Asian Journal of Research in Crop Science

7(4): 1-13, 2022; Article no.AJRCS.92318
ISSN: 2581-7167

Effect of Methanolic Extracts of *Senna didymobotrya* and *Moringa oleifera* on Growth of *Ralstonia solanacearum*

George T. Opande [a*], Buyela D. Khasabulli [b] and David M. Musyimi [b]

[a] Department of Biology and Agriculture, SOSCI, Kaimosi Friends University, Kaimosi, Kenya.
[b] Department of Botany, SPHS, Maseno University, Maseno, Kenya.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRCS/2022/v7i4140

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/92318

Original Research Article

Received 20 July 2022
Accepted 22 September 2022
Published 24 September 2022

ABSTRACT

Tomato is one of the most important vegetables in the world. *Ralstonia solanacearum* causes *a* devastating bacterial wilt that is wide-spread throughout tropical environments that has been difficult to control with chemicals and African cultural practices in poor regions of the world. This study was initiated to determine the inhibitory effect of methanolic root extract of *Senna didymobotrya* and *Moringa oleifera* seed extracts on growth and development of *R. solanacearum.* The study was conducted both in the Laboratory and in the green house in Maseno from where *S. didymobotrya* and *M. oleifera* specimens used were collected from the University Botanic Garden, dried under shade for 30 days, before being ground into a fine powder. 1000 grams of the plant powder was later transferred into a conical flask and covered with 250mls of methanol. Filtration was done and the extracts concentrated. Ten diseased tomato plants showing bacterial wilt symptoms earlier collected from Maseno, Mariwa, Seme and Holo all within Kisumu county (Kenya) were cut to obtain plant sections (0.5–1cm) that were later plated onto Triphenyltetrazolium chloride media. Bacteriostatic activity of the extracts was determined by the disc diffusion method on Mueller Hinton agar. *In vivo* evaluations were conducted in a green house using tomato seedlings. Data collected was subjected to analysis of variance and means separated were compared using least significance difference (P≤ 0.05). Both plants extracts showed inhibitory activity against *R. solanacearum* pathogen (*M. Oleifera*; 8.5 and *S. didymobotrya* 9.2). Based on the outcome of this study, *S. didymobotrya* is recommended as a potential botanical agent suitable for future trials and use in the control of *R. solanacerum* by tomato farmers in Maseno region Kisumu county.

**Corresponding author: E-mail: opandeg@gmail.com;*

Keywords: Tomato; Ralstonia solanacearum; Senna didymobotrya; Moringa oleifera; bacterial wilt.

1. INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most popular vegetables in the world [1]. It is the world's largest consumed vegetable crop after irish potato and also tops the list of canned vegetables [2]. It is a very versatile plant and it could either be grown for fresh market or for processing in which mechanical processes are involved [3]. The popularity of tomato is because of its taste, colour, high nutritive value and its diversified use. Tomato and its products are rich in antioxidants and considered to be a good source of vitamins C, E and carotenoids, particularly lycopene and β-carotene and other phenolic compounds that protect the body against diseases [4,5]. Despite the importance of tomato in many developing and developed countries, this crop has often been neglected, and its production has experienced several constraints like poor soils, use of unimproved varieties, land tenure system, damage by pests and diseases that have led to production below its full potential [6]. Several authors have reported the contributions of many types of diseases affecting tomato production [7-9]; however the effect of bacterium wilt stands out as the most damaging [10].

Bacterial wilt is caused by *R. solanacearum*, an important and wide-spread bacterial [11,12] *R. solanacearum* is an aerobic non-sporing Gram negative bacterium [13]. It is soil borne and motile by means of a polar flagellum tuft and sometimes range from 1 to 4 polar. This organism colonizes the xylem, causing bacterial wilt in a wide range of potential host plants [14,15]. Highly susceptible crops such as potato, tomato, egg plant, chili, bell pepper and peanut among 450 other plants species have been reported as hosts of this pathogen [16]. The disease causes serious economic problems worldwide leading to serious annual losses exceeding USD 950 million [11]. In Kenya the pathogen has been reported at both low and high altitudes [17]. Management is difficult due to high variability of the pathogen, limited possibility for chemical management, high capacity of the pathogen to survive in diverse environments and its extremely wide host range [18], hence the need to come up with a proper control method in order to control the pathogen [19-22].

The use of plant extracts has an important role in the management of bacterial wilt [23]. Plant extracts can help development of alternative management measures or can be integrated with other practices for effective disease management at the field level [24]. Use of plant extracts for control not only suppress the disease and increases the crop yield but is important in preventing the environmental pollution associated to pesticides [25].

The efficacy of plant extracts is associated to secondary metabolites produced by plants such as alkaloids, tannins, flavonoids and phenolic compounds among others [26,27], which possess bacteriostatic properties.

Before this study, information pertaining to trials on the control of *R. solanacearum* on tomato plants in Maseno region by farmers using botanicals was inadequate, moreover, the inhibitory effects of S*. didymobotrya* root extract and *M. oleifera* seed extracts on *R. solanaceaerum* was unknown therefore this study was initiated to answer this study questions of the unknown.

2. MATERIALS AND METHODS

This study was conducted between October 2021 and April 2022 at Maseno University farm and in the Department of Botany Microbiology Laboratory and in a green house that had a day temperature ranged between 25^0C- 40^0C and Night temperatures ranged between 20^0C-30^0C, 14/10h photoperiod and 70-90% humidity. Maseno is situated in Western, Kenya, its geographical coordinates are 0° 10' 0" South, 34° 36' 0" East and the altitude is 1,503 meters or 4,934 feet above sea level (KNBS, report, 2013), it receives both short and long rains averaging 1750mm per annum with mean temperature of 28.7^0C.

2.1 Collection, Preparation and Preservation of Plants Parts

S didymobotrya and *M. oleifera* plants were identified in the field with reference to taxonomic keys [28]. After which all the samples were transported and air-dried in the shade until when completely dry after thirty (30) days [29], ground into a fine powder before being stored in airtight

plastic containers at room temperature (25-30^{o}C) for extractions.

2.2 Plant Extraction using Organic Solvent (Methanol)

Dry weight of one thousand (1000) grams of the powdered root bark of *S. didymobotrya* and seeds of *M. oleifera* were weighed and transferred to two five liter conical flasks (Pyrex) [30] after which 2.5 liters of 99% methanol was added to cover the plant material under a fume hood and left to soak in the solvent at room temperature for 5 days with shaking on rotary shaker with a speed of 20 revolutions per minute. Extracts were filtered through No. 1 Whatman filter paper on a Buchner funnel under vacuum pump (Vacuubrand GMBH). The filtrate was then rotary vapoured using a Rotary vapour pump (Eyela SB-1000) to concentrate the extracts.

2.3 *R. solanacearum* Infected Plant Sample Collection

A total of 10 diseased tomato plants were collected from four sites i.e. Maseno, Holo, Seme and Mariwa all located within Kisumu County, where they were selected on the basis of tomato production. 5 samples were prepare from each plant [31]. Simple random sampling technique was used for collection of samples so as to eliminate selection bias and for accuracy of representation.

2.4 Isolation of *R. solanacearum*

Collected tomato plant materials were surface sterilized with 1% Sodium Hypochlorite (NaOCl) solution for 1 to 2 min, followed by three repeated washings with distilled water and blot dried [32,33]. The plant sections (0.5–1 cm) were then plated onto 2, 3, 5 triphenyl tetrazolium chloride (Kelman's TZC agar) medium (glucose 10 g, peptone 10 g, casein hydrolysate 1 g, agar 18 g, distilled water 1000 ml). 5 ml of TZC solution filter sterilized was added to the autoclaved medium to give final concentration of 0.005%) as earlier described [34]. The plates were incubated at 28^{o}C ± 2°C for 24–48 hr. The virulent colonies in the medium characterized by dull white color, fluidal, irregularly round with light pink centers were further streaked on TZC medium to get pure colonies of the bacterium.

2.5 Preservation of *R. solanacearum*

Two loopfuls of bacterium from 48 hr old colonies grown on Kelman's TZC Agar was transferred to 5 mL of sterile double distilled water in screw capped vials according to the procedure earlier described [34], and stored under refrigeration at 20^{o}C for maintenance of virulence.

2.6 Preparation of *R. solanacearum*

A bacterial suspension was prepared by pouring sterile distilled water over 24hr old bacterial growths on Nutrient agar slants, the suspension was then poured into a test tube and adjusted to optical density (O.D) 0.5 in a spectrophotometer (Novaspec II) in blue filter (425nm) to obtain a bacterial population of 1 x 10^{8} colony forming unit per milliliter of the suspension [35].

2.7 Effects of Plant Extract on Test Bacteria (*In Vitro* Evaluation of the Plant Extracts)

Bacteriostatic activity of the extracts was determined by the disc diffusion method on Mueller Hinton agar according [35]. An overnight culture of the bacterium was diluted to 10^{5} cells/ml using a spectrophotometer (Novespec II) at a wavelength of 625nm. One milliliter of the bacterial suspension was introduced into sterile petri plate and 20 ml of Mueller Hinton agar at 40ºC before it was poured into the inoculated plates. The plates were allowed to cool and solidify. A sterile filter disc (Whatman No. 9) soaked in the different extracts with a concentration of 15%, 10%, 5% and 2.5% respectively were picked with sterile forceps and placed on the surface of a solid inoculated agar plates. The plates were incubated at 37ºC for 24hr. This was carried out in triplicates. The petri plates were then assessed for bacteriostatic activities. The control consisted of the water alone and served as the negative control.

2.8 Effects of Plant Extract on Tomato Wilt (*In Vivo* Evaluation of the Plant Extracts)

In vivo evaluations were conducted in a greenhouse to assess the screened plant extracts using tomato seedlings [36], using susceptible tomato variety Rio Grande. The experimental design was complete randomized design (CRD) with three replications. The soil that was used in these studies was obtained from the top layer (first 15 cm, corresponding to the area of the roots of tomato plants). A sufficient volume of soil was autoclaved at 121°C for 30 min which was then used to fill pots 18cm tall

and 30×13 cm diameter to a height of 15 cm, to which 20 ml of pathogen suspension was added as Inoculum. One week after inoculation, 20-day-old seedlings of wilt-susceptible tomato was transplanted into the pots, grown at 28°C and watered twice daily (morning and late afternoon) using micro-sprinklers. When these plants senescenced due to bacterial wilt disease, new plants were re-transplanted into the same pots so that when the second set of plants died, the soils were considered to be sufficiently infested by the pathogen. These pots were then used for testing the plant extracts capacity of the two plants to control the disease.

To test the selected extracts, three 20-day-old tomato seedlings were planted into each of the pots and grown under the same conditions described herein. Selected plant extracts 15% were applied individually to each pot seven days after transplanting, and arranged in a completely randomized design with three replicates for each treatment. These extracts were again applied after a week. In addition, two control treatments were included: one with no pathogen nor extract, and the other with pathogen, but without an extract.

Plants were examined for disease incidence starting from one week after transplanting and continuing until the end of harvesting time. Disease incidence was assessed using the 1-5 (0-5) scolding scale with modifications suggested for bacterial wilt [12].

1. No visible symptoms; 1-10
2. 2.1-25% of the plants showing wilting; 11-30
3. 26-50% wilting; 31-60
4. 51-75% wilting; more than 60 but less than 100
5. More than 75% wilting.

Disease index (%) = $\lceil \sum (ni\ Xvi) / (V \times N) \rfloor \times 100$.

Where

the *ni*=number of plants with the respective disease rating;
Vi=disease rating;
V=the highest disease rating (5); and
N=the number of plants observed.

Based on disease index collected data, two parameters; disease incidence and control efficacy of extracts was estimated according to the formulae bellow [37].

$$\text{Disease incidence} = \frac{Disease\ index \times number\ of\ diseased\ plants\ in\ this\ index\ \times 100\%}{Total\ number\ of\ plants\ investigated \times the\ highest\ disease\ index}$$

$$\text{Extract control efficacy} = \frac{D.I\ of\ control - D.I\ of\ extract\ treated\ group \times 100\%}{Disease\ incidence\ of\ control}$$

Where

D.I= Disease index.

2.9 Determination of Root and Shoot Length

Tomato plants were removed from the green house, gently washed, then [38] spread on paper for measurement of root and shoot length (cm) using a ruler.

2.10 Determination of Fresh and Dry Shoot and Root Weight

Plants were cut into roots and shoots, and fresh root weight (gm) and fresh shoot weight (gm) was taken using a weighing balance before being recorded [38], prior to drying for 72 h at 60°C before the dry weight was determined using weighing balance and recorded separately as root dry weight and shoot dry weight.

2.11 Determination of Number of Fruits

To obtain total yield, the number of fruits per plant were counted and average yield per plant was determined [39]. The fruits were gathered as soon as they started to ripen. Collection of fruits continued until the end of the harvesting period (three months).

3. RESULTS

3.1 Inhibitory Effects

Generally, bacteriostatic activity results (Table 1) showed that *R. solanacearum* were sensitive to *M. oleifera* and *S. didymobotria* at 15% concentration. All the extracts showed antimicrobial activity by developing clear zones of inhibition (Plates 1 and 2). The presence of inhibition zones depicted the antibacterial activity of *M. oleifera* and *S. didymobotria* plant extracts. There were no inhibition zones in the controls which consisted of sterile distilled water (Plates 3 and 4). Based on the four *R. solanacearum* isolates, *M. oleifera* extract had a higher zone of inhibition for Maseno isolate at (8.8) as compared to *S. didymobotria* at (8.7) (Table 1). *S didymobotria* had higher means of inhibition as compared to the other three isolates i.e. Mariwa, Seme and Holo (Table 1).

Table 1. Mean diameter of zones of inhibition of *M. oleifera* and *S. didymobotria* for the four isolates

Isolate	*Senna didymobotria*	*Moringa oleifera*
	Inhibition means(mm)	**Inhibition means(mm)**
Maseno	8.71b	8.82b
Mariwa	8.78b	7.91c
Seme	8.49b	7.80c
Holo	10.84a	9.55a
LSD	0.6203	0.6598
P.value	0.0001	0.0001
%C.V	16.14784	18.55546

Legend: Means followed by different letter down the column are statistically different at P≤ 0.05by Fisher's protected least significant difference test

R. didymobotria root extract did not have a significant difference (P≥0.05) for Maseno, Mariwa and Seme isolates but there was a significance different (P≤ 0.05) for Holo isolate (Table 1). However *M. oleifera* seed extract showed significant difference between Maseno and Holo isolates but did not show significance difference (P≥0.05) between Mariwa and Seme isolates (Table 1). The overall results showed that *S. didymobotria* performed better than *M. oleifera* in inhibiting the growth of the four *R. solanacearum* isolates (Table 2). There was significance difference (P≤0.05) between *M. oleifera* and *S. didymobotria.*

Table 2. Comparative effect of *M. oleifera* and *S. didymobotria* on *R.solanacearum*

Treatment	Inhibition zones (mm)
M. oleifera seed extract	8.5222b
S. didymobotria root extract	9.2056a
LSD	0.3206
P.value	0.0001

Legend: Values in the same column not sharing the same letter differ significantly at P≤0.05

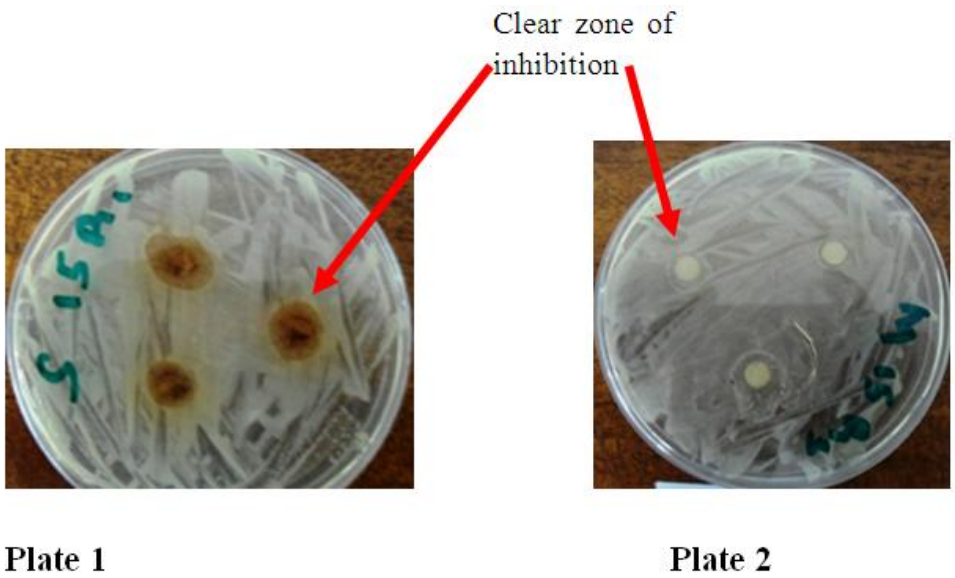

Plate 1. S.didymobotria inhibition zone at 15% of the extract
Plate 2. *M. oleifera* inhibition zones at 15% of the extract

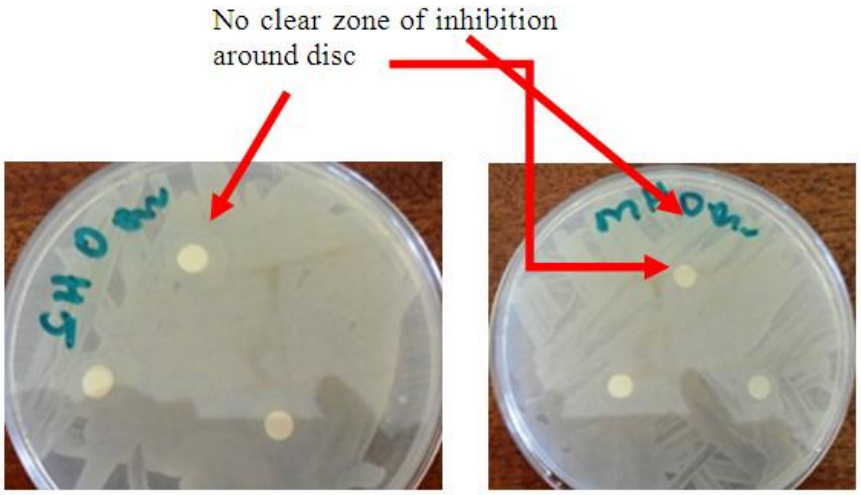

Plate 3 Plate 4

Plate 3. *S. didymobotria* control
Plate 4. *M. oleifera* control

Table 3. Comparative effect of Mean diameter of zones of inhibition of *M. oleifera* and *S. didymobotria* on *R. solanacearum*

Treatment	Inhibition means (mm)				
	0%	2.5%	5%	10%	15%
M. oleifera root extract	0.0000a	8.28b	10.11a	12.03b	12.19b
S. didymobotria seed extract	0.0000a	9.78a	10.61a	12.53a	13.11a
LSD	0.5069	0.5069	0.5069	0.5069	0.5069
P.value	0. 0001	0. 0001	0. 0001	0. 0001	0. 0001
%C.V	17.38336	17.38336	17.38336	17.38336	17.38336

Legend: Means followed by different letter in the same column are statistically different at P≤0.05 by Fisher's protected least significant difference test

Analysis of variance showed that the four different concentrations (2.5, 5, 10 and 15%) of *S. didymobotria* and *M. oleifera* plant extracts exhibited highly significant (P≤0.05) difference on their effect against growth of *R. solanacearum* (Table 3). In both cases, antibacterial activity of the test materials increased as their concentration increased but *S. didymobotria* was more effective than *M. oleifera*.

3.2 Effect of *S. didymobotria* Root Extract and *M. oleifera* Seed Extract on Disease Incidence

At the beginning of the experiment (week one to week four), the effect of *S. didymobotria* and *M. oleifera* treatments did not differ significantly (P≥0.05) on the incidence of bacterial wilt (BW) among the different test isolates. However, the treatments effectively (P≤0.05) reduced BW incidence towards the middle (week five and six) and end of the experiment. At the middle of the experiment (week five) however, the two treatments significantly (P≤0.05) reduced BW incidence relative to control as indicated in Table 4. Throughout the experiment, the interaction effect between the crops and the treatments was significant (P≤0.05). There was a significant difference (P≤0.05) on the incidence of BW between the treatments and the control during the same period as shown in Table 4, *M. oleifera* seed extract treatments recorded higher disease incidence as compared with *S. didymobotria* root extract. The tomato cultivar used, Rio Grande, was very susceptible to bacterial wilt. Disease incidence progressed rapidly beginning from week one after transplanting. Final disease incidence reached 100% after five weeks' time in pots without plant extracts, indicating high inoculum pressure (Table 5). Disease incidence stabilized at week five and continued until harvest. *S. didymobotria* application provided effective protection against bacterial wilt, with only 32.9% of plants wilting,

Table 4. Comparative effect of control efficacy of *M. oleifera* seed extract and *S. didymobotri*a root extract on *R. solanacearum* on tomato plants

Treatment	Disease incidence (%)
Senna didymobotria root extract	32.9 c
Moringa oleifera seed extract	49.5 b
Distilled water(Negative control)	0.0 d
R.solanacearum (Positive control)	82.5 a
LSD	4.6265
P.value	0.0001
%C.V	27.86342

Legend: Means followed by different letter down the column are statistically different at P≤0.05 by Fisher's protected least significant difference test. S. didymobotria and M. oleifera plant extracts in millimeters

Table 5. Weekly disease incidences in tomato plants after treatment with *Senna didymobotria* and *Moringa oleifera* plant extracts

Week	1	2	3	4	5	6	7	8	9	10	11	12
Treatment												
R. didymobotria	0 d	20b	20c	34.75b	40c	40c	40c	40c	40c	40c	40c	40c
M. oleifera	0 d	24b	38.25b	51.25c	60b	60b	60b	60b	60b	60b	60b	60b
Distilled water (-ve control)	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0 d
R. olanacearum (+ve control)	0 d	53a	56.50a	80a	100a	100a	100a	100a	100a	100a	100a	100a
LSD	8.033	8.033	8.033	8.033	8.033	8.033	8.033	8.033	8.033	8.033	8.033	8.033
P.value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
%C.V	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9

Legend: Means followed by different letter in the same column are statistically different at P≤0.05 by Fisher's protected least significant difference test. S. didymobotria and M. oleifera plant extracts in millimeters

Table 6. Effect of, *S. didymobotria* and *M. oleifera* plant extracts at 15% on fresh and dry (stem and root weight), shoot height, root length and yield of tomato under greenhouse conditions after plants were inoculated with *R. solanacearum*

Treatment	Fresh stem weight	Dry stem weight	Fresh root weight	Dry root weight	Shoot height	Root length	Number of fruits
Senna didymobotria	62.23b	12.10 a	14.00a	6.32 a	78.87 a	19.06 b	19.93 b
Moringa oleifera	51.73b	8.95b	8.54 b	2.75b	68.60 b	16.45 c	17.93 c
Control	76.89a	12.21a	15.34a	7.23a	60.00c	21.67a	27.33a
LSD	13.41	2.23	5.02	2.45	4.79	2.41	1.83
P.value	0.0751	0.0689	0.7151	0.2925	0.0001	0.0130	0.0001
%C.V	28.3	27.0	53.4	60.4	9.3	16.9	11.3

Legend: Means followed by different letter in the same column are statistically different at P≤ 0.05 by Fisher's protected least significant difference test. S .didymobotria and M. oleiferaare plant extracts in millimeters

which was significantly lower than the untreated control (82.5%) (Table 5). *M. oleifera* extract also reduced bacterial wilt incidence significantly (P≤0.05) compared with the untreated pots; however, it was less effective than *S. didymobotria*, with approximately half of the plants wilting in *M. oleifera* seed extract treated pots (49.5) (Table 5). Both *S. didymobotria* root extract and *M. oleifera* seed extract treatments significantly protected tomato plants from severe yield loss as a result of the disease.

3.3 Effect of *S. didymobotria* Root Extract and *M. oleifera* Seed Extract on Shoot and Root Biomass

The effects of type of botanicals on above-ground biomass (fresh and dry weight) per plant are presented in Table 6. There was no significance difference (P≥0.05) between *S. didymobotria* root extract (62.23b) *and M. oleifera* seed extract (51.73b) at 15% for above-ground fresh weight (AGFW) but there was a significance difference (P≤0.05) for the above-ground dry weight (AGDW) between *S. didymobotria* (12.10a) root extract and *M. oleifera* seed extract (8.95 b). A significance difference (P≤0.05) was established for the below ground fresh weight (BGFW) and below ground dry weight (BGDW) between *S. didymobotria* root extract (fresh weight 14.00a, dry weight 6.32a) and *M. oleifera* seed extract (fresh weight 8.54b, dry weight 2.75b). Plants treated with *S. didymobotria* root extract had no significance difference (P≥0.05) for AGDW, BGFW and BGDW with healthy control plants. But, plants treated with *M. oleifera* seed extract were less in their shoot fresh and dry weights than healthy control plants (Table 6).

3.4 Effect of *S. didymobotria* Root Extract and *M. oleifera* Seed Extract on Shoot Height and Root Length

There was significant difference (P≤0.05) in shoot height observed among the three treatments (*S. didymobotria*78.87a*, M. oleifera* 68.60b and uninfected Control 60.00c). Tomato plants treated with *S. didymobotria* plant extracts were the tallest (78.9cm) followed by *M. oleifera* plant extract (68.6cm) and uninfected control (60.cm) respectively as indicated in Table 6. Significant interaction effect (P≤0.05) on the shoot height was established between the various treatments and the tomato plants.

There was a significant decrease (P≤0.05) in root length with roots of uninfected tomato plants (21.7a), *S. didymobotria* root extract treated, roots (19.06b) and *M. oleifera* seed extract treated roots (16.45c).

3.5 Effect of *S. didymobotria* Root Extract and *M. oleifera* Seed Extract on the Number of Fruits

For the number of fruits there was a significance difference between the three treatments (water 27.3a, *S didymobotria* 19.9b and *M.oleifera*17.9c).The results clearly indicated that *R. solanacearum* affected production.

There was no correlation between *in vitro* inhibition of *Ralstonia* strains as measured by zones of inhibition and control efficiency in tomato plants challenged with *R. solanacearum*. *S. ditymobotria* was the most antagonistic towards *R. solanacerum* strains *in vitro* and also effective in reducing bacterial wilt when used to treat tomato plants later challenged with the pathogen.

4. DISCUSSION

4.1 Efficacy of Treatments in *in vitro* Experiment on *R. solanacearum*

The results indicate that both plant extracts (*M. oleifera* seed and *S. didymobotria* root) had growth inhibitory activity against *R. solanacearum* but *S. didymobotria* root extract was more efficient in inhibiting the growth of *R. solanacearum.* These results on root extract inhibition of *R. solanacearum* concurred with a previous study [40] where it was reported that *S. didymobotrya* root had significant inhibition against Gram-negative bacteria. Growth inhibition by *S. didymobotria* could be due to presence of secondary metabolites such as saponins, flavonoids, anthraquinones tannins, alkaloids, phenols, terpenoids, steroids, steroidal nucleus and cardiac glycosides as documented [41].

In this study *Moringa oleifera* seed extract was found to significantly P≤0.05 inhibit the growth of *R. solanacearum* thus these results being in agreement with those earlier reported [42,43] but it is importantly to note that other workers while working with *M. oleifera* determined the presence of phytochemicals associated with its medicinal properties [44,45].

4.2 Efficacy of Treatments on Incidence of *R. solanacearum*

This study revealed significant p≤0.05 reduction in the disease incidence by the two plant extracts at 15% among the tomato plants (Table 3). Under greenhouse conditions, *S. didymobotria* and *M. oleifera* plant extracts were evaluated for their efficacy against tomato bacterial wilt. In general, *S. didymobotria* showed a lower disease incidence (32.9%) as compared to *M. oleifera* (49.5%) but both plant extracts consistently restricted disease progression on tomato plants under greenhouse conditions. Overall, the results indicated that drenching topsoil with crude methanolic extracts of *S. didymobotria* and *M. oleifera* had the potential to suppress the bacterial wilt incidence and severity. This results are in agreement with those previously reported by other investigators using plant extracts to control tomato bacterial wilt under greenhouse conditions [12,46-49]. Application of crude methanolic extract of *S. didymobotria* and *M. oleifera* significantly (P<0.05) reduced bacterial wilt incidence. This variation in restricting disease progression between *S. didymobotria* and *M. oleifera* might be due to difference in chemical compositions of the extracts, membrane permeability of the target pathogen, difference in efficacy and durability of extracts in the soil [50]. This was supported by the work earlier workers [51], who reported that soil drenching of some aqueous plant extracts variably and significantly reduced the disease severity of bacterial wilt, caused by *R. solanacearum*, on potato plants compared with inoculated control under both greenhouse and field conditions. An earlier study concluded that antimicrobial activities of plant extracts may exist in a variety of different components, including aldehyde and phenolic compounds [52]. Naturally occurring combination of the secondary compounds can be synergistic and often result in crude extracts having greater antimicrobial activity than the purified, individual constituents [28,53]. Competitive microorganisms, and induction of systemic resistance in host plants is known to result in reduction of disease development [54].

4.3 Effect of Plant Extracts on Plant Growth Parameters

The results of the present study are in agreement with those reported [55] that treated tomato plant with different plant extracts at different application time exhibited lesser shoot fresh and dry weight than healthy plants, inoculated control. This is in accordance earlier findings [55] that reported that some aqueous plant extracts increased plant growth parameters including plant height at varying degrees over the infected control against *R. solanacearum* under field experiment.

5. CONCLUSION

S. *didymobotria* root extract and *M. oleifera* seed extract at 15% were found to inhibit the growth of *R. solanacearum* both *in vivo* and *in vitro*, but *S. didymobotria* root extract was better in action as compared to *M. oleifera* seed extract. *S. didymobotria* plant extract at 15% can be used in the integrated management of *R. solanacearum* disease through soil drenching in tomato because it has a lower disease incidence which in turn increased the yield.
T.
Further studies are required to determine the effectiveness of *S. didymobotria* and *M. oleifera* products in the field and to compare different tomato cultivars using different solvents.

Further studies should be done to determine the qualitative and quantitative amounts of phytochemicals present in the two plant extracts.

DISCLAIMER

This paper is an extended version of a Thesis document of the same author.
The Thesis document is available in this link: http://edocs.maseno.ac.ke/bitstream/handle/123456789/775/Buyela%20D.Khasabulli%20Msc%20thesis.pdf?isAllowed=y&sequence=3
[As per journal policy, Thesis article can be published as a journal article, provided it is not published in any other journal]

ACKNOWLEDGEMENTS

We wish to express our sincere thanks to Kaimosi Friends University and Maseno University for providing all the required support in form of equipment and finance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Salam MA, Siddique MA, Rahim MA, Rahman MA, Goffar MA. Quality of tomato

as influenced by Boron and zinc in presence of different doses of cow dung. Bangladesh J Agric Res. 2011;36(1): 151-63.
2. Olaniyi JO, Akanbi WB, Adejumo TA, OG. Growth, fruit yield and nutritional quality of tomato varieties. Afr J Food Sci. 2010;4(6):398-402.
3. Ayandiji A, OR A, Omidiji D. Determinant post harvest losses among tomato farmers in Imeko-afon local government area of Ogun state, Nigeria. Global. J Sci Frontier Research. 2011;11:312-9.
4. Sen S, Chakraborty R. Food in health preservation and promotion: A special focus on the interplay. Explor Nutr Health Benefits Funct Foods. 2016;1: 265-76.
5. Ilahy R, Hdider C, Lenucci MS, Tlili I, Dalessandro G. Phytochemical composition and antioxidant activity of high-lycopene tomato (*Solanum lycopersicum* L.) cultivars grown in Southern Italy. Sci Hortic. 2011;127(3):255-61.
6. Asgedom S, Struik PC, Heuvelink E, Araia W. Opportunities and constraints of tomato production in Eritrea. Afr J Agric Res. 2011;6(4):956-67.
7. Manila R, Nelson R. Nutrient uptake and promotion of growth by arbuscularmycorrhizal fungi in tomato and their role in Bio-protection against the tomato wilt pathogen. J Microbiol Biotechnol Res. 2017;3(4):42-6.
8. Shin TS, Yu NH, Lee J, Choi GJ, Kim JC, Shin CS. Development of a biofungicide using a mycoparasitic fungus Simplicillium lamellicola BCP and its control efficacy against gray mold diseases of tomato and ginseng. Plant Pathol J. 2017;33(3): 337-44.
9. Bona E, Cantamessa S, Massa N, Manassero P, Marsano F, Copetta A et al. Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads improve yield, quality and nutritional value of tomato: A field study. Mycorrhiza. 2017;27(1):1-11.
10. Yuliar, Nion YA, Toyota K. Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. Microbes Environ. 2015;30(1):1-11.
11. Mansfield J, Genin S, Magori S, Citovsky V, Sriariyanum M, Ronald P et al. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol. 2012;13(6):614-29.
12. Nguyen MT, Ranamukhaarachchi SL. Soil-borne antagonists for biological control of bacterial wilt disease caused by *Ralstonia solanacearum* in tomato and pepper. J Plant Pathol. 2010;4:395-405.
13. Sutariati GA, Ilyas Javanmardi S, Azarpanah A. Bioefficacy and characterization effect of arbuscular mycorrhizae fungi on defence response diseases and soil sickness: A review. Crop Plants, 3. 2015. Biological seed treatment for controlling anthracnose disease of hot pepper. International Journal of Sustainable Tropical Agricultural Sciences. 2014;1(1):110-117:116-121.S.
14. Yadeta KA, Thomma BP. The xylem as battleground for plant hosts and vascular wilt pathogens. Induced Plant Resp Microbes Insects. 2014;1:110-6.
15. Santiago TR, Lopes CA, Caetano-Anollés G, Mizubuti ESG. Phylotype and sequevar variability of Ralstonia solanacearum in Brazil, an ancient centre of diversity of the pathogen. Plant Pathol. 2017;66(3):383-92.
16. Achari GA, Ramesh R. Diversity, biocontrol, and plant growth promoting abilities of xylem residing bacteria from solanaceous crops. Int J Microbiol. 2014;4(2):117-225.
17. Muthoni J, Kabira J, Shimelis H, Melis R. Spread of bacterial wilt disease of potatoes in Kenya: who is to blame? Int J Hortic. 2014;4(3):117-22.
18. Hassan S, Inam-Ul-Haq M, Naz F, Tahir MI, Ali Z. *In vitro* investigations on host specificity of Ralstonia solanacearum among solanaceous crops and its biological control in tomato. Pak J Bot. 2016;48(3):1279-87.
19. Zadehbagheri M, Javanmardi S, Azarpanah A. Bioefficacy and characterization effect of arbuscular mycorrhizae fungi on defence response diseases and soil sickness: A review. Crop Plants. 2014;3:116-21.
20. Abdel-Monaim MF, Abdel-Gaid MA, Zayan SA, Nassef DMT. Enhancement of growth parameters and yield components in eggplant using antagonism of Trichoderma spp. against Fusarium wilt disease. Int J Phytopathol. 2014;3(1):33-40.
21. Hyakumachi M, Nishimura M, Arakawa T, Asano S, Yoshida S, Tsushima S et al. Bacillus thuringiensis suppresses bacterial

wilt disease caused by Ralstonia solanacearum with systemic induction of defense-related gene expression in tomato. Microbes Environ. 2013;28(1):128-34.
22. Abo-Elyousr KAM, Seleim MAA, Abd-El-Moneem KMH, Saead FA. Integrated effect of Glomus mosseae and selected plant oils on the control of bacterial wilt disease of tomato. Crop Prot. 2014;66:67-71.
23. Deberdt P, Perrin B, Coranson-Beaudu R, Duyck PF, Wicker E. Effect of Allium fistulosum extract on Ralstonia solanacearum populations and tomato bacterial wilt. Plant Dis. 2012;96(5): 687-92.
24. Sunder J, Jeyakumar S, Kundu A, Srivastava RC, Kumar DEA. Effect of Moringa citrifolia extracts on in-vitro growth of Ralstonia solanacearum. Arch Appl Sci Res. 2011;3(3):394-402.
25. Shams KA, Abdel-Azim NS, Saleh IA, Hegazy ME, El-Missiry MM, Hammouda FM. Phytochemical, antioxidant and antimicrobial studies of Salvia splendens leaves. J Chem Pharm Res. 2015;7(5):1050-74.
26. Ara I, Bukhari NA, Solaiman D, Bakir MA. Antimicrobial effect of local medicinal plant extracts in the Kingdom of Saudi Arabia and search for their metabolites by gas chromatography-mass spectrometric (GC-MS) analysis. J Med Plants Res. 2012;6(45):5688-94.
27. Kaur L, Joseph L, George M. Phytochemical analysis of leaf extract of Aesculus indica. Int J Pharmarcy Pharm Sci. 2011;3(5):232-4.
28. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564-82.
29. Jeyaseelan EC, Jenothiny S, Pathmanathan MK, Jeyadevan JP. Antibacterial activity of sequentially extracted organic solvent extracts of fruits, flowers and leaves of Lawsonia inermis L. from Jaffna. Asian Pac J Trop Biomed. 2012;2(10):798-802.
30. McCloud TG. High throughput extraction of plant, marine and fungal specimens for preservation of biologically active molecules. Molecules. 2010;15(7):4526-63.
31. Ahmed NN, Islam MR, Hossain MA, Meah MB, Hossain MM. Determination of races and biovars of R. solanacearum causing bacterial wilt disease of potato. J Agric Sci. 2013;5(6):86-91.
32. Singh A, Jain A, Sarma BK, Upadhyay RS, Singh HB. Rhizosphere competent microbial consortium mediates rapid changes in phenolic profiles in chickpea during *Sclerotium rolfsii* infection. Microbiol Res. 2014;169(5-6):353-60.
33. Majeed A, Muhammad Z. 2019An overview of the common bacterial diseases of potato in Pakistan, associated crop losses and control stratagems. J Plant Pathol. 2020;102(1):3-10.
34. Grover A, Chakrabarti SK, Azmi W, Khurana SM. Rapid method for isolation of PCR amplifiable genomic DNA of Ralstonia solanacearum infested in potato tubers. Adv Microbiol. 2012;2:441-6.
35. Mushore J, Matuvhunye M. Antibacterial properties of *Mangifera indica* on Staphylococcus aureus. Afr J Clin Exp Microbiol. 2013;14(2):62-74.
36. Almoneafy AA, Xie GL, Tian WX, Xu LH, Zhang GQ, Ibrahim M. Characterization and evaluation of Bacillus isolates for their potential plant growth and biocontrol activities against tomato bacterial wilt. Afr J Biotechnol. 2012;11(28):7193-201.
37. Groth I, Schumann P, Martin K, Schuetze B, Augsten K, Kramer I et al. Ornithinicoccus hortensis gen. nov., sp. nov., a soil actinomycete which contains L-ornithine. Int J Syst Bacteriol. 1999;49(4):1717-24.
38. Riaz M, Farooq J, Sakhawat G, Mahmood A, Sadiq MA, Yaseen M. Genotypic variability for root/shoot parameters under water stress in some advanced lines of cotton (*Gossypium hirsutum* L.). Genet Mol Res. 2013;12(1):552-61.
39. Lolaei A. Effect of calcium chloride on growth and yield of tomato under sodium chloride stress. J Ornamental Hortic Plants. 2012;2(3):155-60.
40. Kitonde CK, Fidahusein DS, Lukhoba CW, Jumba MM. Antimicrobial activity and phytochemical screening of Senna didymobotrya used to treat bacterial and fungal infections in Kenya. Int J Educ Res. 2014;2(1):1-12.
41. Ngule CM, Swamy A. Phytochemical and bioactivity evaluation of Senna didymobotrya fresenirwin used by the Nandi community in Kenya. Int J Bioassays. 2013;2(7):1037-43.
42. Kalappurayil TM, Joseph BP. A review of pharmacognostical studies on Moringa

oleifera Lam. flowers. Pharmacogn J. 2017;9(1):122-7.
43. Surendra TV, Roopan SM, Arasu MV, Al-Dhabi NA, Rayalu GM. RSM optimized Moringa oleifera peel extract for green synthesis of *M. oleifera* capped palladium nanoparticles with antibacterial and hemolytic property. J Photochem Photobiol B. 2016;162:550-7.
44. Bukar A, Uba A, Oyeyi T. Antimicrobial profile of *Moringa oleifera* Lam. extracts against some food–borne microorganisms. Bayero J Pure App Sci. 2010;3(1):211-9.
45. Rasika J. Antimicrobial activity of Moringa oleifera and its synergism with *Cleome viscosa*. Int J Life Sci. 2013;1(3):182-9.
46. Lee YH, Choi CW, Kim SH, Yun JG, Chang SW, Kim YS et al. Chemical pesticides and plant essential oils for disease control of tomato bacterial wilt. Plant Pathol J. 2012;28(1):32-9.
47. Tahat MM, Sijam K, Othman R. Ultrastructural changes of tomatoes (*Lycopersicon esculentum*) root colonized by Glomusmosseae and Ralstonia solanacearum. Afr J Biotechnol. 2012;11(250):6681-6.
48. Abo-Elyousr KAM, Khalil Bagy HMM, Hashem M, Alamri SAM, Mostafa YS. Biological control of the tomato wilt caused by *Clavibacter michiganensis* subsp. michiganensis using formulated plant growth-promoting bacteria. Egypt J Biol Pest Control. 2019;29(1):54.
49. Gaggìa F, Capece A, Marino G. Antimicrobial and antifungal activity of different extracts from *Melia azedarach* L. on phytopathogenic bacteria and fungi of agro-food interest. Book Admin. 2008;41.
50. Hassan MAE, Bereika MFF, Abo-Elnaga HIG, Sallam MAA. Direct antimicrobial activity and induction of systemic resistance in potato plants against bacterial wilt disease by plant extracts. Plant Pathol J. 2009;25(4):352-60.
51. Mithöfer A, Maffei ME. General mechanisms of plant defense and plant toxins Plant Toxins. 2016:1-22.
52. Ningombam A, Ahluwalia V, Srivastava C, Walia S. Antifeedant activity and phytochemical investigation of Millettia pachycarpa extracts against tobacco leaf eating caterpillar, Spodopteralitura (Fabricius) (Lepidoptera: Noctuidae). J Asia Pac Entomol. 2017;20(2):381-5.
53. Ammon HP. Boswellic acids and their role in chronic inflammatory diseases Anti-inflammatory nutraceuticals and chronic diseases. Adv Exp Med Biol. 2016;928:291-327.
54. Alemu D, Lemessa F, Wakjira M, Berecha G. Inhibitory effects of some invasive alien species leaf extracts against tomato (*Lycopersicon esculentum* Mill.) bacterial wilt (*Ralstonia solanacearum*). Arch Phytopathol Plant Prot. 2014;47(11):1349-64.
55. Abbas M, Shakeel S, Ji P, Zafar-ul-Hye M. Induction of resistance in tomato against Sclerotium rolfsii by application of seaweed extract and chemical compounds. International Journal of Pest Management. 2022;1-2.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/92318