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ORIGINAL RESEARCH ARTICLE

Current status of bacterial wilt (*Ralstonia solanacearum*) disease in major tomato (*Solanum lycopersicum* L.) growing areas in Egypt

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ABSTRACT

A survey was carried out to determine the current status of bacterial wilt (*Ralstonia solanacearum*) disease in major tomato growing areas in Egypt incidence and distribution. In the 2014/2015 cropping season in major tomato growing areas of Upper and Lower Egypt. In upper highest incidence of bacterial wilt occurred in Luxour Governorate (10.29%) and lowest in Al- Giza Governorate (9.41%). Wilt severity was recorded (3.44%) in Luxour Governorate and the lowest (2.60%) was recorded in Sohag Governorate. In Lower Egypt Governorates, the highest disease incidence was recorded in Al- Behiera (15.28%) followed by Al-Daqahlia, (13.23), Al- Gharbia (12.41), Kafr Al- Shikh(12.70) and Al- Sharqia (12.14) Governorates. The lowest disease incidence recorded in Al- Suiz Governorate (10.27%), followed by 11.28, 11.58 and 11.67% in Al- Qalioubia, Al- Ismailia and Al- Minoufia Governorates respectively. The highest bacterial wilt severity was recorded in Al- Behiera Governorate, fields (5.70%), followed by Al- Gharbia (4.60%), while the lowest bacterial wilt severity was recorded in Al- Suiz Governorate (2.63%), followed by 3.30, in Al- Qalioubia Governorate. All the collected plants were subjected to PCR to detect the infected samples. The expected 288 bp amplicon was detected in bacterial wilt infected samples from above mentioned governorates and diseases percentage and severity were calculated.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.), is an economically important crop which belongs to the Family Solanaceae. Leading tomato producing countries include China, USA, India, Turkey, Egypt, Italy, Iran, Spain, Brazil, and Mexico (Desneux *et al.*, 2011). Bacterial wilt of tomato caused by *R. solanacearum*, causes a considerable amount of damage to tomatoes and many other crops in tropical, subtropical and warm temperature regions of the world and limits the produc-tion of many crops e.g. potato, tomato, eggplant and pepper (Williamson *et al.*, 2002; Ji *et al.*, 2005). *Ralstonia solanacearum*, is widespread in tropical and sub-tropical regions. Its harmfulness, wide host range, persistence and huge genome plasticity have made it one of the world's most important phytopathogenic bacteria and one of the most inten-sively studied. Bacterial wilt caused by *R. solanacearum* is one of the major diseases of tomato and the disease causes concern for tomato production because it can drastically reduce tomato up to 90%. Bacterial wilt, caused by members of the *Ralstonia solanacearum* species complex, is a key emergent disease in non-tropical regions of the world. It can infect over 200 plant species representing over 50 botanical families (Abo-Elyousr and Asran, 2009; Milling *et al.*, 2011; Fujiwara *et al.*, 2012; Aslam *et al.*, 2017). Kim *et al.* (2016) reported that, Bacterial wilt of tomatoes caused by *R. solanacearum* is a devastating disease that limits the production of tomato in Korea. *R. solanacearum*, the causal agent of bacterial wilt disease, is considered one of the most destructive bacterial pathogens due to its lethality, unusually wide host range, persistence and broad geographical distribution Wei *et al.* (2018).

The objective of this survey, therefore, was to determine the current distribution and relative importance of bacterial wilt of tomato in Egypt.

MATERIALS AND METHODS

Surveying and sampling

A survey was carried out to know the status of bacterial wilt of tomato in Egypt in terms of its incidence and severity in seventeen governorates viz. Al- Giza, Al- Fayoum, Beni Seuief, Al- Minia, Assiut, Sohag, Qena, Luxor, Al- Qalioubia, Al- Minoufia, Al- Gharbia, Al- Daqahlia, Kafr Al- Shikh, Al- Sharqia, Al- Ismailia, Al- Suiz and Al- Behiera. Field surveys were conducted in the third to fourth week of March 2014/2015 and the last week of November to second week of December in 2015/2016 cropping seasons. At least three counties in each governorate and three to five locations in each counties and three to five farmer's fields from each location were surveyed to record the bacterial wilt incidence and severity. The plants were inspected at the nursery stage, after transplanting, at flowering stage and at the fruiting stage. The total number of healthy and wilted plants was counted in a 1 m^2 area and percentage wilt incidence was recorded. Information on the cultivars grown in the area and related field history was gathered from the farmers.

Field symptomology

For proper key diagnostic identification of *R. solanacearum* in the field and to distinguish bacterial wilt from vascular wilts caused by fungal pathogens, bacterial wilt symptoms was identified by visual observation of typical bacterial wilt disease symptoms such as wilting, vascular discoloration, bacterial streaming in glass of water and browning of the vascular bundles of the tuber. Milky white strands containing bacteria and extracellular polysaccharide was oozed out from the cut ends of the xylem.

Disease samples

The plant and soil samples from wilt affected tomato and potato plants showing typical symptoms of bacterial wilt were collected from farmer's field. At least 10 samples of the diseased plants were collected from each of the surveyed district. The diseased samples were brought to the laboratory and subjected aseptically for detection and confirmation of *Ralstonia solanacearum*.

Assessment of disease incidence and severity

The status of bacterial wilt of tomato and potato was surveyed in terms of its incidence and severity. Data on wilt incidence were recorded in at least three locations from three to five farmer's fields for each district. Then the per cent wilt incidence was calculated by the following formula:

$$\% \text{ Wilt incidence } = \frac{\text{Number of wilted plants in each field}}{\text{Total number of plants in each field}} \text{ X } 100$$

Ten plants were randomly selected from each farmer field from each location to calculate the wilt severity in each district. The severity of bacterial wilt was recorded based on the severity scale as described previously by Horita and Tsuchiya (2001), briefly,

1= No symptom, 2 = Top young leaves wilted, 3 = Two leaves wilted, 4 =Four or more leaves wilted and 5 = Plant dies.

$$\text{Disease severity index (DSI\%)} = \Sigma(pn)100/PN$$

Where's

p= disease severity grade, n= number of plants at the grade, P= the highest disease severity grade, N= number of total test plants.

PCR detection and confirmation of *Ralstonia solanacearu* DNA extraction

DNA was extracted from infected tomato plants using DNeasy plant kit (QIAGEN) following the manufacturer's manual. The DNA concentration was estimated using a biophotometer (Eppendorf), by measuring the absorbance at 260 nm, and adjusted to 25 ng μL^{-1}. DNA was stored at −20 °C. DNA from healthy plants was used as positive control.

PCR detection and confirmation of *R. solanacearum*

The samples were detected by using *R. solanacearum* species complex-specific primer pairs 759/760 (10 µM each per reaction; Opina *et al.* (1997). PCR was set up in a total 25 µL reaction comprising 25 µL of Fermentas 2X PCR enzyme mix (Thermofisher Scientific), 5 µL of Q solution (5X QIAGEN) and 25 ng gDNA on a (BIO-RAD) thermal cycler (C1000) with the following cycling conditions: initial denaturation for 5 min at 95 °C; 30 cycles of 30 s at 94 °C, 1 min at 59 °C and 1 min at 72 °C; and final extension of 10 min at 72 °C. The expected 288-bp reference PCR products from the ribosomal DNA specific of *Ralstonia solanacearum* species were revealed by electrophoresis through 1.5% agarose gel in 50X TAE (Tris-acetate-EDTA) buffer stained with ethidium bromide and gels were imaged in GeneSnap (a Syngene Bioimaging System, Syngene) with UV transilluminator at a wavelength of 302 nm and compared with a 1 Kb Plus marker ladder. A positive identification was based on the presence of a 288 bp amplicon.

RESULTS AND DISCUSSION

Incidence and severity of bacterial wilt a total of selected governorates

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops in Egypt. Soil borne diseases including bacterial wilt caused a considerable loss in quantity and quality of tomato yield. An extensive survey was conducted throughout eight hundred ten fields at thirty two Counties of eight governorates. The results of survived tomato cultivars grown under tested fields at all Counties for the percentage of bacterial wilt disease incidence and percentage of disease

severity are shown in Table (1, 2). The percentage of disease incidence and disease severity were calculated during 2014-2015, transplanting season. Our results revealed that tomato cultivars grown under open field conditions are susceptible to different degrees, disease infection with soil borne diseases. Bacterial wilt disease was distributed over the entire tested tomato field where over all averages of disease incidence and disease severity were 9.88 and 3.01%, in Upper Egypt governorates while in Low Egypt governorates were 12.30 and 4.02%, respectively. Bacterial wilt disease of tomato was found in all districts surveyed but not in all fields (Table 1, 2). Common bacterial wilt symptoms observed in the fields were wilting of one side of a leaf and/ or stem, general whole plant wilting and brown discoloration of the vascular system in young stems (Figure 1 a-c). Wilting was the most obvious systemic symptom in the field. In Upper Egypt Governorates, Luxour Governorate tested fields recorded the highest percentage of bacterial wilt disease being 10.29 followed by Assuit and Al- Minia Governorates 10.21 and 10.10% respectively, while Al- Giza Governorate tested field recorded the lowest significantly percentage of disease incidence being 9.41% followed by Sohag and Al- Fayoum Governorates 9.59 and 9.69%. On the contrary, the highest bacterial wilt severity was recorded in Luxour Governorate (3.44%), followed by Assuit and Al- Minia Governorates which were 3.17 and 3.13% respectively, while Sohag Governorate tested field recorded the lowest significantly percentage of disease severity 2.60% followed by Al- Giza and Al- Fayoum Governorates 2.69 and 2.85%, comparing with the other tested fields.

Table 1. Survey of tomato bacterial wilt disease incidence under Giza governorate conditions during 2014-2015 growing season.

Governorate	County	Infection (%)	
		Disease incidence	Disease severity
Giza	Atfeeh	10.03	2.90
	Al-Saff	8.80	2.45
	Al- Ayatt	9.27	2.54
	Al- Bdrasheen	9.56	2.87
Average		9.41	2.69
Al- Fayoum	Tamya	10.04	2.86
	Sennoures	9.44	2.79
	Abshway	9.22	2.69
	Itsa	11.10	3.50
	Yousef Al-Sediq	10.97	3.33
	Al- Fayoum	7.40	1.90
Average		9.69	2.85
Beni Sueif	Ehnasia	9.56	2.96
	Al-Fashn	11.57	4.05
	Al-Wasta	9.82	3.04
	Nasser	9.07	2.38
	Beni Sueif	9.72	2.97
Average		9.95	3.08
Al- Minia	Matai	9.98	2.88
	Samalout	9.30	2.78
	Al- Minia	10.53	3.42
	Al- Edwa	10.58	3.43
Average		10.10	3.13
Assuit	Al- Qousia	9.56	2.83
	Dayrout	10.52	3.31
	Assuit	10.55	3.37
Average		10.21	3.17
Sohag	Akhmim	10.05	2.83
	Gerga	9.20	2.41
	Dar Al- Salam	9.62	2.63
	Tahta	9.49	2.54
Average		9.59	2.60
Qena	Qena	9.14	2.46
	Al- Waqf	9.28	3.09
	Qous	10.03	3.34
	Qeft	10.67	3.45
Average		9.78	3.09
Luxor	Asfoun	9.22	3.11
	Al- Deer	8.55	2.56
	Al- Nemsa	12.70	4.11
	Isna	10.67	3.77
	Al- Ngoaa Qebly	10.33	3.66
Average		10.29	3.44
General average		9.88	3.01

Figure 1 (a-c). *Naturally infected tomato plants showing bacterial wilt symptoms.*

Table 2. Survey of tomato bacterial wilt disease incidence under Low Egypt Governorate conditions during 2014-2015 growing season.

Governorate	County	Infection (%)	
		Disease incidence	Disease severity
	Banha	10.97	2.93
Al- Qalioubia	Toukh	11.55	3.53
	Qalioub	11.32	3.44
Average		11.28	3.30
	Ashmoun	10.71	2.72
	Albagour	10.86	2.76
Al- Minoufia	Quesna	11.68	3.81
	Minouf	11.27	3.46
	Al- Shouhadaa	13.84	4.27
Average		11.67	3.40
	Tanta	13.05	4.71
	Qtour	12.64	4.34
Al- Gharbia	Kafr Al- Zayat	14.44	4.96
	Santah	13.18	4.71
	Zefta	12.41	4.29
Average		13.14	4.60
	Belkas	12.57	4.18
	Dkerns	11.94	4.04
Al- Daqahlia	Al- Mansoura	13.84	4.88
	Meet Ghamr	13.94	4.98
	Sherbin	13.12	4.47
	Talkha	13.96	4.99
Average		13.23	4.59
	Al- Ryad	11.31	3.82
Kafr Al- Sheikh	Qeleen	12.44	4.40
	Biala	12.70	4.42
Average		12.15	4.21
	Belbis	12.84	4.38
Al- Sharqia	Al- Housainiah	11.49	3.64
	Faqous	12.67	4.03
	Abou -Hammad	11.55	3.67
Average		12.14	3.93
	Al-Qsasin	11.33	3.67
	Al- Salhia	11.52	3.84
Al- Ismailia	Aou Ashour	11.83	3.89
	Al- Zaheria	12.56	4.38
	Al- Qarin	11.89	3.97
	Kafr Al -Shiekh Attia	10.33	3.32
Average		11.58	3.85
	Kafr Alnaggar	10.67	2.83
Al- Suiz	Kafr Al- Arab	9.40	2.78
	Genifa	10.54	2.56
	Kabreet	10.45	2.34
Average		10.27	2.63
	Hosh Eisa	14.66	4.65
	Al- Noubaria	15.25	5.33
	Wadi Al-Natroun	16.33	7.76
Al- Behiera	Al-Mahmoudia	14.73	4.88
	Abou Amtamir	15.50	5.67
	Saft Al- Houria	16.30	6.75
	Al- Tawfikia	14.22	4.87
Average		15.28	5.70
General Average		12.30	4.02

The results presented in Table (1) indicate that the highest average of bacterial wilt disease incidence was recorded in Al- Nemsa County (Luxour Governorate) 12.70%, followed by Al - Fashn (Beni Sueif Governorate) 11.57%and Itsa county (Al- Fayoum Governorate)11.10% while the lowest bacterial wilt incidence (8.55%) was recorded in Al- Deer (Luxour Governorate) followed by 8.80 and 9.07% in Al- Saff and Nasser Counties (Al- Giza and Beni Sueif Governorates) (Table 1). Moreover, 10.67% bacterial wilt disease incidence was recorded in Qeft and Isna (Qwna and Luxour Governorates).

In Al- Nemsa County (Luxour Governorate) fields a higher disease severity was observed than all areas 4.11%, followed by Al- Fashn, Isna and Al- Ngoaa Qebly (Beni Sueif and Luxour Governorates) 4.05, 3.77 and 3.66%, while the lowest severity recorded in Al- Fayoum, Nsser and Gerga Counties Which were 1.90, 2.38 and 2.41% respectively. (Table 1). Moreover, these variations of wilt incidence and severity may be attributed due to the diversity of *R. solanacearum* isolates and also due to the variations in soil factors prevailing in different locations surveyed. A noteworthy, all tested field in Lower Egypt Governorates was found to be attacked by bacterial wilt pathogen. The results in Table (2) clearly demonstrated that, the surveyed plants at early stages (3-6 weeks after transplanting) showed root infections expressed at highest records with bacterial wilt infection. The infection gradually increased in severity reaching the highest level at 8-12 weeks. Also, all the surveyed of tomato cultivars under open field were susceptible to bacterial wilt. In Al- Behiera Governorate, fields rcorded a higher disease incidence and severity than all Governorates, the highest disease incidence were 15.28% followed by Al-Daqahlia, Al- Gharbia, Kafr Al- Shikh and Al- Sharqia Governorates which were 13.23, 13.14, 12.15 and 12.14% respectively, besides, the lowest disease incidence recorded in Al- Suiz Governorate (10.27%), followed by 11.28, 11.58 and 11.67% in Al- Qalioubia, Al- Ismailia and Al- Minoufia Governorates respectively. On the other hand, the highest bacterial wilt severity was recorded in Al- Bhira Governorate, fields (5.70%), followed by Al- Gharbia (4.60%), Al-Daqahlia, (4.59%) and Kafr Al- Shiekh , (4.21%). while the lowest bacterial wilt severity was recorded in Al- Suiz Governorate (2.63%), followed by 3.30, 3.40, 3.85 and 3.93% in Al- Qalioubia, Al- Minoufia, Al- Ismailia and Al- Sharqia Governorates respectively. Data also showed that the highest disease infection percentage on tomato were 16.33% in Wadi Al-Natroun County fields followed by Saft Al- Houria, Abou Al-Mtamier Al- Noubaria, Al- Mahmoudia, Housh Eisa, Al-Tawfikia and Kafr Al- Zayat counties which were (16.30, 15.50 ,15.25 and 14.73, 14.66, 14.44 %), followed by (13.96 and 13.94%) in Talkha and Meet Ghamr respectively. While, Al- Shouhadaa and Al- Mansoura counties recorded the same disease incidence (13.84 %), followed by (13.18 %), in Santah, (13.12%) in Sherbien and (13.05%), in Tanta counties. In Kafr Al- Sheikh Attia, Kabreet and Genifacounties recorded the lowest disease incidence (10.33, 10.45 and 10.54%) respectively, followed by Ashmoun, Al- Bagour and Banha (10.71, 10.86 and 10.97%), respectively. On the contrary, the highest bacterial wilt severity was recorded in Wadi El- Natroun, Saft Al- Houria, Abou Al- Mtamier and Al- Noubaria (7.76, 6.75, 5.67 and 5.33%) respectively, followed by 4.99, 4.98, 4.96% in Talkha, Meet Ghamr and Kafr Al- Zayat respectively, in addition Al- Mansoura and Al- Mahmoudia recorded the same severity 4.88%. while the lowest severity recorded in Kabreet, Ganifa, Ashmoun and Albagour (2.34, 2.56, 2,72 and 2.76%), respectively. During survey it was noticed that, a higher disease incidence was found in old fields compared to new fields. Most farmers did not practice crop rotation and often plant seedling from their own nurseries which was found to increase disease incidences. Poor agronomic practices such as farmers may not scout fields, poor field sanitation and flood irrigation were identified as key factors to spread the disease to new fields. The variation between the disease incidence and severity at all the tested fields probably due to one or more of the following factors, their plantation in heavily infested soil or overwatering or poor drainage; overcrowding or poor ventilation of seedlings and excess application of nitrogen; or existing of pathogenic nematodes are present. Interestingly, this investigation on those tested tomato cultivars in all Governorates was unprecedented and the results obtained could fill a gap in such research domain for more investigations in the near and immediate future.

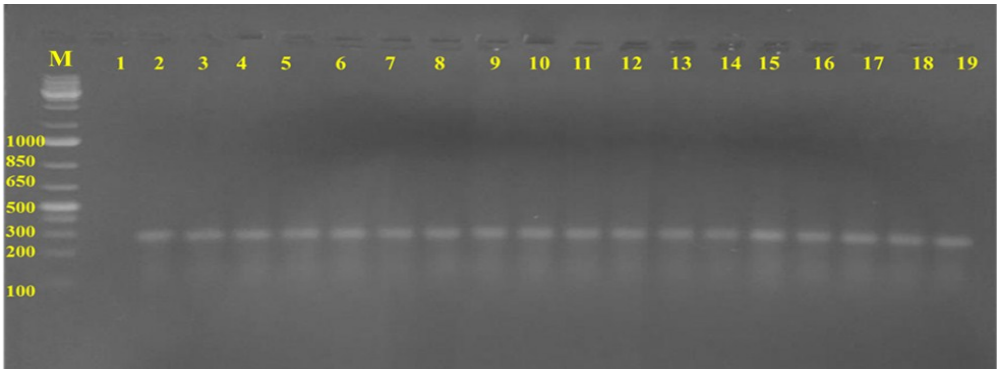

Figure 2 (a). *Single DNA band in the tested plants and the positive control one with very close similarity among them at MW 288 bp.*
Lane 1= Negative control (Healthy plant), 2 = positive control (reference isolate of R. solanacearum identified by the Brown Rot Project, Egypt), lanes, 3= samples of Atfeeh, 4 = samples of Al- Ayatt, 5= samples of Al- Saff, 6= samples of Al- Badrshein, 7= samples of Tmya, 8= samples of Senoures 9= samples of Abshoway, 10= samples of Itsa, 11=samples of Youssef Alsediq, 12= samples of Al- Fayoum, 13= samples of Ehnasia, 13= samples of Al- Fashn, 14= samples of Al- Wasta, 15= samples of Nasser, 16= samples of Beni Suief, 17= samples of Matai, 18= samples of Samalout, 19= samples of Al- Minia.

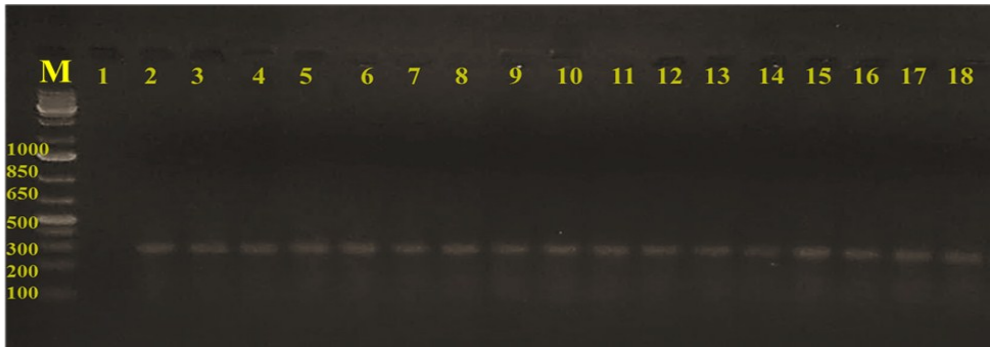

Figure 2 (b). Single DNA band in the tested plants and the positive control one with very close similarity among them at MW 288 bp.
Lane 1= Negative control (Healthy plant), 2 = positive control (reference isolate of R. solanacearum identified by the Brown Rot Project, Egypt), lanes, 3= samples of Al- Edwa, 4 = samples of Al- Qousia, 5= samples of Dayrout, 6= samples of Assuit, 7= samples of Akhmim, 8= samples of Gerga 9= samples of Dar Al- Salam, 10= samples of Tahta, 11=samples of Qena, 12= samples of Al- Waqf, 13= samples of Qous, 13= samples of Qeft, 14= samples of Asfoun, 15= samples of Al- Deer, 16= samples of Al- Nemsa, 17= samples of Isna, 18= samples of Al- Ngouaa Qebly.

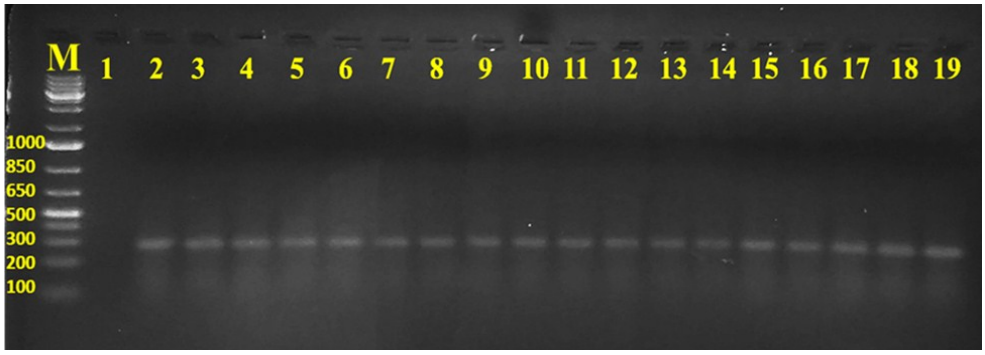

Figure 2 (c). Single DNA band in the tested plants and the positive control one with very close similarity among them at MW 288 bp.
Lane 1= Negative control (Healthy plant), 2 = positive control (reference isolate of R. solanacearum identified by the Brown Rot Project, Egypt), lanes, 3= samples of Banha, 4 = samples of Toukh, 5= samples of Qalioub, 6= samples of Ashmoun, 7= samples of Albagour, 8= samples of Quesna 9= samples of Minouf, 10= samples of Al- Shohadaa, 11=samples of Tanta, 12= samples of Qotour, 13= samples of Kafr Al- Zayat, 13= samples of Santah, 14= samples of Zefta, 15= samples of Belkas, 16= samples of Dekernes, 17= samples of Al- Mansoura, 18= samples of Meet Ghamr. 19= samples of Sherbin.

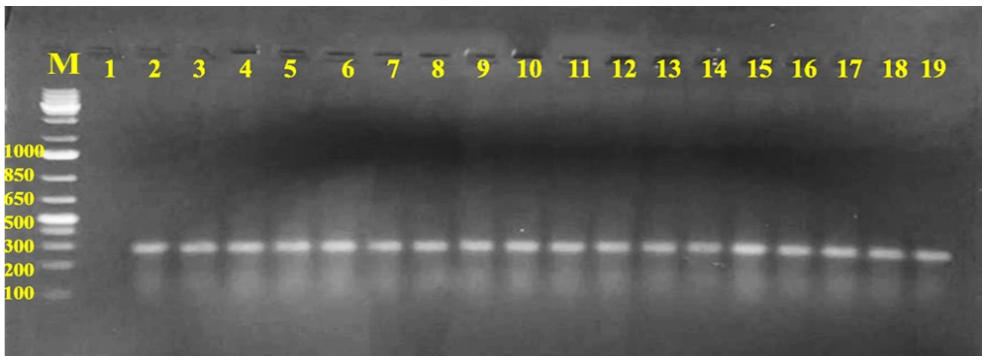

Figure 2 (d). Single DNA band in the tested plants and the positive control one with very close similarity among them at MW 288 bp.
Lane 1= Negative control (Healthy plant), 2 = positive control (reference isolate of R. solanacearum identified by the Brown Rot Project, Egypt), lanes, 3= samples of Talkha, 4 = samples of Al- Ryad, 5= samples of Qeleen, 6= samples of Biala, 7= samples of Belbis, 8= samples of Al- Housainiah, 9= samples of Faqous, 10= samples of Abou- Hammad, 11=samples ofAl- Qsasien, 12= samples of Al- Zaheria, 13= samples of Al- Salhia, 13= samples of Abou Ashour, 14= samples of Al- Qarien, 15= samples of Kafr Al- Shiekh Attia, 16= samples of Kafr Al- Naggar, 17= samples of Kafr Al- Arab, 18= samples of Genifa, 19= samples of Kabreet.

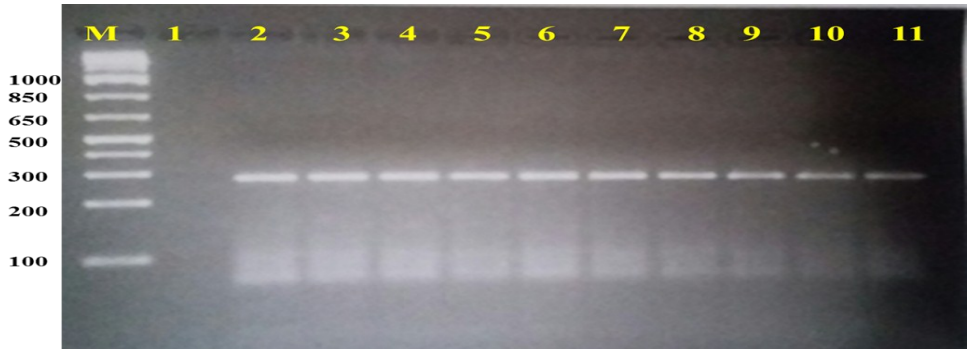

Figure 2 (e). Single DNA band in the tested plants and the positive control one with very close similarity among them at MW 288 bp.
Lane 1= Negative control (Healthy plant), 2 = positive control (reference isolate of R. solanacearum identified by the Brown Rot Project, Egypt), lanes, 3= samples of Housh Eiesa, 4 = samples of Al- Noubaria, 5= samples of Al- Mahmoudia, 6= samples of Abou Al- Mtamier, 7= samples of Saft Al- Houria, 8= samples of Al- Tawfikia, 9= samples of Saft Al- Houria, 10= samples of Abou- Al- Mtamier, 11=samples of Housh Eiesa, 12= samples of Al- Noubaria.

PCR detection and confirmation of *R. solanacearum*

Data in Fig. (2 a, b, c, d, and e) confirmed the infection of bacterial wilt pathogen among the infected plants collected from above mentioned governorates (Table 1, 2) during season 2014/2015 which identified as *R. solanacearum* using PCR technique using two oligonucleotide *R. solanacearum* - specific primers 759/760 (5' GTC GCC GTC AAC TCA CTT TCC 3' and 5' GTC GCC GTC AGC AAT GCG GAA TCG 3') visualized the specific DNA band with molecular weight 288 bp in the tested samples and the positive control. The results revealed that PCR of all plants tested were clearly identical to the positive control (*R. solanacearum* identified by Brown Rot Project in Egypt). 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.

All over the world the disease is very common in tropical regions during warm and humid weather conditions (Temperature range mostly 25-35°C). In Egypt the favorable condition for this disease prevails during monsoon season (July-August when ideal conditions of high humidity and temperature prevail and the crops approach to harvesting time). The data presented in this study substantiate the findings that, the disease is well established in various solanaceous crops grown in variety of vegetable growing areas of the country. Bacterial wilt disease was first observed to cause considerable losses in some fields of the main production areas (Kafr Eddoar and Bosaly in Behara and Etfeeh in Giza) during the 1998-1999 growing season. Disease incidence ranged from 5 to 20% with obvious symptoms of wilt appeared at 6±8 weeks after transplantation. Aly and Abd El Ghafar (2000). Abd-Alla and bashandy, (2008) mentioned that, In Egypt, this disease was first identified in tomato in 2008. The infection symptoms on tomato plants were established at this age, showing stunted growth or completely wilting, resulting in poor fruit quality such as small fruit size and significant yield loss of about 10%. These results are in harmony with those reported by many researchers Singh *et al.* (2010) who conducted a survey to study the status of bacterial wilt of solanaceous crops caused by *R. solanacearum* in Northern and Eastern states of India such as Jammu & Kashmir, Himachal Pradesh, Uttrakhand, Jharkhand and West Bengal. They observed that bacterial wilt disease incidence in tomato and chilli was quite low (1 to 3 %) during summer season, whereas in rainy season, it was (4 to 60 %) in tomato and (3 to 40 %) in brinjal. Disease incidence in tomato crop was higher compared to other solanaceous crops like brinjal, chilli, capsicum, and potato. Also, Mondal (2011) found that, tomato, brinjal and potato were affected by the disease in west Bengal and percentages of damage were 9.86 to 86.45%, to 10.54 to 85.63% and 10.72 to 73.82% respectively. Moreover, Begum *et al.* (2012) found that, bacterial wilt (BW) caused by *R. solanacearum* in different vegetable growing areas of Pakistan (during April-September 2008-09) revealed that sweet pepper was highly susceptible with overall incidence percentage of 21.9% followed by hot pepper (16.6%), tomato (13.3%), potato (10.5%) and brinjal (5.5%). Aggregate incidence in the whole country was 13.8%. Also, Assefa *et al.* (2015) reported that, in Ethiopia percentage wilt incidence of bacterial wilt was as high as 63% on potato, 55% on tomato and 100% on pepper. Also, Kago *et al.* (2016) carried out a survey of bacterial diseases of 10 farms of Potato, tomato and capsicum. A survey was conducted in the period of short rains (October December) in year 2015, covering Kenyan highlands and lowland regions of Nyeri, Kiambu, Nyahururu, Kirinyaga, Murang'a, and Embu counties; the major production zones of potato, tomato and capsicum to determine the prevalence, effects and management measures in practice by farmers in control of *R. solanacearum*. In the survey, farms (75 %) in highlands and 25 % in lowlands had plants with symptoms characteristic of bacterial wilt disease. Bacterial wilt incidences in the survey farms were high in Kirinyanga (26.00%), Murang'a (24.50%), Nakuru (19.00%) and Nyandarua (16.04%); they were low in Nyeri (10.60%) and Embu (13.05%) counties. The survey indicated bacterial wilt as a serious challenge in production of Solanaceae crops in central Kenya. Seleim *et al.* (2014) isolated fifteen *Ralstonia solanacearum* isolates from thirty-three of infected tomato tissues, water and soil samples which were collected from different localities of Al- Minia, As-siut and Sohag governorates, showning bacterial wilt symptoms or taken from location hit by the disease. Regardless of the differences in soil types and cropping patterns, the bacterial wilt disease was prevalent in all the surveyed areas. Moreover Gutarra *et al.* (2017) reported that, the current bacterial wilt infestation was investigated by collecting stem samples from wilted plants and detecting *Ralstonia solanacearum* in total 39 farmers' fields located in the central and northern Peru. *R. solanacearum* was detected in 19 fields, and in 153 out of the 358 samples analyzed, where 43% were found to contain *R. solanacearum*.

Conclusion

The present study was an effort to find out the occurrence of the bacterial wilt disease in major growing tomato areas in Egypt. In Upper Egypt, we found that, the highest disease incidence was recorded in Luxour Governorate (10.29%) and lowest in Al- Giza Governorate (9.41%). The wilt severity was recorded highest level (3.44%) in Luxour Governorate and the lowest (2.60%) was recorded in Sohag Governorate. In Lower Egypt Governorates, Al- Behiera Governorate fields recorded a higher disease incidence and severity than all Governorates, the highest disease incidence was 15.28% followed by Al-Daqahlia (13.23), Al- Gharbia (12.41), Kafr Al- Shikh (12.70) and Al- Sharqia (12.14) Governorates. The lowest disease incidence recorded in Al- Suiz Governorate (10.27%), followed by 11.28, 11.58 and 11.67% in Al- Qalioubia, Al- Ismailia and Al- Minoufia Governorates respectively. The highest bacterial wilt severity was recorded in Al- Behiera Governorate, fields (5.70%), followed by Al- Gharbia (4.60%), while the lowest bacterial wilt severity was recorded in Al- Suiz Governorate (2.63%) followed by 3.30, in Al- Qalioubia Governorate. All the collected plants were subjected to PCR to detect the infected samples. The

expected 288 bp amplicon was detected in bacterial wilt infected samples from above mentioned governorates and diseases percentage and severity were calculated. Intensive study and molecular diagnosis will be needed to supplement the further knowledge.

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