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Surveying and fast detection of *Ralstonia solanacearum* bacterium in some Egyptian governorates

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Abstract

Naturally infected potato plants showing bacterial wilt symptoms were collected from different habitats and used for isolation of *R. solanacearum* pathogen. Also, isolation was carried out from soil, water canals, weeds and some other common crops (Pepper, Tomato, Banana, Snap Bean, Corn, Eggplant, Onion, Cabbage and Clover). All isolates showed typical and atypical morphological growth of *R. solanacearum* on Selective Medium South Africa medium (SMSA)**.** Out of suspected 321 isolates of *R. solanacearum*, only 209 isolates were positive when identified using Immunofluorescence antibody stain test (IFAS) while the rest were negative. When these 209 isolates were identified again by growing on SMSA medium, only 194 isolates were positive as typical forms of *R. solanacearum* while the rest15 isolates appeared as atypical forms of *R. solanacearum*. Selected sixty-six isolates of those identified as typical or atypical forms of *R. solanacearum* were tested for their virulence. Results indicate that all tested 66 isolates could infect potato plants (cv. Spunta), where disease severity (DS %) ranged between 26.4% - 100 % when incubated for 20 days. In this respect, MktT-17 (Menofia - EL Kawady - typical - tuber isolate) isolate was the highest infective one. While, the least infective isolate was BhhaWa-53 (Beheira – Kom Hamada - atypical – water) isolate, at the same period of incubation. Also, the rest of other isolates were infective and caused bacterial wilt symptoms below 98.4% and over 28.0%. All atypical forms of *R. solanacearum* isolates were less infective than the typical forms which were more virulent in this respect. Also, all sixty-six tested isolates of typical or atypical *R. solanacearum* were infective and caused bacterial wilt symptoms of tomato plants (cv. Ponto) which reached 100%DS but they differed in the time of incubation. The typical isolates were more virulent and fast in their infection on tomato plants (cv. Ponto). The highest DS% on tomato plants (cv. Ponto) was recorded with the typical *R. solanacearum* isolate MktT-17 (Menofia - EL Kawady - typical - tuber) to be 100% at 4 days of incubation period. While, the lowest DS% was recorded with MmtOB-47 (Menofia - Om saber – typical – other common crop – Banana) isolate where it recorded 100% DS at 10 days of incubation period. Moreover, the fastest atypical form of *R. solanacearum* was GhzaS-55 (Gharbia - Kafr el Zayat - atypical - Soil) isolate which causing 100% wilt DS on tomato plants (cv. Ponto) at 9 days of incubation period while, the slowest atypical ones were BhhaS-56 (Beheira – Kom Hamada - atypical - soil), GdaS-62 (Giza - Wardan – atypical - soil) and GHzaOT-64 (Gharbia- Kafr el zayat atypical- other common crop - Tomato) isolate which caused 100% wilt DS on tomato plants (cv. Ponto) at 15 days of incubation period.

Key words: Wilt and brown rot diseases, typical and atypical forms, Potato plants, *R. solanacearum*, Virulence.

Introduction

Potato (*Solanum tuberosum* L.) is considered one of the major 10 important food crops around the world with total production of 376 million tons. In Egypt, it represents the second exporting crop after oranges where, about 428 thousand tons were exported to many countries especially the EU countries and Russia **(FAO Stat 2013).** In addition, it ranked as the sixth crop in total production after sugarcane, sugar beet, maize, wheat and rice with total production of 503 thousand tons **(FAO Stat 2016).** Bacterial plant diseases are the most critical factors limiting production of the agricultural sector in most countries and often difficult to control. One of the most important bacterial diseases of potatoes is brown rot disease which caused by *Ralstonia solanacearum* where it predominates in tropical, subtropical and temperate regions and this disease is one of the major constraints to production of solanaceous crops in these regions (**Fahy and Persley 1983, Hayward 1991**). In 1896 the causal agent of bacterial wilt was first identified and described as *Bacillus solanacearum* by Erwin F. Smith. In 1914, Smith changed the name to *Pseudomonas solanacearum* and for almost 80 years, the pathogen was grouped within the genus Pseudomonas. In 1992, the new genus *Burkholderia* was validated and reclassified *P. solanacearum* to become *Burkholderia solanacearum* which changed to *Ralstonia solanacearum* in 1995 (**Smith, 1896 & 1914 and Yabuüchi *et al*. 1992 & 1995)**. *R. solanacearum* is the causative agent of potato bacterial wilt. This pathogen is a heterogeneous and soil-borne plant pathogen, five races have been described revering to the hosts affected and five biovars revering to their ability to utilize and /or oxidize several hexose alcohols and disaccharides (**Hayward, 1991**). In Egypt, the dominant race of *R.*

solanacearum is race 3, biovar 2 which is widely spreading in Europe, suggesting a possible origin of introducing to Egypt with seed tubers (**Farag, 2000**). Currently, the species complex *R. solanacearum* is divided into four phylotypes, characterized by high genetic distances and genomic rearrangements, based on the geographical location; phylotype I (Asia), phylotype IIA and IIB (America), phylotype III (Africa) and phylotype IV (Indonesia). **(Fegan & Prior, 2005).**

This work aimed to study the occurrence of potato brown rot disease in different Egyptian governorates and throw the light on the different hosts of the causal of brown rot pathogen.

Materials & Methods

1. Isolation of *Ralstonia solanacearum* from different habitats:

Out of 221 samples were collected from different habitats (tubers, soil, water canals and weeds) through the following governorates Menofia, Gharbia, Beheira, Beni Suef and Giza.

1.1. Isolation from potato tubers:

Naturally infected samples of potato plants showing external and internal symptoms of potato brown rot disease were collected from potato cultivation sites in different governorates. These samples were investigated in the Potato Brown Rot Project (PBRP), Dokki, Giza, Egypt. In this respect, the infected potato tubers were washed in running tap water, surface sterilized with 90% ethyl alcohol by flaming then the stolon ends were aseptically removed using sharp sterilized knife. Cores of 5-10 mm diameter and 5 mm length, containing mainly vascular and cortical tissues were macerated in 1.0 mL of sterile phosphate buffer; then the suspension was transferred to sterile 1 mL Eppendorf tubes. The suspension was left to stand for 30 min. Plating was made on modified Selective Medium South Africa (SMSA) which consists of basal medium + Crystal violet 0.5 mL, Polymixin-B-sulphate 10mL, Bacitracin 7.5 mL, Chloromphenicol 2.5 mL, Penicillin-G 1 mL and 2,3,5 triphenyltetrazolium chloride salts 5 mL. This medium is considered as semi selective medium for isolation of *R. solanacearum* as described by **Engelbrecht, (1994)** and modified by **Elphinstone, (1996).**

Purification was made by streaking and plating for typical and atypical colonies on basal medium which consists of: 1.0 g casamino acid**,** 10.0 g bactopeptone, 5.0 mL glycerol**,** 20.0 g agar and 1000.0 mL distilled water (pH 6.9). And on King's medium (20.0 g Proteose peptone, 1.5 g K_2HPO_4, 1.5 g $MgSO_4$, 15 mL glycerol, 20.0 g agar and 1000 mL distilled water, pH 7.2) then incubated at 28ºC and daily observed (approx. 3 days) for developing less fluidal, red to dark red center and opaque colonies which considered as atypical colony forms for *Ralstonia solanacearum.* While fluidal, slightly raised, irregular white or white with pink center colonies considered as typical colony forms of *Ralstonia solanacearum*. Colonies were selected, picked up and streaked on glucose nutrient agar medium, incubated for 48hr at 28°C **(Dowson, 1957)** for further studies.

1.2. Isolation from soil:

Soil samples were collected from the same mentioned above governorates to isolate the pathogenic bacteria. About 200g soil was taken from 30 cm depth using a sampling auger from each sampling spot in each field and then mixed together for homogenization. In the laboratory, 10 grams of soil samples were suspended in 250 mL flask contains 90mL of sterilized phosphate buffer (0.01 M) and were shaken at 100 rpm at 15°C for two hrs. Then isolation was carried on SMSA medium by spreading 100 µL per sample and incubated at 28°C for 3 days. **(Wenneker *et al.*, 1999).**

1.3. Isolation from irrigation water:

Water sampling points on streams were chosen near to potato fields and other inspected fields (up and down stream of the potato fields and other inspected fields) 50 mL/sample were collected at a depth of 30-40 cm in 50mL disposable centrifuge tubes then placed in an icebox. In the laboratory, the tubes were centrifuged at 10.000 rpm for 15 minutes at 15°C by using Sigma 3-18K centrifuge. The sediment was resuspended in 1mL sterile phosphate buffer (0.01M) and vortexed for homogenization. Plating was made on modified SMSA medium and incubated at 28°C for 3-5 days. **(Janse, 1988 & 1996; Janse & Schans, 1998 and Wenneker *et al.*, 1999).**

1.4. Isolation from weeds:

Different weed plants were collected from the inspected fields of the governorates mentioned above. Weeds were defined (**Zaki, 1991 & 2000)** as *Rumex dentatus* (toothed dock), *Solanum nigrum* (Black nightshade), *Portulaca oleracea* (little hogweed) and *Malva parviflora* (Cheese weed). Roots plus the crown parts were thoroughly washed under tap water and disinfected in 70% ethyl alcohol. These parts were macerated in 1 mL sterile phosphate buffer 0.1M and left for 10 min then 100µL per sample were spread onto SMSA medium and incubated at 28°C for 3 days as described by **Pradhanang *et al.*, (2000)**.

2. Identification of isolated bacteria:

Different methods were used to identify and study differences between isolates as following:

2.1. Cultural characteristics on SMSA medium:

Isolated bacteria were re-inoculated on SMSA medium to determine the typical and atypical forms of isolated *R. solanacearum* isolates as mentioned by **Engelbrecht (1994) and Elphinstone *et al.* (1996).**

2.2. Immunofluorescence antibody stain (IFAS):

Suspensions of the selected *R. solanacearum* isolates were prepared from grown cultures for 48h and adjusted to a standard optical density at 590 nm to 10^6 colonies forming units (cfu/mL) using Jenway

6300 spectrophotometer, then a standard volume of 20 µL of each suspension was pipetted onto multi-well slides 6 mm window diameter. Slides were left to dry on a Stuart CB500 hot plate at 40°C and gently heat-fixed by flaming **(PBRP protocol, Egypt)**. All windows were covered with 25 µL of the antiserum (anti *R. solanacearum* polyclonal) in four dilutions (1:800, 1:1600, 1:3200 and 1:6400) and incubated in a humid tray chamber for 30 min at room temperature then washed with tween buffer and 0.01 M phosphate buffer (PB). Add 25 µL of anti-rabbit Nordic SW/AR fluorescein isothiocyanate conjugate in a 100-fold dilution (prepared in phosphate buffer saline) to each window and incubate for 30 min in a humid tray chamber. Slides then washed with tween buffer and 0.01 M PB, and the excess moisture was removed carefully by blotting with filter or tissue paper. One droplet of 0.1 mol^{-1} phosphate glycerin buffer (pH 7.6) was added to each window and the slides were covered with long cover glasses. Slides were examined with Olympus bx51 microscope (tube factor 1.25) with an epifluerescent light source and suitable filters with FITC, using a 100 X oil immersion objective and a l0 X eyepiece. At least 20 microscope fields per window were scanned for the presence of morphologically typical fluorescing cells **(Janse, 1988).**

3. Virulence of *R. solanacearum* isolates:

Sixty-six isolates were selected based on their occurrence in each governorate, location, form of each isolate and source of isolates (water, tuber, weed, soil and/or other common crops in the same region). So, all governorates and locations were represented in addition to all other common crop soil isolates. Firstly, pure cultures of the selected 66 bacterial isolates were primary identified and confirmed as *Ralestonia solanacearum* using IFAS and SMSA tests. These isolates were grown on casmino acid peptone glucose (CPG) agar medium (5.0 g Dextrose, 10.0 g Peptone, 1.0 g Casamino acid, 20.0 g Agar and 1000 mL Distilled water, pH 7.2) at 28ºC for 48h and suspended in sterile phosphate buffer 0.05 M pH 7.2. The prepared suspensions were adjusted to a standard optical density 590 nm to obtain 10^7 cfu/mL (**Kelman, 1954**). Potato tubers (cv. Spunta) and tomato plants (cv. Ponto) were examined to be free from *R. solanacearum* using IFAS and planting on SMSA media before using in this experiment. Four weeks old, tomato seedlings were transplanted in 10 cm diameter pots, containing sterilized sand-clay soil mixture (1:1 v/v) while, potato tubers which previously stored at 4ºC were placed in trays at room temperature in dark to stimulate germination. Germinated tubers were planted in pots (30 cm diameter) containing 5Kg sterilized sand-clay soil (1:1 v:v), where one germinated tuber was planted in each pot. Tomato plants (three-weeks after transplanting) were injected at the leaf axis with a sterile fine needle laden with the bacterial growth of the pathogen (**Janse, 1988**). Potato plants were inoculated into the axel of the second and third leaf from the apical meristem by injection of the bacterial suspension (10µL) using a sterile fine needle (**Martin and El-Nashaar, 1992**). The inoculated plants were covered with polyethylene bags for three days at 30°C and 28% relative humidity (RH) in an automated quarantine greenhouse, then bags were removed and pots were irrigated daily.

Wilt symptoms severity were recorded daily using the scale of **Kempe and Sequeira (1983)** where, (0 = no symptoms, 1= up to 25 % wilt, 2 = 26-50 % wilt, 3 = 51-75 % wilt, 4 = 76-100% wilt and 5 = dead plants).

Results & Discussion

1. Isolation of *Ralstonia solanacearum*:

Naturally infected potato plants from different habitats showing brown rot diseases were used for isolation of *R. solanacearum* pathogen. Also, isolation was carried out from soil, water canals and some other common crops which cultivated commonly in the region of potato cultivations (i.e., Pepper=P, Tomato=T, Banana=B, Snap Bean=Sb, Corn=C, Eggplant=E, Onion=O, Cabbage=Ca and Clover=Cl) as clear in **Table 1**. All isolates showed typical and atypical morphological growth of *R. solanacearum* on SMSA medium**.** Where from total of 321 suspected isolates, 21 isolates were isolated from irrigation water, 160 isolates were isolated from potato tubers, 36 isolates of potato cultivation soil and 61 isolates of weed plants which grown in potato fields. Also, 8 suspected isolates were isolated from common field crop plants and 35 isolates were isolated from soil of the cultivated common field crops. The colonies for typical forms were fluidal white with red center while, for atypical forms less fluidal, red to dark red center and opaque colonies**.** These results could be discussed in light the findings of **Abd El-Ghany (2010)** who surveyed the causal of brown rot disease infecting potato cultivations in many localities of four Egyptian governorates. This survey was done on potato tubers, water, soil and some grown weed plants in potato fields and borders of irrigation canals. The highest percentage of positive samples were in Menofia followed by Gharbia and Kalubia while the lowest one was in Ismailia governorate. Also, **Hagag (2015)** used SMSA medium for isolation of *R. solanacearum* from soil, tuber and weed samples of different Egyptian governorates. Moreover, **Ali (2017)** isolated the pathogen on SMSA medium from different samples collected from infected potato tubers, field soils with previous disease history, irrigation water and weeds from Ismailia and Menofia governorates. These isolates show bacteriological characteristic like those described for race 3 biovar 2 *Ralstonia solanacearum* as described in Bergey's Manual of Systematic Bacteriology.

Table 1. General screening of suspected *R. solanacearum* isolates on SMSA medium from different habitats which collected from different governorates:

Governorate	Location	Number of suspected *Ralstonia solanacearum* isolates					
		Potato Fields				Common Crops	
		Wa	T	S	We	Pl	S
Gharbia (Gh)	Kafr EL zayat (z)	3	44	14	15	2T 0E 1O	3T 6E 2O
	Gafarya (g)	2	24	2	14	0C 0Cl	1C 2Cl
Beheira (Bh)	Kom Hamada (h)	9	7	6	7	1T	2T
Menofia (M)	Talia (i)	2	10	2	5	0Ca 0Sb	1Ca 1Sb
	Om Saber (m)	2	20	4	7	0B 0Sb 0C 0Cl	2B 1Sb 1C 2Cl
	El Kawady (k)	3	10	3	6	1P	1P
Beni Suef (Bs)	Kafr Mymon (y)	0	9	2	4	0O 1T	2O 1T
Giza (G)	Wardan (d)	0	36	3	3	1P 1T	3P 4T
Sub-Total		**21**	**160**	**36**	**61**	**8**	**35**
Total				**321**			

Where: Common crops= crops commonly cultivated in the region of potato either pre or post cultivation. Wa=Water, T= Tuber, S= Soil, We= Weeds, Pl= Plant. P= Pepper, T= Tomato, B= Banana, Sb= Snap Beans, C= Corn, E= Eggplant, O= Onion, Ca= Cabbage and Cl= Clover

2. Preliminary identification of suspected *R. solanacearum* isolates using IFAS test and planting on SMSA media:

Preliminary identification was done of suspected isolates from different habitats which isolated on the selective SMSA medium (total 321 isolates) using IFAS test as clear in **Table 2.** In this respect, results show that all 21 bacterial isolates of water were positive as *R. solanacearum* using IFAS test while, among 160 bacterial isolates of tubers only 147 were positive as *R. solanacearum* and 13 isolates were negative. On the other hand, among 36 bacterial isolates of soil, only 24 isolates were positive and 12 isolates were negative. Also, among 61 bacterial isolates of weeds, only 7 isolates were positive as *R. solanacearum*. As for the bacterial isolates of other common field crop plants (**Table 3)**, all 8 isolates were negative while, among 35 isolates of soil of cultivated common field crop, only 10 isolates were positive as *R. solanacearum* using IFAS test. As for the identification of the suspected isolates of *R. solanacearum* (321 isolates) using SMSA medium test, Results in **Tables 4** indicate that among 21 bacterial isolates of water, only 18 isolates were typical form of *R. solanacearum* while the rest 3 isolates were atypical form of *R. solanacearum*. Also, among 160 isolates of potato tubers, only 147 isolates were typical form of *R. solanacearum* while the rest 13 isolates were negative as *R. solanacearum* when confirmed by IFAS test. As for soil isolates, among 36 isolates, only 16 isolates were typical form of *R. solanacearum* while the rest 8 isolates were atypical form isolates in addition to 12 isolates were negative as *R. solanacearum* when confirmed for identification using IFAS test. Also, among 61 isolates of weeds in potato fields, only 6 isolates were typical form of *R. solanacearum* and only one was as atypical form isolate of *R. solanacearum* whereas the rest 54 isolates were negative as *R. solanacearum* when confirmed for identification using IFAS test. Regarding the suspected isolated bacteria from other common field crop plants **(Table 5)**, all 8 isolates were negative as *R. solanacearum* when they confirmed for their identification again using IFAS test where they were not typical or atypical forms on SMSA medium test. Meanwhile, among 35 isolates of soil of cultivated common field crop, 7 isolates were typical form as *R. solanacearum* and 3 isolate were atypical forms of *R. solanacearum* while the rest 25 isolates were negative as *R. solanacearum* when confirmed again for identification using IFAS test. Also, it is clear from data in **Table (6)** that out of suspected 321 isolates of *R. solanacearum*, only 209 isolates were positive when identified using IFAS test while the rest were negative. When these 209 isolates were identified again by growing on SMSA medium, only 194 isolates were positive as typical forms of *R. solanacearum* while the rest15 isolates appeared as atypical forms of *R. solanacearum*. For further studies, sixty-six isolates of those identified as typical

or atypical forms of *R. solanacearum* were selected and grouped as clear in **Table 7** based on governorate and location of isolation, habitats of isolation (water, tubers, soil, weeds) and other crops of isolation (pepper, tomato, eggplant and banana). These isolates were coded as clear in **Table 8**. These results could be interpreting in light the findings of **Atta (2008)** who stated that detection methods by plating on the SMSA medium resulting colonies with irregularly round shape and slimy white color with pink centers, which considered the typical morphology of bacterial colony. Also, **Ali (2017)** stated that culturing on SMSA medium is the best method to detect the pathogen. IFAS is of limited interest in detection in soil because the assayed soil extracts caused a strong background fluorescence that hampered the visualization of target cells by IFAS. Meanwhile, **Mikhail *et al.* (2017)** observed two different types of colony the first, was the virulent type or typical which was highly fluidal, irregular, white or cream with pink centers. While the second, was the avirulent type or atypical was uniformly round, smaller and completely pink to deep red colonies.

Table 2. Preliminary identification of *Ralstonia solanacearum* isolates which isolated from different habitats of potato fields in some Egyptian governorates using IFAS test

Governorate	Location	No. of positive and negative isolates from different habitats											
		Water			Tubers			Soil			Weeds		
		TSI	+	-	TSI	+	-	TSI	+	-	TSI	+	-
Gharbia (Gh)	Kafr EL zayat (z)	3	3	0	44	41	3	14	3	11	15	1	14
	Gafarya (g)	2	2	0	24	22	2	2	1	1	14	2	12
Beheira (Bh)	Kom Hamada (h)	9	9	0	7	6	1	6	6	0	7	0	7
Menofia (M)	Talia (i)	2	2	0	10	10	0	2	2	0	5	1	4
	Om Saber (m)	2	2	0	20	18	2	4	4	0	7	1	6
	El Kawady (k)	3	3	0	10	10	0	3	3	0	6	1	5
Beni Suef (Bs)	Kafr Mymon (y)	0	0	0	9	9	0	2	2	0	4	0	4
Giza (G)	Wardan (d)	0	0	0	36	31	5	3	3	0	3	1	2
Total		**21**	**21**	**0**	**160**	**147**	**13**	**36**	**24**	**12**	**61**	**7**	**54**

TSI= Total suspected isolates, (+) = Positive IFAS, (-) = Negative IFAS

Table 3. Preliminary identification of *Ralstonia solanacearum* isolates of the cultivated common field crops in some Egyptian governorates using IFAS test.

Governorate	Location	No. of positive and negative Isolates from other common field crops					
		Plant			Soil		
		TSI	+	-	TSI	+	-
Gharbia (Gh)	Kafr EL zayat (z)	2T 0E 1O	0	3	3T 6E 2O	1T 2E	8
	Gafarya (g)	0C 0Cl	0	0	1C 2Cl	0	3
Beheira (Bh)	Kom Hamada (h)	1T	0	1	2T	1T	1
Menofia (M)	Talia (i)	0Ca 0Sb	0	0	1Ca 1Sb	0	2
	Om Saber (m)	0B 0Sb 0C 0Cl	0	0	2B 1Sb 1C 2Cl	1B 1Sb	4
	El Kawady (k)	1P	0	1	1P	1P	0

Beni Suef (Bs)	Kafr Mymon (y)	0O 1T	0	1	2O 1T	1T	2
Giza (G)	Wardan (d)	1P 1T	0	2	3P 4T	1P 1T	5
Total		**8**	**0**	**8**	**35**	**10**	**25**

Where: P= Pepper, T= Tomato, B= Banana, Sb= Snap Beans, E= Eggplant
(+) = Positive IFAS, (-) = Negative IFAS.

Table 4. Preliminary identification of *Ralstonia solanacearum* isolates which isolated from different habitats of potato fields in some Egyptian governorates using SMSA medium test

Governorate	Location	No. of typical and atypical isolates from different habitats											
		Water			Tubers			Soil			Weeds		
		TSI	t	a	TSI	t	a	TSI	t	a	TSI	t	a
Gharbia (Gh)	Kafr EL zayat (z)	3	2	1	44	41	0	14	2	1	15	1	0
	Gafarya (g)	2	2	0	24	22	0	2	1	0	14	2	0
Beheira (Bh)	Kom Hamada (h)	9	8	1	7	6	0	6	4	2	7	0	0
Menofia (M)	Talia (i)	2	2	0	10	10	0	2	2	0	5	1	
	Om Saber (m)	2	2	0	20	18	0	4	3	1	7	1	0
	El Kawady (k)	3	2	1	10	10	0	3	1	2	6	0	1
Beni Suef (Bs)	Kafr Mymon (y)	0	0	0	9	9	0	2	1	1	4	0	0
Giza (G)	Wardan (d)	0	0	0	36	31	0	3	2	1	3	1	0
Total		**21**	**18**	**3**	**160**	**147**	**0**	**36**	**16**	**8**	**61**	**6**	**1**

TSI= Total suspected isolates, (t) = Typical *R. solanacearum* form, (a) = Atypical *R. solanacearum* form.

Table 5. Preliminary identification of *Ralstonia solanacearum* isolates of the cultivated common field crops in some Egyptian governorates using SMSA test

Governorate	Location	No. of typical and atypical isolates from other common field crops					
		Plant			Soil		
		TSI	t	A	TSI	T	a
Gharbia (Gh)	Kafr EL zayat (z)	2T 0E 1O	0	0	3T 6E 2O	2E	1T
	Gafarya (g)	0C 0Cl	0	0	1C 2Cl	0	0
Beheira (Bh)	Kom Hamada (h)	1T	0	0	2T	0	1T
Menofia (M)	Talia (i)	0Ca 0Sb	0	0	1Ca 1Sb	0	0
	Om Saber (m)	0B 0Sb 0C 0Cl	0	0	2B 1Sb 1C 2Cl	1B 1Sb	0
	El Kawady (k)	1P	0	0	1P	0	1P
Beni Suef (Bs)	Kafr Mymon (y)	0O 1T	0	0	2O 1T	1T	0
Giza (G)	Wardan (d)	1P 1T	0	0	3P 4T	1P 1T	0
Total		**8**	**0**	**0**	**35**	**7**	**3**

Where: P= Pepper, T= Tomato, B= Banana, Sb= Snap Beans, E= Eggplant, t=Typical *R. solanacearum* form, a=Atypical *R. solanacearum* form.

Table 6. Total number of *Ralstonia solanacearum* isolates, which identified using IFAS test and SMSA medium

Governorate	Source	Habitats	Total samples	Positive sample IF		Positive typical SMSA	
				no.	%	no.	%
Gharbia (Gh)	Potato field	Water	5	5	100.0	4	80.0
		Tuber	68	63	92.6	63	86.7
		Soil	16	4	25.0	3	18.7
		Weed	29	3	10.3	3	10.3
	Other crops	Plant	3	0	0.0	0	0.0
		Soil	14	3	21.4	2	14.2
Beheira (Bh)	Potato field	Water	9	9	100.0	8	88.8
		Tuber	7	6	85.7	6	85.7
		Soil	6	6	100.0	4	66.6
		Weed	7	0	0.0	0	0.0
	Other crops	Plant	1	0	0.0	0	0.0
		Soil	2	1	50.0	0	0.0
Menofia (M)	Potato field	Water	7	7	100.0	6	85.7
		Tuber	40	38	95.0	38	95.0
		Soil	9	9	100.0	6	66.6
		Weed	18	3	16.6	2	11.1
	Other crops	Plant	1	0	0.0	0	0.0
		Soil	9	3	33.3	2	18.1
Beni Suef (Bs)	Potato field	Water	0	0	0.0	0	0.0
		Tuber	9	9	100.0	9	100.0
		Soil	2	2	100.0	1	50.0
		Weed	4	0	0.0	0	0.0
	Other crops	Plant	1	0	0.0	0	0.0
		Soil	3	1	33.3	1	33.3
Giza (G)	Potato field	Water	0	0	0.0	0	0.0
		Tuber	36	31	86.1	31	86.1
		Soil	3	3	100.0	2	66.6
		Weed	3	1	33.3	1	33.3
	Other crops	Plant	2	0	0.0	0	0.0
		Soil	7	2	28.2	2	28.2
Total			**321**	**209**		**194**	

Table 7. Selected *Ralstonia solanacearum* isolates for further tests

Governorate	Typical isolates (t)					Atypical Isolates (a)				
	Wa	T	S	We	O	Wa	T	S	We	O
Gharbia (Gh)	2	5	2	3	2	1	0	1	0	1
Beheira (Bh)	2	2	3	0	0	1	0	2	0	1
Menofia (M)	3	3	3	2	2	1	0	3	1	1
Beni Suef (Bs)	0	4	1	0	1	0	0	1	0	0
Giza (G)	0	6	2	1	2	0	0	1	0	0
Sub-Total	**7**	**20**	**11**	**6**	**7**	**3**	**0**	**8**	**1**	**3**
Total	**66**									

Where: Wa = Water isolates, T= Tuber isolates, S= Soil isolates, We= Weed isolates and O= other common crops

Table 8. Code of selected sixty-six isolates of *Ralstonia solanacearum* isolates

No.	Code	No.	Code	No.	Code	No.	Code
1	GhztWa	18	BsytT	35	MktS	52	GhzaWa
2	GhgtWa	19	BsytT	36	BsytS	53	BhhaWa
3	BhhtWa	20	BsytT	37	GdtS	54	MkaWa

4	BhhtWa	21	BsytT	38	GdtS	55	GhzaS
5	MitWa	22	GdtT	39	GhztWe	56	BhhaS
6	MmtWa	23	GdtT	40	GhgtWe	57	BhhaS
7	MktWa	24	GdtT	41	GhgtWe	58	MmaS
8	GhztT	25	GdtT	42	MitWe	59	MkaS
9	GhztT	26	GdtT	43	MmtWe	60	MkaS
10	GhztT	27	GdtT	44	GdtWe	61	BsyaS
11	GhgtT	28	GhztS	45	GhztOE	62	GdaS
12	GhgtT	29	GhgtS	46	GhztOE	63	MkaWe
13	BhhtT	30	BhhtS	47	MmtOB	64	GhzaOT
14	BhhtT	31	BhhtS	48	MmtOSb	65	BhhaOT
15	MitT	32	BhhtS	49	BsytOT	66	MkaOP
16	MmtT	33	MitS	50	GdtOP		
17	MktT	34	MmtS	51	GdtOT		

Where: Gh= Gharbia, Bh= Beheira, M= Menofia, Bs= Beni Swef, G= Giza, z= Kafr el zayat, g= Gafarya, i= Talia, m= Om saber, k= Kawady, h= Kom Hamada, y= Kafr Mymon, d= Wardan, t= typical form, a= atypical form, Wa= water, T= Tuber, S= Soil, We= Weed, O= other crop soil, E= Eggplant, B=Banana, Sb= Snab Beans, T= Tomato, P= Pepper

3. Virulence of selected sixty-six isolates of *R. solanacearum*:

Data in **Tables (9a&9b)** indicate that all tested 66 isolates of typical or atypical *R. solanacearum* could infect potato plants (cv. Spunta) where they caused disease severity % (DS %) ranged between 26.4% - 100 % when incubated for 20 days. In this respect, MktT-17 (Menofia - EL Kawady - typical - tuber) isolate was the highest infective one where it caused 100% DS followed by MmtT-16 (Menofia- Om Saber -typical -tuber) and GhztT-8 (Gharbia - Kafr el Zayat – typical – tuber) which caused 98.4% DS at 20 days of incubation period. On the other hand, the least infective isolates were BhhaWa-53 (Beheira –Kom Hamada - atypical – water), BhhaS-56 (Beheira- Kom Hamada - atypical - soil) and GdaS-62 (Giza -Wardan - atypical - soil) at the same period of incubation. Also, the rest of other isolates were infective and caused DS% of bacterial wilt symptoms below 98.4% and over 28.0%. It is clear from the obtained results that all atypical forms of *R. solanacearum* isolates were less infective than the typical forms which were more virulent in this respect. On the other hand, all sixty-six tested isolates of typical or atypical *R. solanacearum* were also infective and caused bacterial wilt symptoms of tomato plants (cv. Ponto). In this respect, all tested isolates caused 100% DS of wilt infection but they differed in the time of incubation that taken. Also, the typical isolates were more virulent and fast in their infection on tomato plants (cv. Ponto) where atypical forms took a long time of incubation till the DS reached 100%. The highest DS% on tomato plants (cv. Ponto) was recorded with the typical *R. solanacearum* isolate MktT-17 (Menofia - EL Kawady - typical - tuber) to be 100% at 4 days of incubation period. While, the lowest DS% was recorded with MmtOB-47 (Menofia - Om saber – typical - other crop – Banana) where it recorded 100% DS at 10 days of incubation period. Moreover, the fastest atypical form of *R. solanacearum* was GhzaS-55 (Gharbia - Kafr el Zayat - atypical - Soil) which causing 100% wilt DS on tomato plants (cv. Ponto) at 9 days of incubation period while, the slowest atypical ones were BhhaS-56 (Beheira – Kom Hamada - atypical - soil), GdaS-62 (Giza - Wardan – atypical - soil) and GHzaOT-64 (Gharbia- Kafr el zayat atypical- other crop - Tomato) which caused 100% wilt DS on tomato plants (cv. Ponto) at 15 days of incubation period. These results are in harmony with those obtained by **Aley *et al.* (1994)** who inoculated the purified culture of *R. solanacearum* on King's medium on tomato or potato seedlings to confirm its pathogenicity. Wilting of tomato seedlings may begin in less than a week, but potato seedling will appear within 4 weeks. Also, **Elphinstone *et al.* (1996)** reported that the bioassay test using tomato seedlings is reliable to detect as few as 10^4 cells per ml of suspension of infected potato extract. Tomato seedlings are widely used for both pathogenicity testing. Depending on the inoculum potential of the bacterium and the availability of optimum environmental conditions, typical wilting symptoms are usually apparent within a week of inoculation. However, **El-Ariqi *et al.* (2005)** showed that isolates of *R. solanacearum* displayed varying levels of virulence on potato Spunta and Diamont cultivars. **Mikhail *et al.* (2012)** stated that all the nine isolates of *R. solanacearum* (Six virulent and three avirulent) recovered from the natural habitats (potato tubers, weeds, soil and water) were pathogenic to potato plants causing different symptoms. Also, **El-Haj Saleh (2014)** found that when potato plants were injected with *R. solanacearum* isolates, wilt symptoms appeared compared to non-injected potato plants this results confirms that all the tested isolates are virulent and belong to race 3 biovar 2. **Hagag (2015)** found that number of positive *R. solanacearum* isolates retrieved from infected tubers was higher than isolates retrieved from soil followed by weed isolates also, positive samples were highly recorded in Beheria

governorate followed by Menofia governorate and the lowest recorded was in Ben-Seweif governorate. Moreover, **Mikhail *et al.* (2017)** found that all tested virulent isolates of *R. solanacearum* showed high percentages of infection and disease severity on tomato plants after 5 days while, avirulent isolates showed low percentages of infection and disease severity on tomato plants causing stunting and chlorosis.

Table 9a. Virulence of sixty-six isolates of identified *R. solanacearum* on potato plants (cv. Spunta) under artificial inoculation conditions for 20 days.

No.	Tested isolates	DS%	No.	Tested isolates	DS%	No.	Tested isolates	DS%
1	GhztWa	79.2	23	GdtT	76.0	45	GhztOE	52.8
2	GhgtWa	64.8	24	GdtT	82.4	46	GhztOE	59.2
3	BhhtWa	59.2	25	GdtT	68.8	47	MmtOB	48.8
4	BhhtWa	63.2	26	GdtT	63.2	48	MmOSb	54.4
5	MitWa	80.8	27	GdtT	88.0	49	BsytOT	63.2
6	MmtWa	85.6	28	GhztS	72.0	50	GdtOP	68.8
7	MktWa	87.2	29	GhgtS	68.8	51	GdtOT	59.2
8	GhztT	98.4	30	BhhtS	72.0	52	GhzaWa	30.4
9	GhztT	87.2	31	BhhtS	76.0	53	BhhaWa	26.4
10	GhztT	91.2	32	BhhtS	76.0	54	MkaWa	34.4
11	GhgtT	85.2	33	MitS	85.6	55	GhzaS	48.0
12	GhgtT	87.2	34	MmtS	88.0	56	BhhaS	27.2
13	BhhtT	88.0	35	MktS	92.0	57	BhhaS	30.4
14	BhhtT	87.2	36	GdtS	79.2	58	MmaS	30.4
15	MitT	96.8	37	GdtS	72.0	59	MkaS	54.4
16	MmtT	98.4	38	GhztWe	68.8	60	MkaS	48.8
17	MktT	100.0	39	GhgtWe	80.8	61	BsyaS	36.4
18	BsytT	88.0	40	GhgtWe	80.8	62	GdaS	28.0
19	BsytT	87.2	41	MitWe	72.0	63	MkaWe	48.2
20	BsytT	82.4	42	MmtWe	76.0	64	GhzaOT	40.8
21	BsytT	85.6	43	GdtWe	82.4	65	BhhaOT	54.4
22	GdtT	76.0	44	GhztOE	68.2	66	MkaOP	40.8

Where: DS %= Disease Severity%, Gh= Gharbia, Bh= Beheira, M= Menofia, Bs= Beni Suef, G= Giza, Z= Kafr el zayat, g= Gafarya, i= Talia, m= Om saber, k= Kawady, h= Kom Hamada, y= Kafr Mymon, d= Wardan, t= typical form, a= atypical form, Wa= water, T= Tuber, S= Soil, We= Weed, O= other crop soil, E= Eggplant, B=Banana, Sb= Snap Beans, T= Tomato, P= Pepper

Table 9b. Virulence of sixty-six isolates of identified *R. solanacearum* on Tomato plants (cv. Ponto) under artificial inoculation conditions.

No.	Tested isolates	IP (days)	DS %	No.	Tested isolates	IP (days)	DS %	No.	Tested isolates	IP (days)	DS %
1	GhztWa	7	100	23	GdtT	8	100	45	GhztOE	9	100
2	GhgtWa	8	100	24	GdtT	7	100	46	GhztOE	9	100
3	BhhtWa	8	100	25	GdtT	8	100	47	MmtOB	10	100
4	BhhtWa	7	100	26	GdtT	8	100	48	MmOSb	9	100
5	MitWa	8	100	27	GdtT	6	100	49	BstOT	8	100
6	MmtWa	7	100	28	GhztS	8	100	50	GdtOP	8	100
7	MktWa	7	100	29	GhgtS	9	100	51	GdtOT	9	100
8	GhztT	8	100	30	BhhtS	7	100	52	GhzaWa	10	100
9	GhztT	6	100	31	BhhtS	8	100	53	BhhaWa	14	100
10	GhztT	5	100	32	BhhtS	7	100	54	MkaWa	12	100
11	GhgtT	7	100	33	MitS	6	100	55	GhzaS	9	100
12	GhgtT	6	100	34	MmtS	6	100	56	BhhaS	15	100
13	BhhtT	7	100	35	MktS	6	100	57	BhhaS	14	100
14	BhhtT	6	100	36	GdtS	8	100	58	MmaS	12	100
15	MitT	6	100	37	GdtS	8	100	59	MkaS	12	100

16	MmtT	4	100	38	GhztWe	9	100	60	MkaS	12	100
17	MktT	4	100	39	GhgtWe	8	100	61	BsyaS	14	100
18	BsytT	5	100	40	GhgtWe	8	100	62	GdaS	15	100
19	BsytT	6	100	41	MitWe	7	100	63	MkaWe	14	100
20	BsytT	6	100	42	MmtWe	8	100	64	GhzaOT	15	100
21	BsytT	6	100	43	GdtWe	8	100	65	BhhaOT	12	100
22	GdtT	8	100	44	GhztOE	8	100	66	MkaOP	13	100

Where: DS %= Disease Severity%, IP= Incubation period (days)Gh= Gharbia, Bh= Beheira, M= Menofia, Bs= Beni Suef, G= Giza, Z= Kafr el zayat, g= Gafarya, i= Talia, m= Om saber, k= Kawady, t= typical form, a= atypical form, Wa= water, T= Tuber, S= Soil, We= Weed, O= other crop soil, E= Eggplant, B=Banana, Sb= Snap Beans, T= Tomato, P= Pepper

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الحصر والكشف السريع لبكتيريا رالستونيا سولانسيرم المسبب المرضي لمرض العفن البني علي البطاطس في بعض المحافظات المصرية

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استخدمت نباتات البطاطس المصابة طبيعيا والمأخوذة من بيئات مختلفة وتظهر عليها أعراض الذبول والعفن البني لعزل المسبب المرضي رالستونيا سولانسيرم ، كما تم العزل أيضا من عينات التربة وماء الري التي تروي بها البطاطس في المنطقة وكذلك الحشائش المنتشرة في زراعات البطاطس بالإضافة الي العزل من نباتات المحاصيل الشائع زراعتها في مناطق زراعة البطاطس والتربة المنزرعة فيها مثل الفلفل والطماطم والموز والفاصوليا الخضراء والذرة والباذنجان والبصل والكرنب والبرسيم. وقد أظهرت كل العزلات المعزولة مظاهر نمو مطابقة وغير مطابقة لبكتيريا رالستونيا سولانسيرم علي بيئة (SMSA) وكان من بين 321 عزلة متوقعة لبكتيريا رالستونيا سولانسيرم 209 عزلة فقط ايجابية عندما عرفت باستخدام إختبار (IFAS) وكانت باقي العزلات سلبية. وعندما عرفت الـ 209 عزلة مرة أخري بتنميتها علي بيئة SMSA ، كان فقط 194 عزلة هي الإيجابية وأعطت الشكل المطابق تماما لبكتيريا رالستونيا سولانسيرم بينما كانت الـ 15 عزلة المتبقية غير مطابقة لبكتيريا رالستونيا سولانسيرم. أختبرت القدرة المرضية لـ 66 عزلة المختارة والتي سبق تعريفها كعزلات مطابقة وغير مطابقة لبكتيريا رالستونيا سولانسيرم حيث أظهرت النتائج أن الـ 66 عزلة المختبرة يمكنها أن تصيب نباتات البطاطس صنف سبونتا حيث سبب جميعهم شدة مرضية تراوحت بين 26.4-100% عندما حضنت لمدة 20 يوم. وفي هذا المقام كانت العزلة المعزولة من درنات بطاطس من منطقة الكوادي بالمنوفية والمطابقة لبكتيريا رالستونيا سولانسيرم (MktT-17) هي أعلي العزلات إصابة للبطاطس حيث أعطت شدة إصابة 100% بينما كانت أقل عزلة إصابة للبطاطس هي المعزولة من ماء الري بمنطقة كوم حمادة بالبحيرة وغير المطابقة لبكتيريا رالستونيا سولانسيرم (BhhaWa-53) عند نفس الفترة من التحضين. كانت باقي العزلات المختبرة أيضا قادرة علي إصابة البطاطس وسببت شدة إصابة تراوحت بين بين الأقل من 98.4%- الأعلي من 28%. كانت كل الأشكال غير المطابقة لبكتيريا رالستونيا سولانسيرم أقل في إصابتها للبطاطس من الأشكال المطابقة والتي كانت أكثر مرضية في هذا الشأن. كما كانت أيضا كل الـ 66عزلة المطابق منها وغير المطابق لبكتيريا رالستونيا سولانسيرم أيضا ممرضة للطماطم صنف بونتو ومسببة لأعراض الذبول البكتيري والتي وصلت شدة الإصابة به 100% وكان الإختلاف فقط في فترة التحضين لكل عزلة حتي تصل شدة الإصابة 100%. كانت كل العزلات المطابقة لبكتيريا رالستونيا سولانسيرم هي الأكثر مرضية والأسرع في إحداث العدوي علي الطماطم صنف بونتو ، وقد سجلت العزلة المعزولة من درنات بطاطس من منطقة الكوادي بالمنوفية والمطابقة لبكتيريا رالستونيا سولانسيرم (MktT-17) أعلي شدة مرضية بنسبة 100% بعد 4 يوم من التحضين بينما كانت أقل العزلات مرضية هي العزلة المعزولة من الموز كأحد المحاصيل الشائعة في منطقة زراعة البطاطس بمنطقة ام صابر بالمنوفية والمطابقة لبكتيريا رالستونيا سولانسيرم (MmtOB-47) والتي سجلت 100% شدة إصابة عند 10 يوم فترة تحضين. فضلا عن ذلك فقد كان الشكل غير المطابق لبكتيريا رالستونيا سولانسيرم المعزولة من التربة بمنطقة كفر الزيات بالغربية (GhzaS-55) هي الأسرع في إحداث الذبول علي الطماطم صنف بونتو بنسبة 100% شدة إصابة عند 9 يوم فترة تحضين بينما كانت أبطأ العزلات غير المطابقة هي عزلة الطماطم بمنطقة كفر الزيات بمحافظة الغربية (GHzaOT-64) وعزلة التربة من منطقة وردان بالجيزة (GdaS-62) وعزلة التربة من منطقة كوم حمادة بالبحيرة (BhhaS-56) حيث أعطي جميعهم شدة إصابة 100% علي الطماطم صنف بونتو عند 15 يوم فترة التحضين.