

Effect of methanolic extracts of *Senna didymobotrya* and *Moringa oleifera* on growth of *Ralstonia solanacearum*

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 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 ($P \leq 0.05$). Both plants extracts showed inhibitory activity against *R. solanacearum* pathogen (*M. Oleifera*; 8.5 and *S. didymobotrya* 9.2). From 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. solanacearum* by tomato farmers in Maseno region Kisumu county.

Key words: Tomato, *Ralstonia solanacearum*, *Senna didymobotrya*, *Moringa oleifera*, Bacterial wilt.

1.0 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] [8] [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 between 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][20][21][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, more over, the inhibitory effects of *S. didymobotrya* root extract and *M. oleifera* seed extracts on *R. solanacearum* was unknown therefore this study was initiated to answer this study questions of the unknown.

2.0 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 25⁰C- 40⁰C and Night temperatures ranged between 20⁰C-30⁰C, 14/10h photoperiod and an humidity 70-90%. 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 receives both short and long rains averaging 1750mm per annum with mean temperature of 28.7⁰C.

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 (25-30⁰C) 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] before 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]. 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°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°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 1×10^8 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 40°C 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 37°C 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 senesced 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. 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.

$$\text{Disease index (\%)} = \left[\sum (ni \times Vi) / (V \times N) \right] \times 100.$$

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 below [37].

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

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

Where D.I= Disease index.

2.9 Determination of Root and Shoot length

Tomato plants were removed from the green house, and 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.

2.12 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 ripe. Collection of fruits continued until the end of the harvesting period (three months).

3.0 RESULTS

3.1 Inhibitory effects

Generally, bacterialstatic activity results (Table 1) showed that *R. solanacearum* were sensitive to *M. oleifera* and *S. ditymobotria* at 15% concentration. All the extracts showed antimicrobial activity by developing clear zones of inhibition (plate 1 and 2). The presence of inhibition zones depicted the antibacterial activity of *M. oleifera* and *S. ditymobotria* 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) (table1). *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

	<i>Senna didymobotria</i>	<i>Moringa oleifera</i>
ISOLATE	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 $P \leq 0.05$ by Fisher's protected least significant difference test.

R. didymobotria root extract did not have a significant difference ($P \geq 0.05$) for Maseno, Mariwa and Seme isolates but there was a significance different ($P \leq 0.05$) for Holo isolate (Table 1). However *M. oleifera* seed extract showed significant difference between Maseno and Holo isolates but did not show significance difference ($P \geq 0.05$) 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 ($P \leq 0.05$) between *M.oleifera* and *S.didymobotria*.

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

Treatment	Inhibition zones (mm)
<i>M. oleifera</i> seed extract	8.5222b
<i>S. didymobotria</i> 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 $P \leq 0.05$.



Plate 1

Plate 2



Plate 3

Plate 4

Plate 1. *S.ditymobotriainhibition* Zone at 15% of the extract

Plate 2. *M.oleifera* inhibition zones at 15% of the extract

Plate 3. *S. ditymobotria* control

Plate 4. *M. oleifera* control

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 ($P \leq 0.05$) difference on their effect against growth of *R.solanacearum* (Table3). In both cases, antibacterial activity of the test materials increased as their concentration increased but *S.didymobotria* was more effective than *M. oleifera*.

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%
<i>M. oleifera</i> root extract	0.0000a	8.28b	10.11a	12.03b	12.19b
<i>S. didymobotria</i> 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 $P \leq 0.05$ by Fisher's protected least significant difference test.

3.2 Effect of *S. didymobotria* root extract and *M. oleifera* seed extract on disease incidence

<i>S. didymobotria</i>	0 d	20b	20c	34.75b	40c	40c	40c	40c	40c	40c	40c	40c
<i>M. oleifera</i>	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
<i>R. solanacearum</i> (+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 $P \leq 0.05$ by Fisher's protected least significant difference test. *S. didymobotria* and *M. oleifera* plant extracts in millimeters.

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 ($P \geq 0.05$) 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 ($P \leq 0.05$) 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 ($P \leq 0.05$) 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 ($P \geq 0.05$) 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 ($P \leq 0.05$) 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 ($P \leq 0.05$) on the shoot height was established between the various treatments and the tomato plants.

There was a significant decrease ($P \leq 0.05$) 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.

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
<i>Senna didymobotria</i>	62.23b	12.10 a	14.00a	6.32 a	78.87 a	19.06 b	19.93 b
<i>Moringa oleifera</i>	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 $P \leq 0.05$ by Fisher's protected least significant difference test. *S. didymobotria* and *M. oleifera* are plant extracts in millimeters.

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*. *Senna 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.

4.0 DISCUSSIONS

4.1 Efficacy of treatments in *invitro* 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 $P \leq 0.05$ 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 $p \leq 0.05$ 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] [47] [48] and [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 [53] [28]. 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, uninoculated 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.

5.0 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 *Senna didymobotria* root extract was better in action as compared to *Moringa oleifera* seed extract.

Senna 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.

Further studies are required to determine the effectiveness of *Senna didymobotria* and *Moringa 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.

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