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Detection of *Ralstonia solanacearum* phylotype II, sequevar 1 in seasonal weed plants associated with potato cultivations in Egypt

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Abstract

Weed plants were randomly sampled from different potato fields in some Egyptian villages and were examined for the presence of *Ralstonia solanacearum*. A total of (1609) plants were sampled over two year's period (2017/2018 and 2018/2019). These weeds were found belonging to almost (50) species affiliated to (20) families associated more or less with the potato fields near Al-Kalubeiah, El-Behira, El-Giza, El-Gharbeya, El-Ismailia and El-Menofiya governorates at different village (Digwa, El-Saadany, Al-Rifai, Kafr-Yaqoub, Abu Sawyer and Talia, respectively). The pathogen was successfully recovered from the crown area of 272 plant weeds belonging to 25 plant species of 13 families. The weeds in concern, 70 days after planting potato, were found latently infected and mostly symptomless at the time of sampling. The rates of positive isolation from these weeds were generally low, and account for 16.9%. All isolates of *R. solanacearum* from weeds were confirmed as phylotype II, sequevar 1 (race 3, biovar 2) via PCR. Data revealed that, the highest infection percentage of weed plants were shown from El-Gharbeya and Al-Kalubeiah, governorates (44.7 and 31.7 % respectively) followed by El-Behira governorate (16.1%), whereas the lowest percentage (11.5%) was observed in El-Menofiya governorate. On the other hand, both El-Giza and El-Ismailia governorates showed almost similar percentage of infected weeds (12.7 and 13.9% respectively). The winter annual weeds were the most affected weeds and included 11 different species of weeds (e.g. *Amaranthus ascendens* Lois, *Amaranthus cruentus*, *Chenopodium album* and *Chenopodium mural*) followed by the summer annual weeds that included 7 different species (e.g. *Portulaca oleracea* L. and *Solanum nigrum* L.). While the perennial weeds included 4 species (e.g. *Convolvulus arvensis* and *Dicanthium annulatum*), the biennial, however, weeds included only two species of infected weeds (*Beta vulgaris* L. and *Centaurea calcitrapa* L.). The results denoting that symptomless weeds might be a serious masked source of latent infection for either weeds or exported potato crop and it may be considered as an attractive overwintering hosts of *R. solanacearum*.

Keywords: potato bacterial wilt, immunofluorescence antibody stain, real-time PCR, phylotype analysis, seasonal weed flora.

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1. Introduction

Potato Bacterial wilt (syn. brown rot) caused by *Ralstonia solanacearum* phylotype II (sequevar 1) (previously *Pseudomonas solanacearum*) is one of the most serious plant diseases. It was possibly first reported in Egypt by Briton-Jones (1925) in El-Gemmeiza farm, El-Gharbeya governorate based on symptomology only. Although the first introduction of the disease is not well documented, it has been assumed to coincide with the mass importation of potatoes at the time of Mohamed Ali Pasha (1805 ac), the Ottoman Viceroy also, the French invasion of Egypt by Napoleon Bonaparte (1798 ac) might be another possible mean of introduction from Europe (Farag, 2000). Potato brown rot is being described as a quarantine disease that means no tolerance is allowed (zero tolerance) for any consignment, during export (EFSA Panel on Plant Health, 2019; Anonymous, 2000). *Ralstonia solanacearum* invades intercellular elements of roots where it multiplies before invading xylem vessels and producing exopolysaccharide (EPS), leading to wilt of the infected plant. The vascular element classified the pathogen as causing wilt of potato plants and rotting of tubers and may be described as sore eye or jammy eye (visual symptoms), however it may survive latently in the plants without causing any symptoms (Prior et al., 1998). The pathogen has a wide range of economically important crops including different weeds. It is literally described as vascular disease for more than 450 plant species belonging to 54 different botanical families (Allen, 2005). Many infected plant species do not show symptoms after infection and therefore escape attention as hosts, enabling the pathogen to persist though rotations with non-host crops. Granada and Sequeira (1983) reported that the bacterium of *R. solanacearum* can infect the roots of different plants considered as non-hosts but it does not survive for long periods in vegetation-free soil. The survival of this bacterium can be by infecting susceptible plants or by colonizing the rhizospheres of non-host plants. The bacterium survived for only few weeks in artificially inoculated soils (Balabel et al., 2005; Granada & Sequeira, 1983). However, under field conditions the pathogen survived in the absence of potato for long periods (Pradhanang, 1998a; 1998b). Previous studies have shown that *R. solanacearum* could be infect solanaceous and certain non solanaceous weeds such as *Solanum dulcamara*, *Tropaeolum majus* (Olsson, 1976a; 1976b), *S. cinereum* (Graham & Lloyd, 1978), *Portulaca oleracea*, *Tagetes* sp., *Ipomoea* sp. (Zambrano, 1990), *Urtica dioica* (Van-Elsas, et al., 2001), *Solanum nigrum* and *Rumex* sp. (Balabel et al., 2005). All these weeds may not show wilt symptoms and, thus, were not previously considered as hosts. This study was initiated to identify different weeds that could act as an occasional natural host to *R. solanacearum* race 3 to explain extended natural survival of *R. solanacearum* and to explore the role of weeds in the epidemiology of *R. solanacearum* phylotype II, sequevar 1 (race 3 biovar 2).

2. Materials and methods

2.1 Sampling area unit(s)

All weed flora raised in potato fields, at locations in concern 70 days of planting potato in winter and summer plantation were gently uprooted and subject to botanical identification as well as *R. solanacearum* detection. Sampling was

made in term of uproding all weeds developed per one square meter (Kirat area) and three replicated areas per each feddan (Acre) were considered.

2.2 Survey and sample collection

Samples of different weeds developing seasonally in different old traditional potato villages such as Digwa, El-Saadany, Al- Rifai, Kafr -Yaqoub, Abu Sawyer and Talia at Al-Kalubeiah, El-Behira, El-Giza, El-Gharbeya, El-Ismailia and El-Menofiya governorates respectively, were collected during 2017-2018 and 2018-2019 growing season and were identified according to Zaki (1991).

2.3 Isolation of *R. solanacearum* from weeds

Isolation of *R. solanacearum* was carried out from crown area of the weed stems, as described by Pradhanang et al. (2000). Stems were washed thoroughly with tap water and surface disinfected by flaming. Thin sections were made under aseptic conditions and macerated in 1 ml sterile phosphate buffer (0.01 M) in small sterile plastic bags then allowed to stand for 30 min. The supernatant was plated (0.1ml/plate) on modified Semi Selective Medium of South Africa (SMSA) (Elphinstone et al., 1996). Incubation was made at 28°C for 72 hour. A single typical phenotype colony (reddish, irregular and fluidal) from each sample was selected for further work. All isolates were maintained for long-term duration as a suspensions in sterile tap water and were revived by plating on tetrazolium chloride (TTC) medium (Kelman, 1954), when desired. A total of 75 *R. solanacearum* isolates taken from various 25 plant species and 6 governorates were selected as a representative population. These subcultures were used for identification and DNA extraction.

2.4 Identification of *R. solanacearum* isolates

2.4.1 Immunofluorescence antibody stain (IFAS) test

Typical colonies of the selected isolates were selected and propagated on nutrient agar medium for 48 hours, colony-morphology determination was confirmed by a serological test (IFAS), immunofluorescent antibody staining (Janse, 1988). The polyclonal antibodies (cat. No. 07356) manufactured by Lowe Biochemica GmbH, Germany and produced in goats against *R. solanacearum*, race 3 biotype II, was used while, the anti-rabbit anti-goat (RAG/Ig (H+L) (FITC) antiserum (cat. No. 07200) manufactured by Nordic Immunological Laboratories, Nether-lands was used as a conjugate.

2.4.2 Real-Time PCR (Taq-Man) assay

Identification of *R. solanacearum* via qPCR was performed on the selected isolates according to Weller et al. (2000) by using the apparatus of Applied Biosystems 7500. The reaction mixture consisted of 12.5 µl of master mix, 1 µl of primer forward, 1 µl of primer reverse, 1 µl of probe and 7 µl of water and 2.5 µl of nucleic acid extract. The following program conditions was used: (1) 2 min. at 50 C°, (2) 10 min. at 95 C°, (3) followed by 40 two-step cycles of 10 sec at 95 C° then 1 min. at 60 C°. The sequence of primers and probe used is

shown in Table (1) and were provided by OPRON, USA. Every run included controls, DNA extraction (69/20) provided by Potato Brown Rot Project as a positive control and sterile pure water instead of bacterial suspension as a negative control.

2.5 Differentiation of *R. solanacearum* isolates into biovar(s) and race(s)

2.5.1 Biovar determination

Selected isolates (75) were checked for the ability to oxidize three disaccharides (cellobiose, lactose, maltose) and three hexose alcohols (dulcitol, mannitol, sorbitol) to determine biovar of *R. solanacearum* as described by Hayward (1964).

2.5.2 Race determination

Analyses of selected isolates were performed by using the Opina primers 759/760 as internal markers specific for the *R. solanacearum* strains and a set of four phylotype - specific forward primers with a unique and conserved reverse primer targeted in the 16S-23S Intergentic Spacer region (Opina et al., 1997) that allows discrimination between different races. Information of the primers are presented in Table (2). The reaction mixture prepared by adding: 12.5 µl of ready master mix, 1 µl from each primer, 7.5 µl of water and 2 µl of nucleic acid extract. The following cycling program was used in a thermal cycler (Biometra T personal): (1) 96°C for 5 min. (2) then cycled through 30 cycles of 94°C for 15s, 59°C for 30s and 72°C for 30s, (3) followed by a final extension period of 10 min. at 72°C. 13 µl aliquot of each amplified PCR products was subjected to electrophoresis on 2 % (w/v) agarose gels, stained with ethidium bromide (0.5% µgL-1) and imaged (Sagar et al., 2014).

Table 1: Characteristics of primers and Taq-Man probe used to detect *R. solanacearum* by Real-time PCR.

Primer or probe	Sequence(5'→3')	Length	Dye
RS-I-F	GCA TGC CTT ACA CAT GCA AGTC	22	
RS-II-R	GGC ACG TTC CGA TGT ATT ACT CA	23	
RS-P	AGC TTG CTA CCT GCC GGC GAG TG	23	FAM

Table 2: Bases sequence(s) of used primers and their length(s) for phylotype analysis of *R. solanacearum* by Multiplex- PCR.

Primer	Sequence (5'→3')	Length
759	GTC GCC GTC AAC TCA CTT TCC	21
760	GTC GCC GTC AGC AAT GCG GAA TCG	24
Nmult:21:1F	CGT TGA TGA GGC GCG CAA TTT	21
Nmult:21:2F	AAG TTA TGG ACG GTG GAA GTC	21
Nmult:23:AF	ATT ACS AGA GCA ATC GAA AGA TT	23
Nmult:22:InF	ATT GCC AAG ACG AGA GAA GTA	21
Nmult:22:RR	TCG CTT GAC CCT ATA ACG AGT A	22

2.6 Pathogenic potential of selected isolates

Selected isolates (75) were tested for pathogenic potential(s) via inoculation into young tomato (*Solanum lycopersicum* cv. Pinto) seedlings with a suspension of a 48 hour nutrient agar culture, 10^6 cells ml^{-1} in sterile water (Janse, 1988). Injection was made at the

leaf axis by a needle laden with the bacterial growth of the pathogen. Control treatments were prepared by applying few drops of sterile water instead of bacteria. The inoculated plants were covered with polyethylene bags for one day, kept at 30°C, then bags were removed and pots were irrigated as required and examined for wilting symptoms after bench incubation.

3. Results

3.1 Isolation of *R. solanacearum* from different weeds

Using SMSA medium, seventy five isolates were selected by the characteristics previously reported. They selected from different villages (21 isolates from El-Saadany, 15 isolates from Al- Rifai, 15 isolates from Kafr-Yaqoub, 8 isolates from Abu Sawyer, 8 isolates from Digwa and 8 isolates from Talia). Selected colonies were irregular, reddish, and fluidal white with red center (Figure 1). Out of (1609) samples collected from different weeds only (272) were found infected, the rates of successful isolation from these weeds were generally low, and account for 16.9%. Also, Table (3) shows that, the highest infection percentage of weed plants were shown from El-Gharbeya and Al-Kalubeiah governorates (44.7 and 31.7 % respectively) followed by El-Behira governorate (16.1%), whereas the lowest percentage (11.5%) was observed in El- Menofiya governorate. On the other hand, both El-Giza and El-Ismailia governorates showed almost similar percentage of infected weeds (12.7 and 13.9% respectively).

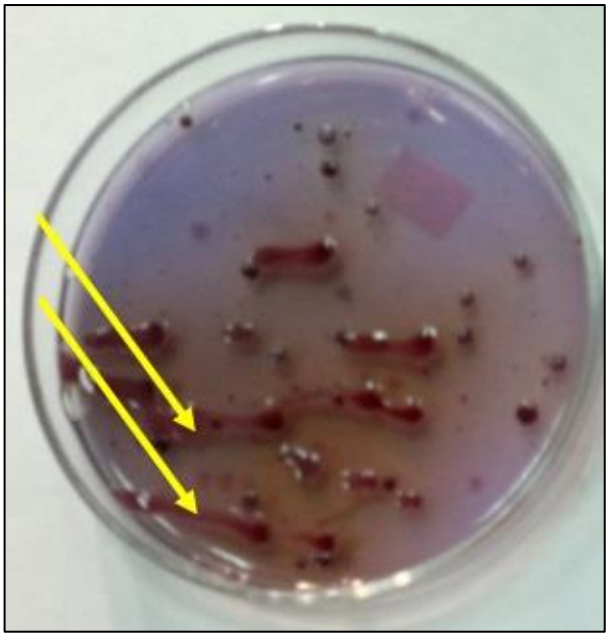

Figure 1: Typical colony with milky white and fluidal central blood red color of *R. solanacearum* on modified Semi Selective Medium of South Africa.

Table 3: Detection of *R. solanacearum* in naturally growing weed species during two growing seasons (2017-2018 and 2018-2019) in plots with potato crop in the six governorates of Egypt.

Governorate (Village)	Total no. of tested plants	Number of healthy plants	Frequency of detection of *R. solanacearum* in stem base	Infection (%)*
Al-Kalubeiah (Digwa)	63	43	20/63	31.7
El-Behira (El-Saadany)	762	639	123/762	16.1
El-Giza (Al-Rifai)	332	290	42/332	12.7
El-Gharbeya (Kafr-Yaqoub)	94	52	42/94	44.7
El-Ismailia (Abu Sawyer)	158	136	22/158	13.9
El- Menofiya (Talia)	200	177	23/200	11.5
Total	1609	1337	272	

* infection % $= \frac{\text{No.of infected plants}}{\text{Total no.of plants}} \times 100$

Data in Table (4) showed that these weeds were belonged to twenty five species affiliated to thirteen families marking the potato fields. The results obtained indicate that, according to the (272) number of infected weeds may be divided into three groups according to their predominance. The first group included species with large numerical numbers such as: *Chenopodium album* L., *Cichorium pamilum, Malva parviflora* L., *Dactyloctenium aegyptium* L., *Cynodon dactylon* L., *Amaranthus ascendens* Lois *and Portulaca oleracea* L. The second group included the infected weeds in medium numbers as: *Brassica nigra* L., *Convolvulus arvensis, Polypogon monspeliensis, Cyperus rotundus, Rumex dentatus* and *Beta vulgaris*. The third group included species of poorly dominated weeds such: *Amaranthus cruentus* L., *Arachis hypogaea, Chenopodium mural* L., *Centaurea calcitrapa* L., *Cyperus difformis* L., *Conyza aegyptiaca* L., *Dicanthium annulatum, Medicago polymorpha* L., *Sonchus oleraceus* L., *Solanum nigrum* L., *Sisymbrium irio* L. and *Urtica urens* L. The results revealed that, the winter annual weeds were the most affected weeds followed by the summer annual weeds while the perennial and biennial weeds included almost the same number of infested weeds 4 and 3, respectively.

3.2 Identification and characterization of *R. solanacearum* isolates

3.2.1 Immunofluorescence antibody stain (IFAS) test

Immunofluorescence antibody stain (IFAS) test was carried out on selected colonies to confirm identity. The cells showed short rod morphology stained evenly as bright green fluorescent (Figure 2).

3.2.2 Real-Time PCR (Taq-Man) assay

Real-time PCR is a sensitive test for detection of low concentrations of *R. solanacearum* and is being considered a confirmatory test in the detection work. The RS primers and probe were employed to detect all biovars and races of *R. solanacearum*. Positive results were noticed with all tested isolates indicating that the 75 isolates were *R. solanacearum*.

Table 4: Common, scientific names and families for different weeds collected randomly from different governorates.

Governorate (Village)	Common name	Scientific name	Family	Growing season	No. of positive plants	Group*
Al-Kalubeiah((Digwa)	Annual sowthistle	*Sonchus oleraceus L.*	Asteraceae	Annual winter	5	3
	Fleabane	*Conyza aegyptiaca L.*	Asteraceae	Annual summer	4	3
	Forssk	*Dicanthium annulatum*	Poaceae	Perennial	2	3
	London rocket	*Sisymbrium irio L.*	Brassicaceae	Annual winter	2	3
	Peanut	*Arachis hypogaea*	Fabaceae	Annual summer	7	3
El-Behira (El-Saadany)	Livid amaranth	*Amaranthus ascendens* Lois	Amaranthaceae	Annual winter	20	1
	Common lambsquarters	*Chenopodium album L.*	Chenopodiaceae	Annual winter	34	1
	Goosefoot	*Chenopodium mural L.*	Chenopodiaceae	Annual winter	4	3
	Small flower umbrella plant	*Cyperus difformis L.*	Cyperaceae	Annual summer	3	3
	Cheese weed (Little Mallow)	*Malva parviflora L.*	Malvaceae	Annual winter	25	1
	Burclover	*Medicago polymorpha L.*	Fabaceae	Annual winter	2	3
	Rabbitfoot grass	*Polypogon monspeliensis L.*	Poaceae	Annual summer	12	2
	Purple nutsedge	*Cyperus rotundus L.*	Cyperaceae	Perennial	10	2
	Black nightshade	*Solanum nigrum L.*	Solanaceae	Annual summer	3	3
	Wild beet	*Beta vulgaris L.*	Amaranthaceae	Annual or biennial	10	2
El-Giza (Al-Rifai)	Crowfoot grass	*Dactyloctenium aegyptium L.*	Poaceae	Annual summer	21	1
	Bermuda grass	*Cynodon dactylon L.*	Poaceae	Perennial	21	1
El-Gharbeya (Kafr-Yaqoub)	Chicory	*Cichorium pamilum*	Asteraceae	Annual winter	25	1
	Common purslane	*Portulaca oleracea L.*	Portulacaceae	Annual summer	17	1
El-Ismailia (Abu Sawyer)	Dock	*Rumex dentatus L.*	Polygonaceae	Annual winter	10	2
	Purple starthistle	*Centaurea calcitrapa L.*	Asteraceae	Biennial	2	3
	Black mustard	*Brassica nigra L.*	Brassicaceae	Annual herb	10	2
El- Menofiya (Talia)	Pigweed	*Amaranthus cruentus* L.	Amaranthaceae	Annual winter	3	3
	Field bindweed	*Convolvulus arvensis* L.	Convolvulaceae	Perennial	11	2
	Burning nettle	*Urtica urens L.*	Urticaceae	Annual winter	9	3

*The isolates were classified according to their predominance into three groups: 1 = greater than or equal 15, 2 = 10 to 14, 3 = 1 to 9.

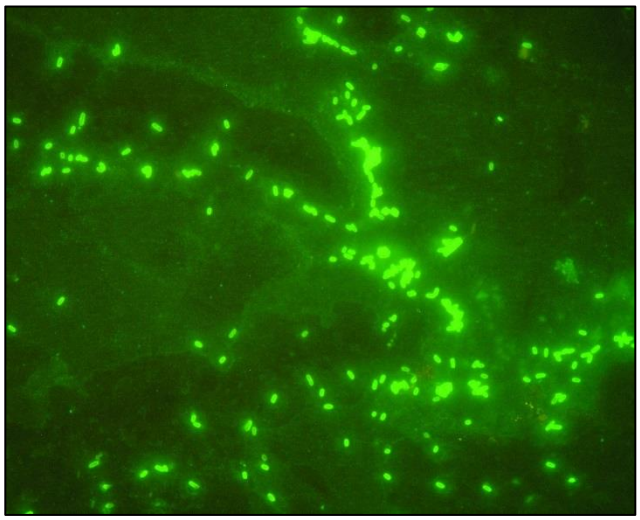

Figure 2: Cell morphology of *R. solanacearum* in the serological immunofluorescent antibody staining (IFAS) test.

3.3 Differentiation of *R. solanacearum* isolates into biovar(s) and race(s)

3.3.1 Biovar determination

The biovars determination was based on the ability of isolates to produce acids from hexose and alcohol sugars. The studied Seventy five isolates were able to produce acids from lactose, maltose, and cellibiose. All isolates, however, were unable to produce acids from sorbitol, mannitol, and dulcitol denoting that, these seventy five isolates were assigned to biovar 2 which is the only race in Egypt described as race 3, biovar II, in previous work (Table 5).

Table 5: Biovar determination of the selected *R. solanacearum* isolates from different villages in Egypt.

Governorate (Village)	Isolate's number	Utilization of (Acid without gas)					
		Maltose	Lactose	Cellobiose	Mannitol	Sorbitol	Dulcitol
El-Behira (El-Saadany)	1	+	+	+	-	-	-
	2	+	+	+	-	-	-
	3	+	+	+	-	-	-
	4	+	+	+	-	-	-
	5	+	+	+	-	-	-
	6	+	+	+	-	-	-
	7	+	+	+	-	-	-
	8	+	+	+	-	-	-
	9	+	+	+	-	-	-
	10	+	+	+	-	-	-
	11	+	+	+	-	-	-
	12	+	+	+	-	-	-
	13	+	+	+	-	-	-
	14	+	+	+	-	-	-
	15	+	+	+	-	-	-
	16	+	+	+	-	-	-
	17	+	+	+	-	-	-
	18	+	+	+	-	-	-
	19	+	+	+	-	-	-
	20	+	+	+	-	-	-
	21	+	+	+	-	-	-
El-Giza (Al-Rifai)	22	+	+	+	-	-	-
	23	+	+	+	-	-	-
	24	+	+	+	-	-	-
	25	+	+	+	-	-	-
	26	+	+	+	-	-	-
	27	+	+	+	-	-	-
	28	+	+	+	-	-	-
	29	+	+	+	-	-	-
	30	+	+	+	-	-	-
	31	+	+	+	-	-	-
	32	+	+	+	-	-	-
	33	+	+	+	-	-	-
	34	+	+	+	-	-	-
	35	+	+	+	-	-	-
	36	+	+	+	-	-	-
El-Gharbeya (Kafr-Yaqoub)	37	+	+	+	-	-	-
	38	+	+	+	-	-	-
	39	+	+	+	-	-	-
	40	+	+	+	-	-	-
	41	+	+	+	-	-	-
	42	+	+	+	-	-	-
	43	+	+	+	-	-	-
	44	+	+	+	-	-	-
	45	+	+	+	-	-	-
	46	+	+	+	-	-	-
	47	+	+	+	-	-	-
	48	+	+	+	-	-	-
	49	+	+	+	-	-	-
	50	+	+	+	-	-	-
	51	+	+	+	-	-	-
El-Ismailia (Abu Sawyer)	52	+	+	+	-	-	-
	53	+	+	+	-	-	-
	54	+	+	+	-	-	-
	55	+	+	+	-	-	-
	56	+	+	+	-	-	-
	57	+	+	+	-	-	-
	58	+	+	+	-	-	-
	59	+	+	+	-	-	-
Al-Kalubeiah (Digwa)	60	+	+	+	-	-	-
	61	+	+	+	-	-	-
	62	+	+	+	-	-	-
	63	+	+	+	-	-	-
	64	+	+	+	-	-	-
	65	+	+	+	-	-	-
	66	+	+	+	-	-	-
	67	+	+	+	-	-	-
El-Menofiya (Talia)	68	+	+	+	-	-	-
	69	+	+	+	-	-	-
	70	+	+	+	-	-	-
	71	+	+	+	-	-	-
	72	+	+	+	-	-	-
	73	+	+	+	-	-	-
	74	+	+	+	-	-	-
	75	+	+	+	-	-	-

3.3.2 Race determination

According to the results of the Pmx-PCR, all selected isolates belonged to the phylotype II sequevar I, as 372- bp amplicon was produced in their reactions (Figure 3).

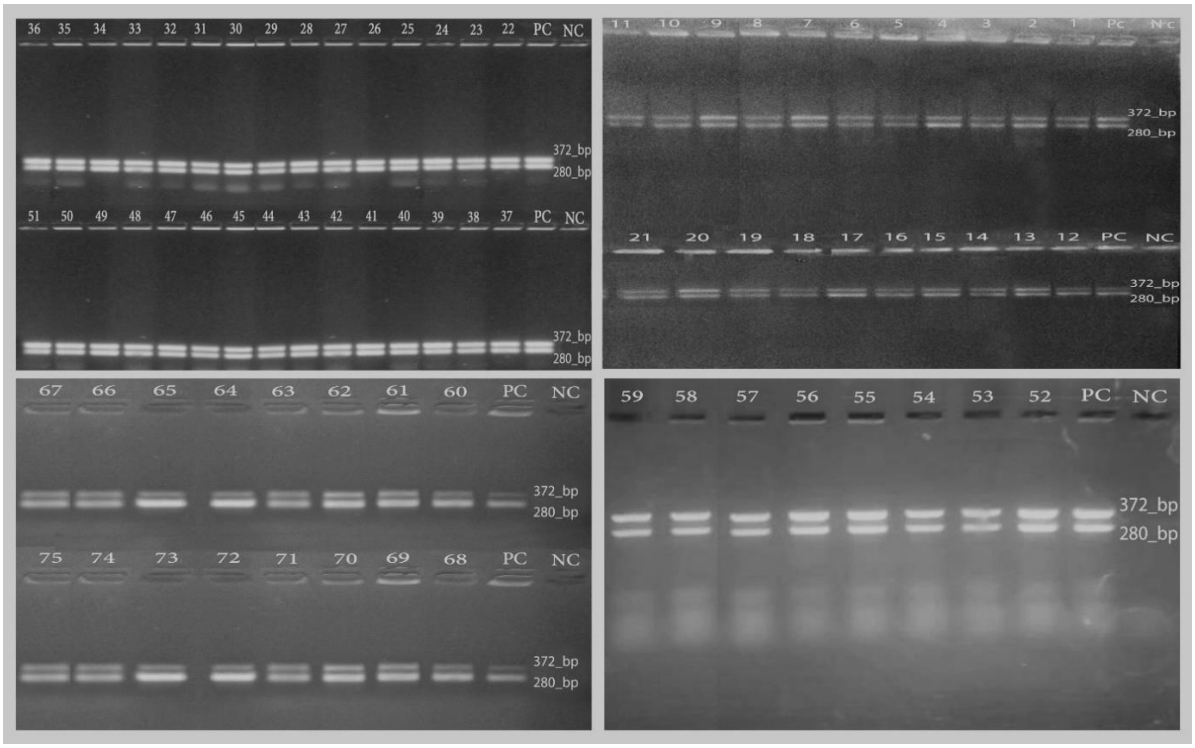

Figure 3: PCR amplification products (280 and 372 bp) to detect *R solanacearum* in weeds from different locations using pmx primers **(**Opina et al., 1997). Lane NC, negative control; lane PC, Positive control; lane 1-75, PCR amplicons derived from 75 separate DNA extracts isolated from different weeds.

3.3.3 Pathogenic potential of representative isolates

The pathogenic potential of randomly selected isolates (75 isolates) was tested for producing wilt to tomato seedlings, 3 days after stem inoculation under greenhouse conditions. The results showed that all tested isolates from different locations were able to wilt tomato seedlings (Figure 4).

Figure 4: Pathogenicity test of *R. solanacearum* isolates from different locations sources on tomato plants.

4. Discussion

Early detection of latent infection with bacterial wilt caused by *R. solanacearum* may be playing an important role to decrease the risk of crop loss. Several detection methods have been developed for *R. solanacearum* such as direct plating on modified (SMSA) medium**,** enzyme-linked immunosorbent assay (ELISA), IFAS, PCR-based methods and bioassay in tomato seedlings (Weller et al., 2000; Elphinstone et al., 1996) and phylotype assignment (Sagar et al., 2014). All selected isolates were collected from the different weeds in this study were identified as *R. solanacearum* using isolation on modified SMSA medium, IFAS test, also real-time PCR assay. On the other hand, the results of biovar determination indicating that all the tested isolates belong to biovar 2 or the so called a member of potato race 3. Moreover, phylotype specific multiplex (Pmx)- PCR revealed that all seventy five isolates of *R. solanacearum* belonged to phylotype II as a 372-bp amplicon was observed for all the tested isolates after electrophoresis (Agarose gel 2% w/v). These results indicating that, the race 3, biovar 2 (phylotype II, sequevar I) is dominant in Egypt, the same results were observed by other searchers (Hanafy et al., 2018; Hassan, et al., 2017; Mikhail et al., 2017). In this study, **t**he pathogenicity tests showed that all the tested isolates were virulent to tomato plants using stem puncture inoculation. The presence of *R. solanacearum* can be detected by SMSA medium at 10^3 CFU /ml (Mikhail et al., 2016). This concentration was adequate for the population analysis of this bacterium in the weed samples. Also, IFAS test is rapid and inexpensive method but lack in sensitivity besides giving false positive results due to cross-reactions with other bacteria (Balabel, 2014). Other methods were able to detect a lower concentration of the bacterium in low densities. Methods such as real time-PCR may be very successful in detecting the bacterium's presence, but preparation, time and money would be needed for this technique. Although PCR as a sensitive and precise detection tool has great potential, it has not been widely used for field samples. Meanwhile, PCR detection results were recorded from plant and soil samples (Elphinstone & Stanford, 1998). Inhibition of the enzymatic PCR reaction by different compounds present in plant and soil samples may be attributable to inconsistent findings (Wilson, 1997; Picard et al., 1992). Also, one of the disadvantages of the PCR method is to obtain false negative results in some cases due to the presence of some inhibitors (Farag & Balabel, 2014; Farag et al., 2010). In this study, randomized weed samples belong to (13) families with (23) genera and (25) species were collected from different potato fields during two successive growing seasons (2017-2018 and 2018-2019) of winter and summer plantation. Seventy five isolates were selected from these weeds and identified as *R. solanacearum*. These results indicate the important role of weeds in overwintering and extended survival of the pathogen in soil. In this point several researchers have clarified the role of the plant weeds. Tusiime et

al. (1998) reported that there are a large number of latently infected non-solanaceous weeds in highland Uganda such as *Amaranthus* spp., *Bidens pilosa*, *Galinsoga perviflora*, *Oxalis latifolia*, *Spergula arvensis, Rumex abyssinicum*, *Tagetes minuta*, and *Stellaria sennii*. Dittapongpitch and Surat (2003) found that weed samples belong to (13) families including (17) genera and (18) species were infected with *R. solanacearum*, among these families: Amaranthaceae, Asteraceae, Chenopodiaceae, Cyperaceae, Portulacaceae and Solanaceae. Also, Hamad et al. (2016) revealed that, *Portulaca olracea*, *Solanum nigrum*, *Rumex dentatus*, *Chenopodium album*, *Brassica kaber* and *Beta vulgaris* are considered as hosts for *R. solanacearum.* These results are consistent with the results of this study. Many studies have suggested the relationship between the presence of weeds and the survival of bacterium is due to infecting susceptible plants or by colonizing the rhizospheres of non-host plants. The pathogen able to colonize the root systems of non-host plants including many weeds, without causing any visible symptoms. In this case weed hosts can act as "Sheltered sites" and this way is considered as one of the ways for the survival of bacteria in the absence of the suitable host (Graham et al., 1979) so, controlling potato bacterial wilt is difficult (Hayward, 1986). Latent infection can play an important role in spreading disease. The bacterium can survive for a long time in soils (Tomlinson et al., 2011), infested surface irrigation water and infected weeds (Tomlinson et al., 2009). From these sources the bacterium can spread from infested to healthy fields by soil transfer on machinery, and surface runoff water after irrigation or rainfall. Also, infected semi-aquatic weeds may spread the pathogen through releasing bacterium from roots into irrigation waters (Hong et al., 2008; Elphinstone et al., 1998). There are a large number of weeds that are considered an alternative host for bacteria in areas planted with potatoes, and as a result, the rate of bacterial growth is slow, which leads to these weeds being a constant source of infection (Pradhanang et al., 2000). So, control of weed hosts and volunteer plants may be the most important ways in control of *R. solanacearum*. It is interesting to note that, there is little information in Egypt that has looked at the species of weeds that *R. solanacearum* can enter in the absence of a suitable host. Most of the weeds reported as alternative hosts of the pathogen including the following species: *Brassica nigra*, *Beta vulgaris*, *Chenopodium album*, *Portulaca oleracea*, *Rumex dentatus* and *Solanum nigrum.* However, in this study many species of weeds were reported. So, it is very important to continue surveys to determine new natural hosts and the role they play in disease spread whereas, weeds can act as host reservoirs of infection. Therefore, elimination of weed hosts could be part of an integrated management strategy for the control of potato bacterial wilt. In order to prevent this source of inoculum, potato growers need to take these findings into account and use management strategies. Further investigations would be needed to carry

out the presence of *R. solanacearum* in weeds, water, and soil and with the potato disease incidence to verify the importance of various inoculum sources.

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