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MANAGEMENT OF POTATO BACTERIAL WILT USING PLANT EXTRACTS, ESSENTIAL OILS, ANTAGONISTIC BACTERIA AND RESISTANCE CHEMICAL INDUCERS.

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Abstract: Twenty isolates of *Ralstonia solanacearum* were isolated from naturally infected potato plants, collected from different localities of Assiut and Sohag Governorates. The isolate M4 exhibited the highest wilt severity followed by M6, M12, A16 and E17 isolates. The effectiveness of plant extracts, essential oils, antagonistic bacteria and resistance chemical inducers in controlling the bacterial wilt disease on potato cv. Diamont under greenhouse and field conditions was evaluated. Among all tested materials, only plant extracts of *Hibsicus sabdariffa*, *Eucalyptus globulus* and *Punica granatum* found to be able to inhibit the growth of bacterial pathogen *in vitro*. In greenhouse experiment, all tested treatments significantly reduced disease severity. Soil drench applications with 50 ml plant extracts/pot of *Eucalyptus globulus*, *Hibiscus sabdariffa* and *Punica granatum* and thyme oil, reduced profoundly disease severity by 94.17, 89.05, 78.99 and 84.83 %, respectively. Application of clove oil, plant extract of *Datura metel* and *Pseudomonas fluorescens* caused intermediate disease severity. Population of *R. solanacearum* was lowest in stems of potato plants treated with plant extracts and thyme oil than in inoculated control, however, other tested treatments caused slight effect. Under field conditions, application of plant extracts, salicylic acid and *Pseudomonas aeruginosa* caused highest reduction in severity of bacterial wilt, marked increase of fresh and dry weight of potato plants and tubers yield. However, application of bacterial suspension of *Pseudomonas fluorescens*, acibenzolar-S-methyl and clove oil showed slight reduction in disease severity and moderate increase in both tuber yield and fresh and dry weights of plants.

Keywords: Potato plants, *Ralstonia solanacearum*, plant extracts, essential oils, resistance chemical inducers.

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Introduction

Bacterial wilt caused by *Ralstonia solanacearum* is an important disease that spreads worldwide and infects hundreds of plant species, such as potato, tomato, banana, pepper and even trees. In potato, *R. solanacearum* not only causes wilt in the above-ground part of the plant but also rotting of the tubers. The tuber symptoms are often described as brown rot (Ooshiro *et al.*, 2004). Potato crop losses up to 75% due to the bacterial wilt have been recorded in many countries (Cook and Sequeira 1994; Castillo and Greenberg 2007). The disease is widely distributed in tropical, subtropical and some warm temperate regions of the world (Hayward, 1991). In Egypt, it is considered as one of the limiting factors to potato production (Messiha *et al.*, 2007). In the last few years, the disease has took more attention as serious problem for potato exportation to Europe and therefore plant quarantines in importing countries are quite alert for the Egyptian potatoes (El-Ariqi *et al.*, 2005).

Control of bacterial wilt is very difficult because *R. solanacearum* survives in the soil for ten years (Abdalla *et al.*, 1999). Some resistant cultivars are available but these are not adapted to different agro-ecological zones and are not effective against all strains of the pathogen (Mendoza, 1994; Lopez and Biosca 2004). Breeding for disease tolerance is not desirable because of a possible correlation between the earliness of a cultivar and low disease tolerance (Farag, 1976). Tolerant varieties could harbour virulent bacteria in a latent form (Priou *et al.*, 1999). Chemical control by soil fumigants, antibiotics, and copper compounds was tried without much success (Farag *et al.*, 1982; Murakoshi and Takahashi 1984; Hartman and Elphinstone 1994). In addition, they have hazardous effects on the environment, non-target beneficial organisms and human health. Therefore, cultural and biological control by using rhizobacteria or *Stenotrophomonas maltophilia* against the disease was tried by many investigators (Michel and Mew 1998; Lemessa and Zeller 2007; Messiha *et al.*, 2007).

Plant treatments with various biotic and abiotic agents can lead to the induction of local and systemic resistance to subsequent pathogen attack (Sticher *et al.*, 1997). Inducible resistance mechanisms such as systemic acquired resistance (SAR) are broadspectrum plant defense responses that can be induced biologically by microorganisms or exposing plants to natural and/or synthetic chemical compounds (Percival 2001). Plant extracts, essential oils and certain chemicals such as DL-3-amino-butyric acid (BABA), acibenzolar-S-methyl (ASM), prohexadione calcium (Regalis®), salicylic acid (SA) and oxlic acid were reported to induce SAR in plants against

plant pathogens (Kessmann *et al.*, 1994; Coste *et al.*, 2001; Oostendorp *et al.*, 2001; Percival 2001; Bowers and Locke 2004; Hassan and Buchenaure 2007).

Therefore, the aim of this study was to evaluate the efficacy of certain plant extracts, essential oils, antagonistic bacterial and resistance chemical inducers on controlling the potato bacterial wilt disease and populations of pathogen in infected treated plants. The effect of the tested materials on plant growth and tuber yield was also investigated.

Materials and methods

1- Isolation of bacterial pathogen

Diseased potato plants showing bacterial wilt and brown rot symptoms were collected from different localities of Assiut and Sohag Governorates. Samples from stem tissues and tubers of diseased plants were washed with tab water several times, surface sterilized for three minutes in 1% sodium hypochlorite solution then rinsed in sterile water. Samples were homogenized in a sterile mortar and pestle with 5 ml of sterile 0.05M potassium phosphate buffer. A loopful of the resulting suspension was streaked onto 2,3,5-triphenyltetrazolium chloride agar medium (TTC) described by Kelman (1954). TTC medium consisted of 250 ml of Casamino-peptone glucose agar (CPG) and 1.250 ml of the stock solution of 0.005% (w/v) 2,3,5 Triphenyl tetrazolium chloride. The CPG agar medium consisted of 5.0 gm dextrose, 10.0 g peptone, 1.0 mg casamino acid, 20 g agar and 1000.0 ml distilled water. Plates were incubated at 27°C for 48 h., and then examined for bacterial growth development. The single colony technique was used to obtain pure culture. Single colonies were subcultured onto the above-mentioned media on tubes and maintained at 4°C for further studies.

2- Potato plants

Healthy tubers of potato plants (*Solanum tuberosum* L.) cv. Diamont were surface sterilized by soaking for 5 min in 1% sodium hypochlorite solution, washed thoroughly with sterilized distilled water and planted directly in sterilized pots (diameter 25 cm), one tuber per pot. Pots and soil were sterilized by 5% formalin and then left for 15 days before planting. The pots filled with 4 Kg of clay and sand mixture (3:1 v/v). The plants were grown in green-house under natural temperature and photoperiods during the growing season. Plants were fertilized every 15 days with urea 46% (20 g/pot) and irrigated with water when necessary. Six weeks old potato plants were used in greenhouse experiments.

3- Pathogenicity test

Stored stock cultures for each isolate was streaked on TTC agar medium in Petri dishes and

incubated at 27°C for 48h. A single colony of the isolates was selected and grown in 250ml Erlenmeyer flasks containing 100 ml of nutrient sucrose broth medium (NSB) and incubated at 27± 2°C for 48h on a rotary shaker at 150 rpm. Bacterial cells suspension was centrifuged (8 min. at 10.000 rpm), the cells resuspended in sterilized distilled water and cell density adjusted to be $1x10^8$ (cfu/ml) using a spectrophotometer at wavelength of 620 nm. Stems of potato plants were injected with 100µl bacterial suspension by syringe 10cm above the soil (Kelman, 1954). Control plants were injected with 100 µl sterilized distilled water. Four replicates were used for each isolate test. The experiment was repeated three times. One month after inoculation, the disease severity index (DSI) was recorded as leaf wilting using the scale of Kempe and Sequeira (1983) as follow: 0 = no symptoms; 1 = slightly to 25%, leaves wilted; 2 = 26-50% leaves wilted; 3 = 51-75% leaves wilted; 4 = more than 75%, but less than 100% of leaves wilted; 5 = all leaves wilted and died.

Disease severity index (DSI) was calculated by following equation:

$$DSI = [\Sigma d / m \times n] \times 100$$

Where: d = the disease rating on each plant

m = the maximum disease rating possible

n = the total number of plants examined in each replicate.

4- Identification of the pathogenic bacteria

The isolated bacteria proved to be pathogenic and cause bacterial wilt of potato plants were identified according to their morphological, cultural and physiological characteristics described by Krieg and Holt (1984) and Brenner *et al.* (2005).

5- Preparation and concentration of plant extracts, essential oils, antagonistic bacteria and resistance chemical inducers

Aqueous extracts of leaves of *Hibiscus sabdariffa, Datura metel, Punica granatum, Eucalyptus* sp., *Rosemarinus officinalis* were prepared from 100 g fresh mature leaves of each plant species. Leave samples were collected, washed with sterile distilled water, ground with 100 ml of sterile water (1:1 w/v), with pestle in mortar and filtered through double-layered chesse cloth, followed by centrifugation at 5000 rpm for 10 min (Kurucheve *et al.*, 1997).

A stable essential oil suspensions from Thyme oil, black cumin oil and clove oil, (El-Yamama Company) were prepared by dissolving 700 µl of essential oil in 6.3 ml of 7% ethanol and detergent at 0.1% in 56 ml of water (Pradhanang *et al.*, 2003).

The antagonistic bacteria, *Pseudomonas fluorescens* and *Ps. aeruginosa* were obtained from stock cultures of Department of Plant Pathology, Faculty of Agriculture, University of Assiut. Isolates were grown at 27°C for 48

hr in NS liquid medium in conical flasks, each containing, 100 ml medium, then centrifuged at 1000 rpm. The optical density of the bacterial suspension was adjusted at 620 wavelength to give $2x10^8$ cfu/ml.

The resistance chemical inducers DL-3-aminobutyric acid (BABA), acibenzolar-S-methyl (ASM), prohexadione calcium (Regalis®), salicylic acid (SA) and oxlic acid were dissolved in distilled water to give 0.5 mg/ml of BABA, oxalic acid and Regalis and 0.2 and 0.7 mg/ml of ASM and SA, respectively.

6- Antimicrobial assay

In vitro, the toxic effects of certain aqueous plant extracts (1:1 w/v), essential oils (10 µl/ml), antagonistic bacteria ($2x10^8$ cfu/ml) and resistance chemical inducers (Regales 0.5 mg/ml, ASM 0.2 mg/ml, BABA 0.5 mg/ml, oxalic acid 0.5 mg/ml and salicylic acid 0.7 mg/ml) were tested against growth of isolate (M4) of *R. solanacreaum* using the impregnated filter paper disk method (Sholberg *et al.*, 2001). One ml bacterial suspension of *R. solanacreaum* isolate M4 ($3x10^9$ cfu/ml) from 48 h old cultures was added to 50 ml of sterzilized TTC agar medium at 47°C and mixed well. The mixture was then poured in sterilized Petri dishes (9ml in diameter). The sterilized Whatman standard filter paper disks (9mm diameter, 1mm thick) were dived in each tested solution and then dried in sterilized empty Petri dishes. Water and streptomycin (1.0 mg/ml) were used as negative and positive control. After one hour when medium was solidified, each disk was placed in the middle of the seeded agar surface. Four replicates were used for each treatment .In order to prolong the diffusion of tested material solutions in agar medium, the plates were first incubated at 4°C for 12 hour and then at 27°C for 48 hour. After incubation, the inhibition zone around each disk was measured and the area of inhibition zone was expressed in cm^2.

7- Control of potato bacterial wilt under greenhouse conditions

Two days before inoculation with the pathogen isolate (M4), 50 ml of each of aqueous plant extracts (1:1 w/v), essential oils (10 µl/ml), antagonistic bacteria ($2x10^8$ cfu/ml) and resistance chemical inducers (Regales 0.5 mg/ml, ASM 0.2 mg/ml, BABA 0.5 mg/ml, oxalic acid 0.5 mg/ml and salicylic acid 0.7 mg/ml), were added to each pot as soil drench. Inoculated and non-inoculated control plants were treated with an equal volume of water. Six weeks after inoculation, observations for development of symptoms were recorded as DSI for each treatment as mentioned previously. The reductions of disease severity were calculated according to the following formula:

$$\text{Reduction of disease severity} = \frac{\text{DSI of inoculated control} - \text{DSI of treatment}}{\text{DSI of inoculated control}} \times 100$$

Four replicates (pots) were used for each treatment and the experiment was repeated twice.

8- Population of *R. solanacearum* *in planta*

For the determination the bacterial multiplication in infected potato plant treated with the above mentioned trials, one gram samples from the lower stem internodes (15 to 20 cm above the soil) of each treatment were taken 6 weeks after inoculation, washed with tap water, surface sterilized with 3% sodium hypochloride and rewashed with sterile water. Samples were homogenized in a sterile mortar and pestle with 10 ml of 0.1 M potassium phosphate buffer (pH 7.0). Stem homogenates were serial diluted from 10^{-1} to 10^{-9} with 0.1 M potassium phosphate buffer. The 200 µl of each dilution were transferred onto TTC medium and spread by using a glass rod. Plates were incubated at 27°C for 48 hr and the number of bacterial colonies was counted (Roberto *et al.*, 2002).

9- Field experiments

This experiment was carried out in the Experimental Farm of Plant Pathology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt. Tested treatments were distributed in a complete randomized block design with four replicates, the experimental plot area was 24.75 m^2 (4.5 X 5.5 meter) containing four rows, each row was 4.5-meter length and distance between rows was 50 cm. Potato seed tubers of Diamont cv. were sown on the middle of the ridge at 40 cm apart. After two months from planting, 100 ml of each treatment was added singly around potato plants 48 hr before the inoculation. The plants were injected with 100µl bacterial suspension of *R. solanacearum* by syringe 10cm above the soil. Disease severity index was recorded 6 weeks after inoculation and the reductions of disease severity were calculated as described before. In the same time, fresh and dry weights of above ground were determined in one half of the treated potato plants by cutting the shoots part of plants above soil level and placed in paper bags. Potato plants washed in running tap water and blotted dry with paper towels, then fresh weights were recorded. Shoots of potato plants were dried in an oven for 4 days at 70°C for determining dry weights. In the other half of treated potato plants, the agricultural practices were carried out as the recommended program of the Egyptian Ministry of Agriculture for potato production. At harvest time (110 days after planting), potato tubers of six plants from each replicate were pulled for the assessment of the total yield of each treatment (ton) per feddan.

Statistical analysis

All experiments were performed twice at different times. A completely randomized design with

four replicates per treatment was used for all experiments. SPSS (Version 11.0J) software was used for statistical analysis. To assess the statistical significance of treatment differences, a one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test (with P set at 0.05) was employed. Means of standard deviation for four plants per treatment are shown.

Results

1-Isolation and pathogenicity tests

Results in Fig. (1) show that the twenty tested isolates of *R. solanacearum* were pathogenic to potato cv. Diamont and produced typical symptoms of leaf wilting on potato plants inoculated by the stem technique method. Also, results indicate that the virulence of the tested isolates significantly varied. Isolate M4 exhibited the highest disease severity, causing a disease severity index of 71.68% followed by isolate M6 which causes DSI of 64.00% and then isolates M12, A16 and E17. Isolate M13 caused the lowest DSI (26.00%). Other tested isolates fell in between.

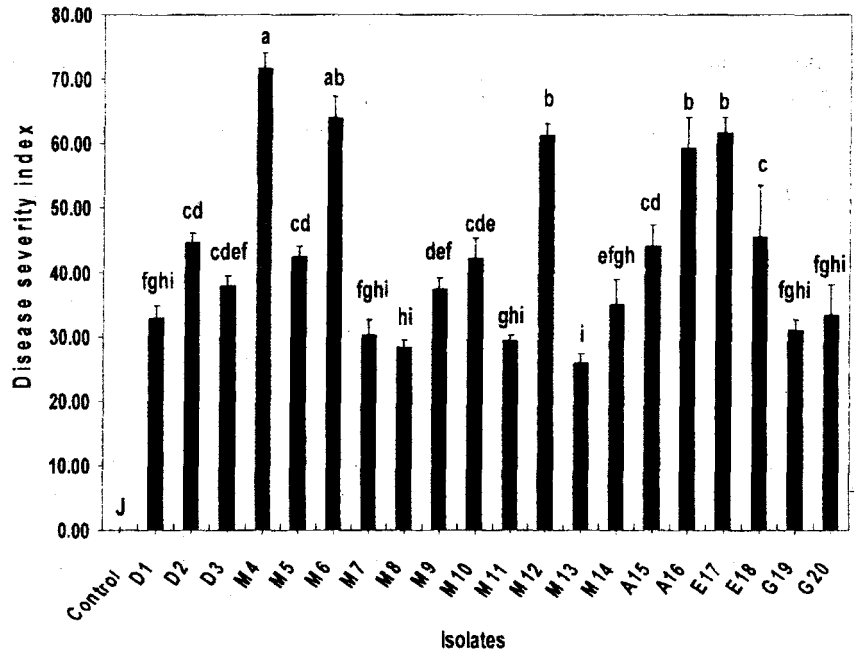

Fig.(1): Pathogenicity of *R. solanacearum* isolates on potato plants of cv. Diamont under greenhouse conditions. Means with the same letters are not significantly different at p = 0.05 (Tukey's test). Means of standard deviation for replicates per isolate are show.

2- Identification of pathogens

All tested isolates were rod-shape, motile, gram negative, non-sporing, oxidase positive, urease positive, catalase positive, growth in NaCl 1% and positive M.R. test did not hydrolize starch, levan negative, did not produce both of hydrogen sulphide, hydrolize aesculin and casein, liquefy gelatin, did not grow at 4°C, 40°C and NaCl 2%, negative V.P., positive (growth) tolerance titrazolium salt 0.1% & 0.02%, negative phenylalanine diaminase test. Colonies were smooth, opaque and highly fluid on CPG medium and they are creamy white with small pink of light red centers on TTC medium. Results of sugars fermentation show that the tested isolates produced acid from maltose, sucrose, fructose, glucose, mannitol and mannose, did not produce acid from lactose, cellobiose and arabiose. Utilization of carbon compounds such as: glucose, fructose, sucrose, lactose, maltose, cellobiose, arabinose, mannose, mannitol. On the basis of the morphological and physiological characteristics of the isolated pathogenic bacteria and according to those reported by Krieg and Holt (1984) and Brenner et al. (2005). It could be stated that all tested isolates are identified as *R. solanacearum*.

3- Effect of certain plant extracts, essential oils, resistance chemical inducers and antagonistic bacteria on growth of *Ralstonia solanacearum in vitro*

The averages of inhibition zones are represented in Table (1). Out of five plant extracts, three only, i.e., *Hibsicus sabdariffa, Eucalyptus globulus* and *Punica granatum* (1:1 w/v) inhibited the growth of *R. solanacearum*. Essential oils, microorganisms and resistance chemical inducers have no antibacterial effects. *Hibsicus sabdariffa* and *Punica granatum* displayed the highest antagonistic activity against *R. solanacearum* (inhibition zone area was 3.14 cm^2), while the *Eucalyptus globulus* slightly inhibited growth (inhibition zone area was 2.01 cm^2).

4- Effect of certain plant extracts, essential oils, antagonistic bacteria and resistance chemical inducers on severity of bacterial wilt disease and population of the pathogen in potato plants under greenhouse conditions

Almost all tested soil drench treatments presented in Table (2) significantly reduced disease severity of potato bacterial wilt compared to infected control, except ASM treatment which showed no effect on disease severity index (DSI). Soil drench applications with 50 ml/pot of plant extracts of *Eucalyptus globulus*, *Hibiscus sabdariffa*, *Punica granatum* and the thyme oil reduced profoundly the DSI by 94.17, 89.05, 84.83 and 78.99% respectively, compared to the infected control (83.7%). Results also showed that clove oil, plant extracts of *Datura metel* and *Pseudomonas fluorescens* caused

intermediate DSI compared with other treatments. Generally, plant extracts showed the highest reduction in DSI followed by essential oil, antagonistic bacteria and finally resistance chemical inducers.

Table(1): Effect of certain plant extracts, essential oils, antagonistic bacteria and resistance chemical inducers on growth of *Ralstonia solanacearum in vitro*.

Treatments	Concentration of tested treatments	Inhibition zone diameter (cm)	Inhibition zone area (cm^2)
Plant extracts			
Hibsicus sabdariffa	(1:1 w/v)	2.0	3.14
Eucalyptus globulus	(1:1 w/v)	1.6	2.01
Rosemarinus officinalis	(1:1 w/v)	0.0	0.0
Datura metel	(1:1 w/v)	0.0	0.0
Punica granatum	(1:1 w/v)	2.0	3.14
Essential oils			
Thyme oil	10 µl/ml	0.0	0.0
Black cumin oil	10 µl/ml	0.0	0.0
Clove oil	10 µl/ml	0.0	0.0
Antagonistic bacteria			
Pseudomonas fluorescens	$2x10^8$ CFU/ml	0.0	0.0
Pseudomonas aeruginosa	$2x10^8$ CFU/ml	0.0	0.0
Resistance chemical inducers			
Regalis	0.5 mg/ml	0.0	0.0
ASM	0.2 mg/ml	0.0	0.0
BABA	0.5 mg/ml	0.0	0.0
Oxalic acid	0.5 mg/ml	0.0	0.0
Salicylic acid	0.7 mg/ml	0.0	0.0
Control			
Streptomycin (positive control)	1.0mg/ml	3.33	8.71
Water (negative control)	—	0.0	0.0

Results in Table (2) also showed that all tested materials significantly reduced the numbers of *R. solanacearum* cells in stems of potato plants as compared with the inoculated control, except of *Pseudomonas fluorescens* and oxalic acid which showed no effect. The population of *R. solanacearum* was lowest in potato plants treated with plant extracts of *Hibsicus sabdariffa* ($4.4x10^5$ cfu/g), *Eucalyptus globulus* ($2.23x10^6$ cfu/g) and *Punica granatum* ($5.1x10^7$ cfu/g) and thyme oil ($5.4x10^7$ cfu/g) than in inoculated control plants ($2.8x10^{10}$ cfu/g). Other tested treatments caused intermediate effect on decreasing the population of the pathogen within host plants.

Table(2): Effect of soil drenching with certain plant extracts, essential oils, antagonistic bacteria and resistance chemical inducers on severity of bacterial wilt disease and population of *Ralstonia solanacearum* in stem tissues of potato plants under greenhouse conditions.

Treatments	Concentration of tested treatments	DSI	Reduction of DSI	Bacterial pathogen population(cfu/g stem tissue)
Plant extracts				
Hibsicus sabdariffa	(1:1 w/v)	09.17 ij	89.05	$4.40x10^{5}$ b
Eucalyptus globulus	(1:1 w/v)	04.88 jk	94.17	$2.23x10^{6}$ b
Rosemarinus officinalis	(1:1 w/v)	42.50 cdc	49.23	$9.93x10^{8}$ b
Datura metel	(1:1 w/v)	35.80 efg	57.23	$4.00x10^{8}$ b
Punica granatum	(1:1 w/v)	17.58 h	78.99	$5.10x10^{7}$ b
Essential oils				
Thyme oil	10 µl/ml	12.70 hi	84.83	$5.40x10^{7}$ b
Black cumin oil	10 µl/ml	62.43 b	25.42	$5.80x10^{8}$ b
Clove oil	10 µl/ml	31.17 fg	62.76	$1.05x10^{9}$ b
Antagonistic bacteria				
Pseudomonas fluorescens	$2x10^{8}$ CFU/ml	37.33 def	55.40	$1.36x10^{10}$ a
Pseudomonas aeruginosa	$2x10^{8}$ CFU/ml	44.30 cd	47.08	$5.70x10^{8}$ b
Resistance chemical inducers				
Regalis	0.5 mg/ml	49.63 c	40.71	$3.90x10^{9}$ b
ASM	0.2 mg/ml	87.3 a	- 04.29	$4.33x10^{7}$ b
BABA	0.5 mg/ml	42.95 cde	48.69	$4.88x10^{8}$ b
Oxalic acid	0.5 mg/ml	48.23 c	35.48	$2.07x10^{10}$ a
Salicylic acid	0.7 mg/ml	28.33 g	66.15	$2.80x10^{8}$ b
Water				
Water (Inoculated control)	—	83.71a	00.00	$2.80x10^{10}$ a
Water(Non-inoculated control)	—	00.00 k	100.00	$0.00x10^{0}$ c

Within each column, values with the same letters are not significantly different at p = 0.05 (Tukey's test).

5- Effect of certain plant extracts, essential oils, antagonistic bacteria and resistance chemical inducers on severity of bacterial wilt disease, fresh and dry weight and tuber yield of potato plants under field conditions

Results in Table (3) revealed that all tested soil drench treatments significantly reduced DSI of bacterial wilt of potato compared to infected control. Soil drenching with 100ml/ plant extracts of *Punica granatum*, *Eucalyptus globulus*, *Hibiscus sabdariffa* and thyme oil reduced the DSI by 68.39, 64.06, 63.23 and 65.93%, respectively. Data also showed that application of *Pseudomonas flurescens*, ASM, Black cumin oil were least effective in reducing disease severity, since they reduced DSI by 10.67%, 14.17% and 15.05%, respectively. In general, application of plant extracts were superior in reduction of bacterial wilt followed by essential oils then other tested treatments.

Table(3): Effect of certain plant extracts, essential oils, antagonistic bacteria and resistance chemical inducers on severity of bacterial wilt, fresh (FW) and dry weight (DW) and tuber yield of potato plants under field conditions.

Treatments	DSI	Reduction of DSI (%)	FW of shoots (g/plant)	DW of shoots (g/plant)	Yield (ton/fed)	Increasing of yield (%)
Plant extracts						
Hibsicus sabdariffa	23.53 gi	63.23	466.75 bcd	135.3 abc	10.20 efg	35.46
Eucalyptus globulus	23.00 gi	64.06	483.50 bc	137.0 ab	12.08 bcd	60.42
Rosemarinus officinalis	27.33 fgi	57.30	366.75 fg	118.0 de	11.29 cdef	49.93
Datura metel	27.60 fgi	56.88	466.75 bcd	119.0 cde	12.24 bcd	62.55
Punica granatum	20.23 i	68.39	533.50 ab	146.3 a	15.07 a	100.13
Essential oils						
Thyme oil	21.80 gi	65.93	400.00 def	124.4 bcd	9.89 fg	31.34
Black cumin oil	54.37 bc	15.05	316.75 g	114.2 def	10.82 defg	43.69
Clove oil	41.17 de	35.67	416.75 cdef	123.8 bcd	10.98 defg	45.81
Antagonistic bacteria						
Pseudomonas flurescens	57.17 ab	10.67	466.50 bcd	126.9 bcd	13.19 b	75.16
Pseudomonas aeruginosa	32.00 f	50.00	450.00 cde	125.8 bcd	12.70 bc	68.65
Resistance chemical inducers						
Regalis	44.30 c	30.78	400.00 def	106.3 ef	10.35 efg	37.45
ASM	54.93 b	14.17	366.75 fg	102.4 ef	13.01 b	72.77
BABA	46.23 cd	27.77	375.00 efg	99.3 f	9.42 g	25.09
Oxalic acid	34.00 ef	46.88	393.75 defg	115.0 def	11.53 bcdef	53.12
Salicylic acid	28.67 fg	55.28	416.75 cdef	116.5 de	11.60 bcde	54.05
Water						
Water (Inoculated control)	64.00 a	00.00	233.25 i	55.8 g	7.53 h	00.00
Water (Non- inoculated control)	0.00 h	100.00	566.50 a	149.3 a	11.29 cdef	49.93

Within each column, values with the same letters are not significantly different at p = 0.05 (Tukey's test)

Data also indicate that the fresh and dry weights of potato plants inoculated by *R. solanacearum* were significantly lower than that of non-inoculated control plants. Plant extracs, essential oils and antagonistic bacteria as well as resistance chemical inducers significantly increased fresh and dry weight of potato plants (g/plant) compared to inoculated control plants. The treatments with aqueous extract of *Punica granatum* and *Eucalyptus globulus* caused the highest increase in both fresh and dry weight of potato plants followed by *Hibsicus sabdariffa*. Other tested treatments had intermediate effect on fresh and dry weights compared with inoculated control plants. In general, plant extracts treatments showed the highest increase of fresh and dry weight of potato plants followed by other tested treatments.

Results in Table (3) also showed that the tested treatments significantly increased the potato tubers yield compared to inoculated control. The treatment with *Punica granatum* extract caused the highest yield of tubers followed by treatments with *Ps. flurescens*, ASM, *Ps. aeruginosai*, *Datura metel and Eucalyptus globules*. Potato tuber yield of inoculated control was significantly lower than that of non-inoculated control plants.

Discussion

Potato bacterial wilt caused by *R. solanacearum* is a major soil borne disease in tropics and subtropics (Hayward, 1991). In the present study twenty isolates of *R. solanacearum* were isolated from naturally diseased potato tubers and plants grown in different localities of Upper Egypt. Obtained isolates were identified as *R. solanacearum* according to their morphological, physiological and biochemical characteristics (Krieg and Holt 1984; Galal *et al.*, 2003; Brenner *et al.*, 2005). Beside traditional methods for identifying *R. solanacearum* several selective media such as TTC medium was used. TTC medium could easily distinguish the suspected *R. solanacearum* from other bacteria, since colonies of virulent isolate appeared pink colour while other bacteria did not. Data agree with those reported by Farag *et al.* (1999) and Galal *et al.* (2003).

R. solanacearum isolates differed in their pathogenicity on Diamont potato cv. Isolates M4, M6, M12, A16 and E17 were more virulent than others. Variations in the virulence of *R. solanacearum* isolates in potato plants have been reported by several authors (Williamson, *et al.*, 2002; Galal *et al.*, 2003; El-Ariqi *et al.*, 2005).

Antibacteterial activity of certain plant extracts, essential oils, antagonistic bacteria and resistance chemical inducers against *R. solanacearum* was investigated *in vitro*. Data revealed that plant extracts of *Hibiscus sabdariffa*. *Eucalyptus globulus* and *Punica*

granatum were able to inhibit the growth of the causal pathogen *in vitro*. However, other tested plant extracts, essential oils, antagonistic bacteria and resistance chemical inducers had no inhibitory effects. Plant extracts of many plant species have been reported to have antibacterial effect against plant pathogenic bacteria and this property could be utilized for management of bacterial diseases (Kagale *et al.*, 2004; Basim *et al.*, 2006). The possibility of a direct effect of certain resistance inducers such as BABA, ASM and SA or essential oils has been tested *in vitro* against many plant pathogens and exhibited no toxic effects against fungal and bacterial plant pathogens (Cohen 1994; Oostendorp *et al.*, 2001; Pradhanang *et al.*, 2003).

In our experiments, under both greenhouse and field conditions all tested treatments gave significant reduction in disease severity. Plant extracts showed the highest reduction of DSI followed by essential oils, antagonistic bacteria and finally chemical inducers. These results are in agreement with those obtained by many researchers (Bowers and Locke, 2004; Kagale *et al.*, 2004; Baysal *et al.*, 2005; Pradhanang *et al.*, 2005; Basim *et al.*, 2006;; Lemessa and Zeller, 2007).

In greenhouse experiments, soil drenching with extracts of *Hibiscus sabdariffa*, *Punica granatum* and *Eucalyptus globulus* and thyme oil reduced profoundly the DSI compared to the untreated inoculated control. These results are in agreement with those obtained by several researches (Baysal and Zeller 2004; Pradhanang *et al.*, 2005). Plant extracts were also reported to induce resistance in plants for other bacterial diseases (Mende *et al.*, 1993; Baysal and Zeller 2004; Kagale *et al.*, 2004).

Results reported herein showed that application of plant extracts increased fresh and dry weights as well as tubers yield of potato plants as compared with untreated control. This may be due to reduction of the disease incidence in addition to the increase of vegetative characters. Such results are agreement with those reported by Abd El-Kareem, *et al.*, (2001) and Kagale *et al.*, (2004). Plant extract or essential oils may be associated with secretion of auxins, gibberellins and cytokinins and suppression of deleterious microorganisms in the rhizosphere as reported by Ji, *et al.*, (2005).

The populations of *R. solanacearum* were lower in potato plants grown in soil treated with plant extracts of *Hibsicus sabdariffa*, *Eucalyptus globulus* and *Punica granatum* and thyme oil than in untreated inoculated control plants, other tested treatments caused intermediate effect on decreasing the population of the pathogen within host plants. Such results indicated that the reduction in bacterial wilt severity of potato

plants was correlated with lower bacterial growth in treated plants by about one third as compared to control plants. This suggests that inhibitors of bacterial growth may be produced as a result of resistance induction already before inoculation or the plants respond quickly to inoculation by production of bacterial growth inhibitors after inoculation. Similarly, Werner *et al.*, (2002) and Baysal *et al.*, (2003) reported that ASM treatment of tomato plants reduced *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) populations and spread of the pathogen in xylem tissue of plants. Also, Hassan and Buchenauer (2007) reported that application BABA combined with ASM reduced bacterial population in apple seedlings. On the basis of obtained results, it may be assumed that reduction of bacterial multiplication in treated plants was accompanied by accumulation of defense constituents in plant tissues especially in the xylem as a result of resistance induction already before inoculation or the plant respond quickly to inoculation by production of bacterial growth inhibitors after inoculation. Antimicrobial compounds, for example acidic PR-proteins, phenolic acids, peroxidases, lignin and other defense compounds may be accumulated in plant tissue treated with plant extracts and thyme oil. These compounds might be involved in retardation of multiplication of bacterial cells. We have shown that natural plant extracts and oils can reduce pathogen populations and disease severity. These materials, however, need to be researched more fully in several pathosystems and the mode of actions of tested materials as well as the interaction between such plants and the pathogens before they may be commercially acceptable.

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مكافحه مرض الذبول البكتيرى فى البطاطس بأستخدام بعض المستخلصات النباتيه و الزيوت المعدنيه و البكتيريا المضاده والمواد المستحثه

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تم عزل عشرون عزلة بكتيرية من نباتات بطاطس مصابه بمرض الذبول البكتيرى من محافظات أسيوط وسوهاج وعرفت على أنها بكتريا رالستونيا سولاناسيرم طبقا لخواصها الفسيولوجية والبيوكيميائية. وأظهرت العزله M4 أعلى شده أصابه للمرض ثم تبتعتها العزلات,A16, M12, M6 وE17. تم تقييم بعض المستخلصات النباتية و الزيوت المعدنيه و الميكروبات المضاده والمواد المستحثه على مكافحه مرض الذبول البكتيرى فى البطاطس صنف الدايامونت تحت ظروف الحقل والصوبه. أوضحت التجارب أن من بين جميع المواد المختبره, المستخلصات النباتية من الكركديه والكافور والرمان فقط لهم القدرة على تثبيط نمو المسبب المرضى تحت ظروف المعمل. تحت ظروف الصوبة أثبتت جميع المعاملات القدرة على خفض شدة الأصابة المرضية بدرجات متفاوته بالمقارنه النباتات الغير معامله (كنترول). ومن أفضل تلك المعاملات هو معامله نباتات البطاطس عن طريق التربه قبل العدوى بمستخلاصات الكركديه، الكافور، الرمان وزيت الزعتر حيث أدت الي خفض شده الإصابة بنسبة 94.17، 89.05، 78.99، 84.83% على التوالي. فى حين أن المعاملة بزيت حبة البركة والمستخلص النباتى للداتوره والبكتيريا البسيدوموناس فلوروسنت أدت الى خفض متوسط للإصابة بالمرض. وأوضحت الدراسة أن جميع المعاملات السابقة أدت إلى خفض أعداد المسبب المرضى البكتيرى (رالستونيا سولاناسيرم) فى سوق نباتات البطاطس المعديه، وبصفة عامة كانت أعداد المسبب المرضى أقل ما يمكن فى حالة النباتات المعاملة بالمستخلصات النباتيه ثم زيت الزعتر على التوالي، فى حين أن المعاملات الأخرى أدت الى خفض بسيط فى أعداد المسبب المرضى البكتيرى. وتحت ظروف الحقل، أدت جميع المواد المستخدمة الى خفض شدة الأصابة بأعراض الذبول البكتيري، ووجد أن المستخلصات النباتية وحمض السالسليك والبكتيريا البسيدوموناس أروجينوزا أكثر قدره فى خفض شده المرض و زيادة فى محصول الدرنات البطاطس والوزن الجاف و الرطب للمجموع الخضرى. أما معامله النباتات بالبكتيريا البسيدوموناس فلوروسنت والبيون وزيت حبه البركه كان له تأثير بسيط على خفض شده الأصابه وفى نفس الوقت تأثير متوسط فى زياده كل من محصول الدرنات والوزن الجاف والرطب للنباتات.