrations and irrigation levels. Yield is influenced by traits including number of fruits per plant and total fruit weight. The results of the current study are in agreement with those of Baba *et al*. (2019), David *et al*. (2016); and Ramada and Ramanathan (2017). Baba *et al.* (2019) reported that leaf extracts of *Allium fistulosum* produced longest pod length and high number of seeds per pod. The significant increase in current study could be explained by increased concentration of *Allium fistulosum* crude extract (20%) and availability of nutrients such as calcium and phosphorous essential for plant growth. David *et al*. (2016), and Ramada and Ramanathan (2017) in their different studies to determine yield of various vegetable crops concluded that low yield was obtained when plants were subjected to water stress (lack or insufficient amount of water) conditions throughout the growing season while deficit irrigation during initial and late stages of growing season did not significantly affect crop yield. Furthermore, Ramesh *et al*. (2016) reported similar findings to those of present study which revealed that highest yield of capsicum crop of 36.17 Kg was recorded with use of moderate irrigation. In this study, low yield was attributed to water stress (lack of water) during foliage growth and reduced photosynthetic activity that contributed to reduced development and enlargement of fruit. Higher irrigation levels helped to increase vegetative growth of plant which in turn improved assimilates available for storage thus increasing yield.

In their studies, Enchalew *et al*. (2016), Gebregwergis *et al*. (2016) and Yetagesu *et al*. (2020) reported that applying low level of water during fruit development and ripening growth stages produced lower number of fruits thus contributing to low fruit yields and that crop response is higher under irrigated conditions than non-irrigated conditions. Results of present study agrees with those of above studies. Ramesh *et al*. (2016) reported that highest

number of fruits (18.26) were recorded on use of 0.5cm irrigation. Highest number of fruits obtained in present study could be due to better micro-climate responsible for efficient water utilization at early crop growth stages which ultimately lead to more number of flowers hence more number of fruits. On the other hand, lower number of fruits per plant in the control treatment could be due to reduced number of flowers. Increase in yield due to application of high water level could be attributed to increased vegetative growth and increased production of assimilates associated with an increase in stem collar diameter and average fruit weight. In addition, Yenus (2013) and Kenneth *et al*. (2017) reported that highest and lowest yield was obtained on application of 1.2 to 0.5 ETc to tomato crop respectively. Birhanu and Tilahun (2010) also found out that total marketable and unmarketable yield of tomato was lowest in treatments which received reduced amount of water that is 75% water deficit.

Total weight of tomato fruits was significantly influenced by *Allium fistulosum* crude extract concentrations combined with varying irrigation levels. In this study, weight of fruits increased with increasing irrigation levels. These observations were similar to those of Birhanu and Tilahun (2010) who reported that fruit weight and fruit size was reduced with reduction in amount of irrigation water applied. This is explained by reduced water to support cell division and increase in fruit weight. On the other hand, high fruit weight could be as a result of cell expansion or a larger number of cells and positive effect of water availability for cell division. This effect has also been reported by Ehret *et al*. (2012) who found out that increase in tomato fruit weight was also due to higher level of irrigation. Opiyo *et al*. (2015) also reported that water stress results in lower fruit water content. Findings of Opiyo *et al*. (2015) also revealed that higher fruit weight in well irrigated fruits was most likely be due to high fruit water content. In their study, Ramesh *et al*. (2016) reported that average fruit weight of capsicum was significantly influenced by irrigation levels. Highest fruit weight of 95.2g was recorded. Gupta *et al*. (2010) also reported highest fruit weight of capsicum with moderate irrigation level of 80% of pan evapo-transpiration through drip irrigation combined with surface irrigation. Sezen *et al*. (2011) observed that increase in water deficit (lack of water) in root zone results in loss in turgidity leading to reduction in average fruit weight.

5.4 Effects of Concentration of *Allium fistulosum* Crude Extract and Irrigation Levels on Quality of Tomato

Plant extracts and irrigation levels during production are vital factors influencing

quality parameters of tomato crop.

5.4.1 Effect on Total Soluble Solids

Allium fistulosum crude extract concentrations combined with irrigation levels in the present study had a significant influence on total soluble solids of tomato fruits. In the present study, total soluble solids of tomato fruits increased with a decrease in irrigation level and decreased with an increase in irrigation level. The significant increase in total soluble solids could be explained by a decrease in irrigation water level which enhanced a water deficit environment that induced a higher starch accumulation during first stage of fruit growth, followed by conversion of starch into sugars during maturation. Decreased irrigation level also induced greater total soluble solids because of a decrease in water accumulation by the fruit without any significant modification in the quantity of accumulated sugars (Patane' *et al*., 2011). Similar findings were obtained by Birhanu and Tilahun (2010) and Ehret *et al*. (2012). They reported that reduced soil moisture levels increase sugar content in tomatoes. On the other hand, lowest total soluble solids recorded in fruits that received high levels of irrigation water level was attributed to the higher water uptake by the plants which lead to a dilution of concentration of total soluble solids of tomato fruits. Subjecting tomato plants to water stress (lack of water or reduced amount of water) enhanced their sweetness by increasing their glucose, fructose and sucrose contents Nahar *et al*. (2011). Birhanu and Tilahun (2010) reported that tomato fruit total soluble solids content increased with an increase in level of water stress (lack of water). This gave an implication that an increase in irrigation water level leads to a decrease in fruit total soluble solids and vice versa. In their study, Nahar *et al*. (2011) also observed that subjecting tomato plants to a water deficit or water stress environment improved quality parameters of tomato crop by increasing total soluble solids content and organic acids. Ramesh *et al*. (2016) also reported that highest total soluble solids of 5.30 degrees brix was recorded in treatment that receive lowest irrigation water (low amount of water). Results of present study disagreed with those of Gupta *et al*. (2010) who reported that highest total soluble solid of capsicum crop was recorded under highest irrigation water.

5.4.2 Effect on Shelf-life

In the current study, different concentrations of *Allium fistulosum* crude extract combined with irrigation levels had a significant influence on shelf-life of tomato fruits. Results of present study concurred with those obtained by Olaleye *et al*. (2014) who used

aqueous extracts of *Piper nigrum*, *Xylopia aethiopica*, *Tetrapleura tetraptera* and *Carica papaya* at concentrations of 0.25mg/ml, 0.5mg/ml, 0.75mg/ml and 1.00mg/ml. Results showed that the extracts extended shelf-life of tomatoes and pepper. In the comparative analysis, shelf-life of pepper was Eight days which was higher than that of tomato (five days). On the other hand, *Carica papaya* extract increased shelf-life of these vegetables by 12 days at the least concentration of 0.25mg/ml. Results from the present study confirmed that increasing the concentration of *Allium fistulosum* crude extract extended shelf-life of tomato fruits. Extension of shelf-life by concentration of 20% of *Allium fistulosum* crude extract was due to presence of phytochemicals such as alkaloids and tannins which possesses antimicrobial activity (Neli *et al*., 2011). Results of the present study were similar to those of Kamal (2020) who carried out a study on Valencia orange fruits and Thompson seedless grapevines they both confirmed treatments with garlic extracts increased shelf-life and thereby increasing storage period. Tunwari *et al*. (2019) reported that tomato fruits treated with ginger rhizome extract highly significantly prolonged shelf-life of fruits from 8 to 10% in comparison to control treatment. Irokanulo *et al*. (2015) on the other hand reported that tomato fruits treated with powders of Moringa oleifera parts had an extended storage life.

5.4.3 Effect on Ascorbic Acid

Allium fistulosum crude extract concentrations combined with irrigation levels increased ascorbic acid of tomato fruits. Ascorbic acid content increased with increase in irrigation level. Ascorbic acid content is an important quality parameter due to plenty of nutritional benefits. Results of present study disagree with those of Lajos *et al.* (2012) who reported that a high level of ascorbic acid content was measured in treatment that received high amount of water. Lower amount of ascorbic acid produced in highly watered treatments could be explained by fact that irrigation has a negative effect on main antioxidant components of tomato crop.

Results of present study concurred with those of Ramesh *et al*. (2016) who observed that ascorbic acid content of 126.19mg/100g was recorded upon usage of 0.75cm irrigation at alternate days while lowest ascorbic acid content of 122.62 mg/100g was recorded upon usage of 0.25cm irrigation at alternate days. Gupta *et al*. (2010) also reported highest ascorbic acid upon usage of 100% evapo-transpiration in comparison to 80% and 60% evapo-transpiration trough drip irrigation in capsicum. Results of present study were in disagreement with those reported by Singh *et al*. (2010) who noted that higher ascorbic acid

content with 0.75 pan evaporation was recorded in comparison to 0.5 and 1.0 pan evaporation in greenhouse grown capsicum crop.

5.4.4 Effect on Lycopene and ß-Carotene

Allium fistulosum crude extract concentrations combined with irrigation levels increased lycopene and ß-Carotene content of tomato fruits. Lycopene and beta-carotene are natural compounds involved in reducing risk of development of certain diseases such as diabetes, gastrointestinal and cardiovascular diseases. Lycopene development depends on cultivar, growing season, agricultural techniques and processing methods. Combination of 20% concentration of *Allium fistulosum* crude extract with half a litre of irrigation water resulted to highest contents of lycopene and ß-Carotene in tomato fruits. This could be explained by a reduction in amount of water given to plants and also capacity of allicin contained in *Allium fistulosum* crude extract in enhancing contents of lycopene and ß-Carotene in tomato fruits. Findings of this study are similar to those of Fahio *et al*. (2009) who reported that accumulation of carotenoids (lycopene and ß-Carotene) under water deficit irrigation was higher than that of tomatoes which were well irrigated. The study also revealed that vitamin C content was also influenced by irrigation practices.

Results of present study were in agreement with those of Sandor *et al*. (2020) who reported that the highest amount of lycopene and beta-carotene was recorded in control treatment (low water supply) while lowest concentration of lycopene and beta-carotene was recorded in treatment that received highest water supply. Low level of lycopene and beta-carotene could be explained by the diluting effect of high amount of irrigation water applied. Lycopene and beta-carotene concentration in control treatment differed significantly from the concentration in other treatments. Results of other studies by Arbex de *et al*. (2017)., Le *et al*. (2018) contradicted with those of the present study whose reports revealed that concentration of lycopene and beta-carotene was highest in treatments that received highest supply of water.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the current study findings, the following conclusions were made:

a) Concentrations of *Allium fistulosum* crude extract inhibits bacterial wilt *In-vitro* with the highest zone of inhibition diameter of 11.467 mm being recorded on use of 20% concentration of *Allium fistulosum* crude extract while lowest zone of inhibition diameter of 5.8 mm recorded under negative control treatment.

b) Concentrations of *Allium fistulosum* crude extract and irrigation levels reduces bacterial wilt incidence and severity in greenhouse. Lowest disease incidence and severity was recorded with the use of combination of 20% concentration of *Allium fistulosum* crude extract with half a litre of irrigation water followed with 20% combined with one litre of irrigation water. On the other hand, highest disease incidence and severity was recorded with a combination of negative control with two litres of irrigation water followed by positive control (Greencop) combined with two litres of irrigation water.

c) Concentrations of *Allium fistulosum* crude extract and irrigation levels improved growth and yield of tomato crop. Tallest plants, highest number of branches and internodes, highest length of internodes and thickest stems were obtained on use of 20% concentration of *Allium fistulosum* crude extract combined with two litres and one and a half litres of water in all days after transplanting. Early flowering was achieved when 20% concentration of *Allium fistulosum* crude extract combined with one litres of water while late flowering was achieved under the negative control treatment combined with two litres of water. 20% concentration of *Allium fistulosum* crude extract combined with two litres of water produced highest total number of tomato fruits and total fruit weight while lowest was obtained under negative control combined with two litres of water.

d) Concentrations of *Allium fistulosum* crude extract and irrigation levels improves quality of tomato. Regarding the quality parameters of tomato fruits, shelf-life of fruits was maintained highest on use of 20% combined with one and a half litres of water while total soluble solids, ascorbic acid content, lycopene content and beta-carotene content of tomato fruits were maintained highest under 20% combined with

one litre and half a litre of water as compared to negative control combined with two litres of water.

6.2 Recommendations

a) Farmers should consider using 20% concentration of *Allium fistulosum* crude extract as an environmental ecofriendly approach in management of bacterial wilt disease as it had the capacity to reduce growth of *Ralstonia solanacearum* pathogen *In-vitro*.
b) Tomato farmers should consider using combination of 20% concentration of *Allium fistulosum* crude extract with one liter of irrigation water as an integrated disease management strategy. This combination reduced disease incidence and severity in greenhouse without negatively affecting growth and yield thus considered as a cost effective and environmentally friendly measure.
c) Tomato farmers should consider using combination of 20% concentration of *Allium fistulosum* crude extract with two litre of irrigation water as an integrated approach in improvement tomato growth and yields.
d) Tomato farmers should consider using combination of 20% concentration of *Allium fistulosum* crude extract with half a litre of irrigation water as an integrated approach in enhancement of tomato quality.

6.3 Suggestions for Further Study

a) Studies should be undertaken on tomato to determine the concentration of allicin in *Allium fistulosum* crude extract that can be able to reduce bacterial wilt disease incidence and severity.
b) Studies should be established to determine effects of *Allium fistulosum* crude extract and irrigation levels on nutritional properties of tomato crop.
c) Studies should be carried out on phytochemical analysis to determine which phytochemicals present in *Allium fistulosum* crude extract which are responsible for reducing bacterial wilt incidence and severity.
d) Studies should be conducted to carry out molecular characterization of *Ralstonia solanacearum* pathogen causing bacterial wilt of tomato.

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APPENDICES

Appendix A. ANOVA Tables

Appendix 1: Diameter of inhibition zone on bacterial growth (mm) ANOVA (Trial one)

Source	DF	SS	MS	F Value	Pr > F
Treatments	5	67.13333333	13.42666667	109.85	<.0001
Error	12	1.46666667	0.12222222		
Total	17	68.6000000			

Appendix 2: Diameter of inhibition zone on bacterial growth (mm) ANOVA (Trial two)

Source	DF	SS	MS	F Value	Pr > F
Treatment	5	55.19777778	11.03955556	242.33	<.0001
Error	12	0.54666667	0.0455555		
Total	17	55.7444444			

Appendix 3: Disease incidence (14 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	24000.00	2666.67	29.09	<.0001
Error	50	4583.33	91.67		
Total	59	28583.33			

Appendix 4: Disease incidence (28 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	29125.00	3236.11	48.54	<.0001
Error	50	3333.33	66.67		
Total	59	32458.33			

Appendix 5: Disease incidence (42 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	29593.75	3288.19	38.50	<.0001
Error	50	4270.83	85.42		
Total	59	33864.58			

Appendix 6: Disease incidence (56 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	1	25927.08	2880.79	28.22	<.0001
Error	50	5104.17	102.08		
Total	59	31031.25			

Appendix 7: Disease incidence (70 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	19010.42	2112.29	20.69	<.0001
Error	50	5104.17	102.08		
Total	59	24114.58			

Appendix 8: Disease severity (14 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	52.00000000	5.7777	55.55	<.0001
Error	50	5.20000000	0.1040		
Total	59	57.20000000			

Appendix 9: Disease severity (28 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	55.72	6.191	30.96	<.0001
Error	50	4.00	0.200		
Total	59	59.72			

Appendix 10: Disease severity (42 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	42.92	4.76	39.67	<.0001
Error	50	2.66	0.12		
Total	59	45.84			

Appendix 11: Disease severity (56 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	48.00	5.33	202.00	<.0001
Error	50	1.32	0.026		
Total	59	49.32			

Appendix 12: Disease severity (70 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	48.00	5.33	202.00	<.0001
Error	50	1.32	0.026		
Total	59	49.32			

Appendix 13: % Disease severity Index (14 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	1142.08	126.89	29.86	<.0001
Error	50	212.50	4.25		
Total	59	1354.58			

Appendix 14: % Disease severity Index (28 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	1312.08	145.79	44.86	<.0001
Error	50	162.50	3.25		
Total	59	1474.58			

Appendix 15: % Disease severity Index (42 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	1067.08	118.56	43.11	<.0001
Error	50	137.50	2.75		
Total	59	1204.58			

Appendix 16: % Disease severity Index (56 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	1160.42	128.94	61.89	<.0001
Error	50	104.16	2.08		
Total	59	1264.58			

Appendix 17: % Disease severity Index (70 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	1160.42	128.94	61.89	<.0001
Error	50	104.17	2.08		
Total	59	1264.58			

Appendix 18: Plant height (14 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	128.73	14.30	47.68	<.0001
Error	50	15.00	0.30		
Total	59	147.73			

Appendix 19: Plant height (28 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	327.27	36.36	58.03	<.0001
Error	50	31.33	0.63		
Total	59	358.60			

Appendix 20: Plant height (42 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	1183.35	131.48	32.73	<.0001
Error	50	200.83	4.02		
Total	59	1384.18			

Appendix 21: Plant height (56 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	3115.27	346.14	56.44	<.0001
Error	50	306.67	6.13		
Total	59	3421.93			

Appendix 22: Plant height (70 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	5346.17	594.00	400.45	<.0001
Error	50	74.17	1.48		
Total	59	5420.18			

Appendix 23: Number of branches (14 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	34.72	3.858	72.52	<.0001
Error	50	2.66	0.0532		
Total	59	37.38			

Appendix 24: Number of branches (28 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	34.72	3.85	12.06	<.0001
Error	50	6.67	0.32		
Total	59	41.29			

Appendix 25: Number of branches (42 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	104.06	11.562	50.052	<.0001
Error	50	10.66	0.2132		
Total	59	114.72			

Appendix 26: Number of branches (56 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	118.66	13.184	70.73	<.0001
Error	50	9.32	0.1864		
Total	59	127.98			

Appendix 27: Number of branches (70 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	180.26	20.029	32.66	<.0001
Error	50	30.66	0.6132		
Total	59	210.92			

Appendix 28: Number of internodes (14 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	34.72	3.85	72.36	<.0001
Error	50	2.66	0.0532		
Total	59	37.38			

Appendix 29: Number of internodes (28 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	34.74	3.86	28.94	<.0001
Error	50	6.67	0.1334		
Total	59	41.41			

Appendix 30: Number of internodes (42 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	104.06	11.562	50.052	<.0001
Error	50	10.66	0.2132		
Total	59	114.72			

Appendix 31: Number of internodes (56 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	118.66	13.184	70.73	<.0001
Error	50	9.32	0.1864		
Total	59	127.98			

Appendix 32: Number of internodes (70 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	180.26	20.029	32.66	<.0001
Error	50	30.66	0.6132		
Total	59	210.92			

Appendix 33: Length of internodes (14 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	29.4	3.26	122.372	<.0001
Error	50	1.332	0.02664		
Total	59	30.732			

Appendix 34: Length of internodes (28 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	49.22	5.469	40.996	<.0001
Error	50	6.67	0.1334		
Total	59	55.89			

Appendix 35: Length of internodes (42 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	105.932	11.7702	147.128	<.0001
Error	50	4.000	0.08		
Total	59	109.932			

Appendix 36: Length of internodes (56 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	122.666	13.6295	102.323	<.0001
Error	50	6.66	0.1332		
Total	59	129.22			

Appendix 37: Length of internodes (70 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	206.934	22.993	143.706	<.0001
Error	50	8.0000	0.16		
Total	59	214.934			

Appendix 38: Stem collar diameter (14 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	12.00000000	1.33	49.813	<.0001
Error	20	1.333	0.02667		
Total	29	13.233			

Appendix 39: Stem collar diameter (28 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	21.82	2.424	30.3	<.0001
Error	50	4.00	0.08		
Total	59	25.82			

Appendix 40: Stem collar diameter (42 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	80.4	8.933	338.37	<.0001
Error	50	1.32	0.0264		
Total	59	81.72			

Appendix 41: Stem collar diameter (56 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	59.26	6.584	49.504	<.0001
Error	20	2.66	0.133		
Total	29	61.92			

Appendix 42: Stem collar diameter (70 days after planting) ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	42.72	4.747	89.23	<.0001
Error	50	2.66	0.0532		
Total	59	45.38			

Appendix 43: Days to 50% flowering ANOVA

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	60.6	6.733	63.117	<.0001
Error	50	5.334	0.10668		
Total	59	65.934			

Appendix 44: Number of fruits ANOVA (Trial One)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	381.3333333	42.3703704	5.25	0.0010
Error	20	161.3333333	8.0666667		
Total	29	542.6666667			

Appendix 45: Number of fruits ANOVA (Trial Two)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	383.3666667	42.5962963	7.10	0.0001
Error	20	120.0000000	6.0000000		
Total	29	503.3666667			

Appendix 46: Total fruit weight ANOVA (Trial One)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	6.84013667	0.76001519	11.03	<.0001
Error	20	1.37760000	0.06888000		
Total	29	8.21773667			

Appendix 47: Total fruit weight ANOVA (Trial Two)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	6.84013667	0.76001519	11.03	<.0001
Error	20	1.37760000	0.06888000		
Total	29	8.21773667			

Appendix 48: Total soluble solids ANOVA (Trial One)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	10.41633333	1.15737037	694.42	<.0001
Error	20	0.03333333	0.00166667		
Total	29	10.44966667			

Appendix 49: Total soluble solids ANOVA (Trial Two)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	8.59366667	0.95485185	1432.28	<.0001
Error	20	0.01333333	0.00066667		
Total	29	8.60700000			

Appendix 50: Tomato shelf-life ANOVA (Trial One)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	250.8000000	27.8666667	278.67	<.0001
Error	20	2.0000000	0.1000000		
Total	29	252.8000000			

Appendix 51: Tomato shelf-life ANOVA (Trial Two)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	220.8000000	24.5333333	122.67	<.0001
Error	20	4.0000000	0.2000000		
Total	29	224.8000000			

Appendix 52: Tomato Ascorbic acid ANOVA (Trial One)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	406.8534533	45.2059393	4.25	0.0034
Error	20	212.7634667	10.6381733		
Total	29	619.6169200			

Appendix 53: Tomato Ascorbic acid ANOVA (Trial Two)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	432.3594833	48.0399426	14.16	<.0001
Error	20	67.8609333	3.3930467		
Total	29	500.2204167			

Appendix 54: Tomato Lycopene ANOVA (Trial One)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	2661.054550	295.672728	62.52	<.0001
Error	20	94.583067	4.729153		
Total	29	2755.637617			

Appendix 55: Tomato Lycopene ANOVA (Trial Two)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	1426.228697	158.469855	55.16	<.0001
Error	20	57.458600	2.872930		
Total	29	1483.687297			

Appendix 56: Tomato Beta-Carotene ANOVA (Trial One)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	73.32256333	8.14695148	605.42	<.0001
Error	20	0.26913333	0.01345667		
Total	29	73.59169667			

Appendix 57: Tomato Beta-Carotene ANOVA (Trial One)

Source	DF	SS	MS	F Value	Pr > F
Treatment	9	56.37594667	6.26399407	515.70	<.0001
Error	20	0.24293333	0.01214667		
Total	29	56.61888000			

Appendix B. Research Permit

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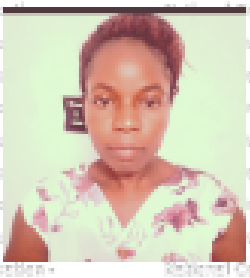

This is to Certify that Ms.. Edinah Musonya Shikoli of Egerton University, has been licensed to conduct research in Kakamega on the topic: INFLUENCE OF BUNCHING ONION (Allium fistulosum) CRUDE EXTRACT AND IRRIGATION LEVELS ON BACTERIAL WILT INCIDENCE, GROWTH, YIELD AND QUALITY OF TOMATO (Solanum lycopersicum L.) for the period ending : 27/September/2022.

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Appendix C: Abstract of First Published Paper

IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 14, Issue 9 Ser. I (September 2021), PP 47-56
www.iosrjournals.org

Allium fistulosum Crude Extract and Optimum Irrigation Levels as Alternative Management Option of Tomato Bacterial Wilt in Greenhouse

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Abstract

Tomato production in Kenya is limited due to abiotic and biotic constraints among them water availability and bacterial wilt caused by Ralstonia solanacearum. A study with objective of determining the effects of Allium fistulosum crude extract concentrations and irrigation levels on Ralstonia solanacearum inhibition in-vitro, and bacterial wilt disease incidence and severity on tomato grown in the greenhouse was conducted at KALRO-Kakamega, Kenya. The experiment employed a single factor treatment design with combination of different levels of Allium fistulosum crude extract and irrigation treated as distinct treatments. A CRD, with three replications were used in both the laboratory and greenhouse experiments. Treatments in the laboratory experiment were; negative control (distilled water), positive control (Greencop at 50g/20L) and Allium fistulosum concentrations at 0%, 5%, 10%, 15% and 20% while in greenhouse experiment were combinations of different levels of Allium fistulosum; 20%, 15%, 0%, positive control with four levels of irrigation, 0.5L, 1L, 1.5L and 2L/pot/week. Data were collected on diameter of zone of inhibition, disease incidence and disease severity and subjected to Analysis of Variance (ANOVA) using PROC GLM of the Statistical Analysis System (SAS) programme version 9.1. Tukey's Honestly Significant Difference (Tukey's HSD) mean separation test was conducted at α=0.05 level. The highest inhibition mean diameter of 11.48mm was obtained under 20% concentration of Allium fistulosum in the in-vitro antibacterial assay while the lowest inhibition mean diameter of 5.8mm was under negative control treatment. In the greenhouse experiment, all combinations of Allium fistulosum crude extract with irrigation levels generally reduced disease incidence and severity of tomato plant compared to positive and negative controls. The lowest disease incidence and severity was recorded with the use of 20% Allium fistulosum crude extract combined with either one litre or a half a litre of water while the highest disease incidence was recorded under positive control (Greencop) and negative control (0% extract) combined with two litres of water. In conclusion, Allium fistulosum crude extract concentration of 20% combined with one litres of water/pot/week is recommended to be used as alternative eco-friendly method in tomato production systems for the management of bacterial wilt. Future Studies should base on determining the concentration of allicin in Allium fistulosum crude extract that can be able to reduce bacterial wilt disease incidence and severity in the field.

Key words*: Tomato, Inhibition, Greenhouse, Ralstonia solanacearum, In-vitro, Incidence, Allium fistulosum, Severity*

Date of Submission: 06-09-2021 Date of Acceptance: 20-09-2021

Appendix D: Abstract of Second Published Paper

E. Afri. Agri. For. J (2021, Volume 85, 1-4, Pg. 31-39)

EFFECTS OF BUNCHING ONION CRUDE EXTRACT AND IRRIGATION LEVELS ON GROWTH AND YIELD OF TOMATO

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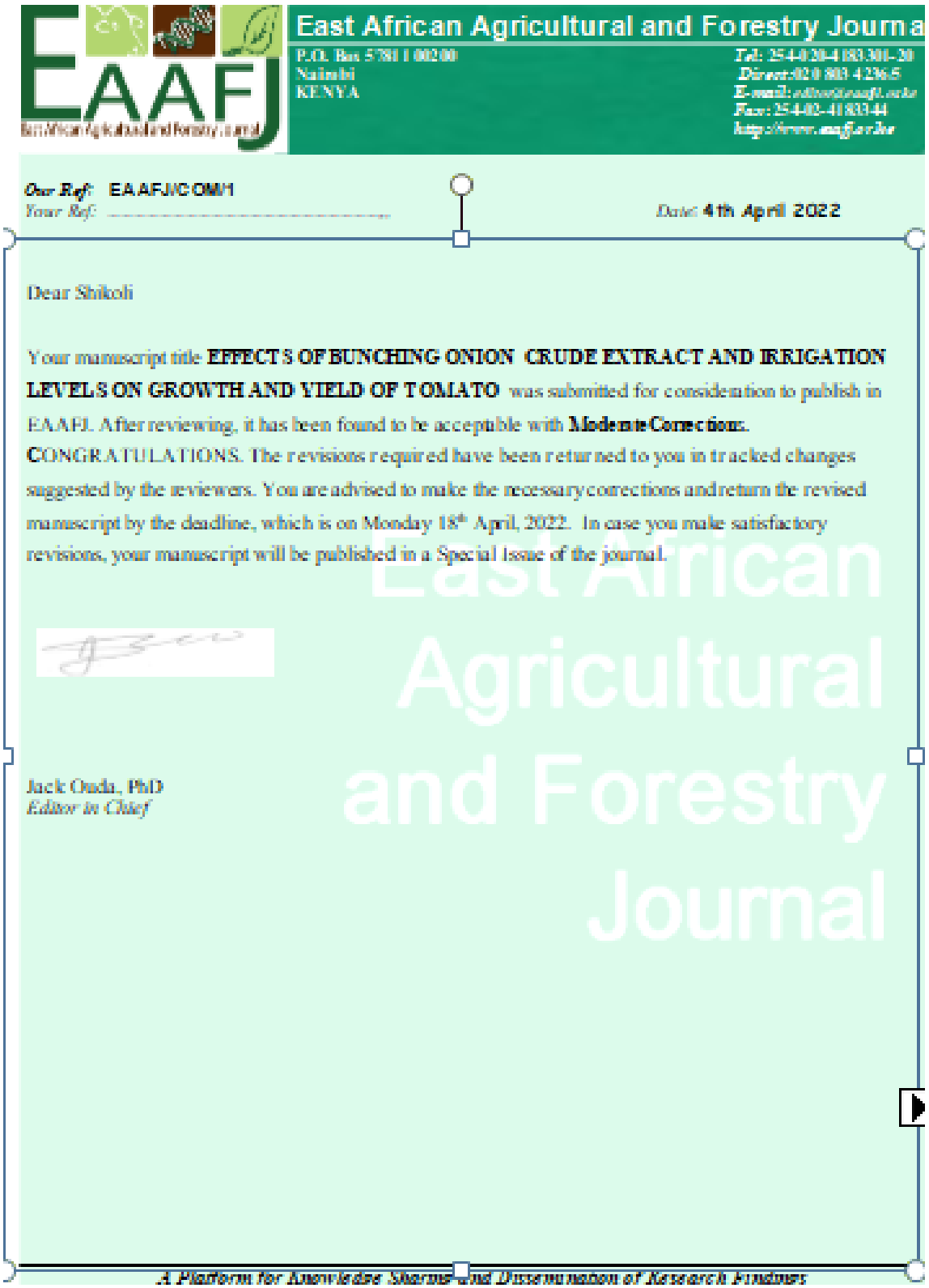
ABSTRACT

Tomato (Solunum lycopersicon L.) which belongs to solanaceae family is the second most important vegetable in Kenya. The objective of this study was to determine the effects of Bunching onion (Allium fistulosum) crude extract concentrations and irrigation levels on growth and yield of tomato. Greenhouse experiment was conducted in a completely randomized design (CRD) with three replications and employed a single factor treatment design with combinations of different levels of Allium fistulosum crude extract and different levels of irrigation treated as distinct treatments. The treatments consisted of combinations of Allium fistulosum at 20% and 15% with 4 levels of irrigation at 0.5 l, 1 l, 1.5 l and 2 l/pot/week and negative and positive control (green cop) combined with 2 l/pot/week. The results from the study showed that tallest plants and thickest stems were obtained under 20% combined with 2 l and 1.5 l of water. Tomato plants flowered early when treated with under 20% diluted in 1 l of water treatment. Highest number of tomato fruits and fruit weight were obtained under 20% combined with 2 l of water while the lowest number of fruits and fruit weight were obtained under the negative control treatment. From this study Allium fistulosum crude extract in combination with irrigation levels improved tomato growth and yield. It was clear that, concentration 20% of Allium fistulosum crude extract combined with in 2 l of water improved tomato growth and yield.

Key words: Tomato, Flowering, Internodes, Irrigation, Allium fistulosum

Appendix E: Letter of Acceptance for Publication of Manuscript



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Date: 4th April 2022

Dear Shikoli

Your manuscript title **EFFECTS OF BUNCHING ONION CRUDE EXTRACT AND IRRIGATION LEVELS ON GROWTH AND YIELD OF TOMATO** was submitted for consideration to publish in EAAFJ. After reviewing, it has been found to be acceptable with **Moderate Corrections.**
CONGRATULATIONS. The revisions required have been returned to you in tracked changes suggested by the reviewers. You are advised to make the necessary corrections and return the revised manuscript by the deadline, which is on Monday 18th April, 2022. In case you make satisfactory revisions, your manuscript will be published in a Special Issue of the journal.

East African Agricultural and Forestry Journal

Jack Onda., PhD
Editor in Chief

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