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Identification and Pathogenicity of Bacterial Caused Wilt of Potatoes in Some Types of Egyptian Soil

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BACTERIAL wilt caused by *Ralstonia solanacearum* - is very damaging especially in tropical and sub tropical humid conuntries. The present investigation was conducted to identify the pathogenicity of bacterial caused brown rot and wilt of potatoes grown in Dakahlia and Sharkia governorates of Egypt.

From twenty isolates, 13 formed white with red center colonies (virulent), where as 7 isolates formed deep red colonies (avirulent) on King's medium amended with (triphenyl terrazolium chloride) (T.Z.C). These isolates were identified as *R.solanacearum*, biovar II.

From the 13, virulent isolate No. 1 was found to produce the highest percentage wilt infection and disease severity. On the other hand, 7 isolates, produced the lowest disease severity on patato cultivar Sponta under green- house conditions.

Susceptibility of five potato cultivars to *P. solanacearum*, was tested in sterile and non- sterile soil under green - house conditions. Cara and Sponta cultivars were susceptible, Draga and Solani cultivars were moderately susceptible, while, Diament cultivar was resistant.

Host- range of *R. solanacearum* was tested against some economic solanaceous plants, two cultivars of tomato (super strain B, super mormand) were susceptible, while, 2 cultivars of pepper (strain 313 A, strain 313 B), were moderately susceptible and 2 cultivars of Eggplant (Baladi, Romy), were resistant. This isolate belong to race3.

Keywords: Bacterial wilt, *P. solanacearum*, Biovar, Potato, Cultivar, Identification, Morphology, Tomato, Pepper, Eggplants, Susceptible, Race.

Potato (*Solanum tuberosum* L.) is considered to be one of the most important vegetable crops in Egypt, since, it is cultivated for local consumption as well as for exportation to European markets.

Potatoes cultivars are infected by brown rot disease caused by Ralstonia solanacearum A.F Smith which is considered to be one of the most important diseases in the world, especially in Egypt, where significant yield losses occur in potato production (Farag, 1970; Mickail *et al.,* 1985 and Abd El-Ghafar *et al.,* 1995).

Many investigators have shown that *R. solanacearum* causes wilt to many solanaceous plants, weeds, and diploid and triploid banana crops (He *et al.,* 1983; Adhikari, 1993 and Abd El-Ghafar *et al.,*1995).

Bacterial colonization and virulence of *R. solanacearum* varied between cultivars. Resistant plants showed a significant decrease in bacterial invasion from collar to mid stem and there was no increase of bacterial density at tap root and collar, in contrast to susceptible plants (Grimault and Prior, 1993 and Grimault *et al.,* 1994).

The objections of this study was to isolate biovar and races of *R. solanacearum* pathogen from different locations in Egypt and to examin pathogenicity, varietal reaction and host-range.

Material and Methods

Isolation and purification of causal pathogen

The causal pathogen was isolated from diseased potato tubers, (Fig. 1 and 2). showing typical brown-rot symptoms collected from El-Dakahlia and El-Sharkia governorates. Tubers were washed in tap water and surface sterilized by soaking in 0.1% sodium hypochlorite solution for 2 min, rinsed twice in sterilized-distilled water, sectioned and white-slime bacterial ooze was suspended in sterilized-distilled water, bacterial suspension was streaked onto King's medium amended with on triphenyl-tetrazolium chloride. Plates were incubated for 48 hr at 30-32°C and examined daily for bacterial growth. Single colonies were restraked to obtain pure cultures of bacteria (Fig. 3, a&b) according to Kelman, (1954) and Adhikari, (1993).

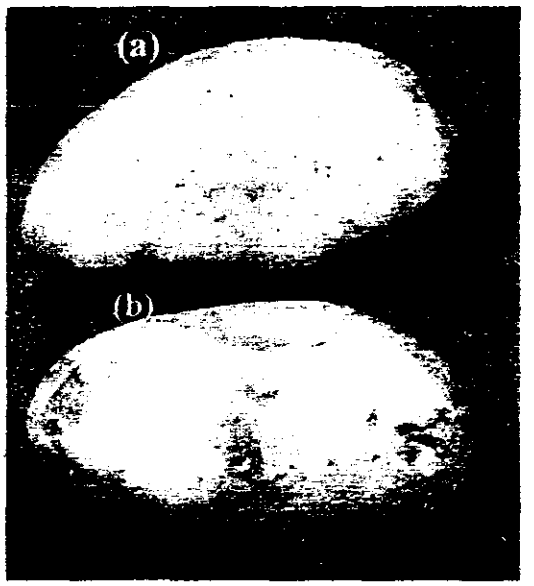

Fig. 1. External view of naturally potato tubers showing (a) naturally healthy potato tubers. (b) naturally infected potato tuber with brown rot disease.

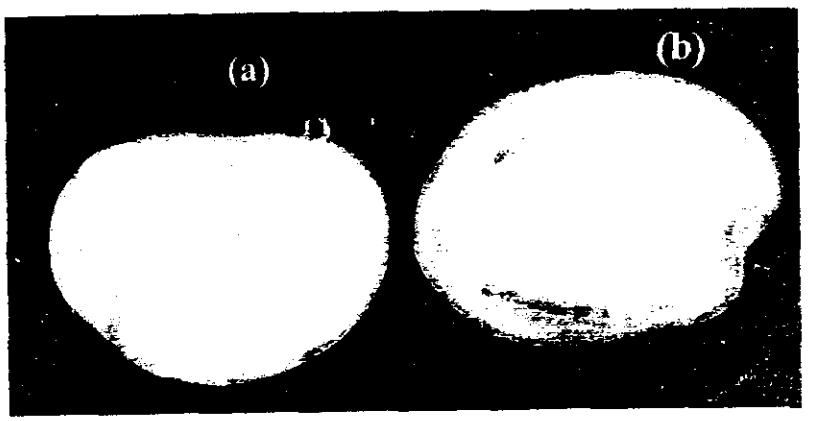

Fig. 2. Internal view of naturally potato tubers showing (a) naturally healthy potato tubers. (b) naturally infected potato tuber with brown rot disease.

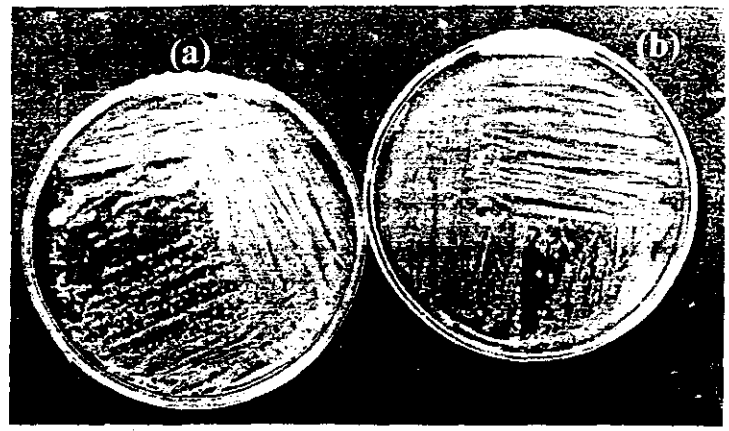

Fig. 3. (a) Color of virulent colonies appeared white with red centers and (b) avirulent colonies appeared as deep red on T.Z.C. medium.

Identification of the isolated bacteria

Morphological, physiological and traditional biochemical tests were performed according to Fahy and Persley (1983); Lelliott and Stead (1987) and Adhikari (1993).

Biovar identification was based on the ability of the isolates under study to oxidize lactose, maltose, sorbitol, mannitol and as well as hydrolysis of both starch and tween-80, these carbohydrates were sterilized as described by He *et al.* (1938) and tested as sole carbon source according to Hayward (1964). Tests were performed at 28-30°.

Pathogenicity test

Thirteen isolates, having white collonies with red centers, were selected from twenty isolates and were tested for pathogenicity on potato "Sponta cultivar" tubers were washed in tap water, surface sterilized by soaking in 0.1% sodium hypochlorite solution for 5 min and then rinsed quickly in ethyl alcohol 95%, then rinsed twice in sterilized distilled water. After surface sterilizing tubers were planted into pots (30 cm in diameter), contain, 2 kg of steam sterilized (2 hr at 121°C). Four pots were used for each treatment as replicates.

All isolates of *P. solanacearum* were grown on sucrose peptone agar medium for 48 hr. The bacterial growth was suspended in sterilized distilled water to

$3x10^8$ c.f.u/ml. The inoculation was carried out according to the method described by Winstead and Kelman (1952), by forcing a sharp needle into the stem through a drop of bacterial suspension placed in the axil of the second or third expanded leaf below the stem apex-of, 17 day old plants. Ten plants (Sponta c.v) were inoculated. Control treatment of the plants were inoculated with sterilize distilled water. After 2 days of inoculation, the percentage of infection and healthy plants were recorded according to the equations of Walker, (1950).

$$\% \text{ of infection} = \frac{\text{Number of infected plants}}{\text{Total No of plants}} \times 100 \text{ , then we take average of 3 replicates.}$$

$$\text{Healty survival} = \frac{\text{Number of healthy plants}}{\text{Total No of plants}} \times 100 \text{, then we take average of 3 replicates.}$$

Disease severity was recorded using the scale adopted by Kempe and sequeira (1983) as follows: 0 = no symptoms, 1 = 1-10% of the foliage wilted, 2= 11-30% of the foliage wilted, 3 = 31-60% of the foliage wilted, 4= more than 60%, but less than 100% of the foliage wilted and 5 = all leaves wilted.

Varietal-reaction

Five potato cultivars namely: Cara, Sponta, Solani, Draga and Diamont were evaluated for their reaction to *R. solanacearum.* Isolate No. (1), which produced the highest disease severity was used in their evoluation. Potato tubers of the five cultivars, were washed in tap water and surface sterilized as above. Subsequently, tubers were rinsed for 3-5 min in a bacterial suspension $3x10^8$ C.f.u/ml of isolate No. (1). Ten treated small tubers of each cultivar were planted in each pot (30 cm in diameter) at the first of November 1999. Twelve pots were used for each culivar, half of which (6), were filled with soil and then autoclaved at 121°C for 2 hr, while another (6) pots were filled with non autoclaved field soil. Each 6 pots was divided into two sets (3 pots/set), one of which was planted by the inoculated tubers, while the other set was planted by non-inoculated tubers.

After 45 days from planting, stems of potato varieties, were inoculated with heavy bacterial suspension to ensure the entrance of the bacteria within the plant tissues.

The percentage of infection and disease severity were calculated as mentioned before.

Host range

Some species from solanaceae *e.g* . Egg plant (*Solanum melongea* L.). Baladi and Romy cultivars, Pepper (*Capsicum annum* L.) blue star strain 313 A and strain 313 B cultivars and tomato (*Lycopersicon esculenitum,* Mill), superstrain B and super-mormand cultivars were used in this study. Seedlings of these plants, were surface sterilized through immersion in 0.1% sodium hypochlorite solution for 2 min, then immersion in ethyl alcohol (95%) for 1 min, followed by washing twice with sterilized distilled water, then transplanted into sterilized soil. Plants were allowed to grow to a height of 20-25 cm before inoculation.

R. solanacearum was grown as mentioned before, inoculation was carried out after wounding the roots by inserting a scaple into the soil near the tap root, the suspension of *P. solanacearum* (5 ml), was poured into the wound area (Kelman and Person, 1961 and Adhikari, 1993). Percentage of wilt infection and disease severity were recorded as mentioned before.

Results and Discussion

Isolation, Purification and Identification of the causal pathogen

Morphological, physiological and biochemical characteristics of the bacterial isolates were similar to the characters of the isolates of *R. solanacearum*. Holt *et al.,* (1994) and Sneath *et al;* (1986) who consulted these results are in agreement with those reported by Shalaby (1979); Fahy and Persely (1983); Adhikarii (1993) and Hsu *et al.*(1993).

Bacteria were isolated from diseased potato plants grown in different locations of El-Dakahlia and El-Sharkia governorates. Data in Table 1 indicate that all the bacterial isolates were able to utilize lactose and maltose, some isolates are able to utilize tween-80. All isolates were unable to utilize sorbitol, mannitol and starch. All were also gram negative and had the ability to reduce nitrate. Colony color of some strains is deep-red and the others were white with red center. These results are similar to that reported by Lelliott and Stead (1987); Adhikari (1993) and Hsu *et al.*(1993).

TABLE 1. Characristics of *P. solanacearum* isolated from El-Dakahlia and El-Sharkia governorates.

Isolates No.	Carbohydrate-utilization					Gram-stain	Tween-80 hydrolysis	Nitrate-reduction	Colony-colour	Locations	Potato-cultivars
	Lactose	Maltose	Sorbitol	Mannitol	Starch						
R1	+	+	-	-	-	Negative	+	+	W.R.C.	Belquas (El-Dakahlia)	Sponta
R2	+	+	-	-	-	-ve	+	+	W.R.C.	El-Tawella (El-Dakahlia)	Sponta
R3	+	+	-	-	-	-ve	+	+	W.R.C.	Belques (El-Dakahlia)	Sponta
R4	+	+	-	-	-	-ve	+	+	W.R.C.	Diast (El-Dakahlia)	Cara
R5	+	+	-	-	-	-ve	+	+	W.R.C.	Kafr-Sakr (El-Sharkia)	Cara
R6	+	+	-	-	-	-ve	+	+	W.R.C.	El-Baklia (El-Dakahlia)	Cara
R7	+	+	-	-	-	-ve	-	+	D.R	El-Salhia (El-Sharkia)	Diamont
R8	+	+	-	-	-	-ve	-	+	D.R	Belbis (El-Sharkia)	Diamont
R9	+	+	-	-	-	-ve	+	+	W.R.C.	Al-Ashraf (El-Sharkia)	Draga
R10	+	+	-	-	-	-ve	+	+	D.R	Tag-El-Ezz (El-Dakahlia)	Draga
R11	+	+	-	-	-	-ve	+	+	W.R.C.	Belquas (El-Dakahlia)	Sponta
R12	+	+	-	-	-	-ve	+	+	W.R.C.	Belquas (El-Dakahlia)	Sponta
R13	+	+	-	-	-	-ve	+	+	W.R.C.	Diast (El-Dakahlia)	Sponta
R14	+	+	-	-	-	-ve	-	+	D.R	Diast (El-Dakahlia)	Solani
R15	+	+	-	-	-	-ve	-	+	W.R.C.	El-Baklia (El-Dakahlia)	Cara
R16	+	+	-	-	-	-ve	-	+	D.R	ElBaklia (El-Dakaahlia)	Diamont
R17	+	+	-	-	-	-ve	+	+	D.R.	Kafr-Sakr (El-Sharkia)	Diamont
R18	+	+	-	-	-	-ve	+	+	W.R.C.	El-Salhia (El-Sharkia)	Sponta
R19	+	+	-	-	-	-ve	-	+	W.R.C.	El-Tawella (El-Dakahlia)	Sponta
R20	+	+	-	-	-	-ve	+	+	D.R.	El-Tawella (El-Dakahlia)	Solani

W.R.C. = While with red center D.R. = Deep-red.

Pathogenicity Test

The pathogenicity of *R. solanacearum* strains, having white with red center colonies on TZC medium, was tested on potato "Sponta cultivar". Data in Table 2 indicated that the highest percentage of wilt infection and disease severity caused by isolate (No. 1), while isolates No: 2, 3, 4 and 5 had moderate percentage of infection and disease severity. On the other hand, isolates No:6, 9, 11, 12, 13, 15, 18 and 19 had the lowest percentage of infection and disease severity. The differences in the percentage of infection and disease severity of tested isolates may be due to the differences in its genetic structures.

TABLE 2. Pathogenicity test of *R. solanacearum* isolates for potato plant (Sponta C.V) in sterile-soil under green-house conditions.

Isolates	% of infection	Healthy-survivals	D.S
1	66.66	33.34	4
2	43.33	56.67	3
3	53.66	46.34	3
4	43.66	56.94	3
5	32.00	68.00	2
6	40.33	59.67	3
9	21.66	80.67	2
11	19.33	98.34	2
12	10.00	90.00	1
13	13.33	86.67	2
15	22.00	78.00	2
18	15.33	84.67	2
19	9.66	90.34	1
Control	0.00	100.00	0.0
L.S.D at 5%	9.63	11.68	1.36

D.S. = Disease severity.

Varietal reaction

Data in Table 3 and Fig. 4, 5 & 6 indicated that potato cultivars tested varied in their resistance to infection by *R. solanacearum*. Cultivar Cara and Sponta exhibited the highest percentage of infection (66.66%) and disease severity (4), followed by Draga and Solani cultivars which exhibited moderate percentage of infection and disease severity, Diamont cultivar, achieved the lowest percentage of infection and disease severity in sterile, and non sterile soil. The percentage of infection and disease severity were higher in sterile soil compared with the corresponding non sterile soil. Such results are in accordance with those reported by Tuthill and Decker (1941); Farag (1970); Cook and Papendick (1972) and Mickail *et al.* (1985).

Host-range

Data in Table 4 and Fig.7 indicated that the highest percentage of infection and disease severity was obtained in tomato plants (60% of cultivare super mormand), while, the percentage of infection and disease severity was moderate in pepper. On the other hand, the egg plant had the lowest percentage of infection and disease severity. Such results are in accordance with those reported by Akiew (1982); Hsu (1991); and Abd El-Ghafar *et al.* (1995). Differences found in reaction among the tested species towards *P. solanacearum*, might be due to genetic make up of the host and strains tested.

According to Hayward's classification scheme (1964), all isolates are biovar 2, were highly virulent on potato, tomato plants, but were less virulent to egg plant and pepper plants.

These results are similar to those reported by Abd El-Ghafar *et al.* (1995) who found that biovar 2, was widely distributed in Egypt. The results of pathogenicity test revealed that these isolates of R. solanacearum belong to race 3 (He *et al.,* 1983). Biological control of these isolates with antagonistic bacteria, actinomycetes, some plant extracts and some volatile oils, will be studied in further study.

TABLE 3. Percentage of infection and disease severity of different potato cultivars infected with *R. solanacearum* under greenhouse conditions.

Potato cultivars	Autoclaved-soil				Field soil			
	Ino. Tubers		Non- Ino.tubers		Ino-tubers		Non-Inoc tubers	
	% of inf.	D.S	% of inf.	D.S	% of inf.	D.S.	% of inf.	D.S
Cara	66.66	4	0.0	0.0	43.33	3	0.0	0.0
Sponta	66.66	4	0.0	0.0	36.66	3	10.00	1
Solani	36.66	3	0.0	0.0	23.33	2	10.00	1
Diamont	33.33	3	0.0	0.0	23.33	2	10.00	1
Draga	53.33	3	0.0	0.0	36.66	3	0.0	0.0
L.S.D at 5%	10.43	N.S	N.S	N.S	8.75	N.S	5.46	N.S

Ino = Inoculated
% of inf. = Percentage of infection
D.S = Disease-severity
N.S = Non-significance

TABLE 4. Percentage of infection and disease-severity of some species from family "Solanaceae" infected with *R. solanacearum* under greenhouse conditions.

Species	Cultivars	% of infection	D.S
(1) Tomato (*lycopersicon esculenitum*)	Super strain B	56.66	3
	Super mormand	60.00	3
(2) Egg-plant (*Solanum melongea*)	Baladi	10.33	1
	Romy	14.66	2
(3) Pepper (*Capsicum annum*)	Strain 313 A	16.33	2
	Strain 313 B	15.66	2
L.S.D at 5%		12.45	1.56

D.S. = Disease severity % of infection = Percentage of infection

Fig. 4. Symptoms of bacterial wilt (brown-rot) disease of potato plant (Draga-cv) infected with *R.solanacearum*.

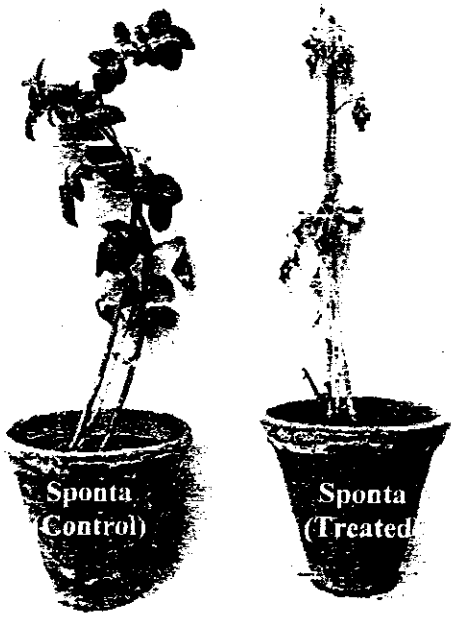

Fig. 5. Symptoms of bacterial wilt (brown-rot) disease of potato plant (sponta potato cultivar) infected with *R.solanacearum* under green-house conditions.

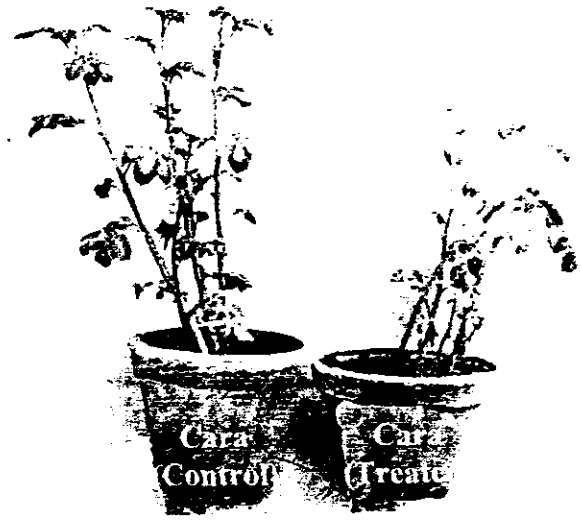

Fig. 6. Symptoms of brown-rot disease of potato plant (Cara potato cultivar).

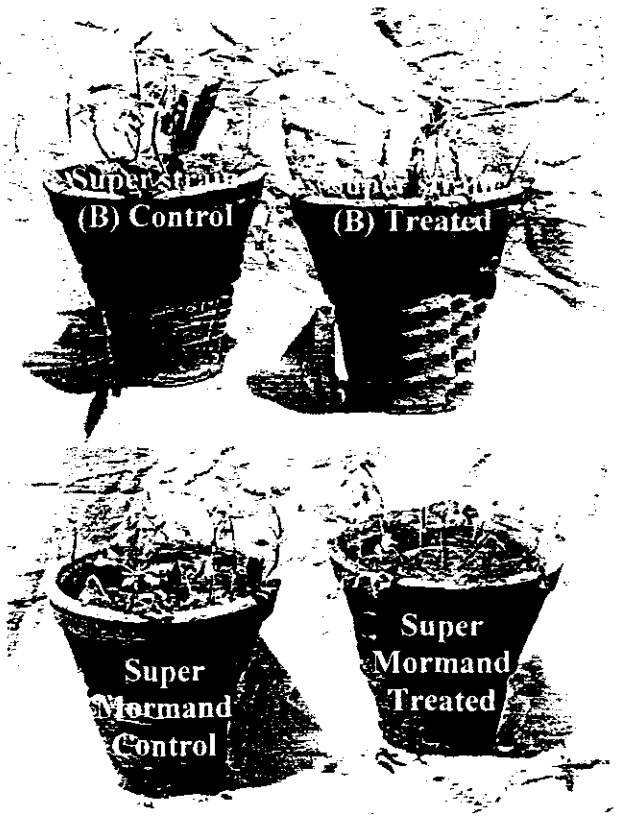

Fig. 7. Symptoms of wilt disease of two strains of tomato plant caused by *R.solanacearum* under greenhouse conditions.

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تعريف ومرضية البكتريا المسببة لمرض الذبول فى نبات البطاطس فى بعض أنواع من التربة المصرية

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مرض الذبول البكتيريا المتسبب عن بكتيريا سيدوموناس سولاناسيرم يسبب خسارة فادحة لاسيما فى البلاد الاستوائية او تحت الإستوائيه وتهدف تلك الدراسة إلى تعريف وتحديد مرضية البكتريا المسببة لمرض العفن البنى والذبول فى نبات البطاطس المنزرعة فى محافظتى الدقهلية والشرقية بمصر . وكانت النتائج المتحصل عليها هى :

١- وجد من بين ٢٠ عزلة بكتيريه ١٣ فيها تبدو مستعمراتها بلون أبيض بمركز أحمر (حادة مرضياً) ، ٧ عزلات تبدو مستعمراتها بلون أحمر غامق (غير حادة مرضياً) وذلك على البيئة الغذائية وهذه المستعمرات عرفت ببكتريا سيدوموناس سولاناسيرم طرز رقم ٢ .

٢- من بين ١٣ عزلة (المرضية) أظهرت العزلة رقم ١أعلى نسبة إصابة وكانت ٥ عزلات متوسطة الإصابة ، وأظهرت ٧ عزلات أقل نسبة إصابة وذلك على الصنف سيونتا تحت ظروف الصوبة .

٣- بأختبار مدى قابلية خمسة أصناف بطاطس لمرض الذبول البكتيرى حسب ظروف الصوبة لوحظ أن :
أ - صنفى كارا وسبونتا أكثر قابلية للإصابة .
ب -صنفى دراجاوسولانى متوسطا القابلية للإصابة .
ج- الصنف دايمونت أكثر مقاومة للمرض وبذلك يعتبر مصدرا للمقاومة تحت الظروف المصرية .

٤- وجد كذلك أن صنفين من الطماطم يصابا بنسبة كبيرة بهذه البكتريا الممرضه ولكن تكون الإصابة ضعيفة فى صنفين من الباذنجان وهما بلدى ورومى وكذا فى صنفين من الفلفل وهما سلالة ٣١٣ أ ، ٣١٣ ب لذا فإن البكتريا تتبع سلالة رقم ٣ .