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Antibacterial Activity of Some Invasive Alien Species Extracts Against Tomato (*Lycopersicon esculentum* Mill) Bacterial Wilt Caused by *Ralstonia solanacearum* (Smith)

[1,2]Derib Alemu, [2]Fikre Lemessa, [2]Mulatu Wakjira and [2]Gezahegn Berecha
[1]Department of Plant Science, Debre Berhan University, P.O. Box 445, Debre Berhan, Ethiopia
[2]Department of Horticulture and Plant Sciences, Jimma University, P.O. Box 307, Jimma, Ethiopia

Abstract: Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetables in the world including Ethiopia. However, its production is constrained by different abiotic and biotic factors. Among biotic factors, bacterial wilt caused by *Ralstonia solanacearum* is one of the most important pathogens, threatening the production of tomato and potato in Ethiopia. So far there is no single means that would totally manage the disease and provide an absolute cure or fully protect host plants against the pathogen. Hence it is important to look for alternative mechanisms of disease management that can be used as an integrated disease management scheme. This study was, therefore, initiated with the objectives of evaluating the antibacterial activities of aqueous and solvent (acetone and methanol) extracts of five invasive alien species (*Eichhorina crassipes, Mimosa diplotricha, Lantana camara* and *Prosopis juliflora*) against *R. solanacearum*. *In vitro* antibacterial test was carried out in disc diffusion sensitivity test in a completely randomized design with three replications. It is evident from the result that most of the plant extracts exhibited significant inhibition of the bacterial growth compared with the control. Aqueous extract of *E. crassipes* provided the highest inhibition zone (26 mm), followed by *M. diplotricha* (14 mm). After *in vitro* screening, four promising invasive alien species extracts (aqueous extracts of *E. crassipes, M. diplotricha, L. camara* and methanolic extract of *P. juliflora*) with inhibition diameter>10 mm were selected and Minimum Inhibitory Concentration was assessed *in vitro*. They were also evaluated on tomato plants by applying the botanicals at three time of application (at the time of inoculation and 2-days before and after inoculation). The result of current study revealed that most of the treatment combinations significantly reduced percent disease severity index, but the inhibitory activities of tested plant species were reliant on type of plant species and their application time. More than 91% reduction in percent severity index of bacterial wilt was observed in tomato plants treated with leaf extract of *E. crassipes* when it was applied at a time of inoculation. The result suggested a need to continue research on invasive alien species extracts and determine their active principles to develop environmentally friendly management approach against bacterial wilt of tomato.

Key words: Antibacterial activity, botanicals, inhibition zone, *Eichhorina crassipes*, *Mimosa diplotricha*, *Lantana camara*, *Prosopis juliflora*, crude extract

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* (Smith), is one of the most destructive soil borne plant diseases. It is predominantly damaging in humid climates and at low and medium elevations in the tropical, subtropical and warm temperate regions of the world (Kurabachew *et al*., 2007; Mwangi *et al*., 2008). The pathogen *R. solanacearum* is highly heterogeneous species and have different host range that differs in geographical distribution, pathogenecity and physiological properties (Hayward, 1991). In Ethiopia, bacterial wilt was first recorded in 1956 on potato and eggplant around Jimma in the western part of the country (Stewart, 1956). Since then it causes a severe loss and becoming the major limiting constraint in increasing the production of tomato and potato in the country (Lemessa and Zeller, 2007b).

So far different control strategies have been employed and suggested such as use of resistant variety, crop rotation, selection of disease free planting material, disinfection of plant materials (Guo *et al*., 2004), microbial antagonists (Lemessa and Zeller, 2007a) and organic soil amendments (Yadessa *et al*., 2010; Getachew *et al*., 2011)

Corresponding Author: Derib Alemu, Department of Plant Science, Debre Berhan University, P.O. Box 445, Debre Berhan, Ethiopia

and other cultural practices as single or integrated disease management. However, managing the disease totally and protecting host plants from infection through employing a single means is still a dream of researchers. Therefore, alternative management options are strongly considered necessary.

Antimicrobial agents which are obtained from plants through secondary metabolism protect themselves from pathogen attack (Macdonald, 2008). The antimicrobial activities of plant products such as plant extracts and essential oils have been reported by different researchers and gaining due attention as they are environmentally safe and economically feasible (Macdonald, 2008; Rhouma *et al*., 2009; Abera *et al*., 2011). Therefore, plant extracts or plant secondary metabolites which are not toxic and specific in their action could be considered as an alternative to synthetic bactericides based on the availability of material, hence our exploration is on more available weedy and invasive alien species.

In Ethiopia, Invasive Alien Species (IAS) is of a major problem and causing a negative impact on the environment, especially on biodiversity of the country, either socially or economically. The reason to choose and work with IAS is that IAS may be such successful competitors due to resistance towards different pathogens. These plant species may therefore contain active principles to resist plant pathogen attack and the IAS are used as raw material for plant derived chemicals then there are large quantities of material readily available for use. Therefore, the objectives of this study were to evaluate the antibacterial activity of some IAS extracts against tomato bacterial wilt caused by *R. solanacearum* under *in vitro* and percent disease severity on tomato plants under *in vivo* conditions.

MATERIALS AND METHODS

Study area: The research was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUAVM), Jimma, Ethiopia, in Plant Pathology laboratory.

Collection and preparation of plant materials: Fresh leaf samples of the five major Invasive Alien Species (IAS) viz. *Parthenium hysterophorus* (Family, Asteraceae); *Lantana camara* (Family, Verbenaceae); *Eichhornia crassipes* (Family, Pontederiaceae); *Mimosa diplotricha* and *Prosopis juliflora*, belonging to the same family, Fabaceae, were collected in July 2011. *P. hysterophorus*, *L. camara* and *M. diplotricha* were collected from natural habitats around Jimma, Ethiopia, in August 2011, while *E. crassipes* and *P. juliflora* were collected from natural habitats around Ziway and Afar, Ethiopia, in July 2011, respectively. The collected samples of each IAS were washed under tap water, surface sterilized with 5% sodium hypochlorite solution for two minutes followed by rinsing thoroughly with sterile water. Then the plant samples were cut in to smaller size of about 2-3 cm long; air dried at room temperature, ground with the help of sterile pestle and mortar in to fine powder and kept in refrigerator until use.

Extraction of plant materials: Crude plant leaf extract was obtained following standard procedures described by Nduagu *et al.* (2008). Fifty gram of each plant material was separately infused in 250 mL sterilized water, acetone (70%) and methanol (70%), separately, to give 20% (w/v) in a 1000 mL conical flask, kept on shaker for 24 h at 121 rpm. The infusion was filtered afterwards through double layer cheesecloth followed by Whatman No. 1 filter paper and the organic solvents were evaporated in hot air oven at 40°C. After solvent evaporation, the remaining crude extract was diluted with sterilized water and kept in air tight bottle and was put in refrigerator until use.

Isolation of the pathogen and pathogenicity test: The pathogen was originally isolated from symptomatic potato tubers collected from local markets and infected tomato plants which were collected from JUCAVM research field, Jimma, Ethiopia following standard procedures described by Abo-Elyousr and Asran (2009). Samples were surface sterilized by soaking in 2% sodium hypochloride solution for 1-2 min, rinsed twice in sterile water, cut in to small pieces and placed in a glass of sterile water. After 3-5 minutes milky exudates were oozed out from infected samples and a loopful of the resulting suspension was streaked over the surface of Casmino acid-Peptone-Glucose CPG (CPG) agar medium and plates were incubated at 28°C for 48 h and examined for colony development. The single colony technique was adopted to obtain pure cultures and identified as *R. solanacearum* based on their morphological and cultural characteristics on CPG agar medium as stated in Klement *et al.* (1990), tomato pathogenicity bioassay and some of the biochemical characteristics. Development of typical wilting symptom was recorded weekly and re-isolation of the bacteria was made on CPG agar medium to confirm the bacteria (Koch's rule).

The isolated bacterial cultures (strain RsJLMp and RsJUCAVMt) proven to be pathogenic and causing wilt symptoms to tomato plants. On CPG agar medium, colonies of both strains of *R. solanacearum* were visible after 24-48 h of incubation at 28°C and had typical characteristics; whitish creamy color, irregularly round,

larger in size, fluidal and opaque. The pure culture was maintained for further bioassays in sterilized water at room temperature as indicated by Kelman and Person (1954) to reduce mutation.

Biochemical tests of the pathogen for identification

Catalase test: Catalase test was performed according to methods described by He *et al*. (1983). One milliliter of a 3% solution of hydrogen peroxide (H_2S) was added to a Petri dish and a loop full of fresh culture grown on CPG agar medium was added into the solution. Release of bubble from the culture was recorded as catalase positive (Sands, 1990). According to this test the isolated strains of *R. solanacearum* were catalase positive; release gas upon addition of H_2S.

KOH solubility test: It was performed according to Fahy and Hayward (1983) using 24-48 h culture. Two to three drops of 3% KOH were put onto glass slide and the colony of test strain was stirred into the solution with clean loop for 5-10 s. When the solution was viscous enough to stick to the loop causing a thin strand of slime, then the test is recorded as KOH soluble (positive). Based on this test both strains of *R. solanacearum* were KOH positive, forming a thin strand of slime and were lifted up with the loop after stirring into the solution.

Carbohydrate utilization and oxidation of alcohols: Utilization of sugars and sugar alcohols was tested by following standard procedures described by Hayward (1964). The basal medium was constitutes: $NH_4H_2PO_4$ (1 g), KCl (0.2 g), $MgSO_4.7H_2O$ (0.2 g), bromothymol blue (0.03 g), agar (3 g) and distilled water 1 L. Before adding the agar the final pH of the medium was adjusted to 7 (an olivaceous green color) by drop wise addition of 40% w/v NAOH solution. Ten milliliter of each sugar and sugar alcohol solutions10% (w/v) were added to 90 mL of molten cooled Hayward's basal medium and 20 mL volumes of the resulting amended medium were dispensed into previously sterilized test tubes. About 4 mL of inoculum suspension of *R. solanacearum* grown on CPG for 48 h at 30°C was made in distilled water (OD = 0.1 at 600 nm) and with a sterile Pasteur pipette 0.1 mL of the prepared inoculum suspension was inoculated to each tube (Hayward, 1976). Three replicates were used and basal medium with no carbohydrate but inoculated with the pathogen was used as a control. then, it was incubated at 30°C and examined at 3, 7 and 14 days for change to acid pH (yellow color) from top downward and yellow color formation was considered as positive reaction (Hayward, 1964; 1976). Both strains of *R. solanacearum* (strain RsJLMp and RsJUCAVMt) utilized carbohydrate sugars (sucrose, maltose, glucose and lactose), but failed to oxidize sugar alcohols (soribitol and mannitol). This indicated that both strains are *R. solanacearum* belonging to Biovar 2 Race 3 (Hayward, 1964; EPPO, 2004).

Media preparation and culturing of the bacteria: The culture medium used was Casmino Peptone Glucose (CPG) agar consisting of 1.0, 10.0, 5.0 and 17.0 g L^{-1} of casmino acid, peptone, glucose and agar, respectively and then the mixture was boiled while stirring with a magnetic stirrer for 15 minutes to completely dissolve the powdered agar. The solution pH was then adjusted to 6.5 to 7.0 by using dilute solution of NaOH and was autoclaved at 121°C for 15 min under autoclave. After autoclaving, the medium was allowed to cool down and poured in to sterilized 9 cm Petri dishes and allowed to solidify. Then after the bacterial suspension was streaked over the already prepared media and the plates were incubated at 28°C for 24-48 h.

Preparation of antibacterial discs: Antibacterial sensitivity testing was carried out using the disc diffusion sensitivity test which is adopted from Opara and Wokocha (2008). Discs (5 mm in diameter) were punctured out from a Whatman No.1 filter paper. Thereafter the discs were placed in Petri dish diameter allowing about 4 mm in between discs and then sterilized in hot air oven at 160°C for 1 h. After allowing the disc to cool, filter paper discs (5 mm) were pipetted with 5 μL of each aqueous and solvent plant extracts. Sterilized water was used as control.

***In vitro* antibacterial assay:** Antibacterial activity of each plant material extracts was determined following the method described by Abo-Elyousr and Asran (2009). Sterile Petri dishes of 9 cm diameter was filled to a depth around 4 mm with CPG agar medium. Thereafter, 100 μL of the bacterial suspension from 24 h old culture was evenly spread over the medium using sterilized cotton swab as described by Wagura *et al*. (2011). The inoculated plates were allowed to dry for approximately 5 min and four antibacterial discs were placed per plate with a needle. The plates were then incubated at 28°C for 48 h. After incubation, diameters of inhibition zone around each disc were measured to the nearest millimeter (mm) with a ruler. Three replicates were used for each treatment and treatments were arranged in completely randomized design.

Determination of minimum inhibitory concentration (MIC): Based on the initial results obtained in *vitro*, four plant extracts that showed the most suppressive effect were further tested at different concentrations to determine the Minimum Inhibitory Concentrations (MIC). Accordingly, most successful IAS extracts that show

higher diameter of inhibition zones (>10 mm) were tested at four concentrations of extracts; 200, 150, 100 and 50 mg mL^{-1}, by dilution methods of the extract (w/v). The least concentration of each plant extract showing a clear zone of inhibition was taken as the MIC (Dikbas *et al*., 2009).

***In vivo* antibacterial assay:** *In vivo* antibacterial potential of selected plant extracts on disease severity was examined on tomato seedlings under greenhouse conditions. Four week tomato seedlings raised in plastic tray were transplanted in to pots having sterilized soil medium. After a week the potted plants were arranged in three groups in a greenhouse. The plants in the first group were inoculated with the pathogens 10^9 CFU mg mL^{-1} (30 mL) (Abo-Elyousr and Asran 2009; Sangoyomi *et al*., 2011) two days before application with plant extract (40 mL of each extract) Abo-Elyousr and Asran (2009). Plants in the second group were inoculated with the pathogen after two days application of plant extracts. Plants in the third group were inoculated with the pathogen at the same time of application as the plant extracts. Inoculated and non-inoculated control pots and plants were treated with an equal volume of distilled water.

Disease assessment and data analysis: Disease severity was assessed at weekly intervals for development of bacterial wilt symptoms. Severity were evaluated based on a six point rating scale (0 to 5) adopted from (Winstead and Kelman, 1952), where: 0 = no wilt symptoms, 1 = one leaf wilted, 2 = two or more leaves wilted, 3 = all leaves except the tip wilted, 4 = whole plant wilted and 5 = death (collapse) of the whole plant. Disease Severity Indexes (DSI) were calculated by following equation adopted from Cooke (2006):

$$DSI=\sum\left[\frac{d\times n}{N\times m}\right]\times 100$$

where, d is the disease rating on each plant, m is the maximum disease rating possible, N is the total number of plants examined in each replicate and n is number of plants in each score.

Finally, reduction of PSI was calculated using:

$$PR=\left[\frac{(PC-PT)}{PC}\right]\times 100'$$

where, PR is percent reduction, PC is percentage value of the control and PT is percentage value of the treatment group as described in Aliye *et al*. (2008).

In the study, diameters of inhibition zone (mm) and disease severity index were recorded and analyzed using analysis of Variance (ANOVA). Statistical analysis were done using SAS software version 9.2 (SAS, 2008). Single and interaction effect of factors were determined using the GLM procedure of SAS. Whenever significant interactions were observed between factors, the level of one factor was compared at each level of other factors and comparison of means at 5% level were made by Tukey's test. Angular transformation was performed for severity data before analysis to normalize variance.

RESULTS

***In vitro* antibacterial assay:** *In vitro* studies of antibacterial activities of aqueous, acetone and methanol leaf extracts of five IAS against the growth of *R. solanacearum* were conducted and found to give significantly different results (Table 1). *In vitro* antibacterial test result confirmed that most of the tested IAS extracts showed antibacterial activity, created inhibition zone around antibacterial discs, against *R. solanacearum*.

All aqueous extracts had significantly inhibited the growth of *R. solanacearum in vitro* compared with control, treated with sterile water. The antibacterial activity of aqueous extract of five IAS extracts was ranged from 8.56 to 26 mm. The highest diameter of inhibition zone was recorded from *E. crassipes* (26 mm) that was statistically different from all other treatment combinations and over the infected control.

The *in vitro* inhibitory activity of methanol extract against the target pathogen (*R. solanacearum*) ranged from inhibition diameter of 0 mm to 11.1 mm. Methanol extract of *P. juliflora* was provided the highest diameters of inhibition zone (11.1 mm) followed by *L. camara* methanol extract (7 mm). Similarly with acetone extract, the highest diameter of inhibition zone was displayed by *P. juliflora* (8.6 mm) that was significantly different over the other selected IAS extracts and control. Diameters of inhibition zone of 7.98, 7.81 and 6.12 mm were offered by acetone extract of *E. crassipes*, *M. diplotricha* and *P. hysterophorous* respectively. While, methanol extracts

Table 1: Interaction effect of invasive alien species (*Eichhornia crassipes*, *Lantana camara*, *Parthenium hysterophorous*, *Mimosa diplotricha* and *Prosopis juliflora*) extracts and three extractants (methanol, acetone and water) on mean diameter of inhibition zone (mm) against *Ralstonia solanacearum in vitro*

	Mean diameter of inhibition zone (mm)*		
	Solvents used for extraction		
Plant species	Methanol	Acetone	Water
E. crassipes	0.00^{i}	7.98^{ef}	26.00^{a}
L. camara	7.00^{g}	0.00^{i}	10.87^{c}
P. hysterophorous	0.00^{i}	6.12^{h}	8.85^{d}
M. diplotricha	0.00^{i}	7.81^{f}	14.00^{b}
P. juliflora	11.10^{c}	8.60^{de}	8.56^{de}
Control	0.00^{i}	0.00^{i}	0.00^{i}

* Data are means of three replicates in two separate experiments. Values in followed by a similar letter (s) are not significantly different at $\alpha = 0.05$ using Tukey test

of leaves of *M. diplotricha, P. hysterophorous* and *E. crassipes* and acetone extracts of *L. camara* were inactive against *R. solanacearum*.

Determination of minimum inhibitory concentration: Results in the prescreening study revealed that aqueous extract of *E. crassipes*, *M. diploterichа*, *L. camara* and methanol extract of *P. juliflora* gives higher diameter of inhibition zone, compared to all the tested IAS by forming inhibition zone of 26, 14, 10.87 and 11.1 mm, respectively (Fig. 1). Accordingly, the antibacterial activities of those extracts were tested at different concentrations (5, 10, 15 and 20%) against *R. solanacearum* to determine the MIC of each extract.

Analysis of variance showed that there was an interaction effect between selected IAS extracts and four concentrations indicating differences in their antibacterial effect against growth of *R. solanacearum* (Table 2). The results of the present study revealed that aqueous extracts of *E. crassipes* at 5, 10, 15 and 20% significantly reduced the growth of *R. solanacearum* compared to the control, treated with sterile water. While, statistically significant differences were not evident between 5% concentration extracts of *M. diplotricha*, *L. camara* and methanol extracts of *P. juliflora* and the control in diameter of inhibition zone. Thus, the Minimum Inhibitory Concentration for DIZ of *R. solanacearum* under *in vitro* condition were 5% for *E. crassipes* aqueous extract and 10% for aqueous extract of *M. diplotricha* and *L. camara* and methanol extract of *P. juliflora*.

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***In vivo* antibacterial assay**

Effect of botanicals on disease severity index: According to the present study, plant extracts, time of application and the interaction of the two had significantly ($p<0.05$) affect bacterial disease development in terms of disease severity index. Though the applications of all the four plant extracts resulted in reductions of wilt incidence and disease severity index, *E. crassipes* leaf extract was found better in restricting the symptom development of the disease than other three tested plants extracts. Accordingly, simultaneous application of aqueous extract of *E. crassipes* with pathogen inoculation resulted in reduction of 91.7% disease severity, compared to infected control (Table 3). Other treatments also resulted in a

Table 2: Antibacterial activity of *Eichhorina crassipes, Lantana camara and Mimosa diploterichа* aqueous extracts and methanolic extract of *Prosopis juliflora* against *Ralstonia solanacearum* at different concentrations under *in vitro* conditions

	Mean diameter of inhibition zone (mm)*			
	Concentration levels (%)			
Plant species	5	10	15	20
E. crassipes	8.84^{ef}	12.10^{c}	13.84^{b}	26.00^{a}
L. camara	0.00^{i}	6.10^{h}	6.50^{h}	10.87^{cd}
M. diplotricha	0.00^{i}	8.42^{fg}	10.33^{de}	14.00^{b}
P. juliflora	0.00^{i}	6.12^{h}	7.10^{gh}	11.10^{cd}
Control	0.00^{i}	0.00^{i}	0.00^{i}	0.00^{i}

*Data are means of three replicates in two separate experiments. Values followed by a similar letter (s) are not significantly different at $\alpha = 0.05$ using Tukey test

Table 3: Interaction effects of selected invasive Alien species extracts and their application times, 2-days pre-inoculation, simultaneously and 2-days post-inoculation, on mean disease severity of bacterial wilt disease on tomato caused by *Ralstonia solanacearum*, under greenhouse condition, pot experiment

	Mean disease severity index^{y} (%)					
	Application times					
	Before 2-days		Simultaneously		After 2-days	
Plant species (botanicals)	DSI	PR	DSI	PR	DSI	PR
E. crassipes	(20.0) 26.56^{cde}	75.0	(6.6) 14.76^{f}	91.7	(16.6) 24.0^{de}	79.2
L. camara	(20.0) 26.56^{cde}	75.0	(15.0) 22.79^{de}	81.2	(16.6) 24.04^{de}	79.2
M. diplotricha	(20.0) 26.56^{cde}	75.0	(11.6) 19.89^{ef}	85.5	(20.0) 26.56^{cde}	75.0
P. juliflora	(30.0) 33.03^{bc}	62.5	(23.3) 28.85^{cd}	70.8	(35.0) 36.27^{b}	56.2
Control^{z}	(80) 63.44^{a}	-	(80) 63.44^{a}	-	(80) 63.44^{a}	-

PR was calculated based on 'PR: [(DSIC-DSIT)/DSIC]×100', where, PR: Percent reduction, DSI: disease severity index, DSIT: disease severity index by the treatment group, DSIC: disease severity index by the control. Percent reductions were calculated from the actual data. ^{y}Values followed by the same letter (s) are not significantly different at ($\alpha = 0.05$) according to Tukey test and values in brackets are actual data before transformation. ^{z}Pathogen inoculated and sterile water treated instead of botanicals. Note: On uninoculated control, healthy plants, no natural infection occurred and wilt symptom development were not recorded, therefore, zero values have been omitted

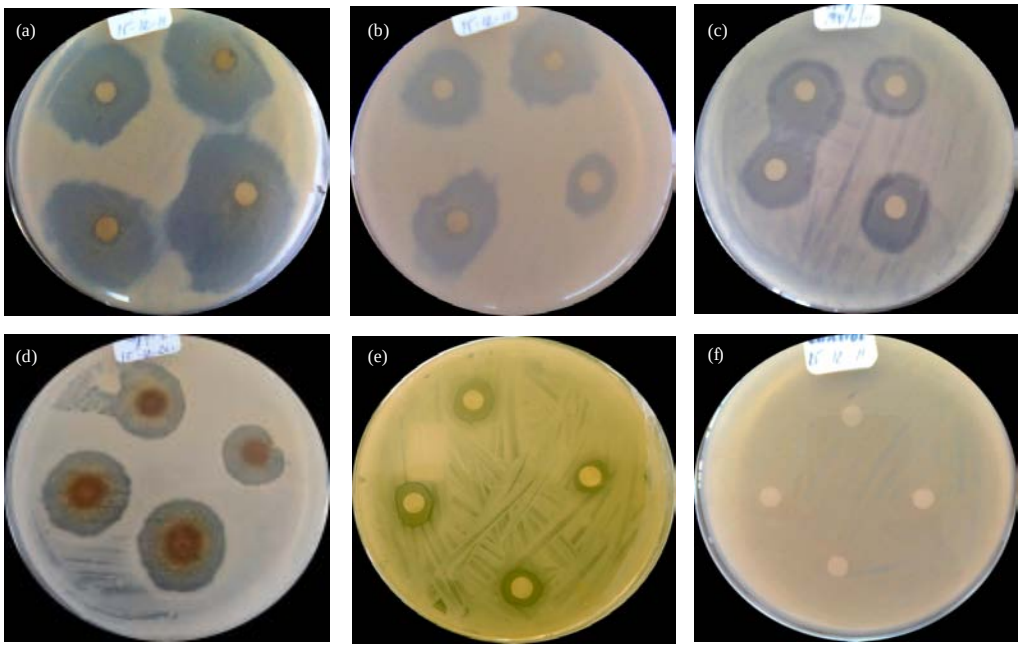

Fig. 1(a-f): *In vivo* antibacterial activity of the most effective invasive alien species extracts against *Ralstonia. solanacearum* (a) *Eichhorina crassipes* aqueous extract (b) *Mimosa diplotricha* aqueous extract (c) *Lantana camara* aqueous extract (d) *Prosopis juliflora* 70% methanol extract (e) *Parthenium hysterophorous* aqueous extract (f) control, untreated plate

significant reduction of mean disease severity index in tomato plants compared to none treated, infected control (Table 3).

DISCUSSION

Green plants have been shown to represents a reservoir of effective chematherapeutants and can provide valuable sources of natural pesticides (Dorman and Deans, 2000). Plants produce antimicrobial agents by secondary metabolism to protect themselves from pathogen attack and therefore many plant species possess substantial antimicrobial activity and have been screened and used in different fields of study (Macdonald, 2008).

In vitro antibacterial test result of the present study revealed that except methanolic extract of *E. crassipes*, *M. diploteric*ha, *P. hysterophorous* and acetonic extract of *L. camara*, the tested IAS extracts were effective for inhibition of bacterial growth verified by disc diffusion sensitivity test. The present study is in accordance with the works of Barsagade and Wagh (2010), who reported that acetone and methanol leaf extracts of common plants and weeds exhibited antibacterial and antifungal activities against one fungal and two bacterial species. Abo-Elyousr and Asran (2009), also reported that extracts of *Allium Sativum* L., *Datura stramonium* L. and *Nerium oleander* L., showed antibacterial activity and significantly inhibited the growth of *R. solanacearum* isolates *in vitro* compared with untreated control. Moreover, the antibacterial effects of crude medicinal plant extract of *Ocimum gratisimum*, *Brassica oleracae* and *Ipomoea batatas* against *Ralstonia solanacearum* were reported by Wagura *et al*. (2011).

Among plant extracts tested aqueous extract of *E. crassipes* was fond to be the best and provided inhibition zone of 26 mm. This result is in agreement with Shanab *et al*. (2010) and Aboul-Enein *et al*. (2011), who reported that the crude extract of *E. crassipes* leaf exhibited antibacterial activities against both the Gram positive and the Gram negative bacteria. The antibacterial activity of *E. crassipes* may be attributed to various chemicals detectable in its extracts. This is supported by many researchers, who reported phytochemical compositions present in crude extracts from *Eichhornia crassipes* are tannins, phlobatannin, steroid, terpenoid, alkaloid, flavonoid, phenolic contents, quinone, anthraqninone and cardiac glycosides (Lata and Dubey, 2010; Aboul-Enein *et al*. (2011).

In vitro antibacterial assay of the aqueous crude extract and methanol extract of *L. camara* showed moderate inhibitory activity against *R. solanacearum.*

But, acetonic extract of the same plant had no antibacterial activity against the test pathogen, suggesting that acetone is failed to solubilize bioactive compounds present in leaves of *L. camara* while they were better extracted in water and methanol. The antibacterial potential of *L. camara* extracts found in this study is supported by different workers which had been demonstrated against human pathogenic and phytopathogenic microorganisms (Sonibare and Effiong, 2008; Sharma *et al*., 2009; Sobia *et al*., 2012). The *in vitro* antibacterial activity assay of the aqueous and two solvent (methanol and acetone) crude extracts of *M. diplotricha* showed aqueous extract was the highest antibacterial activity and created inhibition zone of (14 mm), followed by acetone extract of the plant which was produced inhibition zone of (7.81 mm), confirming that the bioactive compounds are better extracted with water than acetone. Antibacterial compounds present in leaves of *M. diplotricha* were failed to solubilize in 70% methanol for which no inhibition zone was recorded.

Among the five plant species evaluated for their antibacterial activity, *P. juliflora* was the only plant that showed antibacterial activity in all solvents used for extraction. The antimicrobial potential of extracts from *P. juliflora* has been evaluated by many researchers against different microorganisms and supported the finding of this study. *P. juliflora* leaf extracts contained alkaloids; juliflorine, juliflocine and benzene insoluble alkaloidal fraction and all these alkaloids were found to be possessed significant antibacterial activity as reported by Ahmad (1991). Raghavendra *et al*. (2009) indicated methanol extract of *P. juliflora* recorded highly significant antibacterial activity among different solvent extracts tested which supports our finding; phytochemicals present in leaf of *P. juliflora* is relatively more soluble in methanol than acetone and water. They also reported that aqueous, methanol and ethanol extracts of *P. juliflora* leaves showed significant antibacterial activity against all tested phytopathogenic bacteria.

The leaf extracts of plants and weeds have great potential as antimicrobial compounds against different microorganisms. It has been suggested that the antimicrobial activity of plant secondary metabolite is mainly due to the presence of essential oils, flavonoids and triterpenoids and other natural polyphenolic compounds or free hydroxyl groups (Rojas *et al*., 1992). This is supported by Barsagade and Wagh (2010), who indicated antimicrobial activities of plant and weed extracts may exist in a variety of different components, including aldehyde and phenolic compounds. Variation in the effectiveness of the extract against a target microorganism depends upon the chemical compositions of the extracts and membrane permeability of the microbe for the chemicals. Naturally occurring combination of these compounds can be synergistic and often results in crude extracts having greater antimicrobial activity than the purified, individual constituents. The antimicrobial effect of the extracts could be explained by disturbance of the permeability barrier of the living membrane structure. Cowan (1999) came to similar conclusion.

Result from the *in vitro* study revealed that the antibacterial activity of all the selected IAS extracts were significantly ($p<0.0001$) affected by solvents used for extraction against *R. solanacearum*. Water extracts were relatively more effective than acetone and methanol and all aqueous IAS extracts showed antibacterial activity against the target pathogen. Thus, result of present study revealed that most of the active ingredients; secondary metabolites having antibacterial activity against *R. solanacearum*, present in leaves of all tested IAS are soluble in water. Bioactive compounds which have antibacterial activity against the target pathogen contained in *Mimosa diplotricha, Parthenium hysterophorous* and *Eichhorina crassipes* leaves cannot solubilize in methanol, showed no inhibition zone. Acetone also failed to solubilize active ingredients present in *L. camara* leaves against *R. solanacearum*.

According to the present study antibacterial activity results might have been influenced by the solubility of the active compound (s) in extracting solvents, with high solubility of *E. crassipes* compounds than other plant species tested. The greater effectiveness of aqueous extract compared with methanol and acetone extract may be due to differences in constituent and amount extraction of phytochemicals, which are toxic to *R. solanacearum*, present in leaf parts of tested IAS. The result of current study is comparable with Cowan (1999), who reported plant secondary metabolites; starches, polypeptides and lectins which have antimicrobial activity are soluble only in water while, xanthoxyllines, totarol, quassinoids, lactones and phenones are only soluble in methanol. This is corresponding with reports of Sukanya *et al*. (2009), who indicated that antimicrobial activity of various solvent extracts of medicinal plants showed varied level of inhibition against both human and phytopathogenic bacteria. In addition the potency of each extract differs from one another, suggesting that the toxicity of different extracts may be due to their solubility in extracting solvents. Qasem and Aau-Blan (1996) came to the same conclusion.

Analysis of variance showed that there was an interaction effect between selected IAS extracts and concentrations used ($p<0.005$) indicating differences on their antibacterial effect against growth of *R. solanacearum*. The result is in accordance with Balestra *et al*. (2009), who reported that *in vitro* test of

vegetal extracts from cloves of *Allium sativum* and fruits of *Ficus carica* against bacterial pathogens of tomato including *R. solanacearum* showed best antibacterial effect at different concentrations. Abera *et al*. (2011), also found a significant interaction effect between type of plant materials and different concentrations used indicating that the antifungal effect of plant extracts reliant on solvents used for extraction and concentration levels.

The result of the current study revealed that application of crude aqueous extracts of and methanol extract of selected IAS at different time of application significantly reduced bacterial wilt disease severity index, compared to infected control. But, varied results were found based on plant species used and their application time. This variation in restricting disease development between IAS and their application time might be due to the fact difference in chemical compositions of the extracts, membrane permeability of the target pathogen, difference in efficacy and durability of extracts in the soil. This was supported by the work of Hassan *et al*. (2009), 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.

In conclusion, it is interesting to notice that the compounds in crude extracts of *E. crassipes*, *M. diplotricha*, *L. camara* and *P. juliflora* showed antibacterial activity against the test pathogen, *R. solanacearum*. Even though the present study of antibacterial evaluation of IAS plants against *R. solanacearum* gives scientific information for further phytochemical analysis, further research is necessary to determine the identity of the antibacterial compounds and mechanisms of actions should also be studied.

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