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Control of tomato bacterial wilt using certain of plant ethanol extracts

Kamal A.M. Abo-Elyousr[1,2*], Hadeel M.M.K. Bagy[1]

[1]Plant Pathology Department, Faculty of Agriculture, Assiut University, 71526 Assiut, Egypt

[2]Department of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, 80208 Jeddah, Saudi Arabia

Abstract

In this study, the antibacterial properties of ethanol extracts of *Citrus sinensis*, *Citrus reticulate*, *Punica granatum* and *Cinnamomum camphorm* were tested *in vitro* and also under greenhouse conditions against *Ralstonia solanacearum*, the tomato bacterial wilt disease causal pathogen. *In vitro* experiments, *C. sinensis* caused the highest antibacterial activity against bacterial wilt followed by *P. granatum* and *C. camphorm*. All plant extracts increased the seed germination. In greenhouse experiments, the soil application of concentrations of 20% and 15% of the tested plant extracts were arranged in two groups: the first group was two days after inoculation and the second group was two days before inoculation. All plant extracts decreased significantly tomato wilt disease severity. Tomato treated with plant extracts two days before the inoculation caused in higher disease severity reducing compared to two days after inoculation. Generally, the concentration of 20% has significantly reduced bacterial wilt development than 15%. Also, all treatments promoted the tomato plants biomass. In conclusion, we can recommend using of plant extracts of *C. sinensis*, *C. reticulate*, *P. granatum* and *C. camphorm* in the disease control programs of bacterial wilt disease of tomato.

Keywords: *Ralstonia solanacearum*, plant extracts, bacterial wilt, tomato, disease control.

*Corresponding author: Kamal A.M. Abo-Elyousr,
E-mail: elyousr@aun.edu.eg, ka@kau.edu.eg

Introduction

Tomatoes (*Solanum lycopersicum* L.) are considered the maximum global significant vegetable crops after potato (Khoso, 1994). Yield loss of tomato plant 10–20% annually caused by soil borne diseases. *Ralstonia solanacearum* is an important pathogen of many crops (Yabuuchi *et al*., 1995). Also, it is the greatest eradicative soil-borne pathogen that impacts tomatoes causing bacterial wilt disease in all over the world, especially in temperate, subtropical and tropical regions (Yuliar *et al.,* 2015; Champoiseau *et al.,* 2009). Bacteria wilt is a hard control disease. Chemical control of tomato bacterial wilt is dangerous to environment, human and animal as well as raising the pathogens of chemical resistant. So, there is a critical requirement to substitute or at minimize the use of chemicals for reducing of the environment pollution. Besides, the use of chemicals has not been very effective in the control of such disease because the causal pathogen is a soil borne and is systemic in its nature. Antibiotics showed low effect and resistant varieties occurred due to the strain diversity and latent infection of the pathogen (Farag *et al.*, 1982). Use of crop rotation with non-host crops can decrease the populations of pathogen in the soil (Ahmed *et al*., 2000). The use of copper-based bactericides and antibiotics seldom gave satisfactory control. Use of plant extracts could be useful in control of this disease because of their natural origin are biodegradable and they do not leave any toxic residues or by products to accumulate in the environment (Abo-Elyousr & Asran, 2009). Therefore, under this scenario, botanical pesticides seem to be ideal candidate to be exploited in the tomato bacterial wilt control in view of the safety, renewable nature, cost effective and high target specificity. Hence an investigation was conducted to study the effect of extracts from a few important medicinal plants against *R. solanacearum*. Plant products are considered as significant sources for control of certain plant diseases (Abdel-Monaim *et. al.*, 2011; Hassan *et. al.*, 2009; Ji *et. al.*, 2005; Regnault *et. al.* 2005; Kagale *et. al.*, 2004). Plant extracts are actually safe alternatives for environment and it can be included in the disease control programs. Hassan *et. al.* (2009) used the ethanol extracts of *Eucalyptus globules Punica granatum* and *Hibiscus sabdariffa,* against the potato bacterial wilt disease. This study was planned to study the efficiency of *Citrus sinensis*, *Citrus reticulate*, *Punica granatum* and *Cinnamomum camphorm* extracts against the tomato bacterial wilt causal pathogen, and to examine the efficiency of time of applications on the disease severity.

Materials and methods

Source of the pathogen and inoculum production: A virulent isolate of *Ralstonia solanacearum* obtained from previous study (Abo-Elyousr & Asran 2009) was used. The pathogen isolate was grown on nutrient agar medium for 48 h at 28 °C. Pathogen cells were immersed with sterilized distilled water then scraped off the nutrient agar plate using a sterile cotton swab to make a suspension (Wai *et al*., 2013). The bacterial suspension concentration was adjusted using spectrophotometer (OD_{600} = 0.3) to 1.5 × 10^8 CFU/mL (Lin *et al*., 2014; Hindi, 2013) and they utilized in the following experiments.

Plants collection: Four local plants namely, *Citrus sinensis*, *Citrus*

reticulate, *Punica granatum* and *Cinnamomum camphorm* were collected from markets in Assiut, Egypt were used in the current study. The name of plant and its parts were used in this study are presented in Table (1).

Table 1: Plants sampled and parts used.

Botanical name	English Name	Part of plant used
Citrus sinensis	Orange	peel
Citrus reticulate	Tangerine	peel
Punica granatum	Pomegranate	peel
Cinnamomum camphora	Camphor	leaf

Plant extracts preparation: Fresh plant parts of *C. camphorm* and peel of *C. sinensis*, *C. reticulate* and *P. granatum* were carefully washed with tap water to remove the dust and other unwanted materials accumulated on the leaves and peel. The samples were dried under laboratory conditions for 20 days. Then, the dried leaves and peel were powdered using an electric blender. Finally, the powdered materials either leaves or peel were sieved through the strainer and the fine powder was collected and used for the extraction process. Ten grams of the weighed plant materials powder was soaked in 100 ml of ethanol into 200 ml conical flask plugged with sterile cotton and covered with aluminium foil and kept in a reciprocating shaker at 200 rpm for 24 h. Then, the extract was filtered through muslin cloth cloth and this repeated three times, then through Whatman no 1 filter paper. The solvent from the extracts was removed by using rotary vacuum evaporator. As a final point, the residues were collected, and the concentrations of 15 and 20% were prepared and used in the next experiments.

Efficacy of plant extracts against the pathogen *in vitro*: Antibacterial activities of the tested concentrations (15 and 20%) of the ethanol extracts plants were studied *in vitro* using the agar well diffusion method. The NA plate surface is inoculated by spreading a 100 µl of the pathogen suspension (10^8 CFU/ml) over the entire surface (Balestra *et al*., 2009). After that, a hole with a 9 mm diameter is aseptically bored with a sterilized cork borer. Hundred µl of test extracts was added in each well. Water was used as a control. After overnight incubation at 27˚C, inhibition zones were measured by a transparent ruler. The experiment was repeated twice.

Effect of plant extracts on seed germination under laboratory conditions: Tomato seeds cv. Super Marmande were soaked in each tested concentration of plant extracts and then treated with the pathogen. Seeds were carefully shaken for 1 h then, left to dry, and finally plated on wet blotters. Seeds treated only with the pathogen were used as control and the germination was measured according to the filter paper method (Abo-Elyousr & El-Handawy Hoda, 2008).

Effect of plant extracts on the disease severity under greenhouse conditions: Tomato plants cv. Super Marmande were grown in 20 cm sterilized pots filled with sterilized clay and sand mixture (2:1 w/w) under greenhouse conditions and

watered regularly. The plants were fertilized once with 100 mL/plant of micronutrients hydrosol fertilizer one week after transplanting. Plants were inoculated by clipping the lower leaf with scissors dipped in a fresh bacterial suspension of 10^8 CFU/mL (Hindi, 2013). The potted plants were arranged in two groups, the plants in the first group were inoculated with the pathogen two days after application with plant extract (40 ml of each extract) and the plants in the second group were inoculated with the pathogen two days before the plant extract application. Plants within each group were treated only with the pathogen were used as control. Each group was divided into two concentrations 15% and 20%. The experiment was repeated twice. Disease severity percentages were calculated four weeks after the application according to Wai *et al*. (2013). The 1-5 disease severity scale was used where, 1 = no visible symptoms, 2 = one leaf to half of the foliage wilting, 3 = nearly all of the foliage wilting, 4 = the whole plant wilting and dead. isease severity (%) was expressed as formula developed by Bdliya and Dahiru (2006):

$$\text{Disease severity (\%)} = [\sum (ni \times vi) \div (V \times N)] \times 100$$

Where: ni= number of plants with the respective disease rating, vi= the disease rating, V= the highest disease rating (5), and N= the total number of observed plants.

Measuring the fresh and dry weights: For estimating fresh weight and dry weight of all treatments, ten plants of each treatment were randomly taken, washed, left to dry and then their fresh weight was recorded. Then, these plants were dehydrated at 60°C for three days and the dry weight was determined.

Statistical analysis: All recorded data were analyzed statistically, Fisher's least significance difference test at p=0.05 was used to compare the average values of treatments (Gomez & Gomez, 1984).

Results and Discussion

***In vitro* study:** Tested concentrations of the ethanol extracts of *C. sinensis*, *C. reticulate*, *P. granatum* and *C. camphorm* were caused varied pathogen growth inhibition zones *in vitro* (Table 2). In compared with other plants, inhibition zones were the highest in case of use of orange extracts at 20%. *C. reticulate* at 20% caused the least inhibition zones (1.37 mm). Results also, showed that the use of orange produced the highest decreasing of the bacterial pathogen growth (2.2 mm) followed by *P. granatum* and the lowest one is *C. reticulate* extract. These results agree with those reported by others study Abo-Elyousr and Asran (2009), Ranjit *et al*. (2012) and El-Ariqi *et al*. (2005). Numerous investigators indicated that the plant contains a number of chemicals groups such as tannins and flavonoids (Ranjit *et al*., 2012; Kapoor, 2001). The bacterial growth inhibition caused by plant extracts possibly explicated on the basis of the act of antimicrobial secondary metabolites present in the plant.

Influence of certain plant extracts on seed germination percentage: Tested

plant extracts enhanced the tomato seed germination percentage compared to infected control, the treatment with *C. sinensis* extract was the best one which increased the seed germination percentage to 84.0%, followed by *P. granatum* and *C. camphorm* extracts especially at 20% concentrations (Figure 1). Among all tested treatments, *C. reticulate* achieved the lowest increasing percentage of the seed germination (48%).

Table 2: *In vitro* impact of some concentrations of certain plant extract on *Ralstonia solanacearum*.

Treatments	Concentration	Zone of inhibition (mm, dia.)	Mean
C. sinensis, Orange	15	1.8 de	2.2 a
	20	2.6 a	
C. reticulate, Tangerine	15	1.37 f	1.6 d
	20	1.87 d	
P. granatum, Pomegranate	15	1.77 de	2.0 b
	20	2.23 b	
C. camphor, Camphor	15	2.03 c	1.8 c
	20	1.7 e	
Control		0.0 g	0.0 e

Values in the same column have a similar superscript letter are not significantly different according to LSD test at *P*=0.05.

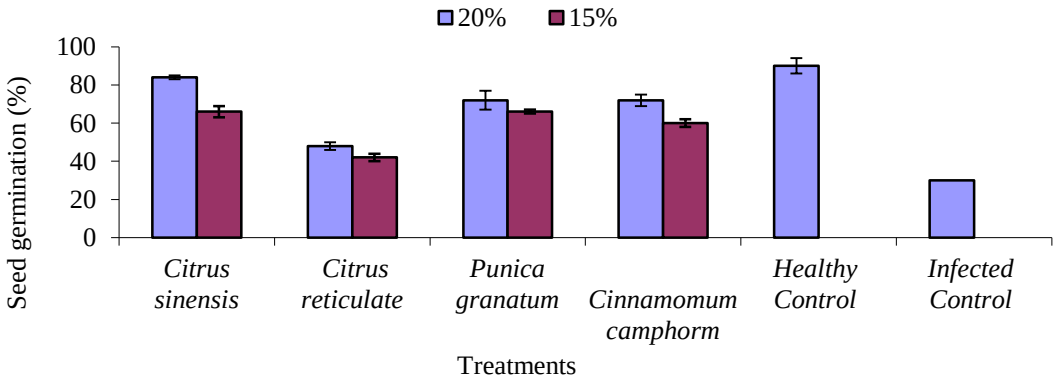

Figure 1: Influence of tested concentrations of certain plant extracts on seed germination percentage of tomato cv. Super Marmande *in vitro*. Bars indicate the standard error.

Effect of certain plant extracts on diseases severity under greenhouse conditions: Data in Table (3) revealed that all treatments either applied after or before inoculation reduced the disease severity compared to control, *C. sinensis* achieved the highest disease reduction percentage (89.6%) followed by *P. granatum* concentration (85.6%) while *C. reticulate* and *C. camphorm* after inoculation recorded the lowest disease reduction percentage (27.5%). Gaind and Budhiraja (1967) mentioned that the extracts of certain plants were found to be effective in inhibiting the activity of a broad spectrum of bacteria. Also, Abo-Elyousr and Asran (2009) found that the application of extracts of datura, garlic and nerium to the soil at two days before inoculation or the time of inoculation, and two days after inoculation by the pathogen, have significantly reduced the

disease severity of bacterial wilt of tomato plants under greenhouse conditions. As well as the use of these plant extracts could induce resistance in the plants against the pathogen or enhanced the peroxidases, phenylalanine ammonia lyase, pathogenicity-related proteins and phenolic substances against the bacterial wilt of tomato (Hassan *et al*., 2009).

Table 3: Efficacy of plant extracts 20% concentrations to control tomato bacterial wilt disease under the greenhouse conditions**.**

Treatments	Time of application from inoculation	Disease incidence (%)	Control efficacy (%)
C. sinensis, Orange	Before	6.8 c	89.6
	After	9.5 c	85.5
C. reticulate, Tangerine	Before	25 b	61.8
	After	47.5 d	27.5
P. granatum, Pomegranate	Before	16.25 c	75.2
	After	8.8 b	85.6
C. camphor, Camphor	Before	25.75 b	60.17
	After	47.5 d	27.5
Infected Control		82.25 a	-

Values in the same column have a similar superscript letter are not significantly different according to LSD test at *P*=0.05.

Table 4: Efficacy of plant extracts concentrations on biomass of tomato plants after inoculation with *Ralstonia solanacearum* under the greenhouse conditions.

Treatments	Time of application from inoculation	Fresh weight	Dry weight
C. sinensis, Orange	Before	43.67 a	16.2 ab
	After	23.08 c	14.77 abc
C. reticulate, Tangerine	Before	23.10 c	11.7 cde
	After	19.73 d	9.495 e
P. granatum, Pomegranate	Before	39.33 b	12.85 bcde
	After	24.93 c	13.3 bcd
C. camphor, Camphor	Before	24.23 c	13.48 bcd
	After	24.67 c	10.99 de
Infected Control		12.05 e	7.95 f
Healthy Control		44.73 a	16.13 ab

Values in the same column have a similar superscript letter are not significantly different according to LSD test at *P*=0.05.

Effect of specific plant extracts on tomato plants biomass under greenhouse conditions: All tested plant extracts in the two different times of application were caused increasing of shoot fresh and dry weights compared with control (Table 4). The highest fresh weight was recorded in tomato plant treated with *P. granatum* followed by orange before inoculation. Also, the highest dry weight was recorded in infected control and *P. granatum*. Our results are agreed with a previous study of Abo-Elyousr and Asran (2009). In conclusion, we can recommend that use of plant extracts of *C. sinensis*, *C. reticulate*, *P. granatum* and *C. camphorm* to control of *R. solanacearum* under greenhouse conditions.

References

Abdel-Monaim MF, Abo-Elyousr KAM, Morsy KM, 2011. Effectiveness of plant extracts on suppression of damping-off and wilt disease of lupine (*Lupinus*

termis Forsik). Crop Protection **30**:185–191.

Abo-Elyousr KAM, Asran MR, 2009. Antibacterial activity of certain plant extracts against bacterial wilt of tomato. Archives of Phytopathology and Plant Protection**42**: 573–578.

Abo-Elyousr KAM, El-Hendawy Hoda H, 2008. Integration of *Pseudomonas fluorescens* and acibenzolar-S-methyl to control bacterial spot disease of tomato. Crop Protection **27**: 1118–1124.

Ahmed AS, Sanchez CP, Candela ME, 2000. Evaluation of induction of systemic resistance in pepper plants (*Capsicum annuum*) to *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidol accumulation. European Journal of Plant Pathology **106**: 817–824.

Balestra GM, Heydari A, Ceccarelli D, Ovidi E, Quattrucci A, 2009. Antibacterial effect of *Allium sativum* and *Ficus carica* extracts on tomato bacterial pathogens. Crop Protection **28**: 807–811.

Bdliya BS, Dahiru B, 2006. Efficacy of some plant extracts on the control of potato tuber soft rot caused by *Erwinia carotovora* ssp *carotovora.* Journal of Plant Protection Research **46**: 285–294.

Champoiseau PG, Jones JB, Allen C, 2009. *Ralstonia solanacearum* race 3 biovar 2 causes tropical losses and temperate anxieties. Plant Health Progress **10**: 1.

El-Ariqi SNS, El-Moflehi M, El-Arbara K, El-Kobati A, El-Shaari A, 2005. Antibacterial activity of extracts from *Withania somnifera* and *Aloe vera* aginst *Ralstonia solanacearum* in potato. Arab Journal of Plant Protection **23**: 95–99.

Farag NS, Lashin SM, Abdel-All RS, Shatta HM, SeifElyazal AM, 1982. Antibiotics and control of potato black leg and brown rot diseases. Agricultural Research Review **60**(2): 149–166.

Gaind KN, Budhiraja RD, 1967. Antibacterial and anthelmintic activity of *Withania coagulans* Dunal. Indian Journal of Pharmacy **29**: 185–186.

Gomez KA, Gomez AA, 1984. Statistical procedures for agricultural research. John Wiley & Sons, New York, USA.

Hassan MAE, Bereika MFF, Abo-Elnaga HIG, Sallam MAA, 2009. Direct antimicrobial activity and induction of systemic resistance in potato plants against bacterial wilt disease by plant extracts. Plant Pathology Journal **25**: 352–360.

Hindi NKK, 2013. *In vitro* antibacterial activity of aquatic garlic extract, apple vinegar and apple vinegar - garlic extract combination. Am J Phytomed Clin Therapeutics **1**: 42–51.

Ji P, Momol MT, Olson SM, Pradhanang PM, Jones J, 2005. Evaluation of thymol as biofumigant for control of bacterial wilt of tomato under field conditions. Plant Disease **89**: 497–500.

Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R, 2004. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. Physiological and Molecular Plant Pathology **65**: 91–100.

Khoso AW, 1994. Growing vegetable in Sindh. 2nd Ed. Allied Printing Corporation, Hyderabad, India, 136 pp.

Lin CH, Tsai KC, Prior P, Wang JF, 2014. Phylogenetic relationships and population structure of *Ralstonia solanacearum* isolated from diverse origins in Taiwan. Plant Pathology **63**: 1395–1403.

Ranjit PM, Santhipriya T, Nagasri S, Chowdary Y, Pasumarthy N, Gopal V, 2012. Preliminary phytochemical screening and antibacterial activities of ethanolic extract of *Calotropis procera* flowers against human pathogenic strains. Asian Journal of Pharmaceutical and Clinical Research **5**(3): 127–131.

Regnault R.C, Philogène BJR, Vincent C, 2005. Biopesticides of Plant Origin. Lavoisier, Paris, France.

Wai KPP, Lee J, Mo HS, Kim BS, 2013. Sources of resistance to bacterial wilt and restorer-of-fertility genotype for cytoplasmic male sterility in *Capsicum pepper*. Horticulture Environment and Biotechnology **54**: 266–271.

Yabuuchi E, Kosako Y, Yano I, Hotta H, Nishiuchi Y. 1995. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. Nov.: Proposal of Ralstonia pickettii (Ralston, Palleroni and Doudoroff 1973) comb. Nov., *Ralstonia solanacearum* (Smith 1896) comb. Nov. and *Ralstonia eutropha* (Davis 1969) comb. Nov. Microbiology and Immunology **39**: 897–904.

Yuliar, Nion YA, Toyota K, 2015. Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. Microbes and Environments **30**(1): 1–11.