Results

Results

1.Isolation and Identification of *R. solanacearum* isolates collected from different Habitats.

Data in Table (1) illustrates the results of isolation and identification of twenty two collected isolates from different localites (Dakahlia, Monufia ,and Ismailia)Governorates and different sources (potato tubers and soil) .13 isolates collected from Dakahlia Governorate from two potato cultivars (Spunta and Draga), 8 isolates from Monufia from two sources that were potato cutivars (Draga, Spunta and Lady rosetta) and clay soil but the only one isolate collected from Ismailia was from potato cultivar (Draga). Only 9 isolates out of the 13 isolates from Dakahlia were found positive result in all carried out tests. While 4 isolates were negative. But only one isolate out of eight isolates isolated from Monufia as well as the isolate from Ismailia gave negative result.

All isolates showed the typical morphological growth on SMSA medium **Fig.(11)** which considered positive result except three isolates K1,K4 and k18 which gave non typical colonies on SMSA media.

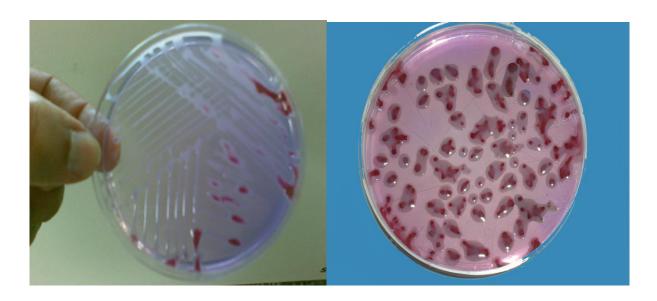


Fig. (11) Colony morphology on SMSA media

positive result Also all isolates gave when tested **IFAS** (Immunoflurescence Microscope Antibody Staining) except 4 isolates K1, K2 K4 and K8 in which they showed no florescence rod shape Fig. (13). However, pathogenicity test Fig. (12) (Bioassay test) for all isolates showed different percentages of infection & disease severity ranging from 0 % infection to 83.8 % based on the scale equation. The most disease severity obtained with K3 isolates isolated from Dakahlia governorate. Re IFAS test for the isolates after isolated from artificial infected tomato plants in pathogenicity test indicated that all bacterial isolates gave positive result IFAS test except four isolates K1, K4, K8 and K18, and the same result obtained by polymerase chain reaction test Fig.(14, 15) compared to the reference isolate of Ralstonia Solanacearum .



Fig.(12)Sever wilt symptoms on tomato and potato plants caused by R. solanacearum

N.C: Negative control (water treatment)

P.C: pathogen control (pathogen treatment)

Results

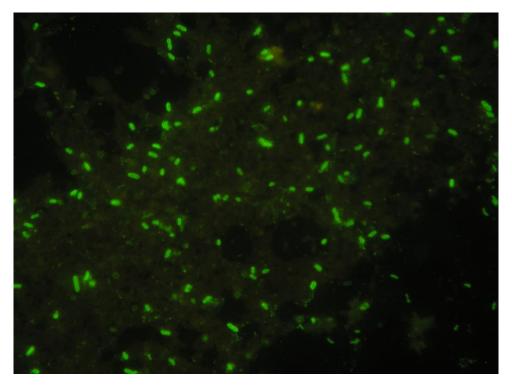
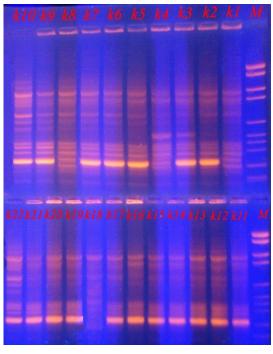


Fig.(13) Cell morphology of R. solanacearum by IFAS testing



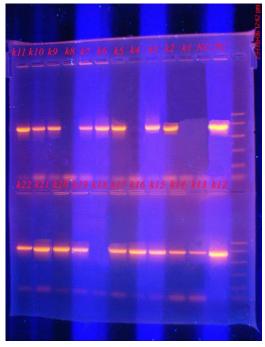


Fig. (14)

Fig. (15)

Agarose gel electrophorasis of Box PCR for Different isolates From K1 to K22

M= Marker

Agarose gel electrophorasis of Bastrik PCR PC. = Refernce control strain N.C= Ultra pure water control

Table (1) Isolation and identification of R. solanacearum isolates collected from different habitats.

^{*}D.S: Disease severity.

G 1	T. 6 1		*G * 4G *	**************************************	Bioassay		*Re IF	*DCD
Code	Type of sample	location	*SMSA	*SMSA *IFAS	% infection	D.S		*PCR
1K	Potato(Draga)	Dakahlia	-	-	0	0	-	-
2K	Potato(Draga)	Dakahlia	+	-	52.6	3	+	+
3K	Potato(spunta)	Dakahlia	+	+	83.8	4	+	+
4K	Potato(Draga)	Dakahlia	-	-	0	0	-	-
5K	Potato(Draga)	Dakahlia	+	+	0	0	+	+
6K	Potato(Draga)	Dakahlia	+	+	68.6	4	+	+
7K	Potato(Draga)	Dakahlia	+	+	66.6	4	+	+
8K	Potato(Draga)	Dakahlia	+	-	0	0	-	-
9K	Potato(Draga)	Dakahlia	+	+	72.6	4	+	+
10K	Potato(Draga)	Dakahlia	+	+	78.4	4	+	+
11K	Potato(Draga)	Dakahlia	+	+	70.3	4	+	+
12K	Potato(Draga)	Dakahlia	+	+	70.2	4	+	+
13K	Potato(spunta)	Monufia	+	+	58.2	3	+	+
14K	Potato(nicola)	Monufia	+	+	54.3	3	+	+
15K	Potato(nicola)	Monufia	+	+	46.2	3	+	+
16K	Potato(ladyrosetta)	Monufia	+	+	76.6	4	+	+
17K	Potato(spunta)	Dakahlia	+	+	74.3	4	+	+
18K	Potato(Draga)	Ismailia	-	+	0	0	-	-
19K	Potato(spunta)	Monufia	+	+	72.4	4	+	+
20K	Clay soil	Monufia	+	+	53.2	3	+	+
21K	Clay soil	Monufia	+	+	70.4	4	+	+
22K	Clay soil	Monufia	+	+	72.3	4	+	+

^{*}PCR : Pastrik PCR & Box PCR

Percent of infection and disease severity according to mentioned equation

2. Identification of R. solanace arum isolates collected from different localites using classical methodology (biochemical test).

^{*}IF:immunofurescent microscope assay staining

^{*}Re IF: confirmation test after it has been isolated from tomato plants in (Bioassay test).

^{*}SMSA: Semi selective Media of South Africa

⁺⁼ positive result

^{- =} Negative result

Results

Based on the biochemical characteristic of bacterial isolates Table (2) illustrates that all isolates were negative to gram stain except three isolates which are gram-positive K1, K4 and K18. Data also illustrated that catalase and oxidase reduction were negative with K1, K8 and K18 while K4 was negative with catalase reduction and the rest isolates were positive catalase and oxidase reduction. The bacterial isolates failed to hydrolyse starch except K1 and K8. The data also indicate that all isolates gave negative results in indole formation test except K4 and K18.

The bacterial isolates reduced nitrate except K1 and K8 and only bacterial isolate K18 could liquefy gelatin however, arginine was hydrolysed by both K4 and K18. All isolates appeared to have a flagella which facilitate their motility except K4 and K8.

Data in **Table** (3) illustrated that some bacterial isolates could utilize sugars as a nutrient source but others could not. All isolates could utilize lactose, maltose, cellobiose, glucose, galactose, mannose and fructose but could not utilize mannitol, sorbitol and dulcitol except K1, K4, K8 and K18 bacterial isolates which showed different utilization of sugars as follow, all of them could not utilize lactose, mannose and fructose but they can utilize glucose. K4, K8 and K18 isolates could utilize mannitol but could not utilize cellobiose. Bacterial isolates K1, K8 and K18 cold not utilize galactose. Bacterial isolate K4 was the only one which could utilize sorbitol sugar.

Results

Table (2) Identification of R. solanace arum isolates collected from different localites using classical methodology (biochemical test).

Isolates	Gram	Catalase	Oxidase	Starch	Indole
	stain	reduction	reduction	hydrolysis	formation
K1	+	-	-	+	-
K2	-	+	+	-	-
К3	-	+	+	-	-
K4	+	-	+	-	+
K5	-	+	+	-	-
K6	-	+	+	-	-
K7	-	+	+	-	-
K8	-	-	-	+	-
К9	-	+	+	-	-
K10	-	+	+	-	-
K11	-	+	+	-	-
K12	-	+	+	-	-
K13	-	+	+	-	-
K14	-	+	+	-	-
K15	-	+	+	-	-
K16	-	+	+	-	-
K17	-	+	+	-	-
K18	+	-	-	-	+
K19	-	+	+	-	-
K20	-	+	+	-	-
K21	-	+	+	-	-
K22	-	+	+	-	-

Cont. Table(2)

Isolates	Nitrate	Gelatin	motility	Arginine
	reduction	liquefaction		dihydrolase
K1	-	-	+	-
K2	+	-	+	-
К3	+	-	+	-
K4	+	-	-	+
K5	+	-	+	-
K6	+	-	+	-
K7	+	-	+	-
K8	-	-	-	-
К9	+	-	+	-
K10	+	-	+	-
K11	+	-	+	-
K12	+	-	+	-
K13	+	-	+	-
K14	+	-	+	-
K15	+	-	+	-
K16	+	-	+	-
K17	+	-	+	-
K18	+	+	+	+
K19	+	-	+	-
K20	+	-	+	-
K21	+	-	+	-
K22	+	-	+	-

Table (3) :Identification of R. solanacearum isolates collected from different sources. (Utilization of different sugars)

Isolates	lactose	mannitol	maltose	cellobiose	sorbitol
K1	-	-	+	+	-
K2	+	-	+	+	-
К3	+	-	+	+	-
K4	-	+	+	-	+
K5	+	-	+	+	-
K6	+	-	+	+	-
К7	+	-	+	+	-
K8	-	+	-	-	-
К9	+	-	+	+	-
K10	+	-	+	+	-
K11	+	-	+	+	-
K12	+	-	+	+	-
K13	+	-	+	+	-
K14	+	-	+	+	-
K15	+	-	+	+	-
K16	+	-	+	+	-
K17	+	-	+	+	-
K18	-	+	+	-	-
K19	+	-	+	+	-
K20	+	-	+	+	-
K21	+	-	+	+	-
K22	+	-	+	+	-

Con. Table (3)

Isolates	glucose	galactose	mannose	fructose	dulcitol
K1	+	-	-	-	+
K2	+	+	+	+	-
К3	+	+	+	+	-
K4	+	+	-	-	+
K5	+	+	+	+	-
K6	+	+	+	+	-
K7	+	+	+	+	-
K8	+	-	-	-	-
К9	+	+	+	+	-
K10	+	+	+	+	-
K11	+	+	+	+	-
K12	+	+	+	+	-
K13	+	+	+	+	-
K14	+	+	+	+	-
K15	+	+	+	+	-
K16	+	+	+	+	-
K17	+	+	+	+	-
K18	+	-	-	-	+
K19	+	+	+	+	-
K20	+	+	+	+	-
K21	+	+	+	+	-
K22	+	+	+	+	-

3. Sensitivity of different potato cultivars to the infection by *Ralstonia* solanacearum.

Data in table (4) reported that the potato cultivar type (Spunta) were the most sensitive cultivar to *Ralstonia solanacearum* which showed (% of infection 88.4, 69.1 and disease severity 4) cultivated in both infested soil and infested field soil respectively, followed by Lady rosetta cultivar which showed (% infection 67.8, 54.2 and disease severity 4, 3) in both types of soils. The least sensitive cultivar to infection by *Ralstonia solanacearum* was Nicola cultivar where infection percent was 57.1, 50 and disease severity 3. Also there are differences in the bacteria pathogenicity to potato cultivars planted in infested soil previously sterilized and infested field soil where the sensitivity of potato cultivars decreased in infested field soil than infested soil previously sterilized.

Table (4) Sensitivity of different potato cultivars to the infection by *Ralstonia* solanacearum.

Potato cultivars	Infested soil *		Infested Field soil **		Control soil***	
	% infection	Disease severity	% infection	Disease severity	% infection	Disease severity
Spunta	88.4	4	69.1	4	0	0
lady rosetta	67.8	4	54.2	3	0	0
Nicola	57.1	3	50	3	0	0

4.Count of *Ralstonia solanacearum* bacteria ($x10^3$)/ 100 μ l attracted by different potato cultivar root exudates on (SMSA) media.

Data in Table (5) represent that the root exudates collected after 40 days from potato plantation obtained from Spunta cultivar cultivated in non infested soil (sterilized) attracted large number of pathogen *Ralstonia solanacearum* with the value $84.2 \text{ x} 10^3 \text{ CFU}/100 \text{ }\mu\text{l}$ root exudates than both Lady rosetta and Nicola cultivars root exudates which attracted less number of bacteria with values 51.58, $20.06 \text{ CFU}/100 \text{ }\mu\text{l}$ root exudates respectively cultivated in the same type of soil.

The data also illustrated that the attractive force of potato cultivar root exudates to the pathogen *Ralstonia solanacearum* decreased when potato cultivated in both infested soil and field soil as well as when the root exudates collected after 20 days from potato plantation. The number of attracted bacteria by the root exudates decreased to values 13.1 , 11.03 and 9.77 CFU/ 100 μ l root exudates for Spunta , Lady rosetta and Nicola root exudates respectively cultivated in sterilized soil. However ,in case of water control where water is used instead of potato root exudates attracted only 0.008 $\times 10^3/100~\mu$ l root exudates.

^{*} infested soil : previously sterilized then infested with Ralstonia solanacearum bacteria.

^{**} infested field soil : not sterilized but infested with Ralstonia solanacearum directly .

^{***} control soil : sterilized soil only.

Table (5)Count of *Ralstonia solanacearum* bacteria ($x10^3$)/ 100 μ l attracted by different potato cultivar root exudates on semi selective media of south africa (SMSA).

Potato cultivars	After 20 days*			After 40 days*		
	Sterilized soil	Infested soil**	Field soil	Sterilized soil	Infested soil	Fiel
Spunta	13.1	0.43	0.3	84.2	1.7	0.8
lady rosetta	11.03	0.22	0.1	51.58	0.5	0.3
Nicola	9.77	0.11	0.07	20.06	0.05	0.3
Water (control)	0.008	0.008	0.008	0.008	0.008	0.0

^{*} after 20 and 40 days : from potato plantation

^{**} infested soil : previously sterilized soil then infested with *R. solanacearum*

5.Effect of different concentrations of salicylic acid on the pathogenicity of *Ralstonia solanacearum* for tomato plants after 10 days from soil infestation and count of bacteria on SMSA media for two bacterial load.

It is clear from the data represented in **Table (6)** that different salicylic acid concentrations reduced both percent of infection and disease severity of the pathogen as well as count of bacteria in tomato roots; As the salicylic acid concentration increased, the values of percent of infection and disease severity decreased, the highest concentration 8mM reduced percent of infection and disease severity caused by bacterial load *Ralstonia solanacearum* (10^8 and 10^6 cfu/ml) to zero value as well as count of bacteria reached zero in tomato roots while the pathogen (control) represented 92.4and 64.2 % infection, 4 and 4 disease severity respectively and the count of bacteria in tomato roots reached its highest value in both bacterial load (48.3×10^8 and 113×10^3 CFU/g root respectively) in case of treatment pathogen (control).

Table (6) Effect of different concentrations of salicylic acid on the pathogenicity of *Ralstonia solanacearum* for tomato plants after 10 days from soil infestation and count of bacteria on SMSA media for two bacterial load.

Salicylic acid	% infection		Disease severity		Count CFU/g root ×1	
concentrations	10 ⁸ Cfu/ml*	10 ⁶ Cfu/ml*	10 ⁸ Cfu/ml	10 ⁶ Cfu/ml	10 ⁸ Cfu/ml	10 ⁶ Cfu
3mM	44.2	16.8	3	2	6.5	4.9
4mM	26	8	2	1	0.6	0.34
5mM	18	4	2	1	0.02	000
6mM	8	3	1	1	0.005	0.000
7mM	2	0	1	0	0.002	0
8mM	0	0	0	0	0	0
Water control	0	0	0	0	0	0
Pathogen control	92.4	64.2	4	4	48.3×10 ⁶	113×1

^{*} 10^8 Cfu/ml and 10^6 Cfu/ml two concentration of Ralstonia solanacearum suspention

6.Effect of different concentrations of salicylic acid on the pathogenicity of *Ralstonia solanacearum* for potato plants After 14 days from soil infestation .

The data in **Table** (7) showed that different concentrations of salicylic acid reduced the pathogenicity of the pathogen as indicated by the percent of infection and disease severity, by increasing the concentration of salicylic acid the pathogenicity of pathogen decreased. in case of 5mM concentration the % infection and disease severity with all potato cultivars (Spunta, Laddy rosetta and Nicola) were 29.5, 14.8 and 12.3 % infection and 2 disease severity respectively while the concentration increased to 7mM the % infection reduced to 9, 4 and 2.5% with 1 disease severity respectively. The highest concentration 9mM caused completely inhibition to *Ralstonia solanacearum* that reduced the pathogenicity to the three potato cultivars to zero value of % infection and disease severity compared to pathogen control. The data also reported that Spunta cultivar showed less sensitivity to induction by salicylic acid than Lady rosetta and Nicola cultivar only in case of low concentrations 5mM and 7mM.

Table (7) * Effect of different concentrations of salicylic acid on the pathogenicity of *Ralstonia solanacearum* for potato plants After 14 days from soil infestation .

Cultivar Spunta Lady rosetta Nicola	
-------------------------------------	--

Salicylic acid treatments	% infection	Disease severity	% infection	Disease severity	% infection	Disease severity
5mM	29.5	2	14.8	2	12.3	2
7mM	9	1	4	1	2.5	1
9mM	0	0	0	0	0	0
Water control	0	0	0	0	0	0
Pathogen control	100	5	94.3	4	90.8	4

^{*} disease severity and %infection recorded after 14 days from soil infestation

7. Count of *Ralstonia Solanacearum* in potato cultivars roots after salicylic acid concentrations treatment on SMSA media.

It is appear from the data in **table (8)** that different concentrations from salicylic acid reduced the number of bacteria in the three potato root cultivars compared to pathogen control. As the salicylic acid concentration increased the count of bacteria in potato root cultivars decreased .The highest concentration 9mM of salicylic acid decreased the number of bacteria to zero value with all potato cultivars which resembling water control result . Spunta cultivar showed highest number of bacteria in the root than Lady rosetta and Nicola culivars in case of low concentrations 5mM and 7mM.

Table (8) Count of *Ralstonia Solanacearum* in potato cultivars roots after salicylic acid concentrations treatment on SMSA media.

Salicylic acid	Count CFU/g root x10 ²					
concentrations	Spunta	Lady rosetta	Nicola			
5mM	590	330	254			
7mM	21.2	9.8	0.34			
9mM	0	0	0			
Water control	0	0	0			

Pathogen control	60×10 ⁶	105×10 ⁵	88×10 ⁵
i amogen control	00 /10	105/10	00/10

^{*} bacterial count after 14 days from soil infestation .

8.Effect of different concentrations of β amino butyric acid on *Ralstonia Solanacearum* pathogenicity in tomato plants after 5 and 10 days from soil infestation and count of bacteria in tomato roots on SMSA media.

The data in **table** (9) represented that different concentrations of β - amino butyric acid played an effective role in reducing *Ralstonia solanacearum* pathogenicity to tomato plants as well as count of bacteria invading tomato roots . Both percent of infection and disease severity reduced by increasing concentration of β - amino butyric acid as in case of 20 µg/mL the percent of infection reduced from 50 %to 20% and disease severity from 3 to 2 after 5 and 10 days from soil infestation respectively by increasing the β - amino butyric acid concentration to 40 µg/mL the percent of infection from 13.3 % to 6.67 % infection and disease severity reduced from 2 to 1 .

However three concentrations 60,80 and 100 $\,\mu g/mL$ protect tomato roots against infection by bacteria where the percent of infection and disease severity reduced to zero value after 10 days from soil infestation as in case of water control also count of bactertia in tomato roots also affected by increasing β amino butyric acid concentration that reached zero value compared to pathogen control . The lowest concentration that able to completly protect tomato plants were 80 $\mu g/mL$.

Table (9) Effect of different concentrations of β -amino butyric acid on R. Solanacearum pathogenicity in tomato plants after 5 and 10 days from soil infestation and count of bacteria in tomato roots on SMSA media.

Concentrations	After 5 days from	soil infestation	After 10 day infests	ount /g root X 10 ²	
μg/mL	% infection	Disease severity	% infection	Disease severity	fter 10 days from soil infestation
20	50	3	20	2	14.6
40	13.3	1	6.67	1	0.5
60	4.2	1	0	0	0
80	0	0	0	0	0
100	0	0	0	0	0
βABA control	0	0	0	0	0
Pathogen control	52	3	100	5	136.2×10 ⁷
Water control	0	0	0	0	0



Fig. (16) Effect of different concentrations of β amino butyric acidon Ralstonia solanacearum in tomato plants

N.C: water control P.C: pathogen control



Fig. (17) Effect of soil amended with (calcium oxide with urea) on *Ralstonia*solanacearum pathogenicity on tomato plants
N.C: water control
P.C: pathogen control
9.Effect of different concentrations of β-ABA on *Ralstonia Solanacearum*pathogenicity in potato plants after 7 and 14 days from soil infestation .

The data in table (10) showed that different concentrations of β - amino butyric acid decreased *both* percent of infection and disease severity of *Ralstonia solanacearum* bacteria potato plants as well as the count of bacteria invading the potato roots ,both of them reduced by increasing concentration of β amino butyric acid as in case of 20 µg/mL the percent of infection reduced from 43.3 % to 23.3 % and disease severity from 3 to 2 after 7 and 14 days from soil infestation respectively. By increasing β - amino butyric acid concentration to 40 µg/mL percent of infection reduced from 12.4 % to 8.6 % infection and disease severity ffrom 2 to 1.

However the three concentrations 60.80 and $100~\mu g/mL$ reduced the percent of infection and disease severity to zero value after 7 and 14 days from soil infestation resembling water control also count of bactertia in potato roots also affected by increasing β -amino butyric acid concentration that reached zero value compared to pathogen control .The lowest concentration that able to completely protect potato plants were $60~\mu g/mL$.

Table (10) Effect of different concentrations of β -ABA on *Ralstonia Solanacearum* pathogenicity in potato plants after 7 and 14 days from soil infestation .

Concentrations	After 7 days from	soil infestation	After 14 day infesta	ount /g root X 10 ²	
μg/mL	μg/mL % infection Di		% infection	Disease severity	fter 14 days from soil infestation
20	43.3	3	23.3	2	126.3
40	12.4	2	8.6	1	0.26
60	0	0	0	0	0
80	0	0	0	0	0
100	0	0	0	0	0
βABA control	0	0	0	0	0
Pathogen control	56.3	4	97.8	4	129.3×10 ⁶
Water control	0	0	0	0	0

10.Effect of soil amended with (calcium oxide with urea) on R. solanacearum pathogenicity on tomato and potato plants after different periods from soil infestation.

It is clear from the data in **table** (11) that the application of mixture consist of CaO and Urea to soil cultivated with potato or tomato plants are leading to reduce the virulence of the pathogenicity of *R. Solanacearum* and that decrease both the percent of infection and disease severity in both plants. In soil amended with CaO and Urea cultivated with tomato plants the percent of infection and disease severity reduced to 26% & 2 respectively compared to pathogen control which showed 96 %, 4 after 10 days from soil infestation, while water control gave 0% of infection and disease severity, also soil cultivated with potato plants amended with CaO and Urea showed 22%, 2 while pathogen control represent 100 % infection and 5 disease severity after 14 days from soil infestation.

Table(11)Effect of soil amended with (calcium oxide with urea) on *Ralstonia solanacearum* pathogenicity on tomato and potato plants after different periods from soil infestation.

		Tomato plants				Potato plants			
Treatments	After 5 days from soil infestation		After 10 days from soil infestation		After 7 days from soil infestation		After 14 days from soil infestation		
	% infection	Disease severity	% infection	Disease severity	% infection	Disease severity	% infection	Disease severity	
Infested soil* with CaO+Urea	20	2	26	2	12	2	22	2	
Pathogen control**	94	4	96	4	100	5	100	5	
Water control	0	0	0	0	0	0	0	0	

^{*} infested soil : previously sterilized then infested with bacteria and Cao+Urea were applicated ** Pathogen control:tomato plants in Ralstonia solanacearum infested soil.

11.Count of *Ralstonia solanacearum* bacteria in soil amended with (calcium oxide with urea) cultivated with potato plants after time course on SMSA media.

The data in **table (12)** illustrated that the logarithmic count of bacteria in soil amended with CaO ad Urea decreased with time compared to pathogen control. the count of bacteria reduced slightly till reaching Log ₁₀ 2 Cfu / ml for soil infested with *Ralstonia Solanacearum* and amended with CaO and Urea after 28 days from soil infestation while pathogen control (soil cultivated with potato infested with bacteria) reaching Log ₁₀ 8.4Cfu / ml after 28 days from soil infestation.

Table (12) Count of $Ralstonia\ solanace arum\$ bacteria in soil amended with (calcium oxide with urea) cultivated with potato plants after time course on SMSA media.

		Log ₁₀ Count of bacteria on SMSA media Cfu / g dry soil							
Treatment	Time	0time	3 days	7 days	10 days	14 days	21days	28 days	
Infested soil* Cao+Ure		10.4	8.01	7.1	5.9	5.2	3.4	2	
Water cont	rol	0	0	0	0	0	0	0	
Pathogen con	trol**	10.4	10.1	9.02	8.3	9.5	9.1	8.4	

12.Effect of both Chinese chive and sweet basil plants on population of *Ralstonia solanacearum* by counting bacteria in infested soil with time course.

The data in **table** (13) indicated that both Chinese chive and sweet basil plants have high effectiveness on population of *Ralstonia solanacearum* in the soil. They reduced the count of bacteria in the soil with time course till reaching zero in count after 28 days from soil infestation resembling water control result while the count of bacteria in pathogen control treatment remained high till 28 days from soil infestation. No significant differences between Chinese chive and sweet basil plantation on the population of bacteria however both of them causing reduction in the number of bacteria in cultivated soil.

Table (13) Effect of both Chinese chive and sweet basil plants on population of *Ralstonia* solanacearum by counting bacteria in infested soil with time course.

		Lo	g ₁₀ Count / g	dry soil on S	MSA media	ı			
Plants	Days soil after soil infestation								
	0 time	3 day	7 day	10 day	14 day	21 day	28 day		
Chinese Chive	10.47	8.01	6.4	5.19	4.9	2.1	0		
Sweet Basil	10.4	8.1	7.1	4.9	4.7	3.8	0		
Water control	0	0	0	0	0	0	0		
oathogen control	10.5	10.4	9.4	9.2	8.9	6.9	5.8		

13. Studying effect of Chinese chive and sweet basil root exudates on cultured *Ralstonia Solanacearum* in basal media

The data in **table (14) (Fig.18,19,20)** indicated the both root exudates of Chinese chive and sweet bail causing an inhibition zones around discs suspended with these root exudates against *R. solanacearum* bacteria compared to water control discs. The diameter of the inhibition zones were 10.4 mm for Chinese chive root exudates and 10.2 mm for sweet basil root exudates . while the water control doesn't possess any inhibition zone. There were slightly difference between the inhibition zones caused by both root exudates .

Table (14) Effect of Chinese chive and seet basil root exudates on cultured *Ralstonia Solanacearum* on basal media

Root exudates	Diameter of inhibition zone range
Chinese chive	10.4mm
Sweet basil	10.2mm
Water control	0

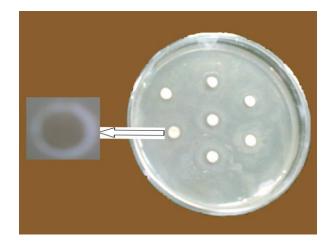


Fig.(18) inhibition zone caused by sweet basil root exudates

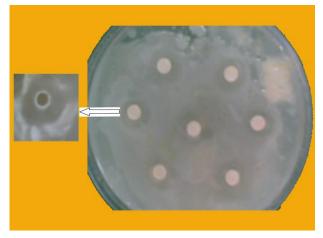


Fig. (19) inhibition zone caused by Chinese chive root exudates



Fig. (20) No inhibition zone in water control

Table (15) Chemical analysis of root exudates of different potato cultivar in different type of soil by GC mass detector.

It is clear from the result in **Table 14** that

Composition of root exudates of different potato cultivars collected after 20 days from potato culivation showed variation in different types of soils, this variation in composition illustrated that spunta cultivar cultivated in soil sterlilized but infested with *R.solanacearum* secrete wide variety of root exudates that differed from those secreted in (sterilized soil) non infested soil, compound such as ethanethioc acid s-tetradecyl ester ,cyclotetradecane, hexadeconic acid methyl ester showed high concentration in spunta root exudates cultivated in infested soil collected after 20 days from potato plantation as follow (34.3%, 32.1 and 24.6%) consequently, while spunta cultivar cultivated in (sterilized soil) non infested soil secreted haxanoic acid and hexanoic acid methyl ester with highest concentration (37.8% and 8.25%) respectively. Spunta root exudates cultivated in field soil (non sterilized soil) infested with R. solanacearum showed difference in its composition than cultivated in field soil only as they secrete glycerol tricaprylate, 2,4 -di--t-butylphenol and O-Menth-2-ene,4-isopropylidene-1-vinyl with highest concentration as (27.7%, 12.85% and 12.4%) respectively while in field soil they secrete neophytadiene and 1-nonadecene (cas)1-docosanol acetatehentriacontane with concentration (8.71% and 8.25%) consequently. In case of lady rosetta root exudates showed different composition when cultivated in soil previosly sterilized then infested with R. solanacearum than cultivated in non infested soil .They secret 4-methyl-1-(1-methylethyl)3-cyclohexene-1-ol and 2,4 -Dinitroacetanilide with concentration (16.31%, 8.3%) in infested soil while in non infested soil they secrete octadecane, butyl citrate and hexanoic acid with concentration (26.4, 17.2 and 13.79 %) however lady rosetta cultivar root exudates in infested field soil contain 1,2-benzenedicarboxylic acid butyl octyl ester, tricontane, dibutyl phthalate and cyclic tetramethylene sulfone with highest concentration (40.12%, 29.56%, 25.46% and 21.47%) respectively but in field soil they revealed the highest concentration of linoleic acid, palmitic acid methyl ester (53.98%, 21.5%) consequently. Nicola culivar roots also secreted (7-

methyl-3H-phenoxazin -3-one , bis (2-ethylhexyl) phthalate and palmitic acid,methyl ester with highest concentration (18.22%, 17.32% and 14.69%) when cultivated in infested soil ,but when cultivated in non infested soil they secreted bentafluroaniline , cyclotetradecane and tributyl acetyli citrate with concentration (18.46, 16.7 and 14.65) , compound such as Linoleic acid methyl ester , Bis (2-ethylhexyl) phthalate were secreted with highest concentration (40.78%, 36.44%) . When Nicola cultivar cultivated in infested field soil , compound such as P-methoxyphenol secreted with concentration 19.12 %.

Generally the amount of compounds found in root exudates increased in infested soil than that found in non infested soil .

The data in Table (15) also illustrated that root exudates collected after 40 days from potato cultivation revealed significant difference in its composition than collected after 20 days.

Spunta root exudates cultivated in infested soil contain compounds such as diisooctyl phthalate with the highest concentration 60.61% while that cultivated in non infested soil phthalic acid diisobutyl ester secreted with concentration 36.76%. In field soil infested with *R.solanacearum* root exudates of Spunta , Laddy rosetta contained compound such as diisooctyl phthalate with highest concentration 52.93% , 73.53% while exudates of Nicola contain hexadecanoic acid , Transphytol with concentration 39.7%,18.36% respectively. However in non infested soil lady rosetta root exudates contain diisooctyl phthalate with conc. 30.61% while Nicola cultivar exudates contain methyl pyroglutamate and 2-propoxymethanol 3,3-DL-glutamic acid with conc. 16.97% and 15.71% . In field soil (non infested) exudates of Spunta and lady rosetta contain diisooctyl phthalate with conc. 30.61% and 59.62 % while exudates of Nicola contain hexadecanoic acid and trans-phytol with conc. 27.14% and 18.1% .

Table (16) Chemical analysis of root exudates of Spunta Cultivar after different soil amendments by GC mass detector.

Data in **Table (15)** illustrated that in case of Salicylic acid amended to soil cultivated with Spunta potato cultivar , root exudates revealed different composition than Spunta pathogen control (infested soil) in **Table (15)** so that diisooctyl phthalate , octanoic acid triglyceride , dicyclohexyl phthalate and dodecenyl succinic anhydride appeared in root exudates with concentration (26.94%, 17.4%, 14.12% and 11.75%) respectively. When the soil amended with β- amino butyric acid Spunta root exudates contain 1-octadecanol, 8-octadecanoic acid methyl ester , trans-phytol , gamma- aminobutyric with conc. (30.34%, 18.01%, 15.9% and 12.04%) consequently. However root exudates in soil amended with CaO with Urea showed 6-phenyl-dodecane , P-chloro-m-xylenol and 2-phenyl-Dodecane with concentration (25.08%, 23.29% and 15.82%).

Result in **Table** (**16**) also revealed that nonadecane, 3-methyl-5-propyl-2-furannonanoic acid and octadecamethyl cyclononasiloxane with highest concentration (42.38%, 14.2% and 9.81%). While analysis of sweet basil root exudates by GC mass detector showed that rosmarinic acid, 3-allyl-6-methoxyphenol and 1,2-benzene dicarboxylic acidphenyl methyl ester found with concentration (32.19%, 14.33% and 10.01%) respectively.

Chinese chive root exudates contain 1-