Zagazig J. Agric. Res., Vol. 35 No .(4) 2008 769-788

DETECTION OF *RALSTONIA SOLANACEARUM* ON SOME CROPS AND WEEDS UNDER EGYPTIAN CONDITIONS

Hamad, Y. I.[1], M.R.A.Tohamy[2], and G.A. El-Morsy[3]

1. General Administration of Plant Quarantine, Ministry of Agric., Cairo, Egypt.
2. Agric. Bot. & Pl. Pathol. Dept., Fac. Agric., Zagazig Univ., Zagazig, Egypt.
3. Plant Pathology Research Institute, ARE. Giza.

Accepted 21 /7/2008

ABSTRACT: The present investigation was carried out to identify weed and crop hosts of *Ralstonia solanacearum* Yabuuchi *et al.,* the causal agent of potato brown rot, under El-Sharkia and El-Ismailia Egyptian governorates. Obtained results revealed that weed species *Solanum nigrum* L. (black nightshad), *Portulaca oleracea* L. (purslane), *Brassica kaber* (DC) L.C. (black mustard), *Chenopodium album* L. (lamb`s paurters, *Beta vulgaris* L. (curly dock), *Rumex dentatus* L. (sorrel) are considered as hosts for *R. solanacearum.* On the other hand, *Amaranthus sylvestris* L. (pig weed), *Cichorium endivia* L. (wild chicory), *Conyza discorids* L., *Conyza linfolia* (wild.) Tackhi, *Sonchus oleraceus* L., *Convolvulus arvensisn* L. (field bind), *Sisymbrium irio* L., *Medicago capulina* L., *Plantago major* L. (great plantain) and *Urtica urens* L. (small nettle) not considered hosts for *R. solanacearum.*

Twelve isolates of *R. solanacearum* were isolated from weeds species. Isolates *R. solanacearum* (race 3 biovar 2) were identified using morphological, physiological and biochemical tests. Also advanced Immunofluorescence Antibody Stain (IFAs)and Polymerase Chain Reaction (PCR) techniques were applied and confirmed that the isolates are related to *R. solanacearum.*

Key words: Detection, survey, *Ralstonia solanacearum,* crops, weeds.

INTRODUCTION

The bacterial wilt disease is a major constraint for vegetable growers, especially potato and tomato farmers in many region of the world. Estimation of yield losses due to the disease incidence ranged from 15 to 90% according to Hayward and Hartman (1994). The number of weed varieties as hosts for *R. solanacearum* is very great.It causes disease at least of 200 different plant species, including herbaceous plants,shrubs and trees(Castillo and Greenberg 2007). Now, *Ralstonia solanacearum* (Yabuuchi *et al.*, 1995) is known to infect a wide range of economically important cash crops (Robinson *et al.*, 1995; Elphinstone *et al.*, 1998 and Pradhanang *et al.,* 2000). El-Didamony *et al.* (2003) surveyed *R. solanacearum* (race 3 biovar 2) from potato tubers cultivated in EL-Dakahlia and EL-Sharkia governorates, Egypt. They also surveyed the pathogen from aubergine, tomato and pepper. Also, Farag *et al.* (2004) surveyed the bacterial wilt of potato (*R. solanacearum*, race 3, biovar 2) and showed that the pathogen can however, be introduced into an area by planting infected potato tubers and grown infected weed hosts such as *Rumex dentatus* L. and *Solanum nigrum* L. in Egypt. Moreover, Balabel, (2005) collected weed samples from potato fields in El-Ismailia and El-Minufiya governorates, found that the most pathogenic isolates were recovered from potato tubers and weeds. Meanwhile, soil, water and potato stem isolates were moderate in this regard. *Rumex dentatus* L. and *Solanum nigrum* L. were found as alternative hosts for *R. solanacearum* race 3 biovar 2 in Egypt.

Balabel (2005) used PCR technique in identification of sixteen *R. solanacearum* isolates derived from different habitats in Talia Village, El-Minufyia governorate. PCR analysis showed very close similarity of the sixteen isolates under investigation. Therefore, the present study aimed to survey and isolate the causal organism of potato brown rot disease caused by *R. solanacearum* from different crops and weeds present in El-Sharkia and El-Ismailia governorate fields. Identification of the pathogen was carried out using traditional and molecular biology techniques.

MATERIALS AND METHODES

Detection of the Causal Organism

In El-Sharkia governorate

Thirty five samples of potato tubers were collected during season 2000/2001 at various pivots at El-Salhia locality (59L13 ,72L14 ,68L14 ,39L11 and 60L11 Pivots) and 408 sample of weeds were also collected from potato fields at various localities of El-Sharkia governorates i.e; Kafr El-ashraf and El-Salhia (39L11 ,60L11 ,59L13 and 72L14 Pivots) during three successive seasons (2002 / 2003, 2003 / 2004 and 2004/2005). Plant samples were collected with roots and transferred to the laboratory in separate labeled plastic bags using an ice box. All samples randomly collected from potato fields, during the period from July in the first year to May of the next year.

In El-Ismailia governorate

Fifty samples of potato tubers were collected during season 2000/2001 at various localities of El-Shapap (26L8, 27L8, 28L8, 29L9, 30L9, 14L5, 11L3 and 8L2 Pivots), and 408 sample of weeds were collected from potato fields at various localities of El-Ismailia i.e; El-Tall El-Kabeer, Tosson village, Sarabion and El-Shapap (8L2, 11L2, 14L5,26L8, 27L8, 28L8, 29L9 and 30L9 Pivots) during the same aforementioned seasons. Samples were collected and transferred as mentioned before.

Isolation and Identification of the Causal Organism

From potato tubers

Randomly samples of infected potato tubers (200 tubers /five feddan) washed in tap water and surface disinfected by immersion in alcohol and flaming (Janse, 1988). The epidermis around the heel end was removed. Small tissue cores (5-10 mm diameter per tuber), containing mainly vascular corticle tissues were removed from the heel end using a regular scalpel. After removal of heel end, cutted tubers were observed for the presence of brown rot symptoms in vascular bundles (Fig. 1). Heel ends were then kept in plastic cups for each sample with addition of 25 ml of sterile phosphate buffer (pH 7). Resulted suspension was shaked for 3h at 100 rpm. Approximately 0.1 ml of the obtained suspension was spread on the surface of tetrazolium chloride (TZC) medium (Kelman, 1954).

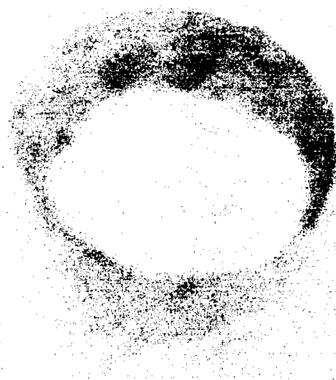

Fig.1. Typical symptoms of the disease on tuber showing distinct brown vascular dissoloration and ooze of bacterial masses

From weed root tissues

Randomly samples of weed roots (1-gram root for each sample), were washed in tap water and surface disinfected by immersion in alcohol and flaming. Sample (one-gram root of weeds) was macerated by hammer in sterile plastic bags then left for half an hour in 9 ml of sterile phosphate buffer (pH7) and allowed to stand for 30 minuets. Approximately 0.1 ml from each obtained suspensions was spread on the surface of tetrazolium chloride (TZC) medium (Kelman, 1954) and incubated at 28°C for 3 days (Kelman, 1954; The plates were examined daily for the appearance of the developed colonies. The appearance colonies were examined morphologicaly, biochemicaly and physiological. characteristics (Kelman, 1954; King *et al.,* 1954; Fahy and Persley, 1983; Krieg and Holt, 1984; Lelliot and Stead, 1987; Engelbrecht, 1994; Elphinstone *et al.* 1996) and tested with IFAS test (Janse, 1988). Weeds are described and named according to Muenscher (1955) and Radosevich and Holt (1984).

Polymerase Chain Reaction (PCR) technique was conducted in Brown Rot Project; A.R.E. according to the method described by Seal *et al.*, (1993) using PCR technique with *R. solanacearum.* Specific oligonucleotide primer OLI-1 (5, GGG GGT AGC TTGA CCTG CC3,) and non-specific primer Y-2, (5, CCC ACT GCT GCC TCC CGT AGG AGT3,)

were used following the original reaction conditions.

Pathogenicity of *R.solanacearum* isolates recovered during isolation from different materials confirmed by inoculating tomato plants cv. Gs (3 leaves stage /seedling). One seedling was grown in pots 5 cm diameter under greenhouse conditions, using the stem puncture technique (Janse, 1988). Injection was made at the leaf axis by a needle laden with the bacterial growth of the pathogen (10^8 CFU/ml concentration). Control treatments were prepared by applying few drops of sterile water instead of bacteria. The inoculated plants were covered with polyethylene bags for three days to ensure the pathogenic potentially, at 30°C, then bags were removed and pots were irrigated daily. Symptoms observed on plants under natural conditions after ten days from inoculation.

RESULTS

Detection of the Causal Organism

In El-Sharkia governorate

Data in Table 1 based on SMSA test reveal that, 14 samples of potato tubers of El- Sharkia governorate showed positive reaction towards *R. solanacearum*; 3 samples for Sponta cultivars in 59L13 pivot; 8 samples of Nicola cultivar in 59L13 , 68L14 and 60L11 pivots, with values of 2, 4 and 2, respectively .One sample of Hirms cultivar and tow sample for Vivalde cultivars in 72L14 pivot showed a low positive reaction. Concerning IFAS tests 6 samples were positive including 2 samples for each of Sponta and Nicola cultivars in 59L13 location. One sample from each of Hirms in 72L14 and Nicola cultivars in 60L11 pivot. Pathogenicity test indicated that three positive samples towards *R. solanacearum* were pathogenic to different potato cultivars i.e.; 2 samples for Sponta cultivars in 59L13 and one sample for Nicola cultivars in 59L13. The total number of samples were 35 included 21, 14, 6 and 3 samples with positive reaction for NA, SMSA, IFAS, and pathogenicity tests. Growth on NA was more better than the other media tested.

Data in Table 2 show that the isolated *R. solanacearum* from *Solanum nigrum* L. (Solanaceae), *Portulaca olracea* .L. (Portulaceae), *Chenopodium album* L., *Beta vulgaris* L. (Chenopodiaceae), *Arachis hypogea* L. (Leguminosae), *Brassica*

Table 1. Detection of *Ralstonia solanacearum* in different potato (cultivars) fields, during growing season 2000/2001, using different methods, at El-Sharkia governorate

Pivots	Potato cultivar	No. (+) samples using				
		Total samples	N.A	S.M.S.A	IFAS	P.T.
59 L 13	Sponta	5	3	3	2	2
59 L 13	Nicola	5	5	2	2	1
72 L 14	Hirms	5	2	1	1	0
72 L 14	Vivalde	5	3	2	0	0
68 L 14	Nicola	5	4	4	0	0
39 L 11	Lady Roasita	5	2	0	0	0
60 L 11	Nicola	5	2	2	1	0
Total		**35**	**21**	**14**	**6**	**3**

NA: Nutrant Agar medium.

SMSA: Semi-Selective Medium of South Africa.

IFAS: Immunofluorescence Antibody Stain.

P.T.: pathogenicity test on tomato seedlings.

Table 2. Detection of *Ralstonia solanacearum* in roots of some weed plants using different methods during three successive growing seasons (2002-2003, 2003-2004, 2004-2005) at El-Sharkia governorate

Time of sampling		2002/2003				2003 / 2004				2004 / 2005				Mean of SMSA test	Mean of IF test	Mean of P.T.
Family	Weed spp.	No. samples	No. (+) samples usingr			No. samples	No. (+) samples using			No. samples	No. (+) samples using					
			SMSA test	IF test	P.T.		SMSA test	IF test	P.T.		SMSA test	IF test	P.T.			
Amaranthaceae	*Amaranthus sylvestris* L.	8	1	0	0	8	0	0	0	8	1	0	0	0.6	0	0
Chenopodiaceae	*Beta vulgaris* L.	8	1	1	1	8	2	1	1	8	2	1	1	1.6	1	1
	Chenopodium album L	8	3	1	1	8	2	1	1	8	2	1	1	2.3	1	1
Compositae	*Cichorium endivia*L	8	1	0	0	8	2	0	0	8	1	0	0	1	0	0
	Conyza dioscorids (L.) Deaf	8	1	1	0	8	2	1	0	8	2	1	0	1.6	1	0
	Conyza linifolia(wild.) Tackhi	8	2	0	0	8	0	0	0	8	1	0	0	1	0	0
	*Sonchus oleraceus*L.	8	1	0	0	8	2	1	0	8	1	0	0	1.3	0.3	0
Convolvulaceae	*Convolvulus arvensis* L.	8	0	0	0	8	0	0	0	8	0	0	0	0	0	0
Cruciferae	*Brassica kaber* (DC) L.C.	8	1	1	0	8	1	0	0	8	3	1	1	1.6	0.6	0.3
	Sisymbrium irio L.	8	1	0	0	8	0	0	0	8	0	0	0	0.3	0	0
Leguminoseae	*Arachis hypogea* L.	8	2	1	1	8	2	1	1	8	2	1	1	2	1	1
	Medicago capulina L.	8	1	0	0	8	1	0	0	8	0	0	0	0.6	0	0
Plantagonaceae	*Plantago major* L.	8	1	0	0	8	0	0	0	8	0	0	0	0.3	0	0
Polygonaceae	*Rumex dentatus* L.	8	6	2	2	8	5	3	1	8	4	3	2	5	2.6	1.6
Portulacaceae	*Portulaca oleracea* L.	8	2	2	1	8	2	2	1	8	4	3	1	2.6	2.3	1
Solanaceae	*Solanum nigrum* L.	8	4	3	3	8	3	2	1	8	3	2	2	3.3	2.3	2
Urticaceae	*Urtica urens* L.	8	0	0	0	8	0	0	0	8	0	0	0	0	0	0
	total	136	28	12	9	136	24	12	6	136	26	13	9			

SMSA: Semi-Selective Medium of South Africa.

IFAS: Immunofluorescence Antibody Stain.

P.T.: pathogenicity test on tomato seedlings.

kaber (DC) L.C. (Cruciferae) and *Rumex dentatus* L. (Polygonaceae) showed positive reaction on SMSA, IFAS and pathogenicity tests during the three tested seasons. However, *Brassica kaber* (DC) L.C. (Cruciferae) reveal positive reactions towards *R. solanacearum* on SMSA, IFAS and pathogenicity tests during 2004 / 2005 season; SMSA and IFAS tests in 2002 / 2003 season as well as SMSA test only in 2003 /2004. Furthermore, *Amaranthus sylvestris* L. (Amaranthaceae), shows positive reaction to *R.solanacearum* on SMSA media only during both 2002/2003 and 2004/2005 seasons. The results also stated that the highest number of positive pathogenicity test for *Solanum nigrum* L. followed by *Rumex dentatus* ,L. *Portulaca olracea* L., *Arachis hypogea* L., *Beta vulgaris* L. , *Chenopodium album* L. and *Brassica kaber (*DC) L.C. with mean values of 2 , 1.6 , 1, 1, 1, 1 and 0.3 isolates, respectively. The highest values of positive reaction over all the three seasons on SMSA for *R. solanacearum* have been recorded for *Rumex dentatus* L. with mean of 5, whereas *Solanum nigrum* L. and *Portulaca olracea* L. showed moderate mean values of 3.3 and 2.6, respectively. On the other hand, the lowest value was recorded by *Sisymbrium irio* L. and *Plantago major* L. with mean value of 0.3 over the three seasons. Concerning IFAS test the results indicate that the highest values of positive reaction towards *R. solanacearum* was recorded for *Rumex dentatus* L. followed by *Solanum nigrum* L. and *Portulaca olracea* with mean values of 2.6 , 2.3 and 2.3 , in the same respective order. Whereas, *Chenobodium album* L., *Beta vulgaris* L., *Conyza dioscorids (*L.) Deaf., *Arachis hypogea* L., *Brassica kaber* (DC) L.C. and *Sonchus oleraceus* L. exhibited 1, 1, 1, 0.6 and 0.3 mean values, respectively, over the three seasons. Negative reactions were recorded with IFAS test for *Sisymbrium riro* L., *Convolvulus arvensis* L. , *Amaranthus sylvestris* L., *Conyza linifolia* (wild.) Tackhi, *Cichorium endivia* L., *Medicago capulina* L., *Plantago major* L. and *Urtica urens* L.

In El-Ismailia governorate

Data in Table 3 based on SMSA test reveal that, 26 samples of potato tubers showing positive reaction towards *R. solanacearum* including 5 samples for Sponta cultivars in Pivot 8L2 location , 14 samples for Nicola cv. in Pivots 27L8, 29L9, 30L9 , 14L5 and 8L2 locations with values of 3, 2, 3, 2 and 4, respectively. Mondial cultivar with 2 samples in pivot 28L8

Table 3. Detection of *Ralstonia solanacearum* in different potato (cultivars) fields, during growing season 2000/2001, using different methods, at El-Ismailia governorate

Pivots	Potato cultivar	No. (+) samples using				
		Total samples	N.A	S.M.S.A	IFAS	P.T.
26 L 8	Lady Roasita	5	5	2	2	0
27 L 8	Nicola	5	4	3	3	1
28 L 8	Mondial	5	2	2	1	0
29 L 9	Nicola	5	3	2	0	0
30 L 9	Nicola	5	4	3	1	1
14 L 5	Hirms	5	2	1	0	0
14 L 5	Nicola	5	2	2	1	1
11 L 3	Diamond	5	3	2	2	1
8 L 2	Nicola	5	5	4	4	2
8 L 2	Sponta	5	5	5	5	5
Total		**50**	**35**	**26**	**19**	**11**

NA: Nutrant Agar medium.

SMSA: Semi-Selective Medium of South Africa.

IFAS: Immunofluorescence Antibody Stain.

P.T.: pathogenicity test on tomato seedlings

location, Hirms cv with one samples in 14L5 location, and Diamond cultivars with 2 samples in Pivot 11L3 location. Concerning IFAs tests 19 samples were positive incloding 5 samples for Sponta cultivars in 8L2 location; For Nicola cultivars 9 samples in Pivots 27L8, 30L9, 14L5and 8L2 locations with values of 3, 1, 1 and 4, respectively; For Mondial cv. with one sample in Pivot 28L8 location ; For Diamond cultivars 2 samples in Pivot 11L3 location and for Sponta cultivars 5 samples in Pivot 8L2 location.

Pathogenicity tests indicated that, 11 positive samples reaction towards *R. solanacearum* of different potato cultivars i.e.: Five samples for Sponta cultivars in Pivot 8L2 location, Nicola cultivars 5 samples in Pivots 27L8, 30L9, 14L5 and 8L2 locations with values of 1, 1, 1 and 2 samples and one for Diamond cultivars in Pivot 11L3 location. The total number of samples were 50 included 35, 26, 19 and 11 samples with positive reaction for NA, SMSA, IFAs, and pathogenicity test. Growth on NA was more better than the other media tested.

Data in Table 4 show that *Solanum nigrum* L. (Solanaceae), *Portulaca olracea* L. (Portulaceae), *Arachis hypogea* L. (Leguminoseae), *Brassica kaber* (DC) L.C. (Cruciferae) and *Rumex dentatus* L. (Polygonaceae) showed positive reactions on SMSA, IFAs and pathogenicity tests during the three successive seasons. However, *Chenopodium album* L., (Chenopodiacea), reveal positive reactions towards *R. solanacearum* on SMSA , IFAs and pathogenicity tests during 2002 / 2003 season. The results also revealed that the highest value of positive pathogenicity tests for *Solanum nigrum* L., *Portulaca olracea* L. and *Arachis hypogea* L. being 2.6, 2 and 2 isolates, respectively. The highest values of positive reaction over all the three seasons on SMSA for *R. solanacearum* have been recorded for *Arachis hypogea* L. being 7.3, followed by *Portulaca olracea* L. 5.6, *Solanum nigrum* L. 5.3, *Brassica kaber* (DC) L.C. 4.6 *Rumex dentatus* L. 4 and *Chenopodium album* 3. Whereas, *Beta vulgaris* L., *Conyza linifolia* (Wild.) Tackhi, *Conyza dioscorides* (L.) Deaf. and *Amaranathus sylvestris* L., showed moderate mean values being 1.6, 1.6, 1.3 and 1 isolates, respectively. On the other hand, the lowest mean values were recorded by *Sonchus oleraceus* L., *Cichorium endivia* L., *Plantajo major* L., *Medicago capulina* L.

Table 4. Detection of *Ralstonia solanacearum* in roots of some weed plants using different methods during three successive growing seasons (2002-2003, 2003-2004, 2004-2005) at El-Ismailia governorate

Time of sampling		2002/2003				2003 / 2004				2004 / 2005				Mean of SMSA test	Mean of IF test	Mean of P.T.
Family	Weed spp.	No. samples	No. (+) samples using			No. samples	No. (+) samples using			No. samples	No. (+) samples using					
			SMSA test	IF test	P.T.		SMSA test	IF test	P.T.		SMSA test	IF test	P.T.			
Amaranthaceae	*Amaranthus sylvestris* L.	8	3	0	0	8	0	0	0	8	0	0	0	1	0	0
Chenopodiaceae	*Beta vulgaris* L.	8	4	2	2	8	0	0	0	8	1	1	0	1.6	1	0.6
	Chenopodium album L.	8	3	2	1	8	3	3	2	8	3	1	1	3	2	1.3
Compositae	*Cichorium endivea* L.	8	2	0	0	8	0	0	0	8	0	0	0	0.6	0	0
	Conyza dioscorids (L.) Deaf.	8	1	1	0	8	2	1	0	8	1	1	0	1.3	1	0
	Conyza linifolia(wild.) Tackhi	8	0	0	0	8	3	0	0	8	2	0	0	1.6	0	0
	Sonchus oleraceus L.	8	2	0	0	8	0	0	0	8	0	0	0	0.6	0	0
Convolvulaceae	*Convolvulus arvensis* L.	8	0	0	0	8	0	0	0	8	0	0	0	0	0	0
Cruciferae	*Brassica* kaber(DC) L.C.	8	3	1	1	8	5	2	0	8	6	2	2	4.6	1.6	1
	Sisymbrium irio L.	8	1	0	0	8	0	0	0	8	0	0	0	0.3	0	0
Leguminoseae	*Arachis hypogea* L.	8	8	4	2	8	6	4	2	8	8	4	2	7.3	4	2
	Medicago capulina L.	8	0	0	0	8	1	0	0	8	0	0	0	0.3	0	0
Plantagonaceae	*Plantago major* L.	8	2	0	0	8	0	0	0	8	0	0	0	0.6	0	0
Polygonaceae	*Rumex dentatus* L.	8	3	2	1	8	4	3	1	8	5	2	2	4	2.3	1.3
Portulacaceae	*Portulaca oleracea* L.	8	5	3	2	8	7	3	2	8	5	3	2	5.6	3	2
Solanaceae	*Solanum nigrum* L.	8	6	4	3	8	6	4	2	8	4	3	3	5.3	3.6	2.6
Urticaceae	*Urtica urens* L.	8	0	0	0	8	0	0	0	8	0	0	0	0	0	0
	total	136	43	19	12	136	37	20	9	136	35	17	12	-	-	-

SMSA: Semi-Selective Medium of South Africa.
IFAS: Immunofluorescence Antibody Stain.
P.T.: pathogenicity test on tomato seedlings.

and *Sisymbrium irio* L. with values of 0.6, 0.6, 0.6, 0.3 and 0.3, respectively. Concerning IFAs test, the results indicated that, the highest mean values of positive reaction of *R. solanacearum* on IFAS test, overall the three seasons were recorded for *Arachis hypogea* L., *Solanum nigrum* L., *Portulaca olracea* L. and *Chenopodium album* L. with values of 4, 3.6, 3 and 2, respectively. Whereas *Brassica kaber* (DC) L.C., *Conyza* dioscorids (L.) Deaf., Beta *vulgaris* L. exhibit the lowest value being less than 2.

It is important to mention that, negative reaction with IFAs test were recorded for *Sisymbrium irio* L., *Convolvulus arvensis* L., *Amaranthus sylvestris* L., *Conyza linifolia* (wild.) Tackhi, *Sonchus oleraceus* L., *Cichorium endivia* L., *Medicago capulina* L., *Plantago major* L. and *Urtica urens* L. It can be conclud, that negative reactions were detected under pathogenicity test for *Sisymbrium irio* L., *Convolvulus arvensis* L., *Amaranthus sylvstris* L., *Conyza linifolia* (wild.) Tackhi, *Conyza dioscorids* (L.) Deaf, *Sonchus oleraceus* L , *Cichorium endivia* L. , *Medicago capulina* L. , *Plantajo major* L. and *Urtica urens* L. Whereas *Brassica kaber* (DC) L.C. reveal positive reaction towards *R. solanacearum* on SMSA, IFAs and pathogenicity tests during 2002 / 2003 season, SMSA and IFAs tests during 2003 / 2004 season as well as SMSA test only during 2004 / 2005. Further more, *Amaranthus sylvestris* L., showed positive reaction to *R. solanacearum* on SMSA media only during 2002/ 2003 and 2004 / 2005 seasons.

Identification of the Causal Agents

Twelve pathogenic bacterial isolates obtained from different weed species of El – Sharkia and El – Ismailia regions, are short – rods, non-spore forming and gram-negative reaction. The formed colonies on nutrient agar (NA) medium are irrgularly round, convex, with smooth surface, entire margin translucent and yellowish brown in color. Moreover, these colonies are whitish gray in color on King's B (KB) medium and fluidal white with pink center on tetrazollium chloride (TZC) medium and on Semi-Selective Medium of South Africa (SMSA). The typical colonies on SMSA medium are shown in Fig. (2) are milky white, irregular and fluidal with blood red coloration in the center. The atypical forms developed less fluidal colonies which are

completely pink to red on such medium. The morphological typical colonies are the main type on SMSA isolation plates from different sources.

All tested isolates, were positive reaction for oxidase reaction and produce acid from glucose, fructose, maltose, xylose and lactose. The isolates showed nigative reaction with Arginine, Gelatine, H2S production, Indole production, KOH 3%, Starch hydrolysis, trehalose, Mannitole, arabinose and ribose.

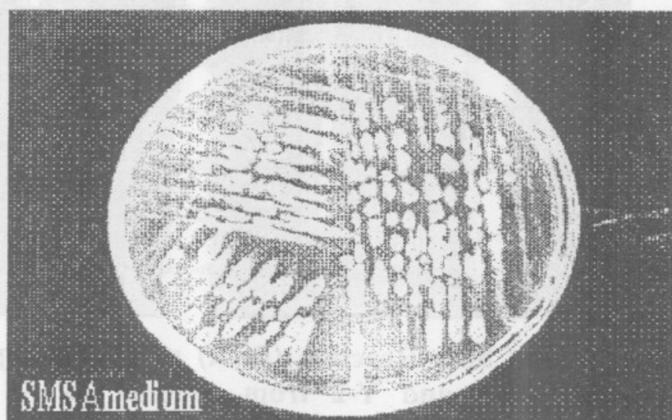

Fig. 2. Typical colonies of *R. solanacearum* on SMSA medium

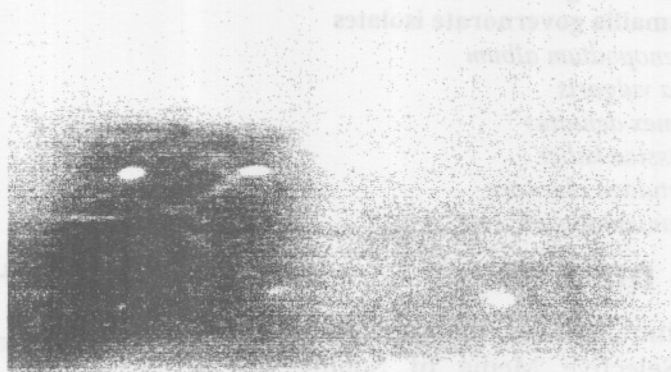

Fig. 3. Cells of *R. solanacearum* under immunofluorescent (IFAs)microscope (3200X)

Isolates of *R. solanacearum* were tested by IFAs. Tested isolates showed as Fig. (3) rod–shaped cells of *R.solanacearum* are clear bright fluorescent and completely react with antiserum in 1:6400 dilutions. The tested isolated bacteria identified as *R. solanacearum* on the base of cell shape and positive reaction with IFAs. All tested isolates were positive in their reaction to Polymerase Chain Reaction (PCR) technique (Fig. 4).

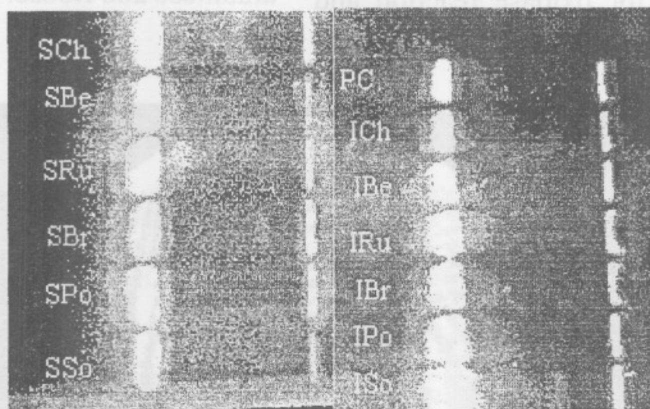

Fig.4. Polymerase Chain Reaction (PCR) products amplified using primers OLI-1 and Y-2 from genomic DNA of different isolates from different habitats

S: El-Sharkia governorate isolates
I: El-Ismailia governorate isolates
Ch : *Chenopodium album*
Be : *Beta vulgaris*
Ru : *Rumex dentatus*
Br : *Brassica kaber*
Po : *Portulaca oleracea*
So : *Solanum nigrum*

DISCUSSION

In the present investigation, Semi Selective Media of South Africa used in survey study for detecting viable cells of *R. solanacearum* from different habitats. This medium has been also recommended by many investigators (Okabe, 1969; Nesmith and Jenkins, 1979 and Hara & Ono, 1983). The typical and atypical forms of the pathogen can be easily differentiated by

Kelman's medium (Kelman, 1954 and Engelbrecht, 1994) as well as Elphinstone *et al.* (1996 and 1998). It is worthy to note that, negative reactions were detected on SMSA test for *Convolvulus arvensis* L. and *Urtica urens* L. Therefore, they can not be expressed as hosts for *R. solanacearum*.

Based on pathogenicty tests as well as growth on SMSA medium and IFAS tests, different percentage of positive reactions were detected in *Solanum tuberosum* L., *Solanum nigrum* L., *Portulaca oleracea* L., *Brassica kaber,* (DC) L.C. *Chenopodium album* L., *Beta vulgaris* L., *Arachis hypogea* L. and *Rumex dentatus* L. cultivated under both El-Sharkia and El-Ismilia governorates. These results could be discussed on the basis that these weeds are hosts for *R. solanacearum* where their structures and chemical components are in compatibility with the needs of the pathogen . In this connection, several authors reported that *R. solanacearum* is known to infect a wide range of economically important cash crops and weeds such as *Arachis hypogea* L. (peanut) (Robinson *et al.,* 1995 and Bulbul and Main 2001); *Solanum nigrum* L. (blak nightshad) and *Portulaca oleracea* L. (purslane) (Elphinston *et al.,* 1998). The obtained results might be explained on the basis that such hosts might provide the investigated organisms with the nutrional needs, or they might not have preventing barriers that, suitable for the organism i.e. *Rumex dentatus* L. and *Solanum nigrum* L. (Farag *et al.,* 2004 and Balabel, 2005). While, *Sisymbrum irio* L., *Convolvulus arvensis* L., *Amaranthus sylvestris* L., *Conyza linifolia* (wild.) Tackhi, *Conyza dioscorids* (L.) Deaf, *Sonchus oleraceus* L., *Cichorium endivia* L., *Sonchus oleraceus* L., *Medicago capulina* L., *Plantajo major* L. and *Utrica urenus* L. showed negative reaction, therefore it can not be expressed as hosts for *R. solanacearum*.

Identification and race determination of the previously isolated *R. solanacearum* isolates were performed by pathogenicity tests using recent accurate microbiological tests for the diagnosis the causal agent in potato tubers, *Arachis hypogea* L. (peanut) and different tested weeds.

Different detection methods of *R. solanacearum* were concerned including the most sensitive and rapid techniques. Data showed that using indicator plants such as tomato seedling still has high

accuracy, for detection the pathogen. All selected bacterial isolates (12 isolates) caused wilt symptoms on tomato seedlings. Morphological, physiological and biochemical characters of all tested isolates reacted similarly. According to the obtained data, these tested bacterial isolates could be identified as *R. solanacearum* race 3 biovar 2. It is worthy to mention that, the morphological, physiological and bacteriological reactions of the isolates are in concern along with the pathological reactions confirmed with those at *R. solanacearum* described by Krieg and Holt (1984).

In addition to traditional methods for detection *R. solanacearum,* several new techniques are applied to confirm such results including Semi Selective Medium of South Africa (modified by Elphinstone *et al.,* 1996) easily distinguished the suspected *R. solanacearum* than other types of bacteria. Data obtained are in agree with those recorded by Predhanag *et al.,* (2000) and Van Broekhuizen (2002).

However, immunofluorescent staining (IFAs) assay is frequently the most sensitive of serological test for detecting the bacteria (DeBoer *et al.,* 1996). Data showed that tested the bacterial isolates gave typical character of *R. solanacearum* under IFAS within 24h (Elphinstone *et al.,* 1996). This procedure is valid to confirm occurrence of *R. solanacearum* but does not valid to detect races or biovars of bacteria and its not completely reliable due to possible cross – reaction with some other harmless bacteria in soil, water and weed samples (Janse, 1988).

PCR procedure was also valid to confirm occurrence of *R. solanacearum* but it does not detect races or biovars. Detection of *R. solanacearum* races and biovars still depends on host plants and reaction of bacteria to disaccharides and hexose alcohols (Hayward and Hartman, 1994). Only solanaceous plants, i.e. eggplant, pepper and tomato were infected with the confirmed race 3 of *R. solanacearum*. Such data are in agreement with (Hayward and Hartman, 1994). Many investigators, used PCR technique to identify several isolates of *R.solanacearum* isolated from different hosts (Timms *et al.,* 2001; Kehil, 2002 and Balabel, 2005).

REFERENCES

Balabel, Nagla, .M.A. 2005. Persistance of *Rolstonia solanacearum* (Syn. *Pseudomonas solanacearum*) in different habitats in Egypt. Ph.D. Thesis, Microbiology Dept, Fac. Agric., Ain Shams University.

Bulbul, M.A. and I.H. Main .2001. Occurrence of races and biovars of *Ralstonia solanacearum* in Bangladesh Journal of Plant Pathology.17: (1 – 2) : 5 – 12.

Castillo D.A. and J.T Greenberg. 2007. Evolutionary dynamics of *Ralstonia solanacearum*. Appl Environ. Microbial., 73 (4): 1225 – 1238.

De Boer, S.H., D.A. Cuppels and R.D. Gitaitis. 1996. Detection latent bacterial infections. Advances in Botanical Research, 23 : 27 – 46.

El-Didamony, G., A.E.A. Ismail, M. Sarhan and S.S. Abdel-Azez .2003. Idenification and pathogenicity of bacterial wilt of potatoes in some types of Egyptian soil. Egyptian J. of Micropiology 38 (1): 89 – 103.

Elphinstone, J.G., H.M. Stanford and D.E. Stead. 1998. Detection of *Ralstonia solanacearum* in potato tubers. *Solanum dulcamara*, and associated irrigation water In : Prior P. Allen C, Elphinstone, J.G. (eds.) Bacterial Wilt Disease : Molecular and Ecological Aspects. Springer, Berlin. Germany, pp. 133 – 139.

Elphinstone, J.G.J., J.K. Hennessy Wilson and D.E. Stead. 1996. Sensitivity of different methods for the detection of *Rolastonia solanacearum* in potato tuber extracts . Bulletin OEPP/EPPO, Bulletin 26 : 663 – 678 .

Engelbrecht, M.C. 1994. Modification of a semi-selective medium for the isolation and quantification of *Pseudomonas solanacearum*. ACIAR Bacterial Wilt Newsletter, 10 : 3 – 5.

Fahy, P.C. and G.J. Persley. 1983. Plant Bacterial Disease. A Diagnostic Guide. Academic Press, New York.

Farag, N.S., W.E. Eweda, M.I. Mostafa and Nagla, M.A. Balabel. 2004. Preliminary observations on the bacteriology and pathology of *Ralstonia solanacearum.* Egyptian Journal of Agricultural Research, 82: 1519 –1523. of the First Scientific Conference of Agricultural

Sciences., Fac. Agric., Assiut Univ. December 13 – 14, 1997 Vol. 1. 625 – 648.

Hara, H. and K. Ono. 1983. Ecological studies on the bacterial wilt of tobacco caused by *Ps. solanacearum* E.F. Smith. Bull. Okayama Tob. Exp. Stn. 42 : 127 – 138.

Hayward, A.C. and G.L. Hartman. 1994. Bacterial wilt. The disease and its causative agent, *Ps. solanacearum.* " CAB " International. Wallingford , UK.

Janse, J.D. 1988. A detection method for *Pseudomonas solanacearum* in symptomless potato tubers and some data on its sensitivity and specificity. Bulletin OEPP / EPPO Bulletin 18 : 343 – 351.

Kehil, Y.E.I. 2002. Studies on the pathological relationship between potato and brown rot bacteria *Ralstonia solanacearum.* Ph.D. Thesis, Plant Pathology Dept., Fac. Agric., Minia Univ., Egypt.

Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology, 44 : 693 – 695.

King, E.O., M.K. Ward and D.E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med., 44 : 301 – 307.

Krieg, N.R. and J.G. Holt. 1984. *Ps. solanacearum*. Bergey's Manual of Systematic Bacteriology, Vol. 1, pp 141–214. Williams and Wilkins, Baltimore, London.

Lelliot, R.A. and D.E. Stead. 1987. Methods for diagnosis of bacterial diseases of plants. Methods in Plant Pathology, vol. 2, Ed. T.F. Preece. Blackwell Scientific Publication 216 pp.

Muenscher, W.C. 1955. Weeds. Cornell Univ., Ithaca, New york.

Nesmith, W.C. and S.F. Jenkins. 1979. A selective medium for the isolation and quantification of *Ps. solanacearum* from soil. Phytopathology, 69 : 182 – 185.

Okabe, N. 1969. Population changes of *Ps. solanacearum* and soil microorganisms in artificially infested natural field soils. Bull. Fac. Agr. Shizuoka Univ., 19 : 1 – 29.

Pradhanang, P.M., J.G. Elphinstone and R.T.V. Fox.

2000. Identification of crop and weed hosts of *Ralstonia solanacearum* biovar 2 in the hills of Nepal. Plant Pathology 49 : 403 – 413.

Radosevich, S.R. and J.S. Holt. 1984. Weed ecology. John wiley and Sons. Brisban.

Robinson, S.A., P. Jones, J.G. Elphinstone and S.M. Forde. 1995. Production of antibodies to *Pseudomonas solanacearum*, the causative agent of bacterial wilt. Food and Agricultural Immunology 7: 67 – 79.

Seal, S.E.; L.A. Jackson, J.P.W. Young and M.J. Daniels. 1993. Differentiation of *Pseudomonas solanacearum, Pseudomonas syzygii, Pseudomonas pickettii* and the blood disease bacterium by partial 16S rRNA sequencing: construction of oligonucleotide primers for sensitive detection by polymerase chain reaction. Journal of General Microbiology, 139:1587-1594.

Timms, W.T.M., K. Bryant and M.J. Bailey. 2001. Strain characterization and 16s – 23s probe development for differentiating geographically dispersed isolates of the phytopathogen *Ralstonia solanacearum.* Environmental-Microbiology. 3 (12):785 –797.

Van Broekhuizen, W., L. Korsten and P.S. Hammes. 2002. Development of an alternative method for the detection of *R. solanacearum* in naturally infested soil. 3rd International Bacterial Wilt Symposium, February, 4 – 8, p. 49.

Yabuuchi, E., Y. Kosaka, I. Yano, H. Hotta and Y. Nishiuchi. 1995. Transfer of two *Burkholderia* and *Alcaligenes* species. To *Ralstonia* gen. Nov.; Proposal of *Ralstonia picketii* (Ralston, Palleroni and Doudoroff 1973) Comb. Nov.; *Ralstonia solanacearum* (Smith, 1896) Comb. Nov. and *Ralstonia eutropha* (Davis 1969) Comb. Nov. Microbial. Immunol., 39 (11) : 897 – 904.

الكشف عن بكتيريا راستونيا سولاناسيرم علي بعض المحاصيل والحشائش تحت الظروف المصريه

ياسر إبراهيم حمد[1] – محمد رضا احمد تهامي[2] – جمعة عنتر المرسي[3]

١- الإدارة المركزية للحجر الزراعي- وزارة الزراعة- الجيزة- مصر.

٢-قسم النبات الزراعي وامراض النبات- كلية الزراعة- جامعة الزقازيق- مصر.

٣- قسم امراض البقوليات- معهد بحوث أمراض النبات- مركز البحوث الزراعية- الجيزة- مصر.

يعتبر مرض العفن البني (الذبول) واحداً من أهم الأمراض التي تسبب مشاكل كبيره للعديد من محاصيل الخضر الاقتصاديه الهامه و علي وجة الخصوص محاصيل البطاطس و الطماطم في العديد من مناطق العالم و تؤدي الي حدوث خسائر شديده في هذه المحاصيل تتراوح نسبتها فيما بين ١٥-٩٠% لذا تم تصميم هذا البحث لحصر بعض المحاصيل والحشائش العائلة لمسبب مرض الذبول البكتيري (العفن البنى) فى البطاطس وهي بكتريا راستونيا سولاناسيرم *Ralstonia solanacearum* وانتقاله من خلال حقول البطاطس الموبؤة بالحشائش فى محافظتى الشرقية والإسماعيلية فى مصر.

ولقد أظهرت نتائج الدراسة أن الفول السودانى والبطاطس من المحاصيل التى تصاب بشده بمسبب مرض الذبول البكتيري *Ralstonia solanacearum* وأن أنواع الحشائش عنب الديب والرجلة والكبر والزربيح والسلق والحميض تعتبر من العوائل الهامه لهذا المسبب. وقد أمكن عزل أثنى عشر عزلة من المسبب من مختلف أنواع الحشائش وتم تعريفها كسلالة ٣ طراز بيولوجى ٢ باستخدام الاختبارات المورفولوجية والفسيولوجية والكيموحيوية. كما أفاد استخدام الطرق المتقدمة مثل اختبار طريقه الأجسام المضادة المعلمة فلورسنتيا وتحليل تفاعل الــبلمرة المتسلسل فى تعريف عزلات المسبب المرضى *Ralstonia solanacearum*.