In vivo studies on the effect of *Warburgia ugandensis* crude extracts against bacterial wilt in tomato

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Abstract

Tomato plants are affected by *Ralstonia solanacearum* which causes bacterial wilt, a devastating soil-borne disease that has no treatment. *Warburgia ugandensis* crude extract have exhibited biocontrol properties against pathogenic fungi and bacteria in animals but in plant the information is limited. The current study was done to evaluate the in vivo efficacy of *W. ugandensis* crude extracts against *R. solanacearum* in tomato plants. *W. ugandensis* leaf and stem bark crude extracts were obtained using ethanol, methanol, hexane, and dichloromethane. The obtained crude extracts were tested against *R. solanacearum* in the greenhouse. Tomato plants established in soil inoculated with *R. solanacearum* and treated with dichloromethane crude extract of *W. ugandensis* stem bark showed no sign of bacterial wilt disease and were comparable to the positive control. Tomato plants established in soil inoculated with *R. solanacearum* but treated with *W. ugandensis* leaf ethanol crude extract had the highest average height of 62.50 cm which was similar to positive control. The study proposed that *W. ugandensis* crude extract has the ability to be used as antibacterial biocontrol against *R. solanacearum*. Further research is important to determine the bioactive compounds against *R. solanacearum*.

Keywords

Tomato, *Ralstonia solancearum* and *Warburgia ugandensis*

1. Introduction

Tomato (*Solanum lycopersicum*) ranked fourth among the leading vegetables in the world (FAOSTAT, 2020) is native to South America (Jenkins, 1948; Razifard et al., 2020). It is a widely grown and consumed vegetable in Kenya with about 28.3 hectares under production that account for 599.458 tonnes (FAOSTAT, 2020). It is an income-generating crop in high-potential and peri-urban areas. Main production counties in Kenya include Meru, Kirinyaga, Kajiado, Taita Taveta, Bungoma, and Kwale (Avedi et al., 2022).

Tomatoes' fruits are used as salad, cooked as vegetables or processed as tomato source, tomato paste, ketchup and juice (Agus et al., 2023). It is nutritionally important as source of vitamins and minerals. The main varieties grown in Kenya can be categorised as those grown in greenhouses and those grown in the field. Varieties grown in green houses include Tylka FI, Riogrande, Claudia F1, among others. Those grown under field condition include Libra F1, Kentum F1, Prostar F1, Cal J among others. Although greenhouse production is slowly gaining popularity, most of tomato production in Kenya is done under open field conditions. Thus, tomato production is hugely affected by numerous agro ecological factors such as climatic and soil conditions. It is now well established under optimal rainfall conditions with supplemented irrigation, tomato does well under average temperatures of between 20 °C-27 °C in a wide range soil conditions such as well drained, deep and uniform clay and silt or loam soils with an average pH of 6.0 to 7.0 (Kumar et al., 2020; Ochieng et al., 2016).

However, because of its versatile potential to grow in a wide range of agro ecological conditions, the tomato crop attracts numerous pests and diseases. Among the major pests that infests tomato crop include white flies, spider mites, nematodes, thrips, aphids, and leaf miners (Mani, 2022) that act as disease vectors. Additionally, tomato crop is affected by various diseases such as fusarium wilt, yellow leaf curl virus, leaf spot, powdery mildew, bacterial spot, bacterial wilt, and late blight (Solankey et al., 2021). Among these, bacterial wilt has been shown as the most lethal because the affected plant has no known effective control. Bacterial wilt is a lethal vascular soil-borne disease caused by *R. solancearum* (Dickstein et al., 2023; Yabuchi et al., 1995). It is known to occur in a wide range of agro ecological zones globally including; sub-tropical, tropics, and warm temperate. The disease is also known to affect more than 450 species in 54 different families with disease incidence ranging from 63% to 100% (Elphinstone, 2005; Kurabachew and Ayana, 2017; Lebeau et al., 2011; Manda et al., 2020). Affected plants easily manifest symptoms mid production cycle such as a flaccid appearance on the young leaves especially in normal warm environmental conditions and subsequently wilt to death (Jiang et al., 2017). The vascular tissue of the stem shows a brown discoloration and when cut cross-section drops of white or yellow bacterial ooze may be visible (Heikrujam et al., 2020).

Despite decades of global efforts in finding an effective control strategy for bacterial wilt in order to reduce losses incurred by farmers, still, no effective control mechanism has so far been reported. Currently, existing control strategies include; cultural, chemical, biological, host resistance, or a combination of all to form an integrated disease management mechanism (Benti, 2023; Schaad et al., 2003)

Traditionally, plants affected by bacterial wilt are rogued and destroyed because no chemical control agents are available to treat and heal the plant. Additionally, it is recommended that plots with affected bacterial plants should be put on a rotational regime with alternative crops from unrelated families. Previous reports had also shown efforts toward breeding for resistance while biotechnology geared towards genetic manipulations has produced some successful candidates (Patil et al., 2012; Vailleau & Genin, 2023).

Chemical control has so far been restricted to soil fumigation using inorganic chemicals such as propiconazole, carbendazim, benomyl, flubendazole, or methyl bromide (Manda et al., 2020) which has since been withdrawn from the market because of its negative impact on the environment. Others include sodium hypochlorite which indiscriminately eradicates microflora and reduces soil health conditions. All these methods lead to long-term soil degradation and are expensive.

In the recent past, a wide range of biocontrol strategies have been reported. Among these include plants based biochemicals that can be used as biochemicals to control diseases. Plant biochemical derivatives have been shown to be more environmentally friendly than synthetic chemicals (Hernández-Díaz et al., 2021). In vitro and in vivo investigations have reported some plants with antimicrobial potential in Salvia, Organum, and Thymus genera (Hachlafi et al., 2023; Jabeen et al., 2008). *Azadirachta indica* methanol extract has antimicrobial properties against *Escherichia coli*, Salmonella, and Streptococcus in animals and humans (Hemdan et al., 2023; Maragathavalli et al., 2012). *Moringa oleifera* leaf and seed extracts have antibacterial properties against *Micrococcus kristinae*, *Aeromonas caviae*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus vulgaris*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Vibrio cholera* and *E. coli* pathogenic bacterial in animals and human (Jabeen et al., 2008; Jikah & Edo, 2023). Ibrahim and Kebede, (2020) also asserted that *M. oleifera, Lepidium sativum,* and *A. indica* leaf extracts had antimicrobial activity against *Shigella boydii*, *Salmonella Typhi*, *Streptococcus agalactiae,* and *S. aureus* pathogenic bacteria in human. Nevertheless, there is limited information about the effect of plant extracts against plant pathogens. *A. indica*, *Tithonia diversifolia,* and *Allium sativum* extracts were used in vitro treatment to manage common bean *Phaeoisariopsis griseola* (Chauhan & Tapwal, 2023). *Rosemarinus officinalis* crude extract was used to manage plant diseases such as *Xanthomonas oryzae pv. Oryzae*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Alternation alternate* and *Colletotrichum graminicola,* and even *R. solanacearum* (Abdallah et al., 2019; El-Wahed et al., 2023). Deberdt et al., (2012) opined that *Allium fistulosum* extract was effective against *R. solanacearum* in both in vivo and in vitro treatments.

Extracts obtained from *Warburgia ugandensis* have been used traditionally in Africa as medicine to treat ailments such as toothaches, constipation, and fever among others (Okello and Kang, 2021). *W. ugandensis* has been reported to have bioactive compounds that confer antimicrobial properties against early and late blight pathogens in tomatoes (Prieto et al., 2013). A series of unique sesquiterpentine 1-4 dialdehydes isolated from *W. ugandensis* have been shown to have broad antibacterial and antifungal activities (Ikeagwuonu & Adjeroh, 2024). Polygodial, warbuganal, and muzigadial obtained from these plants show similar antibacterial spectra (Nkqenkqa & Mundembe, 2023). Nevertheless, there is limited information on the utilization of *W. ugandensis* stem bark and leaf crude extracts to control plant pathogens. The study assessed the efficacy of *W. ugandensis* stem barks and leaf crude extract obtained using organic solvents such as ethanol, methanol, hexane, and dichloromethane as potential in controlling *R. solanacearum* which cause bacterial wilt in tomatoes.

2. Materials and methods

2.1 Sample collection

Leaves and stem barks of *W. ugandensis* were collected from the identified trees at Meru University of Science and Technology (MUST). Debarking was done using a machete for stem bark which was cut into small pieces and leaves were plucked then washed under running tap water and then with sterile distilled water. Leaves and stem barks were dried under shade for three weeks and milled into a fine powder using an electrical blender and subjected to the extraction protocol.

2.2 Organic solvents extraction of *W. ugandensis* leaf and stem bark

Fifty grams of *W. ugandensis* leaf and stem bark powder was transferred into one litre conical flask. Sequential extraction was done with 80% organic solvents viz. dichloromethane, methanol, hexane, and ethanol. Two hundred millilitre of each organic solvent was added to the conical flask and placed on a shaker and soaked for 72 hours. The samples were filtered using Whatman filter paper (No. 1). The filtrate was concentrated using a rotary evaporator at their respective organic solvent boiling points. The concentrate was sterilized by vacuum filtration. The crude extracts were transferred into sterile falcon tubes that were labeled and caped tightly. The crude extracts were stored in a refrigerator at 4 ^{o}C.

2.3 Isolation of R. solanacearum

A survey was done at MUST demonstration farm in Meru County to identify affected tomato plants. The preliminary test of observing wilting of the plants was done. The soil in the rhizosphere of affected tomato plants was collected.

R. solanacearum was isolated from soil in the vicinity of affected Irish tomato plants at MUST biological laboratory. Ten grams of dry soil was placed in a beaker and 100 ml of sterilized distilled water was added. The sample was agitated for 20 minutes and serial dilutions were then carried out by adding 1 ml of the sample to 9 ml of sterilized distilled water to a dilution of 10^{-9}. One hundred microlitres of the solution was spread on the Kelman's 2, 3, 5- Triphenyl tetrazolium chloride (TZC) medium that was dispensed in a Petri plate and incubated at 28 ^{o}C for 24 h to 48 h

2.4 Subculturing to obtain a pure culture

The single colony technique was adopted to obtain pure cultures and identified virulent *R. solanacearum* isolates grown on Kelmans 2, 3, 5-TZC medium as stated in Bergey's Manual of Systematic Bacteriology (Aslam & Mukhtar, 2023; Sneath et al., 1986). The microorganisms of interest were picked by sterilized inoculating wire loop from a mixed culture streaked on a Petri plate containing Kelman's 2, 3, 5-TZC medium and incubated at 28 °C. The observation was made after 12 hours to 48 hours, restreaking on fresh Petri plates containing 2, 3, 5-TZC medium was done to ensure purity. *R. solanacearum* was suspended and stored in distilled water at room temperature (15 ºC min. and 28 ºC max.) and restreaked every six months to maintain virulence (García et al., 2019; Shahbaz et al., 2015).

2.5 Confirmatory tests for *R. solanacearum*

The tests below were carried out to determine the presence of *R. solanacearum.*

2.5.1 Gram staining test for *R. solanacearum*

A loop full of the bacteria was spread on a glass slide and fixed by heating on a very low flame. Aqueous crystal violet solution (0.5%) was spread over the smear for 30 seconds and then washed with running tap water for one minute. It was then flooded with iodine for one minute, rinsed in tap water, and decolorized with 95% ethanol until colourless runoff. After washing the specimen was counter-stained with safranin for approximately 10 seconds, washed with water, dried, and observed microscopically at 10X, 40X, and 100X using oil (Aslam & Mukhtar, 2023; Khasabulli et al., 2017).

2.5.2 Catalase oxidase test for *R. solanacearum*

Young agar cultures (18-24 hrs) and 3% hydrogen peroxide (H_2O_2) were used to observe the production of gas bubbles. A loop full of bacterial culture was mixed with a drop of H_2O_2 on a glass slide and observed for the production of gas bubbles with the naked eye and under a dissecting magnification of 25X (Aslam & Mukhtar, 2023; Khasabulli et al., 2017).

2.5.3 Potassium hydroxide test for *R. solanacearum*

Bacteria were aseptically removed from Petri plates with an inoculating wire loop, placed on a glass slide in a drop of 3% KOH solution, stirred for 10 seconds, and observed for the formation of slime threads (Aslam & Mukhtar, 2023; Khasabulli et al., 2017).

2.6 Preparation of bacterial inoculum

Inoculum of the *R. solanacearum* was prepared by culturing it on Kelman's 2, 3, 5-TZC medium (1 g of Casamino acids, 10 g of peptone, 5 g of glucose in 1000 ml of distilled water. To control saprophytic bacteria and fungi there was addition of 100mg polymyxin ß sulfate,25mg bacitracin 5mg chloromycetin, 0.5g Penicillin and 100mg cycloheximide and 30 minutes prior to use 5 ml of 70% ethanol was dissolved) (Aslam & Mukhtar, 2023; Champoiseau et al., 2009; García et al., 2019; Kelman, 1954; Khasabulli et al., 2017). Cultures were suspended in distilled water and were adjusted to 1×10^{8} CFU ml^{-1} (colony forming unit) using 0.5 Mac Farland solution.

2.7 In vivo efficacy of *W. ugandensis* crude extract against R. solanacearum

The experiment was conducted in the greenhouse using Riogrande tomato variety which Chengo et al., (2022) reported that is susceptible to bacterial wilt and had a germination and purity percentages of 96% and 99.9% (KEPHIS lot NR: 18-20558) respectively. Germination was done in germination trays using Hygromix media and watered when necessary. Transplanting media (soil and sand) were sterilized by autoclaving at 121 $^{\circ}$C for 15 minutes to eliminate contamination. The transplanting media and D.A.P fertilizer were thoroughly mixed and placed in 3 L pots. Holes were made in the soil in the pots and prepared in four categories: non-inoculated with *R. solanacearum,* inoculated with 1 ml of *R. solanacearum* solution (1×10^{8} CFU $ml^{-1)}$ and treated with 1 ml of sodium hypochlorite (1%)(positive control) through drenching, inoculated with 1ml of *R. solanacearum* solution (1×10^{8} CFU $ml^{-1)}$ and treated with 1 ml of Dimethyl sulfoxide (DMSO) (negative control) through drenching and inoculated with 1 ml of *R. solanacearum* solution (1×10^{8} CFU $ml^{-1)}$ and treated with 1 ml of *W. ugandensis* stem bark and leaf crude extract obtained either using ethanol, methanol, dichloromethane or hexane . Randomized complete design (RCD) was used to allocate the pots for each treatment in three replicates. One tomato plant was transplanted in each pot 5 weeks after germination.

Two weeks after transplanting, a teaspoonful of urea (46% N) was applied in each pot followed by a teaspoonful of Calcium Ammonium Nitrate (CAN) in the third week and in weeks six and nine. Foliar feed Wuxal® was applied weekly at 50 ml/20 L water. The crop was kept free of weeds by hand weeding and uprooting of weeds. Irrigation was carried out once every two days. Crop support (staking) was carried out to allow free air movement and reduce moisture accumulation thus reducing disease incidences. Pruning to remove side shoots, laterals, old leaves, diseased leaves, and branches was done by hand (thumb and finger). Standard pest and disease management program was used except for the management of *R. solanacearum.*

2.8 Assessments of bacterial wilt incidence and severity

Disease incidence was assessed at weekly intervals for the development of bacterial wilt symptoms and calculated as the percentage of wilted plants within each treatment according to the formula:

$$WI = \left[\frac{NPSWS}{NPPT}\right] X\ 100$$

Where % WI = percentage wilt incidence,

NPSWS = number of plants showing wilt symptoms and

NPPT = number of plants per treatment (Ayana et al., 2011; Shikoli et al., 2022).

Disease severity scoring of tomato plants affected by bacterial wilt was done on a six-point scale (0= No wilt symptom, 1= One leaf wilted, 2= 2 or more leaves wilted, 3 = all leaves except the tip wilted, 4= Whole plant wilted, 5= Death (collapse) of the whole plant). The six-point scale was proposed by Ayana et al., (2011).

Percentage Severity Index (PSI) as described by (Cooke, 2006; Khairy et al., 2021; Shikoli, 2022) was calculated using the formula: $PSI = \frac{\sum Scores \text{ X } 100}{NPR\ X\ MSC}$

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

2.9 Assessments of tomato plant height, number of branches, plant diameter, and number of fruit sets

Tomato plants' heights were measured by use of measuring tape, the number of branches and the number of fruits were physically counted and plant diameter was measured by use of a Vernier calliper.

2.10 Data collection and analysis

Data was collected weekly through direct observation and by taking photographs. The following parameters data was taken: growth data (stem diameter, height, and number of branches) assessment of disease incidence and severity, number of days taken to show symptoms of infection, and number of fruits set per plant after the growth cycle. Data collected of bacterial wilt incidence, severity stem diameter, height, and number of branches and fruits set was subjected to analysis of variance (ANOVA) at a 5% level of significance. Tukey's test was used in separation means significant difference at a 5% level. The general linear model procedure of the Statistical Analysis System (SAS) program was used to analysed the data.

3. Results and discussion

3.1 Colony Morphology of *R. solanacearum*

R. solanacearum was isolated from the soil in the vicinity of wilted tomato plant samples collected from Meru County during field survey. Fig. 1(A and B) showed virulent isolates grown on Kelmans 2, 3, 5-TZC medium were highly fluidal, white coloured with a light pink centre and round to irregular margin (Aslam & Mukhtar, 2023; Khasabulli et al., 2017).

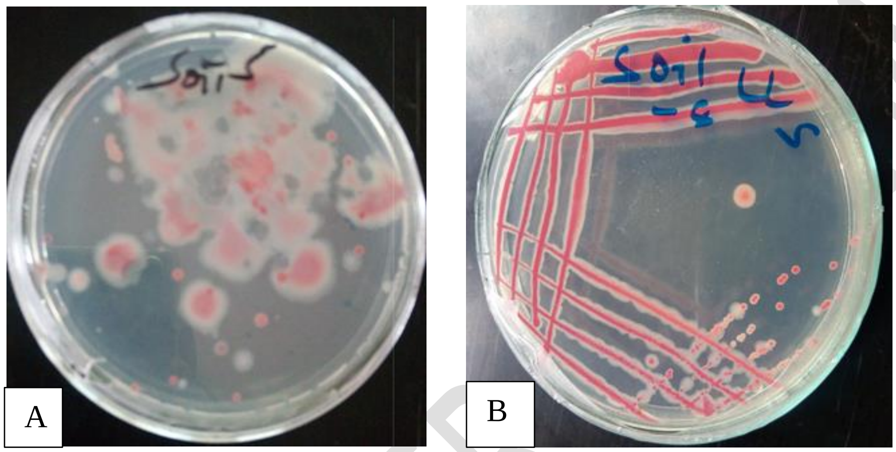

Fig. 1: Colony morphology of R. solanacearum. (A) Represents colony morphology of R. solanacearum isolated cultures grown on 2, 3, 5-TZC medium at incubator for 18 hours and (B) represents colony morphology of R. solanacearum subcultures grown on 2, 3, 5-TZC medium at incubator for 18 h.

3.2 Biochemical Confirmatory Test for *R. solanacearum*

Morphological observations revealed that cells of *R. solanacearum* were straight rod-shaped, with circular ends, cells emerged singly or in pairs, red colouration, and encased when viewed under a compound microscope at 100X magnification with oil immersion. The isolated *R. solanacearum* bacteria were pink in colour in Gram staining reaction under the compound microscope at 100X magnification(Fig. 2A), confirming that they were Gram-negative (Aslam & Mukhtar, 2023; Khasabulli et al., 2017). Fig. 2 (B) *R. solanacearum* showed a positive response to the catalase oxidase test, evidenced by the generation of air bubbles upon inoculation to the medium (Aslam & Mukhtar, 2023; Khasabulli et al., 2017). Additionally, the bacterial culture of *R. solanacearum* produced a string-like viscous material on a glass slide in the KOH test which further confirmed its Gram-negative characteristics (Aslam & Mukhtar, 2023; Khasabulli et al., 2017). Based on the morphological and biochemical traits observed, the bacteria isolated was *R. solanacearum*.

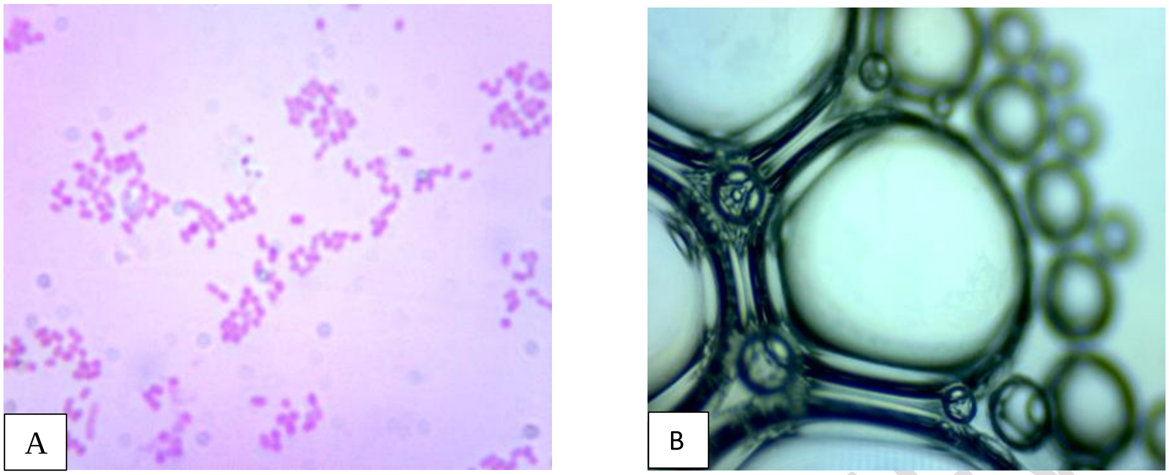

Fig. 2: Gram staining and Catalase Oxidase tests. (A) shows Gram staining of *R. solanacearum*. Pink colour indicates rod shaped cells of *R. solanacearum* at 100X magnification, and (B) show production of air bubbles when a loop full of *R. solanacearum* bacterial culture was mixed with a drop of H_2O_2.

3.3 The effect of soil treatment with *W. ugandensis* crude extracts on tomato Bacterial wilt disease incidence

Tomato plants established in *R. solanacearum* inoculated soil but treated with leaf and stem bark hexane crude extract showed 50% disease incidence (Fig.3). Similarly, tomato plants established in *R. solanacearum* inoculated soil but treated with leaf dichloromethane crude extract had 50% disease incidence (Fig.3). Astonishingly, tomato plants established in *R. solanacearum* inoculated soil but treated with stem bark using dichloromethane crude extract showed 10% disease incidence suppression (Fig.3). Equally, tomato plants established in *R. solanacearum* inoculated soil but treated with the leaf and stem bark using methanol crude extract both showed suppressed disease incidence of 16.67% and 33.33% respectively (Fig.3). Tomato plants established in *R. solanacearum* inoculated soil but treated with ethanol crude extract from both leaf and stem bark showed the lowest disease incidence suppression of 83.33% and 66.67% respectively (Fig.3). Plant crude extracts have been used in greenhouse tomato plants to manage bacterial wilt (Lee et al., 2012; Wamani et al., 2023). Previously, *W. ugandensis* stem bark and leaf crude extracts have shown to contain mukaadial, muzigadial, polygodial, ugandensidial, ugandensolide, and warburganal metabolites that are active against *R. solanacearum* which may be the reason why bacterial wilt incidence was suppressed (Vu et al., 2017). Similar findings have also been reported by Deberdt et al., (2012) and Shikoli et al., (2022) with *A. fistulosum* crude extract showing reduced tomato plant bacterial wilt incidence and severity. As expected, DMSO (-VE) did not show any disease suppression (Fig. 1& 2) while tomato plants established in non-inoculated soil and soil treated with sodium hypochlorite(+VE) did not show any disease incidence at all (Fig.3 & 4).

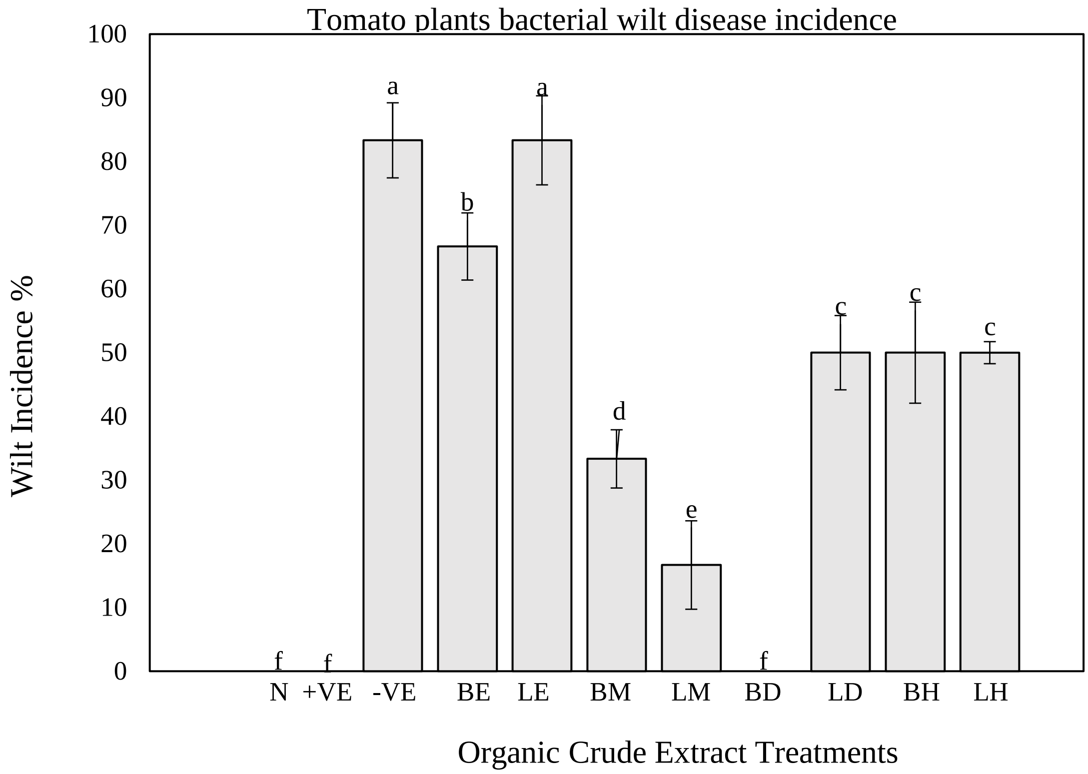

Fig.3: Disease wilt incidence percentage at the terminal stage of development. Rs represents *R. solanacearum* while Wu represents *W. ugandensis*. N (sterilized soil media with no Rs inoculation), +VE (soil media inoculated with Rs and then treated with sodium hypochlorite), -VE (soil media inoculated with Rs and then treated with DMSO), BE (soil media inoculated with Rs and then treated with Wu stem bark ethanol crude extract), LE (soil media inoculated with Rs and then treated with Wu ethanol leaf crude extract), BM (soil media inoculated with Rs and then treated with Wu stem bark methanol crude extract), LM (soil media inoculated with Rs and then treated with Wu methanol leaf crude extract), BD (soil media inoculated with Rs and then treated with Wu dichloromethane stem bark crude extract), LD (soil media inoculated with Rs and then treated with Wu dichloromethane leaf crude extract), BH (soil media inoculated with Rs and then treated with Wu hexane stem bark crude extract and LH (soil media inoculated with Rs and then treated with Wu hexane leaf crude extract). Different letters show significant difference. The statistical significance $p \leq 0.05$.

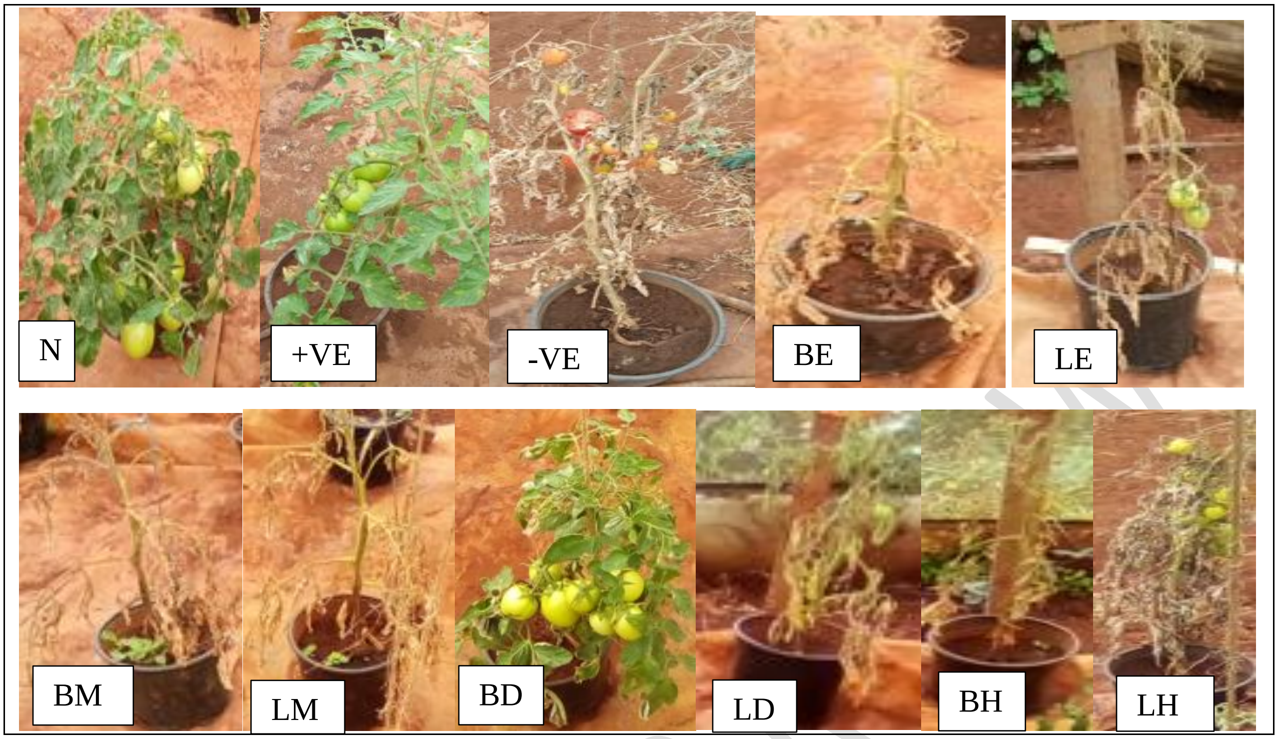

Fig. 4: *W. ugandensis* leaf stem bark and leaf crude extract suppressive effect on *R. solanacearum* of tomato plants. Rs represents *R. solanacearum* while Wu represents *W. ugandensis*. N (sterilized soil media with no Rs inoculation), +VE (soil media inoculated with Rs and then treated with sodium hypochlorite), -VE (soil media inoculated with Rs and then treated with DMSO), BE (soil media inoculated with Rs and then treated with Wu stem bark ethanol crude extract), LE (soil media inoculated with Rs and then treated with Wu ethanol leaf crude extract), BM (soil media inoculated with Rs and then treated with Wu stem bark methanol crude extract), LM (soil media inoculated with Rs and then treated with Wu methanol leaf crude extract), BD (soil media inoculated with Rs and then treated with Wu dichloromethane stem bark crude extract), LD (soil media inoculated with Rs and then treated with Wu dichloromethane leaf crude extract), BH (soil media inoculated with Rs and then treated with Wu hexane stem bark crude extract and LH (soil media inoculated with Rs and then treated with Wu hexane leaf crude extract).

3.4 The effect of soil treatment with W. ugandensis crude extracts on tomato disease severity

Tomato plants established in soil inoculated with *R. solanacearum* treated but with *W. ugandensis* leaf and stem bark hexane crude extract obtained showed 41.67% disease severity (Fig.5). Similarly, tomato plants established in *R. solanacearum* inoculated soils but treated with *W. ugandensis* leaf dichloromethane crude extract had 41.67% disease severity (Fig.5). Surprisingly, tomato plants established in *R. solanacearum* inoculated soil but treated with *W. ugandensis* stem bark dichloromethane crude extract did not show any disease severity (Fig.5). Tomato plants established in *R. solanacearum* inoculated soils but treated with *W. ugandensis* leaf and stem bark methanol crude extracts showed suppressed disease severity of 13.89% and 27.78% respectively (Fig.5). Tomato plants established in *R. solanacearum* inoculated soils but treated with *W. ugandensis* leaf and stem bark crude ethanol extracts showed the highest disease severity of

69.44% and 55.56 respectively (Fig.5). Tomato plants established in *R. solanacearum* inoculated soils but treated with DMSO did not express any disease (Fig.5). These results concur with (Ahmad et al., 2023; Li et al., 2016) reported that DMSO had no impact on *R. solanacearum*. Similar to plants established in non-inoculated soil and *R. solanacearum* inoculated soil but treated with sodium hypochlorite. This study concurs with Mithöfer and Maffei, (2016); Musyimi et al., (2022) that bacterial wilt disease severity was variably and significantly reduced by some aqueous plant crude extracts applied through soil drenching. Previous findings deduced that plant crude extract biochemical compounds that contain antimicrobial properties may be in different forms including phenolic and aldehyde (Nkqenkqa & Mundembe, 2023). Secondary compounds combined naturally in plant crude extract have a higher antimicrobial activity that might be due to synergism than individual constituents that are purified (Liu et al., 2022; Ningombam et al., 2017). The disparity in restraining disease progress by use of *W. ugandensis* crude extracts obtained using different organic solvents could be due to target pathogen membrane permeability, difference in active biochemical compositions of the crude extracts, efficacy difference and extracts durability in the soil (Musyimi et al., 2022). Tomato plants established under negative control and in *R. solanacearum* inoculated soil but treated with *W. ugandensis* organic solvents crude extracts had different disease severity percentage may be due to agronomic practices regulations, genetic interventions, physical measures, and host immunity enhancement, can be employed alone or in tandem to mitigate pathogens, boost host resistance, or alter the conditions under which host-pathogen interactions take place affected the disease development. These results agree with Dossoumou et al.,(2023) and Siddique et al., (2024) that bacterial wilt severity is influenced by factors such as crop type, pathogen characteristics, location, technological resources, and regulatory policies..

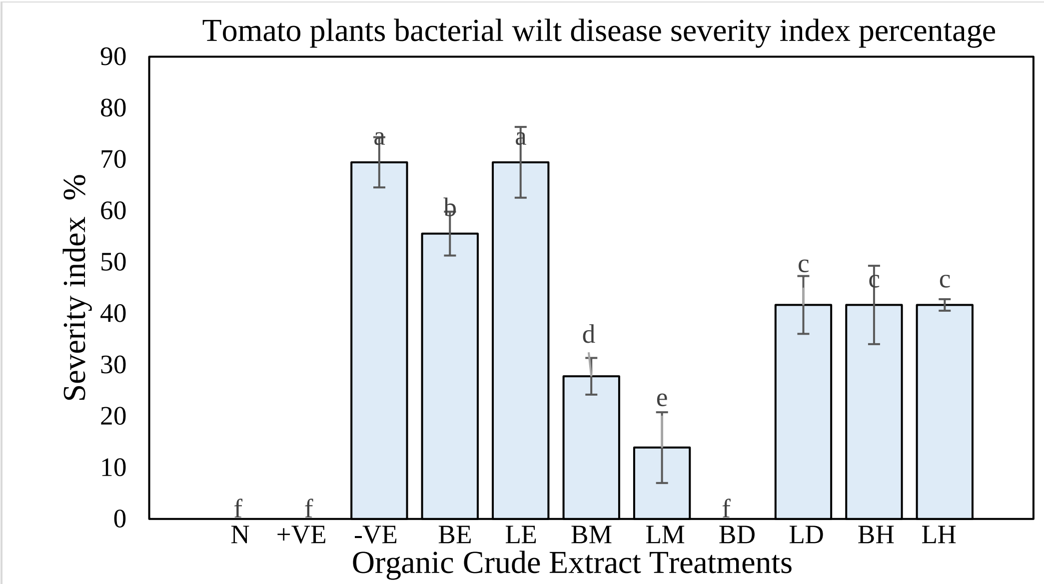

Fig. 5: Disease severity percentage at terminal stage of development. Rs represents *R. solanacearum* while Wu represents *W. ugandensis*. N (sterilized soil media with no Rs inoculation), +VE (soil media inoculated with Rs and then treated with sodium hypochlorite), -VE (soil media inoculated with Rs and then treated with DMSO), BE (soil media inoculated with Rs and then treated with Wu stem bark ethanol crude extract), LE (soil media inoculated with Rs and then treated with Wu ethanol leaf crude extract), BM (soil media inoculated with Rs and then treated with Wu stem bark methanol crude extract), LM (soil media inoculated with Rs and then treated with Wu methanol leaf crude extract), BD (soil media inoculated with Rs and then treated with Wu dichloromethane stem bark crude extract), LD (soil media inoculated with Rs and then treated with Wu dichloromethane leaf crude extract), BH (soil media inoculated with Rs and then treated with Wu hexane stem bark crude extract and LH (soil media inoculated with Rs and then treated with Wu hexane leaf crude extract). Different letters show significant difference. The statistical significance $p \leq 0.05$.

3.5 The effect of soil treatment with W. ugandensis crude extracts on tomato plants mean height, number of branches and diameter

Tomato plants' average height was significantly different in some treatments (Table1). Tomato plants established in *R. solanacearum* inoculated soils but treated with DMSO showed the lowest average height of 49.00 cm which was significant from other treatments. These results agree with Hacisalihoglu et al., (2007) and Wang et al., (2023) reported that *R. solanacearum* retarded tomato plant height. Tomato plants established in *R. solanacearum* inoculated soil but treated with *W. ugandensis* leaf and stem bark ethanol crude extracts showed no significant difference in average height. Similarly, tomato plants established in soils treated with *W. ugandensis* stem bark and leaf methanol and hexane crude extracts also had no significant difference in the average height. Tomato plants established in non-inoculated soil and plants established in *R. solanacearum* inoculated soil but treated with *W. ugandensis* leaf ethanol crude extract had the highest average height of 62.50 cm. Tomato plants established in *R. solanacearum* inoculated soils but treated with sodium hypochlorite had an average height of 55.17 cm which was significantly different from tomato plants established in non-inoculated soil and tomato plants established *R. solanacearum* inoculated in soil but treated with *W. ugandensis* leaf ethanol crude extract. *W. ugandensis* crude extract obtained from the stem bark and leaf using different organic solvents may have had a synergistic effect by stimulating tomato plants' growth through decreased retarding effect of *R. solanacearum*. These results concur with those of Gao et al., (2021) and Marey & Elmasry, (2024) that treatment of tomato plants with *A. fistulosum* crude extract increased the height of tomato plants.

Tomato plants established in soils treated with DMSO had the lowest average number of 17.67 branches which was significant difference from other treatments (Table1). Tomato plants established in *R. solanacearum* inoculated soils but treated with *W. ugandensis* crude extract obtained from the stem bark and leaf using ethanol, methanol, dichloromethane, and hexane showed no significant difference in the average number of branches. Similarly, tomato plants established in *R. solanacearum* inoculated soil but treated with sodium hypochlorite showed an average number of 22.50 branches that were not significantly different from tomato plants established in *R. solanacearum* inoculated soil but treated with *W. ugandensis* crude extract obtained from the stem bark and leaf using ethanol, methanol, dichloromethane, and hexane. Tomato plants established in non-inoculated soil showed the highest average number of 24 branches. This study concurs with previous findings of Abbas et al., (2022) that reported application of seaweed crude extracts against *Sclerotium rolfsii* increased tomato plant's growth parameters.

Tomato plants established in *R. solanacearum* inoculated soils but treated with DMSO had the smallest plant diameter of 10.33 mm which was significantly different from the other treatments (Table1). Tomato plants established in *R. solanacearum* inoculated soils but treated with *W. ugandensis* crude extract obtained from the stem bark and leaf using ethanol, methanol, dichloromethane and hexane showed no significant difference in the average plant diameter. Similarly, tomato plants established in non-inoculated soils and *R. solanacearum* inoculated soil but treated with sodium hypochlorite showed no significant difference in average plant diameter with tomato plants established in *R. solanacearum* inoculated soil but treated with *W. ugandensis* crude extract obtained from the stem bark and leaf using ethanol, methanol, dichloromethane, and hexane. Tomato plants established in *R. solanacearum* inoculated soil but treated with *W.*

ugandensis stem bark ethanol crude extract showed the largest average plant diameter of 13.00 mm. Generally, tomato plants established in *R. solanacearum* inoculated soils treated with *W. ugandensis* crude extract obtained from both the stem bark and leaf using different organic solvents increased growth of tomato plant height, branches, and stem diameter. The results of the current study agree with those of Deberdt et al., (2012) and Fadia et al., (2023) reported that tomato plant growth parameters increased when treated with different plant crude extracts.

Table1: Means and standard error (SE) of tomato plants height, number of branches and plant diameter from eleven different organic extract treatments

Treatment	Plant height (cm)	Number of branches	Plant diameter(mm)
N	62.50 ±1.75a	24.17 ±0.65a	12.77 ±0.53ab
+VE	55.17 ±3.09bcd	22.50 ±0.60abc	12.07 ±0.37ab
-VE	49.00 ±0.50d	17.67 ±0.42f	10.33 ±0.20c
BE	60.50 ±0.13ab	22.00 ±1.08bcd	13.00 ±0.26a
LE	62.50 ±2.96a	23.67 ±0.55ab	11.67 ±0.26b
BM	56.75 ±3.61ab	21.67 ±0.33cde	11.83 ±0.05b
LM	61.80 ±1.90ab	21.33 ±0.88cde	11.93 ±0.30ab
BD	58.33 ±3.02ab	20.67 ±0.67de	12.17 ±0.40ab
LD	50.00 ±1.42cd	20.83 ±0.21cde	11.87 ±5.04ab
BH	56.11 ±1.15abc	20.00 ±0.95cde	12.50 ±0.76ab
LH	56.67 ±3.33abc	20.50 ±0.42de	12.33 ±0.21ab
LSD	6.72	1.68	1.17
P	<0.0052	<0.0052	<.0052
CV	10.14	6.78	8.32

Rs represents *R. solanacearum* while Wu represents *W. ugandensis*. N (sterilized soil media with no Rs inoculation), +VE (soil media inoculated with Rs and then treated with sodium hypochlorite), -VE (soil media inoculated with Rs and then treated with DMSO), BE (soil media inoculated with Rs and then treated with Wu stem bark ethanol crude extract), LE (soil media inoculated with Rs and then treated with Wu ethanol leaf crude extract), BM (soil media inoculated with Rs and then treated with Wu stem bark methanol crude extract), LM (soil media inoculated with Rs and then treated with Wu methanol leaf crude extract), BD (soil media inoculated with Rs and then treated with Wu dichloromethane stem bark crude extract), LD (soil media inoculated with Rs and then treated with Wu dichloromethane leaf crude extract), BH (soil media inoculated with Rs and then treated with Wu hexane stem bark crude extract and LH (soil media inoculated with Rs and then treated with Wu hexane leaf crude extract). Mean values in the same column followed by the same letter are not significant at p≤0.05.

3.6 The effect of soil treatment with *W. ugandensis* crude extracts on tomato plants number of fruits set

Tomato plants established in *R. solanacearum* inoculated soil but treated with *W. ugandensis* stem bark and leaf using hexane crude extracts showed a significant average number of fruits set of

10.67 and 16.50 respectively (Fig. 6). Tomato plants established in *R. solanacearum* inoculated soil but treated with *W. ugandensis* leaf dichloromethane crude extract showed the average number of fruits set of 21.60 (Fig. 6). Similarly, tomato plants established in non-inoculated soils had a 21.33 average fruit set (Fig. 6). Tomato plants established in *R. solanacearum* inoculated soils but treated with *W. ugandensis* leaf and stem bark using methanol crude extracts showed a significant average number fruits set of 13.50 and 22.00 respectively (Fig. 6). Tomato plants in *R. solanacearum* inoculated soil but treated with *W. ugandensis* ethanol crude extract from stem bark had average number of fruits set of 22.00 (Fig. 6). Similarly, tomato plants established in *R. solanacearum* inoculated soil treated with sodium hypochlorite showed the highest average number of fruits set of 22.33 (Fig. 6). Tomato plants established in *R. solanacearum* inoculated soil treated with DMSO showed average number of fruits set of 16.00 (Fig. 6). Tomato plants established in *R. solanacearum* inoculated soils but treated with *W. ugandensis* leaf dichloromethane and stem bark ethanol crude extracts had a significant increase in the average number of fruits set compared to *W. ugandensis* other organic solvents extracts and negative control. This could be because of the applied *W. ugandensis* crude extracts that increased chlorophyll content hence promoting number of tomato fruits set. Similarly Hussain et al., (2021) study shown that the seaweed extracts promoted the following tomato growth parameters: foliage dry weight, root lengths and roots and numbers of flowers and flower clusters. Further demonstrations conducted by Gholizadeh et al., (2017); Yuan et al., (2016) shown that application of seaweed extracts remarkably increased tomato leaves chlorophyll content at the stage of flowering when evaluated by SPAD testing, trial used to determine nitrogen content status in a plant.Tomato plants established in *R. solanacearum* inoculated soils but treated with *W. ugandensis* stem bark dichloromethane extracts had the lowest disease severity index percentage but the number of tomato fruit sets were not significantly different to the negative control. These could be due to water and nutrient that play pivotal roles in influencing both the productivity and quality of crops. These study results are contrary with those of Kusampudi et al., (2023) reported that tomato plants treated *Euphorbia hirta* crude extract obtained using showed average resistance against *R. solanacearum* hence tomato yield increased.

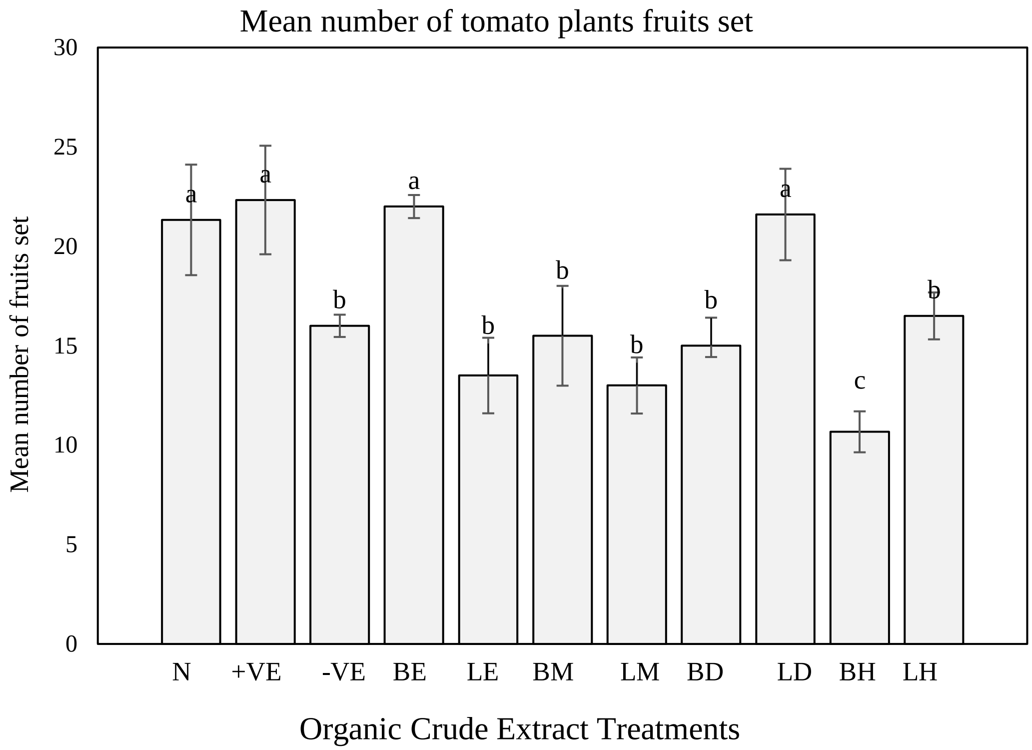

Fig. 6: Mean number of tomato plants fruits set at terminal stage of development. Rs represents *R. solanacearum* while Wu represents *W. ugandensis*. N (sterilized soil media with no Rs inoculation), +VE (soil media inoculated with Rs and then treated with sodium hypochlorite), -VE (soil media inoculated with Rs and then treated with DMSO), BE (soil media inoculated with Rs and then treated with Wu stem bark ethanol crude extract), LE (soil media inoculated with Rs and then treated with Wu ethanol leaf crude extract), BM (soil media inoculated with Rs and then treated with Wu stem bark methanol crude extract), LM (soil media inoculated with Rs and then treated with Wu methanol leaf crude extract), BD (soil media inoculated with Rs and then treated with Wu dichloromethane stem bark crude extract), LD (soil media inoculated with Rs and then treated with Wu dichloromethane leaf crude extract), BH (soil media inoculated with Rs and then treated with Wu hexane stem bark crude extract and LH (soil media inoculated with Rs and then treated with Wu hexane leaf crude extract). The error bars show the standard errors (SE) of the means. Different letters show significant difference. The statistical significance $p \leq 0.05$.

4. Conclusion

This study shows that *W. ugandensis* stem bark and leaf crude extracts can reduce bacterial wilt disease incidence and severity and enhance the growth parameters of tomato plants. Tomato plants established in soil treated with of *W. ugandensis* stem bark dichloromethane crude extract showed bacterial wilt disease incidence and severity of 0% suggesting that crude extracts may contain *R. solanacearum* sterilizing biochemical. Consequently, tomato farmers should examine treating the soil with *W. ugandensis* stem bark dichloromethane crude extract as an integrated strategy in managing bacterial wilt infected greenhouses to promote growth and yields of tomato.

Recommendation

W. ugandensis stem bark dichloromethane extracts have active compounds that may be isolated to be used in the place of synthetic pesticides against *R. solanacearum*.

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