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

SHIKOLI, EDINAH. MUSENYA. [1*] OGWENO, JOSHUA. OTIENO. [1*]
MWANARUSI, SAIDI. [1*] AND WAYUA, FRANCIS. OBUORO. [2*]

[1] *Department of Crops, Horticulture and Soils, Faculty of Agriculture, Egerton University. P.O. Box 536-20115, Egerton, Kenya.*

[2] *Kenya Agricultural and Livestock Research Organization Kakamega- Non Ruminant Research Institute. P.O Box 169-50100, Kakamega, Kenya.*

Corresponding Author

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 fistulosumcrude 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 experimentwere; 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

I. Background

Tomato (*Solanum lycopersicon* L.) is the second most important vegetable in Kenya after potato.Productionis mainly carried out by small-scale growers with land sizes of between 0.5 to 2.5 Ha (Mbaka et al. 2013). The demand for the vegetable is on the rise, which has made farmers adopt high yielding varieties and modern technologies like greenhouse production to ensure all year roundproduction (HCD, 2019). The fruit contains ß-carotene, ascorbic acid and phenolic compounds which have nutritional benefits. Despite the increase in yields and area under production due to enhanced irrigation and expansion of greenhouse production, the prevalence of diseases such as bacterial wilt in many of the producing regions in the Kenya, remains as one of the major challenge to tomato production in the country (HCD, 2019).

Tomato bacterial wilt disease caused by *Ralstonia solanacearum* is one of the most devastating and important bacterial disease that affects many other crops such as potato, eggplant, geranium, weeds and wild plants. The pathogen can survive in surface water, soil, plant debris and vascular bundles that have wounds formed by lateral root emergency (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 soil borne populations of the pathogen and application of chemicals. However, these methods have had challenges because the pathogen can survive in soil in association with weed hosts, thus inhibiting the effect of crop rotation. In addition, the available land owned by small scale farmers is not enough for practicing rotational programmes (Fajinmi and Fajinmi, 2010). Application of chemicals is also another alternativecontrol method is on the other hand quite expensive and unaffordable to small scale farmers who rely on tomatoes as a source of livelihood. The chemicals are also 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 given unsatisfactory control (Fajinmi and Fajinmi, 2010). The above reasons has made farmers to look for alternative methods of controlling the disease.

The use of plant extracts plays a major role in management of bacterial wilt (Buyela, 2017). According to Balestra et al. (2009), plant extracts of *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. *Allium fistulosum* extracts are also rich in a wide range of secondary metabolites which are a major source of bioactive substances including phytochemicals such as alkaloids, tannins, flavonoids and phenolic compounds (Lee and Mitchell, 2011). The plant extracts not only suppress the disease and increase crop yield but also prevents environmental pollution,are locally available, and there is no buildup of resistance as a result of their repeated application, as a result of usage of pesticides(Buyela, 2017). These properties of *Allium fistulosum* plant extract therefore, makes them to offer a potential ecofriendly alternative for controlling tomato bacterial wilt.

Irrigation is the backbone of greenhouse agriculture.Irrigation 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 growth stage of 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 2.7 liters per plant per day (Georgios et al. 2018).

Studies by Agather et al. (2017), reported that *Ralstonia solanacearum* depends on water for proliferation and infection and that the 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 is waterborne. The objectives of this study were to determine the effects of *Allium fistulosum* crude extract concentrations on inhibition of *Ralstonia solanacearum in-vitro* and to determine the effects of combination of *Allium fistulosum* crude extract concentrations and optimum irrigation levels on bacterial wilt disease incidence and severity on greenhouse grown tomato.

II. Methods

Experimental Site Description

Two trialswere conducted in a greenhouse and plant pathology and molecular laboratory of Kenya Agricultural & Livestock Research Organization (KALRO)-Kakamega, Kenya. KALRO-Kakamega 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 m above sea level (Jaetzold and Schmidt, 2012).

Laboratory Experiment

The aim of laboratory experiment wasto determine the effects of *Allium fistulosum* crude extract concentrations on inhibition of *Ralstonia solanacearum in-vitro.*

Media Preparation (TZC- Triphenyl Tetrazolium Chloride)

In a litre of distilled water solution, 1 g casamino acid, 10 g Peptone, 5g glucose and 30 g agar were added to make triphenyl tetrazolium chloride. The solution was then autoclaved at 121 ℃ for 20 minutes. The media was cooled to 55 ℃ and 0.05 mg of tetrazolium chloride(TTC) was added. It was then further autoclaved for 5 minutes at 121 ℃ and left to cool 25 ℃for experimental use.

Isolation and culture of *Ralstonia solanacearum*

Diseased tomato plants were collected from infested fields in Kakamega. Plants that showed characteristic symptoms of wilting of youngest leaves during hottest time of the day and brown spots on stem were examined

for disease symptoms. Presence of the pathogen in the crop was confirmed by an ooze test Kumar et al. (2017).Isolation was done on solidified triphenyl tetrazolium chloride (TZC) agar medium as shown in figure 1 below. The plant parts were taken to the laboratory and section of roots and stems were cut into 1 cm portionswith aid of a scalpel, then surface sterilized in 0.5% sodium hypochloride for three minutes and then rinsed in three series of sterile water to remove traces of sodium hypochloride. One gram of infected tissue pieces were macerated in 1 ml of sterile distilled water and a loopful of resulting suspension was streaked over surface of TZC agar media in autoclaved petri dishes using a sterile loop. The aim here was to achieve uniform spread so that individual colonies are formed. The spread was done by sterilizing the inoculating loop with a flame and spreading out the initial in a successive cycle over the growth media (Thomas et al. 2015). The plates were then incubated at 33 ℃ for 48 hours and well separated virulent colonies were observed. A single colony obtained from above culture was then sub cultured on TZC agar media to obtain a pure culture used for serial dilutions according to Kumar et al. (2017).

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

Preparation of Onion Extracts

Plant extracts from bunching onions collected from farmers fields in Kakamega were extracted using water as per the procedure described by Odey et al. (2012). Collected plant parts (roots and leaves) were washed using distilled water and then chopped into small pieces using a knife. The plant parts were then placed in an oven to dry for 48 hours at a temperature of 65℃ thereafter ground into fine particles (powder). The different concentrations of water extracts were prepared by mixing 0 g (0:100), 5 g (1:20), 10 g (1:10), 15 g (1:6.7) and 20 g (1:5) of each plant parts (roots combined with leaves) in 100 ml sterile distilled waterto produce extract concentrations of 0%, 5%, 10%, 15% and 20%, respectively. The extracts were then sieved in cheese cloth and stored in clean containers for experimental use.

Preparation of Bacterial Inoculum to be used for Inoculation

With the aid of a micro-pipette, 25µl of *Ralstonia solanacearum* stock suspension was added to 10 ml of 2, 3, 5-triphenyl tetrazolium chloride (TZC) agar medium suspended in each of the 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 concentrated, 1×10^1,1×10^2,1×10^3,1×10^4, 1×10^5, 1×10^6 1×10^7 and1×10^8 colony forming unit per milliliter of bacterial suspension by use of an inoculating loop(Pontes et al. 2017). The desired concentration of 1×10^8 cfu/ml was adjusted with the aid of a tally counter(Pontes et al. 2017). 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).

Paper Disc Diffusion Test

Antibacterial activity of crude extracts of *Allium fistulosum* at 0%, 5%, 10%, 15%, 20% (w/v) and positive control (Greencop at 50g/L) was detected by use of the paper disc diffusion method described byTeng et al.(2010). Sterilized filter paper discs of 5 mm diameter soaked in one ml of prepared *Allium fistulosum* were placed in the middle of 90 mm diameter petri dishes containing 10 ml of TZC agar media suspended with 25 µl of bacterial suspension at a concentration of 1 $x10^8$ Colony Forming Unit per ml. Bacterial suspension was spread evenly with aid of a sterilized metallic spreader which was done separately for each treatment. The plates were then incubated at 33 ℃ for 48 hours. Inhibition zones around the paper discs were measured using a standard vernier caliper and recorded in millimeters.

Experimental Design and Treatments

The experiment was set up in a Completely Randomized Design (CRD) with three replications. The experiment was repeated once in the laboratory.There were six treatments for laboratory study namely 0%

(negative control), Greencop 50g/20L (positive control), *Allium fistulosum* (5%), *Allium fistulosum* (10%), *Allium fistulosum* (15%) and*Allium fistulosum*(20%).Each petri dish represented a treatment giving a total 18 petri dishes in each the first and second trial.

Data Collection and Analysis

Data was collected on the diameter of zone of inhibition for growth of *Ralstonia solanacearum* pathogen. The diameter of zone of inhibition wasmeasured in millimeters (mm) using a vernier caliper and recorded as a measure of the antibacterial activity of each treatment.

Plant Materials

The tomato variety used in the study was Sodagar F1 an indeterminate variety 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 and Dienya, 2015). Bunching onion plant material was used in the study as it has antibacterial properties that have potential for controlling various plant pathogens. The Sodagar F1 variety and bunching onion was sourced from farmers' fields in Kakamega.

Greenhouse Experiment

The aim of the greenhouse was to determine the effects of combination of *Allium fistulosum* crude extract concentrations and optimum irrigation levels on bacterial wilt disease incidence and severity on greenhouse grown tomato.

Soil Steam Sterilization

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. 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).

Crop Establishment and Maintenance

Tomato seedlings were established in a nursery until they attained the stage of three to four 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. 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 five 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. Most vigorous and disease free tomato seedlings dipped in the bacterial inoculum solution were then transplanted late in the evening to minimize transplanting shock where one seedling was transplanted in each pot. 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 and 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. 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 the materials at a temperature of 121℃ for 15 minutes in order to kill the pathogen.

Experimental Design and Treatments

The experiment was set up in a Completely Randomized Design (CRD) with three replications. The experiment was repeated once in the greenhouse.In greenhouse study, 10 treatments were used namely *Allium fistulosum* (15%) + Water Level of 0.5/pot/week, *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 which were the most effective in the laboratory experiment were used in greenhouse experiment.

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. Each replicate therefore consisted of 10 experimental units with each represented by 5 pots.

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 since bacterial wilt 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 were done showed absence of bacterial wilt (Gelsomino et al. 2010).

Data Collection and Analysis

Data was collected on the disease incidence and disease severity. The diameter wasmeasured in mm using a vernier caliper and recorded as a measure of the antibacterial activity of each treatment. Disease incidence was assessed as a percentage of wilted plants within each experimental unit and calculated according to Getachew et al. (2011) as:

$DI = \frac{\text{NPSWS}}{NPPT} \times 100\%$.....Equation (1)

where;DI = Disease Incidence, NPSWS = Number of plants showing wilt symptoms and NPPT = Number of plants per treatment

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 and 5 = death (collapse) of whole plant.

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

$PSI = \frac{\sum scores \times 100}{NPR \times MSC}$.....Equation (2)

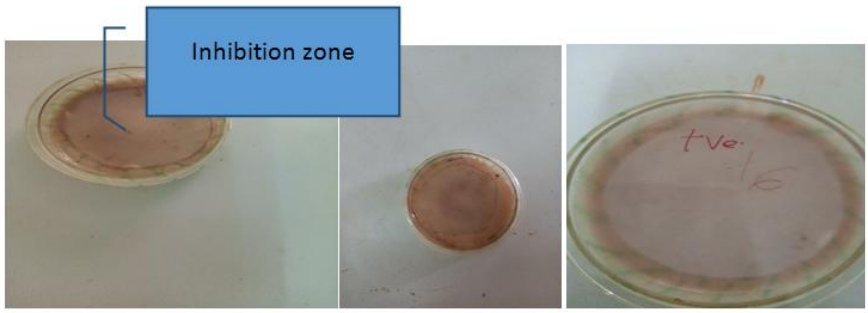
where;PSI = Percent severity index, NPR = Number of plants rated and MSC = Maximum scale of the scores

Data collected was subjected to analysis of variance (ANOVA) at5% level of significance. The Tukey's Honestly Significant Difference (Tukey's HSD) test at 5% level of significance means was used separation. The general linear model procedure of the Statistical Analysis System (SAS) program, SAS version 9.1 (SAS institute Inc, 2010) was used in the analysisthe data.

III. Results

Laboratory Experiment

Use of *Allium fistulosum* crude extractsignificantly inhibited*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 (Figure2 and Table1). 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). Furthermore, the results from 5% concentration and Positive control (Greencop) were not significantly different from each other (P=0.05). However the negative control treatment resulted in significantly lower inhibition diameter compared to all the other treatments in both trials.



10 % 5 % negative control

Figure 2: Effect of different concentrations of *Allium fistulosum* crude extract (20%, 15%, 10%, 5 %, positive and negative control) on diameter of zone of inhibition

Table 1: 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)

Greenhouse Experiment

Bacterial wilt disease incidence of tomato was significantly influenced by the use of *Allium fistulosum* crude extract in combination with and irrigation at different levels as a result of the concentration used (Table 2). In both the trials, 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 except at 28 days after transplanting in trial one. 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 compared to the other treatments but with no significant difference (P=0.05) in both trials. In the second trial 20% and 15% concentrations with all the combinations of irrigation water was significantly different with both positive and negative control in all the days of data collection. Furthermore, the lowest levels of disease incidence in these treatments were recorded on the 56 and 70 days after transplanting (DAT) in both trial one and two by application of *Allium fistulosum* crude extract at 20% combined with all the irrigation water levels.

Table 2: Effect of *Allium fistulosum* crude extract concentrations and irrigation levels on for bacterial wilt disease incidence in %

Treatments	Trial 1					Trial 2				
	14dat	28dat	42dat	56dat	70dat	14dat	28dat	42dat	56dat	70dat
-ve +2L	91.7a*	83.3a*	75.0a*	66.7a*	58.3a*	75.0a*	75.0a*	75.0a*	66.7a*	58.3a*
+ve +2L	50.0b	50.0b	41.7b	41.7b	33.3b	50.0b	50.0b	41.7b	41.7ab	33.3ab
15%+2L	33.3bc	25.0c	25.0bc	25.0bc	25.0bc	33.3bc	25.0c	16.7bc	16.7bc	16.7bc
15%+1.5L	33.3bc	25.0c	25.0bc	25.0bc	25.0bc	25.0cd	25.0c	8.3c	8.3c	8.3bc
15%+1L	25.0bc	25.0c	25.0bc	16.7cd	16.7bcd	25.0cd	25.0c	8.3c	8.3c	8.3bc
15%+0.5L	25.0bc	25.0c	16.7cd	8.3cd	8.3cd	25.0cd	16.7cd	0.0c	0.0c	0.0c
20%+2L	25.0bc	25.0c	8.3cd	0.0d	0.0d	25.0cd	16.7cd	0.0c	0.0c	0.0c
20%+1.5L	25.0bc	16.7cd	0.0d	0.0d	0.0d	16.7cde	0.0d	0.0c	0.0c	0.0c
20%+1L	25.0bc	8.3cd	0.0d	0.0d	0.0d	8.3de	0.0d	0.0c	0.0c	0.0c
20%+0.5L	16.7c	0.0d	0.0d	0.0d	0.0d	0.0e	0.0d	0.0c	0.0c	0.0c

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

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 (Table 3 and 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 sampling days. 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 both 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) which recorded lowest severity and percentage severity index. Disease severity was generally higher in trial one than in trial two which could be attributed to the high temperatures experienced in the greenhouse that enhanced the thriving of the disease.

Table 3: Effect of *Allium fistulosum* Crude Extract Concentrations and Irrigation Levels on Bacterial Wilt Disease Severity (scale of 0-5)

Treatments	Trial 1					Trial 2				
	14dat	28dat	42dat	56dat	70dat	14dat	28dat	42dat	56dat	70dat
-ve +2L	4.0a*	3.7a*	3.0a*	3.0a*	3.0a*	3.3a*	3.3a*	3.0a*	3.0a*	3.0a*
+ve +2L	1.7b	1.3b	1.0b	1.0b	1.0b	1.3b	1.3b	1.0b	1.0b	1.0b
15%+2L	1.3b	1.0b	1.0b	1.0b	1.0b	1.3b	1.0bc	1.0b	0.7bc	0.7bc
15%+1.5L	1.0b	1.0b	1.0b	1.0b	1.0b	1.0bc	1.0bc	0.7bc	0.3bc	0.3bc
15%+1L	1.0b	1.0b	1.0b	0.7b	0.7b	1.0bc	1.0bc	0.3bc	0.0c	0.0c
15%+0.5L	1.0b	1.0b	0.7bc	0.0c	0.0c	1.0bc	0.7bc	0.3bc	0.0c	0.0c
20%+2L	1.0b	1.0b	0.3bc	0.0c	0.0c	1.0bc	0.7bc	0.0c	0.0c	0.0c
20%+1.5L	1.0b	0.7bc	0.0c	0.0c	0.0c	0.7bc	0.0c	0.0c	0.0c	0.0c
20%+1L	0.7b	0.0c	0.0c	0.0c	0.0c	0.3bc	0.0c	0.0c	0.0c	0.0c
20%+0.5L	0.7b	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c

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

Table 4: Effect of *Allium fistulosum* Crude Extract Concentrations and Irrigation Levels on Bacterial Wilt Disease Severity Index (%)

Treatments	Trial 1					Trial 2				
	14dat	28dat	42dat	56dat	70dat	14dat	28dat	42dat	56dat	70dat
-ve +2L	20.0a*	18.3a*	15.0a*	15.0a*	15.0a*	16.7a*	16.7a*	15.0a*	15.0a*	15.0a*
+ve +2L	8.3b	6.7b	5.0b	5.0b	5.0b	6.7b	6.7b	5.0b	5.0b	5.0b
15%+2L	6.7b	5.0b	5.0b	5.0b	5.0b	6.7b	5.0bc	5.0b	3.3bc	3.3bc
15%+1.5L	5.0b	5.0b	5.0b	5.0b	5.0b	5.0bc	5.0bc	3.3bc	1.7bc	1.7bc
15%+1L	5.0b	5.0b	5.0b	3.3b	3.3b	5.0bc	5.0bc	1.7bc	0.0c	0.0c
15%+0.5L	5.0b	5.0b	3.3bc	0.0c	0.0c	5.0bc	3.3bc	1.7bc	0.0c	0.0c
20%+2L	5.0b	5.0b	1.7bc	0.0c	0.0c	5.0bc	3.3bc	0.0c	0.0c	0.0c
20%+1.5L	5.0b	3.3bc	0.0c	0.0c	0.0c	3.3bc	0.0c	0.0c	0.0c	0.0c
20%+1L	3.3b	0.0c	0.0c	0.0c	0.0c	1.7bc	0.0c	0.0c	0.0c	0.0c
20%+0.5L	3.3b	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c

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

IV. Discussion

Use of plant extracts in management of crop diseases especiallybacterial wilt of tomato can be attributed to a significant supply of anti-microbial and antibacterial compounds that have properties that can 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 and Ayaz, 2010). This makes use of plant extracts as a cheap, environmental 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* used in this study as it can offer 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 *Ralstonia solanacearum in-vitro*. Diameter of zone of inhibition remained highest on application of 20% of *Allium fistulosum* crude extract concentration compared to the negative control which

gave the lowest diameter on zone of inhibition for growth of bacteria. This reduction was attributed to 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).

Treatment of 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 which was not significantly different from 10% at P=0.05. 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*. These 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 5%, positive control 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 them at P=0.05.

The presence of *in- vitro*growth inhibition by the treatment of 20% extract may be further explained by the fact that there was sufficient concentration of secondary metabolite in the *Allium fistulosum*plant material. Furthermore, the presence of Allicin a sulphur volatile compound in the extract played a major role in enhancing the anti-microbial properties responsible for inhibition of the growth of the bacteria(Hussein et al. 2017). Furthermore, 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 and Mitchell, 2011). The results of the present study are in agreement with those of Deberdt et al. (2012) and Wagura et al. (2015), where application of *Allium fistulosum* extracts inhibited the growth of *Ralstonia solanacearum* Phylotype IIB/4NPB *In-vitro* and crude medicinal plant extract of *Ocimum gratisimum*, *Brassica oleracae* and *Ipomoea batatas*also managed*Ralstonia solanacearum* pathogen *In- vitro.*

Furthermore, the results of present study are also in agreement with those of Anton et al. (2021), they reported that use of plant extracts from *Lantana camara*, *Allium sativum*, *Azadirachta indica* and *Solanum incanum* controls *Ralstonia solanacearum* causing bacterial wilt in tomatoes (Solanum lycopersicum). Their 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). Their studies showed 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. Results obtained by Mitali et al. (2012) confirmed that plant extracts of *A. conyzoides* to controlled*Clavibacter michigenesis* andthe diameter of zone of inhibition of 10.67 mm was recorded with a concentration of 15%. The results of the study are also in consonance with the current study where a higher concentration of 20% plant extract resulted in highest zone of inhibition of 11.8cm in diameter.

Findings of the present study are also 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 fresh *Allium sativum* suppressed growth rates of *Pyricularia oryzae* and *Phytophthora cactorum* at the 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.

Greenhouse 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 treatment with (20%+0.5L) of irrigation water and higher in the positive control (Greencop at a recommended rate of 50g/L)(ve+2L) and negative control (-ve+2L). These results of the present study were conforming to those by(Balestra et al. 2009 Hassan et al 2013;Kamal et al. 2020). Application of plant extracts combined with optimum water level was reported to reduce a number of crop diseases due to presence of sulfur volatiles produced upon degradation of allium tissues. Extracts also contains allicin with potential antibacterial properties for controlling a number of bacterial and fungal diseases. The mechanism for control of *Ralstonia solanacearum* is through the presence of active ingredients in plant extractsthat reduced disease incidence and severity by acting on pathogen directly, through stimulation or induction of systemic resistance in tomato plant andinduction of some antioxidant enzymes that reduced number of pathogens in the tissues, therefore reduced disease incidence and severity. In addition, plant extracts are of natural origin, biodegradable, produce non-toxic residues, noaccumulation on the environment and therefore are superior in comparison with use of chemicals.

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 and Oduor 2016). In the current 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.

V. Conclusions

The study findings revealed that 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. Furthermore, concentrations of *Allium fistulosum* crude extract and irrigation levels reduces bacterial wiltincidence and severity in greenhouse, therefore, farmers using greenhouse for tomato production in Kenya can usea combination of 20% concentration of *Allium fistulosum* crude extract with half a litre of irrigation water per plant per week as alternative management for *Ralstonia solanacearum*.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.

List of Abbreviations

CRD: Completely Randomized Design DAT: Days after Transplanting GLM: General Linear Model; HCD: Horticultural Crop Directorate; HSD: Honesty Significant Difference; KALRO: Kenya Agricultural and Livestock Research Organization; SAS: Statistical Analysis System.

Acknowledgements

I gratefully acknowledge the financial support of Kenya Climate Smart Agriculture Project (KCSAP) and which lead to success of this research project. I also express my sincere gratitude to the entire team of Kenya Agricultural and Livestock Research Organization (KALRO KAKAMEGA), for providing laboratory where the research was conducted and the necessary equipments in the laboratory.

Authors' contributions

All authors jointly facilitated the design of experiments. SEM conducted laboratory in-vitro antibacterial bioassay and greenhouse study, conducted data collection and analysis, drafted the manuscript with inputs from all authors. OJO, MS and WFO worked in collaboration with SEM right away from design of experiment to data analysis. All authors read and approved the final manuscript.

Funding

The study was funded by KCSAP (Kenya Climate Smart Agriculture Project) Reference Number KCSAP/COMP.2/VOL. (68).

Availability of data and materials

The datasets collected and analysed during the present study area available from the corresponding author on reasonable request.

Ethical approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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SHIKOLI, EDINAH. MUSENYA, et. al. "Allium fistulosum Crude Extract and Optimum Irrigation Levels as Alternative Management Option of Tomato Bacterial Wilt in Greenhouse." *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 14(9), 2021, pp. 47-56.