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Virulence of *Ralstonia solanacearum* the causal of potato brown rot disease under Egyptian conditions

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Abstract

Ten isolates of *Ralstonia solanacearum* bacterium were isolated from potato tubers and soil samples which were collected from Qalubiya and Beheira governorates. The obtained isolates were identified initially according to their morphological, physiological and biochemical characteristics then, immunofluorescent antibody stains (IFAS) and polymerase chain reaction (PCR) techniques were used to confirm the identification of *R. solanacearum* bacterium. All tested isolates caused symptoms of bacterial wilt disease on potato plants compared with the un-inoculated control with superiority of *R. solanacearum* (R6) which recorded the highest percentage of infection and disease severity of brown rot disease at 35 day post inoculation the sterilized soil with brown rot pathogens. All tested potato cultivars were susceptible to infect with *R. solanacearum* when cultivated in sterilized soil and un-sterilized soil. In the sterilized soil, isolate R6 recorded the highest percentage of infection and disease severity on cvs. Gelabica and Cara whereas, the isolates R2 and R1 were the most virulent on cvs. Barren and Spunta, respectively. However, isolate R6 on cvs. Gelabica and isolate R4 on Cara, and R3 on Barren and R4 on Spunta recorded the highest disease severity in the un-sterilized soil.

Key words: Potato, brown rot disease, *Ralstonia solanacearum*, identification, virulence, pathogenicity, varietal reaction.

Introduction

The genus *Ralstonia* was proposed in 1995 to accommodate the generically misplaced species *Burkholderia pickettii*, *Burkholderia solanacearum* and *Alcaligenes eutrophus* **(Yabuuchi *et al.*, 1995)**. Species of the genus *Ralstonia* occupy diverse ecological niches. *Ralstonia solanacearum* is an important phytopathogen that has an unusually broad host range and causes bacterial wilt on a variety of economically important crops **(Hayward, 1991)**. Potato brown rot disease caused by *Ralstonia solanacearum* is one of the serious bacterial diseases attacking potatoes in the world. It represented the second major constraint to potato production in tropical, subtropical and warm temperate regions worldwide after late blight**.** Also, the disease is considered a serious economic problem in several countries where potatoes are cultivated over large areas and economical exportation crop **(Hayward, 1994, Yabuuchi *et al.* 1995 and Hayward *et al.* 1998).**

Triphenyl tetrazolium chloride (TZC) medium was used for isolation of *R. solanacearum* bacteria **(Sumithra *et al.*, 2000)**. Characterization of *Ralstonia solanacearum* strains, the causal agent of potato bacterial wilt disease in Nepal and Thailand was performed based on their pathogenicity, biochemical, physiological and serological tests **(Shambhu *et al.*, 2001)**. **Al-Ani *et al.* (2004)** isolated three isolates of *Ralstonia solanacearum* (R_1, R_2 and R_3) from roots and stems of tomato wilting plants which collected from fields of Iraq. These isolates formed white, raised and shiny colonies with rose center on TZC medium. The genus *Ralstonia* was identified according to the visible characteristics of colonies on nutrient agar (NA) and potato dextrose agar (PDA) media, cell shape and its ability to grow on King's B (KB) selective medium and to produce oxidase, catalase and acids was also reported. The species *solancearum* was identified on the basis of its ability to reduce nitrate, produce H_2S, and produce ammonia from peptone and on its ability to assimilate certain carbohydrates. **Balabel *et al* (2006)** suggested that plating bacterial suspensions of *R. solanacearum* from different sources revealed virulent and avirulent forms, the virulent form appeared as milky, white, flat, irregular and fluidal with red coloration in the center whereas, the avirulent form was less fluidal or a fluidal colony which is completely pink to red. **Pastrik *et al.* (2002)** used the PCR technique as one of the rapid, highly specific and sensitive tests for detection and identificantion of *R. solunacaarum* which isolated from different sources.

The early symptoms of brown rot disease were wilting of the lower leaves with rolling of the leaf margins, subsequently leaves showed sectorial chlorosis and eventually papery brown necrosis. Sometimes only one part of the stem showed wilting symptoms. On tubers, external symptoms may or may not be visible according to the stage of disease development, which depends highly on age of the host plants and environmental factors **(Dean *et al.*, 2006)**.

Gabr and Saleh (1998) screened 10 potato cultivars against brown rot disease, they found cultivars i.e., Alpha, Diamont, Gigant and Turbo were highly susceptible whereas the cultivars of Accent, Agria, Aziza, Desia, Gazria and Mirakel

showed moderate susceptibility to the isolated bacteria. **Badr (2006)** found that the tested potato cultivars *i.e.*, Diamont, Braka, Picasso and Fabula were susceptible with potato wilt disease. **Aissata (2007)** recorded that the Appoline, Claustar and Spunta potato varieties showed a better tolerance in presence of high bacterial wilt disease pressure compared to Mondial, Daifla and Liseta cultivars. **Hajhamed (2011)** evaluated six potato cultivars against bacterial wilt disease using two different inoculation methods i.e. stem injection method and soil drench method. The cultivars of Diamont and Lady balfor were resistance while, Nicola and Lady rosetta were highly susceptible to bacterial wilt disease but Santana and Valor were moderately resistance to the disease.

The present study aimed to evaluate the pathogenic abilities of the obtained isolates of *Ralstonia solanacearum* and evaluating responses of potato cultivars against infection with the potato brown rot pathogenic bacteria.

Materials and Methods

Isolation and identification of *R. solanacearum* isolates:

Potato tubers of wilted potato plants as well as soil samples were collected from different locations of potato cultivations in Qalubiya (Beltan and El-Haddadeen) and Beheira (El-Tawfikia and Hosh-Eisa) governorates, Egypt during the 2009 growing season for isolation of the bacterial disease agent.

For isolation from tubers, samples were washed in tap water, surface sterilized by dipping into 70% alcohol and flaming **(OEPP/EPPO, 1990)** and the epidermis around the heel end were removed using a regularly disinfected scalpel. Tubers were cut then to small tissue cores (diameter 5-10 mm, length 5 mm) taken from the discolored vascular tissues with bacterial oozes and put in sterile water in sterilized bottle. The tuber pieces were maintained in the sterilized water for 30 min **(Wullings *et al.*, 1998)** to diffuse the bacteria into the water. On the other hand, isolation from soil was achieved by placing soil samples (10 g each) into conical flask (250 ml) containing 90 ml of sterilized distilled water and agitated vigorously for 15 minutes then left to set for another 15 minutes. Then, 2 loopfuls of the tuber and soil water suspensions were streaked individually on triphenyl tetrazolium chloride (TTC) medium **(Kelman, 1954)** and incubated at 28°C for 48 h. After 48 h of incubation, purification of *R. solanacearum*-looking colony was made.

Colonies of *Ralstonia solanacearum* were characterized on the selective media of South Africa "SMSA" suggested by **Elphinstone** *et al.*, **(1996)**. However, identification of isolates based on their morphological, physiological and biochemical characteristics (cell shape, sporulation, Gram staining, motility, aerobic growth, potato soft rot, gelatin liquefaction, growth at 41°C, casein hydrolysis, H_2S production, starch hydrolysis, nitrate reduction, catalase activity, and ability isolates to produce acid and gas from; glucose, mannose, fructose, maltose, lactose, mannitol and dulicitol, were achieved as described by **Schaad, (1980), Fahy and Persley, (1983), Bergy's manual (1984), Lelliott and Stead, (1987)** and **Adhikari, (1993).** Identification of *R. solanacearum* isolates was confirmed using immunofluorescent antibody stains (IFAS) and polymerase chain reaction (PCR) techniques according to **Robinson (1993) and Pastrik *et al.* (2002) respectively**.

Virulence of different *R. solanacearum* isolates:

Potato (*Solanum tuberosum* L. "Spunta") was used for pathogenicity tests. Potato intact sprouted tuber seeds were sown in plastic pots (25 cm Φ containing 6 kg of sterilized sandy loam soil), one seed/pot. All pots were kept in a greenhouse at 26 to 30°C and 60 to 80% relative humidity. Seedlings were watered daily. For inoculation, the bacteria were grown on glycerol nutrient agar (**Kelman, 1954)** for two to three days at 28°C, suspended in sterile distilled water and adjusted to 10^9 cfu/ml. Plants were inoculated at the stem base with bacterial suspension (10^9 cfu/ml) of the tested *R. solanacearum* isolates (**Kelman, 1954).** Plants inoculated with sterile water served as negative control. The percentage of infection as well as severity of wilting was recorded at weekly intervals **(He *et al.*, 1983; Horita and Tsuchiya, 2001)** after inoculation on the following scale: 1 = no symptom, 2 = leaf at above soil level turned yellow and wilted, 3 = two or three leaves wilted, 4 = four or more leaves wilted, and 5 = plant died.

Varietal reaction against *R. solanacearum* isolates:

This experiment was carried out under greenhouse conditions for evaluation of four potato cultivars namely Spunta, Berren, Cara, and Gelabica cultivars against infection with *Ralstonia solanacearum*-isolates (R1, R2, R3, R4 and R6). All isolates were streaked on glycerol nutrient broth (GN) medium **(Kelman, 1954)** and incubated at 28°C for 3 days. Potted sterilized and un- sterilized soil (6 kg /pot 25cm diameter) were used in this study. The potted soil were inoculated by bacterial suspension (10^9 cfu/ml) of a known bacterial isolate at rate 100 ml/pot. Pots inoculated with sterilized GN medium at rate 100ml/pot served as control. Pots in each treatment were sown with potato tuber seeds of a given potato cultivar (**Fahmy and Mohamed, 1990)**. Three replicates were used for each treatment. Disease severity and percentage of infection were measured as mentioned before.

Statistical analysis:

The obtained data were statistically analyzed using the analysis of variance and the least significant difference at 0.05 was calculated as mentioned by **Snedecor and Cochran, (1989).**

Results

1- Isolation of *R. solanacearum* bacterium:

As clear in **Table (1),** ten isolates of *Ralstonia solanacearum* bacterium were isolated from potato tubers and soil samples collected from Qalubiya and Beheira governorates. In this respect, five isolates i.e., R2, R4, R6, R8 and R10 were isolated from Qualubia (Beltan and El-Hadadeen) while, the other five isolates i.e., R1, R3, R5, R7 and R9 were isolated from Beheira governorate (El-Tawfikia and Hosh-Eisa). As for isolates of Qalubia, three isolates (R2, R4 and R6) were isolated from potato tubers and two isolates (R8 and R10) were isolated from soil samples. Regarding isolates of Beheira, four isolates (R1, R3, R7 and R9) were isolated from potato tubers and one isolate was isolated from soil samples. It is interest to state that *R. solanacearum* could not be isolated from soil samples collected from Hosh-Eisa (Beheira governorate).

Table 1. Isolated bacteria from infected potato tubers and soil samples of different localities in Egypt

Isolates code	Qalubiya localities				Beheira localities			
	Beltan		El-Haddadeen		El-Tawfikia		Hosh-Eisa	
	Tubers	Soil	Tubers	Soil	Tubers	Soil	Tubers	Soil
R_1	-	-	-	-	+	-	-	-
R_2	+	-	-	-	-	-	-	-
R_3	-	-	-	-	-	-	+	-
R_4	-	-	+	-	-	-	-	-
R_5	-	-	-	-	-	+	-	-
R_6	+	-	-	-	-	-	-	-
R_7	-	-	-	-	+	-	-	-
R_8	-	-	-	+	-	-	-	-
R_9	-	-	-	-	-	-	+	-
R_{10}	-	+	-	-	-	-	-	-

+ = Positive - = Negative R= *Ralstonia solanacearum*

2- Identification of *R. solanacearum* bacterium:

Results presented in Tables (2a) show that, all *R. solanacearum*-isolates showed short rod cells, no sporulating, motile, aerobes, gram negative, and gave positive reaction with tests of catalase activity, nitrate reduction and growth at 1% NaCl. However, all isolates of *R. solanacearum* gave negative reactions when tested for production of fluorescent on KB, gelatin liquefaction, starch hydrolysis, levan production, potato soft rot, H_2S production, growth at 41^oC and growth at 2% NaCl. The results presented in Table (2b) indicate also that, all tested isolates of *R. solanacearum* were able to ferment sugars and produce acid from glucose, mannose, fructose while some *R. solanacearum* were able to produce acid from one or more of the following sugars i.e. Lactose, Maltose, Mannitol, Sorbitol and Dulicitol. The isolates colonies of *R. solanacearum* produced irregularly round or typical, smooth surface, fluidal, white colonies with or without red centers on glycerol nutrient agar (GNA) medium containing tetrazolium chloride (TZC) and SMSA media **(Fig. 1)**.

2- Confirmation the identification of *R. solanacearum* isolates using some modern techniques:

2. a-- Immunofluorescent antibody staining (IFAS):

Identification of tested bacterial isolates using immunofluorescent antibody staining technique (IFAS) gave positive results where it confirmed that these tested isolates are *R. solanacearum*. In this respect, the morphology of bacterial cells appeared as short rod shape and green fluorescent with specific fluorescent-labaled antiserum as cleared in **Fig. (2)**.

Table 2a. Morphological and physiological characteristics of *Ralstonia solanacearum* isolates which isolated from infected potato tubers and soil samples.

Character	Tested isolates									
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
Cell shape	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR
Gram staining	-	-	-	-	-	-	-	-	-	-
Spores formation	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+
Production of fluorescent on KB	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-
Reduction of nitrate	+	+	+	+	+	+	+	+	+	+
Levan production	-	-	-	-	-	-	-	-	-	-
H_2S production from peptone	-	-	-	-	-	-	-	-	-	-
Potato soft rot	-	-	-	-	-	-	-	-	-	-
Growth at 41^oC	-	-	-	-	-	-	-	-	-	-
Growth in 1% NaCl	+	+	+	+	+	+	+	+	+	+
Growth at 2 % NaCl	-	-	-	-	-	-	-	-	-	-

SR= shot rod, + = positive reaction, - = negative reaction.

Table 2b. Utilization of different carbohydrate sources by tested *Ralstonia solanacearum* isolates.

Carbohydrate	Tested isolates									
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R9	R10
Glucose	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+
Lactose	+	-	-	-	+	+	-	-	-	+
Maltose	-	-	+	-	-	+	-	-	-	+
Mannitol	+	+	+	+	-	+	+	+	+	-
Sorbitol	+	+	+	-	+	+	+	+	-	+
Dulicitol	+	+	-	-	+	+	+	-	-	+

- = Negative reaction, + = Only acid production without gas.

GNA+TZC medium SMSA **medium**

Fig. (1): Colonial types of *Ralstonia solanacearum* grown on glycerol nutrient agar medium containing triphenyl tetrazolium chloride (TZC) and selective media of south Africa (SMSA) medium.

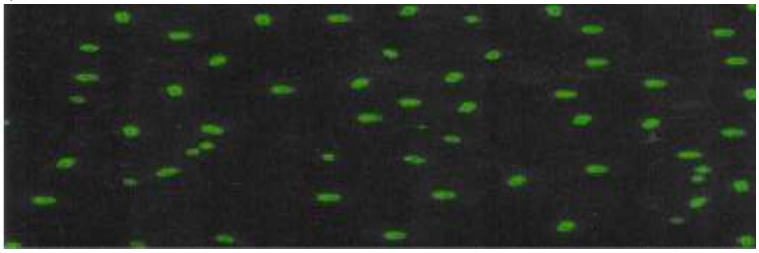
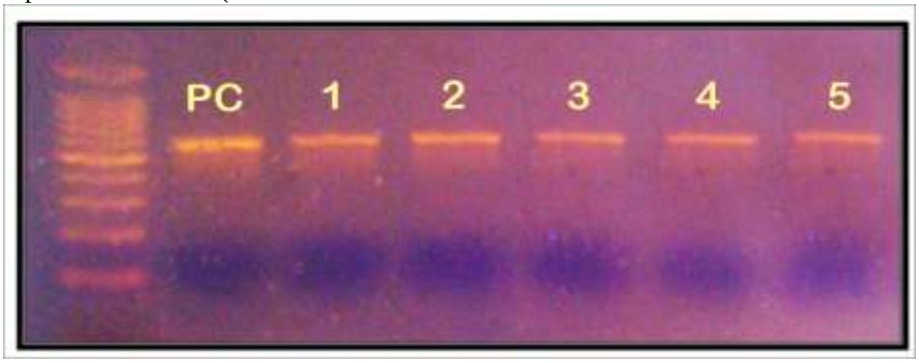


Fig.2: Cell morphology of *Ralstonia solanacearum* using the serological immunofluorescent antibody staining (IFAS) technique where cells appeared as short rods and stained evenly as bright green fluorescent under IF microscope (600X).

2. b -- Polymerase chain reaction (PCR):

Data in **Fig. (3)** confirmed the identification of five bacterial isolates among the ten isolates of those isolated from Beheira and Qalubiya governorates during season 2009 which previously identified as *R. solanacearum* using the traditional and IFAS techniques. In this respect, PCR technique using two oligonucleotide primers namely forward primer Rs-l-F (5\ ACT AAC GAA GCA GAG ATG CAT TA-3\) and reverse primer RS- 1-R (5\ CCC AGT CAC GGC AGA GAG T- 3\) visualized the specific DNA band with molecular weight 288 bp in the five tested bacterial isolates and the positive control one under UV light. The results revealed also that there were very close similarity without any variation among the five tested isolates and the positive control (*R. solanacearum* identified by Brown Rot Project in Egypt) one under investigation to confirm that these five tested bacterial isolates are *R. solanacearum*.



Lane Pc = positive control (reference isolate of *R. solanacearum* identified by the Brown Rot Project, Egypt), lanes 1, 3=R1 & R3 isolates of Beheira governorate**.** While, lanes 2, 4 & 5= R2, R4 & R6 isolates of Qalubiya governorate**.**

Fig. (3): Single DNA band in the five tested *Ralstonia solanacearum* isolates and the positive control one with very close similarity among them at MW 288 bp.

3- Virulence of different *R. solanacearum* isolates (Pathogenicity test):

Data in **Table (3)** reveal the virulence of ten *R. solanacearum* isolates on potato plants (cv. Spunta) in sterilized soil. All tested *R. solanacearum* isolates caused bacterial wilt disease symptoms on potato plants compared with the un-inoculated control. In this respect, *R. solanacearum* (R6) recorded the highest infection % where it gave 100% with 5% disease severity at 35 day post inoculation the sterilized soil with brown rot pathogens. *R. solanacearum* (R3) came in second rank where it caused 62.67% infection with 4.67% disease severity at 35 day post inoculation followed by *R. solanacearum* (R1). The least infection% was recorded by *R. solanacearum* (R8) where it recorded 37.33% followed by *R. solanacearum* (R5). The determined disease severities % were increased as time increased after inoculation from 7 to 35 days compared with the un-inoculated control under greenhouse conditions

Table 3. Virulence of ten *Ralstonia solanacearum* isolates on potato plants (cv. Spunta) in sterilized soil under greenhouse conditions.

Tested isolate	Disease severity % after days of inoculation						Infection %
	7	14	21	28	35	Mean	
R_1	1.33	2.33	3.33	3.67	5.00	3.13	60.00
R_2	1.33	2.33	3.33	3.67	5.00	3.13	58.67
R_3	1.33	2.67	3.00	3.33	4.67	3.00	62.67
R_4	2.33	2.33	2.67	3.67	5.00	3.20	58.66
R_5	1.33	1.33	2.00	2.67	4.33	2.33	41.33
R_6	2.00	3.67	4.00	5.00	5.00	3.93	100.0
R_7	1.00	1.67	2.67	3.33	4.33	2.60	46.67
R_8	1.00	1.00	2.00	3.00	4.33	2.27	37.33
R_9	1.00	1.33	2.67	3.33	4.33	2.53	44.00
R_{10}	1.00	1.00	2.67	3.33	4.33	2.47	41.33
Control	1.00	1.00	1.00	1.00	1.00	1.00	0.00
Mean	1.33	1.88	2.67	3.27	4.30		
L.S.D. at 5%							
Isolates			0.368				
Weeks			0.248				

Interaction	0.822

As cleared in **Fig. (4)**, symptoms of brown rot disease caused by *Ralstonia solanacearum* on infected potato plants (cv. Spunta) under greenhouse conditions could be seen in form of yellowing or sudden leaf wilting with down bending of these leaves before death of the whole potato plants. Also, whitish exudates could be seen when the cut surface of tuber was squeezed, a wet breakdown initiated at the point of attachment of the stolon and the eyes of tubers. A light-brown breakdown of water-conducting tissues would be seen in tuber crosses. Light milky fluid is squeezed from this discolored area in infected potato tubers.

Control (un-infected) **Infected with *R. solanacearum***

Fig. (4): Symptoms of brown rot disease (*Ralstonia solanacearum* No. 6) on plants (above) and tubers (below) of potato (cv. Spunta) under greenhouse conditions.

4- Varietal reaction of four potato cultivars against different isolates of *R. solanacearum* in sterilized and un-sterilized soil under greenhouse conditions

The results about responses of the tested potato cultivars *i.e.*, Spunta, Berren, Cara, and Gelabica) to infection with certain isolates of *R. solanacearum* are illustrated in **Table (4a&b)**. Under stress of the pathogenic bacteria, all potato cultivars were susceptible to infection either grown in sterilized and un-sterilized soil. However, the tested potato cultivars were significantly varied in this respect. Also, the infectivity of a tested *R. solanacearum* isolate seemed to be varied in the sterilized and un-sterilized soil. As for sterilized inoculated soil, data in **Table (4a)** reveal that the highest scores of disease severity on the potato cultivars Gelbica (3.4), Cara (3.05), Barren (2.27) and Spunta (2.25) were recorded by the isolates i.e., R6, R6, R2 and R1, respectively. On the other hand, the least virulent isolates were R6 isolate on cvs. Spunta (1.98) and Barren (1.67), R2 isolate on cv. Cara (2.4), and R3 isolate on cv. Gelabica (2.33). Regarding the interaction between the pathogenic isolate and potato cultivars in the un-sterilized soil, data in **Table (4b)** proved that, the highest disease severity was recorded in the following interactions: R4/Spunta (2.58), R6/Barren (2.49), R4/Cara (3.11) and R6/Gelabica (3.4). However, the lowest scores of disease severity in the same soil were recorded by the following interactions: R1/Spunta (1.87), R4/Barren (2.25), R2/Cara (2.45) and R1/Gelabica (1.91).

Table 4a. Varietal reaction of four potato cultivars against different isolates of *Ralstonia solanacearum* in sterilized soil under greenhouse conditions.

Tested isolate	Disease severity on tested cultivars				Mean	Infection % on tested cultivars				Mean
	Spunta	Barren	Cara	Gelabica		Spunta	Barren	Cara	Gelabica	
R1	2.25	2.07	2.45	2.45	2.31	46.7	46.7	53.3	53.3	50.00
R2	2.10	2.27	2.40	2.40	2.29	46.7	46.7	60.0	53.3	51.68
R3	2.11	2.07	2.69	2.33	2.30	40.0	40.0	60.0	46.7	46.68
R4	2.05	1.80	2.73	2.47	2.26	46.7	26.7	60.0	53.3	46.68
R6	1.98	1.67	3.05	3.40	2.53	46.7	33.3	73.3	73.3	56.65
Control	1.00	1.00	1.00	1.00	1.00	0.0	0.0	0.0	0.0	0.00
	1.92	1.81	2.39	2.34		37.80	32.23	51.10	46.65	
L.S.D. 5%										
Cultivars			0.19					7.89		
Isolates			0.23					9.66		
interaction			1.03					22.91		

Table 4b. Varietal reaction of four potato cultivars (cvs. Spunta, Berren, Cara and Gelabica) against different isolates of *Ralstonia solanacearum* in un-sterilized soil under greenhouse conditions

Tested isolate	Disease severity on tested cultivars				Mean	Infection % on tested cultivars				Mean
	Spunta	Barren	Cara	Gelabica		Spunta	Barren	Cara	Gelabica	
R1	1.87	2.33	2.67	1.91	2.20	40.0	46.6	60.0	40.0	46.65
R2	2.40	2.27	2.45	2.16	2.32	53.3	53.3	52.3	46.7	51.40
R3	2.00	2.45	2.82	2.07	2.34	46.7	53.3	73.3	46.7	55.00
R4	2.58	2.25	3.11	2.07	2.50	66.7	46.7	73.3	60.0	61.68
R6	2.47	2.49	2.89	3.40	2.81	53.3	60.0	66.7	73.3	63.33
Control	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00
	2.05	2.13	2.49	2.10	2.19	43.33	43.32	54.27	44.45	46.34
L.S.D. 5%										
Cultivars			0.16					8.97		
Isolates			0.19					10.99		
interaction			0.87					21.98		

Discussion

In this study, ten bacterial isolates were isolated from potato tubers and soil. Using the traditional identification methods based on morphological, physiological and biochemical characteristics of bacteria, all isolates of bacterial were identified as *R. solanacearum*. The results are in agreement with **Sumithra *et al.*, (2000)** who isolated *R. solanacearum* bacterium on tetrazolium chloride medium (TZC) from infected plants, symptom less plants, seeds extracted from fruits of wilted and stem inoculated plants developed with symptoms. The characterization of *Ralstonia solanacearum*, the causal agent of potato bacterial wilt disease, was also performed based on their pathogenicity, biochemical, physiological and serological tests **(Shambhu *et al.*, 2001** and **Al-Ani *et al.*, 2004)**.

Using immunofluorescent antibody staining technique (IFAS) exhibited the morphology of isolated bacterial cells as short rod shape and green fluorescent with specific fluorescent-labaled antiserum which confirm identification of bacterial isolates as *R. solanacearum*. PCR technique using two oligonucleotide primers namely forward primer Rs-l-F (5\. ACT AAC GAA GCA GAG ATG CAT TA-3\) and reverse primer RS- 1-R (5\ CCC AGT CAC GGC AGA GAG T- 3\) visualized the specific DNA band with molecular weight 288 bp in the five tested bacterial isolates and the positive control one under UV light. These results are in harmony with the obtained results of **Pastrik *et al.* (2002)** who reported that PCR is one of the rapid, highly specific and sensitive tests used for detection and identification of *R. solunacaarum* from different sources**.**

Symptoms of brown rot disease caused by *R. solanacearum* on infected potato plants (cv. Spunta) grown under greenhouse conditions appeared in form of yellowing or sudden leaf wilting with down bending of these leaves before appearance the whole death onto potato plants. Also, whitish exudates might be seen onto the cut surface of tubers, a wet breakdown initiated at the point of attachment of the stolon and the eyes of tubers. A light-brown breakdown of water-conducting tissues would be seen in tuber crosses. Light milky fluid is squeezed from this discolored area in infected potato tubers. The recorded symptoms of brown rot disease are similar to those recorded by **Dean *et al.*, (2006)**. All tested *R. solanacearum* isolates caused bacterial wilt disease symptoms on potato plants compared with the un-inoculated control. Also, all tested potato cultivars were susceptible to different extents and responded differently against infection with most tested isolates of *R. solanacearum*. These results

could be interpret in light the findings of **Badr (2006)** who found that all tested cultivars *i.e.,* Diamont, Braka, Picasso and Fabula were susceptible against potato wilt disease. Also, **Hajhamed (2011)** evaluated six potato cultivars against bacterial wilt disease using two different inoculation methods i.e. stem injection method and soil drench method. The cvs. *i.e.,* Diamont and Lady balfor were resistance to bacterial wilt disease. While, Nicola and Lady rosetta cvs., were highly susceptible to bacterial wilt disease.

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ضراوة بكتيريا رالستونيا سولانسيرم المسببة لمرض العفن البني في البطاطس تحت الظروف المصرية

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تم عزل عشرة عزلات من بكتيريا رالستونيا سولانسيرم من درنات بطاطس وعينات تربة تم جمعها من محافظات القليوبية والبحيرة ، وقد تم تعريف تلك العزلات باستخدام الطرق التقليدية المبنية علي خصائصها المورفولوجية والفسيولوجية والبيوكيميائية كما استخدمت تقنيات PCR, IFAS في تأكيد هوية البكتيريا المعزولة علي أنها رالستونيا سولانسيرم. وقد وجد أن كل العزلات المختبرة من بكتيريا رالستونيا سولانسيرم كانت قادرة علي إحداث مرض الذبول البكتيري علي نباتات البطاطس المختبرة مقارنة بالكنترول الغير ملقح مع تفوق العزلة R6 من بكتيريا رالستونيا سولانسيرم والتي سجلت أعلي معدل إصابة وشدة مرضية لمرض العفن البني بعد 35 يوم من تلقيح التربة المعقمة بمسبب العفن البني. وكانت جميع أصناف البطاطس المختبرة حساسة للإصابة ببكتيريا رالستونيا سولانسيرم عندما زرعت في التربة المعقمة والغير معقمة. وقد سجلت أعلي شدة مرضية ونسبة إصابة مع العزلة R6 لبكتيريا رالستونيا سولانسيرم علي صنفي البطاطس جيلابيكا وكارا تلتها في ذلك العزلات R3 على برن و R4 على اسبونتا في التربة غير المعقمة.