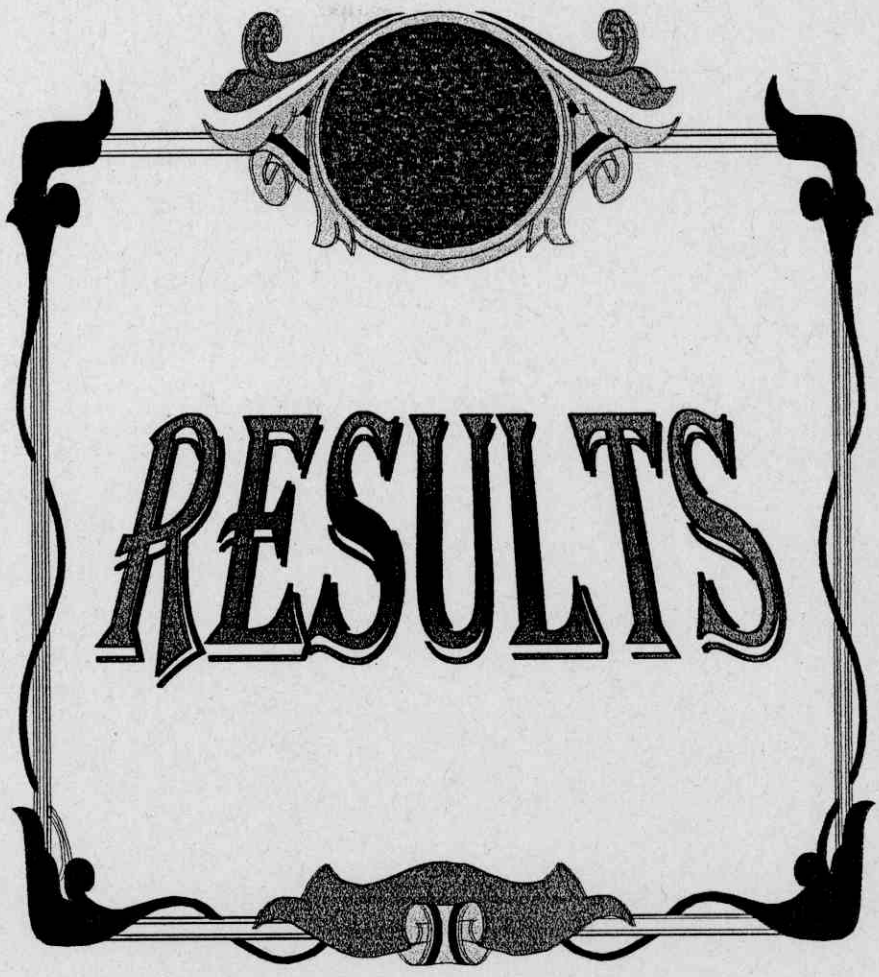
RESULTS

RESULTS

1. Isolation of fungi and bacteria associated to rotted dahlia tubers

Sum of 107 fungal and 55 bacterial isolates were obtained from naturally infected dahlia tubers in three locations i.e. Moshtohor, El-Dair and El-Manashy in Kalubia Governorate. Data shown in **Table (4)** indicate that the isolated fungi belong to 6 genera and 7 species. Fungi were identified as: *Sclerotium rolfsii*, *Fusarium* sp., *Rhizoctonia solani*, *Aspergillus niger*, *Aspergillus flavus*, *Penecillium digitatum* and *Rhizopus* sp.

S. rolfsii was more occurrence in all tested samples comparing to the other isolated fungi. The highest isolation number and frequency % of *S. rolfsii* was recorded in El-Dair location (70.0%) followed by El-Manashy (60.0%) and Moshtohor (54.1%) locations. The other fungi were isolated within small numbers and frequency % affecting location. On the other hand, the isolated bacteria from rotten dahlia tubers were identified and discussed for their pathogenic abilities in further part. The highest frequency% was recorded in El-Manashy followed by Moshtohor and El-Dair locations i.e. 42.2%, 32.7% and 27.3% respectively.

2. Pathogenicity test of fungal pathogens

Data in **Table (5)** show that *S. rolfsii* is the most pathogenic of tested fungi in inducing rotting of dahlia tubers where the infection area increased gradually to decay 17 mm after the first day from infestation, this area reached 50mm after three days of infestation with 34-100 infection % respectively.

Meanwhile, *R. solani* followed by *Fusarium* spp. revealed infection % ranged between 22-28 and 20-22%, for both fungi after the first and third day, respectively.

Table (4): Frequency (%) and percentage of fungi and bacteria isolated from dahlia tubers represent three different locations in Kalubia governorate.

Isolated microorganisms	Location of isolation						
	Moshtohor		El-Dair		El-Manashy		Total
	Fre.	%	Fre.	%	Fre.	%	
Fungi:	37	67.27	40	72.73	30	57.69	107
Sclerotium rolfsii	20	36.36	28	50.91	18	34.62	66
Fusarium spp.	0	0.00	1	1.82	0	0.00	1
Rhizoctonia solani	5	9.09	0	0.00	2	3.85	7
Aspergillus niger	3	5.45	2	3.64	2	3.85	7
Aspergillus flavus	2	3.63	2	3.64	2	3.85	6
Penecillium digitatum	3	5.45	3	5.45	3	5.77	9
Rhizopus sp.	4	7.27	4	7.27	3	5.77	11
Bacteria	18	32.70	15	27.27	22	42.31	55
TOTAL	55		55		52		162

Table (5): Pathogenic abilities of three chosen fungi isolated from rotted dahlia tubers.

Pathogenic fungi	1 day		3 days	
	*IA (mm)	%	IA (mm)	%
Sclerotium rolfsii	17	34.0	50	100.0
Fusarium spp.	10	20.0	11	22.0
Rhizoctonia solani	11	22.0	14	28.0

*IA = infected area (mm)

3. Physical factors affecting growth and sclerotial formation of *S. rolfsii:*

3.1. Different media

Data presented in **Table (6)** clearly show that *S. rolfsii* was able to grow on most of the tested media. The highest average of linear growth was obtained on PDA where the fungus filled the plate after 4-days post inoculation, followed by dahlia tuber agar, Brown's media, while the lowest average of linear growth occurred on Czapek's medium.

Concerning sclerotial formation, media of PDA followed by dahlia tuber and peptone were the most favourable media for producing sclerotia of *S. rolfsii*. Sclerotia were also produced on the other tested media but with small numbers ranged between 5-11 sclerotia/plate. On the other hand, it is clear that the highest weight of five sclerotia (g) was obtained on Brown's medium, while the least weight was in cases of peptone and dahlia tuber media.

The first sclerotium formed after 3 days on Brown's and Baren's media, while it formed after 9 days in case plain agar medium. Also, the first sclerotium was formed on PDA, dahlia tuber and peptone media after 7 and 8 days within the highest numbers.

Table (6): Effect of different media on the growth and sclerotium formation of *S. rolfsii*.

Media	Growth (mm) after days		No. of sclerotia/ plate	Weight of 5 sclerotia (g)	The first sclerotium formed after (days)
	2	4			
PDA	39	90	168	0.006	7
Dahlia tuber	36	84	67	0.003	7
Peptone	33	60	55	0.003	8
Czapek's	25	44	7	0.008	4
Brown	37	62	10	0.019	3
Baren's	27	56	11	0.009	3
Conne	36	61	6	0.011	8
Plain agar	34	56	5	0.013	9

3.2. Temperature

Seven temperature degrees were studied for their effect on the growth, sclerotia yield and formation of the first sclerotium of *S. rolfsii*. Data in **Table (7)** show that *S. rolfsii* could be grow within 15 - 40°C, while, no growth was recorded below 15°C. Maximum growth of the fungus was obtained at 30°C whereas, minimum growth was at 15°C.

The highest number of sclerotia was formed at 30°C (263 sclerotium/plate) and at 25°C (243 sclerotium/plate), while, the least number was at 40°C (94 sclerotium/plate). Also, no sclerotia were formed below 15°C. Maximum weight of 5 sclerotia obtained at 30 and 35°C (0.009 g) and 0.008 g at 25°C.

The minimum weight of 5 sclerotia was 0.007 g at 40°C. Therefore, the first sclerotium formed after 4 days at 40°C, but 15°C was the least favourable temperature degree tested for formation of sclerotia where, the first sclerotia appeared after 11 days.

Table (7): Effect of different temperature degrees on the growth and sclerotium formation of *S. rolfsii*.

Temperature (°C)	Growth (mm) after days		No. of sclerotia/ plate	Weight of 5 sclerotia (g)	The first sclerotium formed after (days)
	2	4			
10	0	0	0	0.0	0
15	15	37	108	0.009	11
20	18	42	162	0.007	7
25	25	83	243	0.008	7
30	37	90	263	0.009	5
35	39	60	112	0.009	5
40	27	50	94	0.007	4

3.3. Relative humidity

Results in **Table (8)** indicate that *S. rolfsii* could be grow at RH values ranged between 14-100%, however, the average linear growth has gradually increased by increasing RH values from 14 to 100% after four days incubation. The maximum growth was recorded between 80 -100% RH, resulting 67-63 mm after 4 days incubation. Meanwhile, 90% RH gave the best linear growth (71 mm). On the other hand 14 and 50% RH were not favourable for growth of *S. rolfsii*. In this respect also, all tested RH values were not suitable for sclerotial formation.

Table (8): Effect of different levels of relative humidity on the growth of *S. rolfsii.*

RH (%)	Growth (mm) after days		No. of sclerotia/ plate	Weight of 5 sclerotia (g)	The first sclerotium formed after (days)
	2	4			
100	48	63	0.0	0.0	0.0
95	47	66	0.0	0.0	0.0
90	49	71	0.0	0.0	0.0
85	41	65	0.0	0.0	0.0
80	46	67	0.0	0.0	0.0
74	41	62	0.0	0.0	0.0
50	34	53	0.0	0.0	0.0
14	29	46	0.0	0.0	0.0
Control	39	90	168	0.006	7

3.4. pH values

Data in **Table (9)** show that, acidity and alkalinity of the culture media play an important role on growth and sclerotial formation of *S. rolfsii*. In this respect, the optimum pH for growth of the fungus was pH 5 where the maximum growth scored after 6 days incubation (90 mm). Meanwhile, the lowest growth was obtained at pH 4 and 8 after 6 days incubation. Also, the results indicate that *S. rolfsii* could be grow at different pH values ranging from pH 3 to 8.

Concerning sclerotial formation, the best pH values for producing the highest number of sclerotia were pH 5 and 6 (172 and 120 sclerotium/plate), respectively. Meanwhile, the least number was scored at pH 8.0.

Moreover, the highest weight of 5 sclerotia was occurred when the fungus grown at pH 5 and 4 where the weight were 0.024 and 0.021 g, respectively, while, the least weight was at pH 7. Additionally, the first sclerotium formed after 6 days in medium adjusted at pH 8, followed after 7 days at pH 5.

Table (9): Effect of different pH values on the growth and sclerotial formation of *S. rolfsii*.

pH value	Growth (mm) after days			No. of sclerotia/ plate	Weight of 5 sclerotia (g)	The first sclerotium formed after (days)
	2	4	6			
4	28	47	70	112	0.021	8
5	31	55	90	172	0.024	7
6	31	53	75	120	0.017	8
7	21	41	59	97	0.016	8
8	12	19	28	55	0.019	6

3.5. Carbon sources

Data presented in **Table (10)** show that *S. rolfsii* isolate use many different carbon sources in nutrition such as glycerol, sucrose, glucose, starch, maltose, lactose, dextrin and arabinose. Glucose and lactose were the best carbon sources for growth of the fungus where, its growth was 90 and 83 mm, respectively after 6 days post inoculation. Glycerol and dextrin were the lowest favourable in this respect. It is clear also that starch, arabinose, maltose and sucrose were moderately affected growth of *S. rolfsii*. On the other hand, glucose, lactose and starch were the best carbon sources for sclerotial formation where, the formed sclerotia were 140, 134 and 72 sclerotium/plate,

respectively. Therefore, the highest weight of 5 sclerotia was in case of arabinose followed by glucose and sucrose respectively. Also, the first sclerotium formed on media containing dextrin after 2 days, while in presence of maltose or arabinose the first sclerotium appeared after 9 days post inoculation.

Table (10): Effect of different carbon sources on the growth and sclerotium formation of *S. rolfsii.*

Carbon Sources	Growth (mm) after days			No. of sclerotia/ plate	Weight of 5 sclerotia (g)	The first sclerotium formed after (days)
	2	4	6			
Glycerol	24	31	38	15	0.016	8
Sucrose	24	36	46	13	0.018	5
Glucose	38	65	90	140	0.019	6
Starch	30	54	68	72	0.013	7
Maltose	24	44	56	56	0.006	9
Lactose	35	61	83	134	0.018	6
Dextrin	18	25	28	10	0.013	2
Arabinose	28	41	61	41	0.020	9
Without C	19	20	20	9	0.008	2

3.6. Nitrogen sources

Results in **Table (11)** indicate that yeast extract, peptone, asparagine and gelatin were the most favourable nitrogen sources for growth of *S. rolfsii* where its growth was 90, 55, 52 and 45 mm, respectively, after four days post inoculation. Meanwhile, media containing casein, urea, sodium nitrate were not favourable for growth of the fungus. Media containing yeast extract, peptone and asparagine as sole nitrogen sources were the best for sclerotial formation of *S. rolfsii,* respectively. While the

lowest sclerotial numbers were produced on media containing ammonium nitrate, urea, sodium nitrate. On the other hand, the highest weight of five sclerotia was in case of yeast extract, peptone, asparagine, beef extract and gelatin as sole nitrogen source, respectively. While, the least weight was in case of urea and sodium nitrate. Additionally, the first sclerotium formed after 4 days post inoculation when used ammonium nitrate, urea, peptone and sodium nitrate, while it formed after 6 days in cases of casein and gelatin as nitrogen sources.

Table (11): Effect of different nitrogen sources on the growth and sclerotium formation of *S. rolfsii.*

Nitrogen Sources	Growth (mm) after days		No. of sclerotia/ plate	Weight of 5 sclerotia (g)	The first sclerotium formed after (days)
	2	4			
Casein	25	30	27	0.024	6
Ammonium nitrate	30	42	23	0.027	4
Beef extract	27	42	45	0.021	5
Urea	0	18	12	0.009	4
Yeast extract	43	90	232	0.007	5
Peptone	28	55	75	0.013	4
Asparagin	30	52	68	0.014	5
Gelatin	31	45	30	0.022	6
Sodium nitrate	22	38	12	0.006	4
Without N	31	47	5	0.013	4

3.7. Light colors

Six different light colors were tested for their effect on growth and sclerotial formation of *S. rolfsii.* In this respect, results in **Table (12)** revealed clear differences between the

different colors in their effect on growth of *S. rolfsii*. The highest linear growth was obtained on black color, while the lowest growth was recorded on green color.

Also, black, blue and yellow colors were the best for sclerotial formation (184, 91 and 62 sclerotia/plate, respectively).

The highest weight of five sclerotia was obtained in case of red, green and black colors which, were 0.019, 0.018 and 0.013 g). While, the minimum weight was in case of yellow and blue colors. In addition the first sclerotium formed with using black color after 5 days, followed by white and yellow colors after 6 days, while it formed after 8 days with blue and red colors.

Table (12): Effect of different light colors on the growth and sclerotium formation of *S. rolfsii*.

Light colors	Growth (mm) after days		No. of sclerotia/ plate	Weight of 5 sclerotia (g)	The first sclerotium formed after (days)
	2	4			
White	34	66	50	0.019	6
Green	33	63	53	0.018	7
Blue	35	79	91	0.010	8
Yellow	36	72	62	0.010	6
Red	37	77	44	0.019	8
Black	45	90	184	0.013	5

4. Factors affecting disease incidence on dahlia tubers infected with *S. rolfsii*:

4.1. Effect of wounding

Six wound levels were made into dahlia tubers before inoculation with an equal disc (6 mm) of *S. rolfsii* to test their relations with infection. In this respect, data in **Table (13)** show that there are positive relation between wounding and appearance of infection with *S. rolfsii* on dahlia tubers. The infected area was increase gradually by increasing the wound levels from needle wound level till 10 mm cyclic wound at all incubation days from the 3^{rd} – 11^{th}. The highest infection area were 94mm and 91mm which were recorded onto dahlia tubers wounded at levels 10 and 5 mm cyclic wounds respectively after 11 days incubation. The infected area in case of needle wound onto dahlia tubers reached 64 mm after 11 days incubation. The results indicate that the fungus was able to infect dahlia tuber naturally wounded and its infection increased gradually to reach 61mm after 11 days incubation.

The results clearly show that infection with *S. rolfsii* reduced the weight of dahlia tubers and these reduction were affected by incubation period and wound level where the reduction in infected dahlia tubers increase gradually from the 3^{rd} till 11^{th}. The highest reduction in infected dahlia weight was 22.3% at 10 mm cyclic wound after 11 days incubation, while, the least reduction in weight of infected dahlia tubers was 14.9% after 11 days in case of needle wound comparing with control (non-wounded/non-inoculated).

Table (13): Effect of wounding dahlia tubers on infection with *S. rolfsii* under *in vitro* conditions.

Depth of wound	Days post inoculation with *S. rolfsii*									
	3		5		7		9		11	
	*IA	*RW	IA	RW	IA	RW	IA	RW	IA	RW
Needle	13	2.8	25	5.9	34	8.9	50	12.7	64	14.9
Superficial	19	3.6	32	7.3	48	9.8	62	12.9	76	15.3
3mm-depth cyclic	23	4.1	46	7.5	55	10.5	65	13.1	81	16.5
5mm-depth cyclic	28	4.5	51	8.6	62	10.7	77	13.5	91	16.8
10mm-depth cyclic	33	5.1	59	9.1	71	13.2	82	16.8	94	22.3
Naturally wounded	10	2.5	23	5.6	31	7.2	43	10.4	61	13.5
Control	0.0	1.5	0.0	2.3	0.0	3.0	0.0	3.5	0.0	4.6

*IA= infected area (mm)

RW= reduction in weight of dahlia tuber infected with *S. rolfsii* (g).

4.2. Effect of inoculum density:

4.2.1. On dahlia tubers under *in vitro* conditions.

Data in **Table (14)** reveal that all used inoculum density of *S. rolfsii* (1-10 sclerotia/wound) had the ability to induce infection on wounded dahlia starting from the 5th day. This infection was developed gradually by increasing incubation period. The highest infected area reached 92 mm after 11 days using inoculum density 10 sclerotia/wound, meanwhile, the least infected area was 46 mm after the same time using one sclerotia/wound.

Also, this infection reduced the weight of infected dahlia gradually in correlation to the used inoculum density, where the highest reduction percentage was 10.2% after 11 days by using 10 sclerotia/wound. On the other hand, the least reduction

percentage in weight was 6.4% using one sclerotia/wound. The reduction % values between the tested 1-10 sclerotia/wound were gradually increased affected by sclerotia number and incubation period.

Table (14): Relationship between inoculum density of *S. rolfsii* and disease incidence on wounded dahlia tubers (10-mm cyclic wound) under *in vitro* conditions.

No. of Sclerotia/ wound	Period after inoculation with sclerotia of *S. rolfsii* (days)									
	3		5		7		9		11	
	*IA	RW	IA	RW	IA	RW	IA	RW	IA	RW
1	0	0.0	13	2.7	23	3.8	33	5.2	46	6.4
2	0	0.0	19	2.8	30	3.9	43	5.3	52	6.5
3	0	0.0	21	3.0	33	4.3	45	5.5	55	7.3
4	0	0.0	24	3.2	35	5.0	47	6.3	59	7.9
5	0	0.0	26	3.5	40	5.3	50	6.5	64	8.4
6	0	0.0	28	3.7	45	5.5	63	6.8	73	8.7
7	0	0.0	30	3.8	48	5.8	66	7.2	77	9.0
8	0	0.0	32	4.0	51	6.1	68	7.6	81	9.3
9	0	0.0	35	4.2	53	6.3	70	8.1	89	10.1
10	0	0.0	39	4.5	59	6.5	74	8.3	92	10.2
Control	0	0.0	0.0	2.5	0.0	3.0	0.0	3.7	0.0	4.5

*IA= infected area (mm)

RW= reduction in weight of dahlia tuber infected with *S. rolfsii* (g).

4.2.2. As soil treatment under greenhouse conditions.

Data in **Table (15)** indicate that, infesting soil with different inoculum levels of *S. rolfsii* has a great effect on surviving of dahlia plants. In this respect, increasing of inoculum potential from 1 –5 % increased gradually the dead

plants where 5% inoculum potential gave the highest death percentage of dahlia plants which was 87.5%, followed by 4% inoculum were 62.5% whereas, 1% gave the least percentage of dead plants of dahlia.

Table (15): Effect of inoculum potential (IP) of *S. rolfsii* on disease incidence in dahlia tubers.

*IP (%)	2000/2001 season	
	Dead Plant (%)	Survived Plant (%)
1	16.6	83.4
2	37.5	62.5
3	54.1	45.9
4	62.5	37.5
5	87.5	12.5

*IP= inoculum potential

5. Pathogenicity and host range of isolated bacteria

Results in **Table (4)** show that bacterial isolation trial from rotted dahlia tubers resulted in 55 bacterial isolates. Out of these, 7 isolates (No. 4, 7, 9, 10, 11, 12 and 14) were only pathogenic and caused soft rot on dahlia tubers, meanwhile, the remained isolates were not pathogenic and showed no symptoms on dahlia tubers when tested for their pathogenicity. The pathogenic ability of bacteria isolates (7 isolates) was tested on different plant hosts as shown in **Table (16)**. All these tested isolates were highly pathogenic on dahlia and potato tubers and never affected sugar beet and taro. On the other hand, isolate No. 12 was highly pathogenic on all tested host plants except sugar beet roots and taro-(corms). While, isolate 4 was highly pathogenic only on potato, squash, dahlia and cucumber,

meanwhile, it was moderately affected sweet potato and carrot. Also, isolate No. 7 was highly pathogenic on potato, carrot and dahlia and moderately affected sweet potato, squash, cucumber and onion. Meanwhile, isolate No. 9 was highly pathogenic on potato, carrot, dahlia and low affected squash, cucumber and onion, but moderately affected only sweet potato. Isolate No. 10 revealed highly pathogenic ability on potato, carrot, squash and dahlia, while, it was moderately pathogenic on sweet potato, cucumber and onion. Moreover, isolate No. 11 was highly pathogenic only on potato and dahlia but it was moderately pathogenic on sweet potato, carrot, squash and onion and low affecting cucumber. The last isolate (No. 14) was highly affected potato, squash, dahlia and moderately affected carrot and onion, while it was low affected sweet potato and cucumber.

Table (16): Reaction of different plant hosts to inoculation by the pathogenic bacteria isolates of dahlia tuber rot.

Plant – (organ)	Pathogenic bacterial isolates						
	4	7	9	10	11	12	14
Potato – (tuber)	H	H	H	H	H	H	H
Sweet potato- (tuber root)	M	M	M	M	M	H	L
Carrot – (root)	M	H	H	H	M	H	M
Squash – (fruit)	H	M	L	H	M	H	H
Dahlia – (tuber)	H	H	H	H	H	H	H
Sugar beet – (root)	-	-	-	-	-	-	-
Cucumber – (fruit)	H	M	L	M	L	H	L
Onion – (bulb)	M	M	L	M	M	H	M
Taro – (corm)	-	-	-	-	-	-	-

- = Non pathogenic, H= highly pathogenic, M = moderate pathogenic and L = low pathogenic.

6. Identification and classification of pathogenic bacterial isolates

6.1. Traditional techniques

Out of 55 bacterial isolates, 7 isolates only were pathogenic. These pathogenic isolates were identified and classified based on their morphological and physiological properties. In the first scheme **(Table, 17)**, the pathogenic bacterial isolates were classified in three groups based on Gram stain and spore formation. The first group included two (2) isolates which are long rod shaped, Gram positive (G+) and spore formers, growing under aerobic condition. The aforementioned properties indicate that these two isolates belong to the genus Bacillus. The second group consisted two isolates which were short rod shaped Gram negative (G-), white colonies, no diffusible pigment, non-spore former, so it may belong either to the genus *Erwinia* or *Pseudomonas*. The second scheme **(Table, 18)**, classified the Bacillus isolates for two species according to their reactivity with different bio tests. The isolates No. 7 and 9 belonged to the species *Bacillus polymyxa.* While the third scheme **(Table, 19)** show that both isolate No. 4 and 12 were short rod shape, Gram negative (G-), positive for potato soft rot incidence, gelatin liquefaction and producing acid from lactose, so they were classified as *E. carotovora*. Fourth scheme **(Table, 20)** indicated that the isolates No. 10, 11 and 14 might be identified as *Ps. cepacia*. On the basis of host plant reaction and source of isolation (dahlia tuber), this isolate was identified as *Ps. cepacia*.

Table (17): Classification of pathogenic bacterial isolates (Scheme-1*).

Identification Tests	Isolate No.						
	4	7	9	10	11	12	14
Gram reaction	-	+	+	-	-	-	-
Growth on common media	+	+	+	+	+	+	+
Size	Short	Long	Long	Short	Short	Short	Short
Spore production	-	+	+	-	-	-	-
Pigment K.B.	-	-	-	-	-	-	-
Starch hydrolysis	-	+	+	-	-	-	-
Gelatin liquefaction	+	+	+	+	+	+	+
Yeast extract dextrose $CaCO_3$	+	+	+	+	+	+	+
Pectate degradation	+	+	+	+	+	+	+
Growth on peptone yeast extract agar (PYEA)	+	+	+	+	+	+	+
Growth on MS medium	+	+	+	+	+	+	+
Fried egg on PDA	+	-	-	-	-	+	-
Tolerance to NaCl 5%	H	H	M	M	M	H	H
Tolerance to NaCl 7%	M	M	L	L	L	M	M
Reducing substance from sucrose after:							
2 min.	+	+	+	-	-	+	-
10 min.	-	+	+	-	+	-	-
Acid production from glucose	-	+	+	-	+	-	+
Acid production from lactose	+	+	+	+	+	+	+
Gas production from lactose	-	-	-	-	-	-	-
Anaeorbic production of gas from glucose	+	-	-	-	-	+	-
Relation to O_2	F.	A.	A.	A.	A.	An.	A.
Catalase activity	+	+	+	+	+	+	+
**Bacterial genera	*E.*	*B.*	*B.*	*P.*	*P.*	*E.*	*P.*

* Based on classification tests suggested by **Schaad (1980), Fahy and Persley (1983)** and **Lelliott and Stead (1987).**

** *E.= Erwinia, B.= Bacillus* and *P.= Pseudomonas.*

Table (18): Classification of the rod shape, Gram positive (G^+) and spore former bacterial isolates (*Bacillus* classification) Scheme-2.

Identification Tests	Isolate No.	
	7	9
Gram reaction	+	+
Shape	rod	rod
Spore production	+	+
Pigments K.B.	-	-
Starch hydrolysis	+	+
Catalase activity	+	+
Gelatin liquefaction	+	+
Tolerance to NaCl 5%	+	+
Tolerance to NaCl 7%	+	+
Anaerobic production of gas from glucose	-	-
Growth at 50°C	-	-
Bacterial isolate identified	*Bacillus polymyxa*	

Table (19): Classification of the rod shape and Gram negative (G^-) bacterial isolates (*Erwinia* classification) Scheme-3*.

Identification Tests	Isolate No.	
	4	12
Potato soft rot	+	+
Potato soft rot	+	+
Gelatin liquefaction	+	+
Starch hydrolysis	-	-
Catalase activity	+	+
Pectate degradation	+	+
Fried egg on PDA	+	+
Growth on MS medium	+	+
Yeast extract dextrose $CaCO_3$	+	+
Tolerance to NaCl 5%	+	+
Reducing substance from sucrose after 10min.	-	-
Anaerobic production of gas from glucose	+	+
Acid production from lactose	+	+
Relation to O_2	F.	An.
Bacterial isolate identified	*E. carotovora*	*E. carotovora*

* Based on classification tests suggested by **Schaad (1980), Fahy and Persley (1983)** and **Lelliott and Stead (1987).**

Table (20): *Pseudomonas* classification, the non fluorescent group, short rod shape and Gram negative (G^-) Scheme-4*.

Identification Tests	Isolate No.		
	10	11	14
Pigments diffusible	Non	Non	Non
Non diffusible pigments	-	-	-
Starch hydrolysis	-	-	-
Gelatin liquefaction	+	+	+
Pectate degradation	+	+	+
Peptone yeast extract agar (PYEA)	+	+	+
Fried egg on PDA	-	-	-
Tolerance to NaCl 5%	M	M	H
Tolerance to NaCl 7%	L	L	M
Potato soft rot	+	+	+
H_2S production	-	-	-
Aerobiosis	A.	A.	A.
Reducing substance from sucrose	+	+	+
Hypersensitivity reaction	-	-	-
Maximum temp. for growth (°C)	35	35	35
pH of glucose nutrient broth (after 72)	7.0	7.0	6.9
Growth on KB Medium	-	-	-
Pectate gel liquefaction	+	+	+
Levan type	+	+	+
Bacterial isolate identified	*Pseudomonas cepacia*	*Pseudomonas cepacia*	*Pseudomonas cepacia*

* Based on classification tests suggested by **Schaad (1980)** and **Fahy and Persley (1983)**.

6.2. Electrophoretic analysis of protein patterns

Data in **Fig. (1)** and **Tables (21 & 22)** show that protein bands derived from the electrophoretic gel of the soluble proteins of the seven pathogenic bacterial isolates which infected dahlia tubers show that the two bacterial isolates *E. carotovora*-4 (Lane 1) and *E. carotovora*-12 (Lane 2) which, identified previously by the traditional techniques are similar to each other in 10 molecular weights, i.e., 189.6, 166.5, 158.8, 143.4, 95.4, 73.3, 65.6, 56.1, 53.03 and 51.3 KDa with similarity coefficient 0.374. Meanwhile, *E. carotovora*-4 (Lane 1) is found high similar to the bacterial isolate (Lane 6) which previously identified as *Ps. cepacia*-10 by traditional techniques where the similarity coefficient is 0.401. On the other hand, bacterial isolates which previously identified as *B. polymyxa*-7 & -9 (Lanes 3 & 4) by the traditional techniques were found similar to each other in 5 molecular weights, i.e., 151.1, 143.4, 107.9, 86.5 and 78.4 KDa with similarity coefficient 0.227. On the other hand, the last 3 isolates which, identified previously as *Ps. cepacia*-10, -11 & -14 (Lanes 5, 6 &7) are similar somewhat to each other in some molecular weights. In this respect, *Ps. cepacia*-10 (Lane 5) is similar *to Ps. cepacia*-11 (Lane 6) in molecular weights, i.e., 128.1, 112.9, 75.8, 63.05, 57.6 and 49.55 with similarity % 0.194, while, the similarity coefficient of *Ps. cepacia*-10 (Lane 5) and *Ps. cepacia*-14 (Lane 7) was 0.230. Meanwhile, it is clear from the results that the similarity coefficient between *Ps. cepacia*-11 (Lane 6) and *Ps. cepacia*-14 (Lane 7) was low (0.107) where the two isolates are similar in only 4 molecular weights of total resulted protein bands.

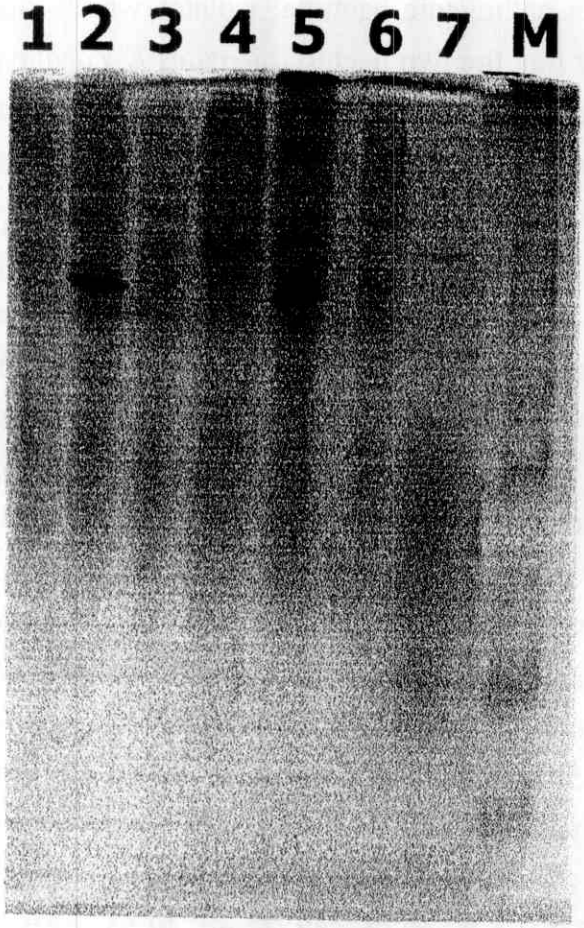

Fig. (1): SDS-PAGE protein pattern of 7 bacterial isolates infected dahlia tubers.

Lane 1 =*Erwinia carotovora*-4
Lane 2 =*Erwinia carotovora*-12
Lane 3 = *Bacillus polymyxa*-7
Lane 4 = *Bacillus polymyxa*-9
Lane 5 = *Pseudomonas cepacia*-10
Lane 6 = *Pseudomonas cepacia*-11
Lane 7 = *Pseudomonas cepacia*-14

Table (21): Molecular weights of the seven tested pathogenic bacterial isolates infected dahlia tubers.

Molecular weights	*Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
212.7	0	0	1	0	0	0	0
205.0	0	1	0	0	1	0	0
189.6			0	0	0	0	0
181.9	0	1	1	0	0	1	0
174.2	0	0	0	1	1	0	0
166.5			0	0	0	1	0
158.8			0	0	1	0	0
151.1	0	0			0	1	0
143.4					0	1	0
128.1	1	0	0	1			0
112.9	0	1	0	0			0
107.9	0	0			0	0	0
101.5	1	0	1	0	0	0	0
99.3	0	0	0	0		0	
95.4			0	1	0		
91.5	0	1	0	0	1	0	0
90.3	0	0	0	1	1	0	0
86.5	1	0			0	1	0
80.9	1	0	0	0		0	
78.4	0	0			1	0	0
75.8	0	0	0	0			0
73.3			0	1		0	
65.6				0	0	1	0
63.05	1	0	0	0			
62.2	1	0	0	0	0	0	0
57.6	1	0	0	0			
56.1			0	0	0	1	0
54.6	0	1	0	0	1	0	0
53.03			0	0	0	1	0
52.2	0	0	0	0		0	
51.3			0	1	0	0	0

Table (21): Molecular weights of the seven tested pathogenic bacterial isolates infected dahlia tubers (continued).

Molecular weights	*Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
49.55	0	0	0	0			0
48.8	0	0	0	1	1	0	0
48.1	1	0	0	0	0	0	1
46.7	0	1	0	1	0	0	0
45.8	0	0	0	0	0	1	0
44.03	1	0	0	0	0	0	1
42.3	0	0	1	0	0	1	0
37.98	0	1	1	0	0	0	1
37.2	0	0	1	0	0	0	0
35.9	0	0	1	0	1	0	0
34.15	1	0	0	0	0	1	0
32.84	0	0	1	0	0	0	1
30.35	0	0	0	0	0	0	1
28.32	0	0	0	0	0	0	1
Total Bands	**20**	**17**	**14**	**13**	**19**	**18**	**13**

*Lane 1 =*Erwinia carotovora*-4
Lane 2 =*Erwinia carotovora*-12
Lane 3 = *Bacillus po;ymyxa*-7
Lane 4 = *Bacillus polymyxa*-9
Lane 5 = *Pseudomonas cepacia*-10
Lane 6 = *Pseudomonas cepacia*-11
Lane 7 = *Pseudomonas cepacia*-14

Table (22): Similarity index matrix among seven bacterial isolates based on SDS-PAGE.

	1	2	3	4	5	6	7
1	1.0	0.374	0.097	0.179	0.182	0.401	0.269
2		1.0	0.148	0.200	0.161	0.296	0.111
3			1.0	0.227	0.065	0.231	0.038
4				1.0	0.185	0.192	0.083
5					1.0	0.194	0.230
6						1.0	0.107
7							1.0

7. Biochemical changes in infected dahlia tubers:

In this experiment, biochemical changes in infected dahlia tubers and control was carried out to determine sugars contents (total, reducing and non-reducing) and phenols (total, conjugated and free) in order to explain their role in infection.

7.1. Sugars content

Data in **Table (23)** reveal that sugar contents was affected as a result for infection with bacterial pathogens and *S. rolfsii*. In this respect its clear that the highest amount of total sugars in infected dahlia tubers was 79.8 mg/g fresh weight with *S. rolfsii*, followed by *Ps. cepacia* 51.7 and 42.7 mg/g with *E. carotovora*-12, meanwhile the least amount of total sugars was 30.2 mg/g (f.w.) in case of *E. carotovora*-4. Also, its clear from results that all pathogenic bacteria did not have a great effect on reducing sugars, while the fungus *S. rolfsii* reduced sugars in infected dahlia to high level where the reduction reached 58.8 mg/g (f.w.) comparing with control (un infected tubers) and bacterial inoculated tubers. On the other hand, all non-reducing sugars were determined in low amounts in all infected dahlia tubers with bacterial pathogenic and *S. rolfsii* comparing to control treatment. The same trend was recorded during replaying this experiment and gave similar results although the readings were different to somewhat.

Table (23): Effect of artificial inoculation with tuber rot pathogens on sugar content (mg/g fresh weight) of tubers incubated *in vitro*.

Pathogens	Total sugars	Reducing sugars	Non-reducing sugars
Experiment I			
Erwinia carotovora- 4	30.2	16.7	13.5
Erwinia carotovora-12	42.7	14.0	28.7
Pseudomonas cepacia	51.7	23.5	28.2
Sclerotium rolfsii	79.9	58.8	21.1
Control	85.9	26.7	59.2
Experiment II			
Erwinia carotovora-4	42.3	9.1	33.2
Erwinia carotovora-12	56.3	13.4	42.9
Pseudomonas cepacia	51.2	11.8	39.4
Sclerotium rolfsii	73.5	39.4	34.1
Control	78.4	11.3	67.1

7.2. Phenols content

Data in **Table (24)** indicate that infection with the fungus *S. rolfsii* increased total and conjugated phenols in dahlia tubers to high extent comparing with bacterial infection during the two separately experiments. In this respect, total and conjugated phenols were 29.4 and 8.4 mg/g fresh weight in infected dahlia tubers with the fungus followed by 33.5 and 10.9 mg/g (f.w.) during the first and second experiment, respectively. On the other hand, all conjugated phenols determined in infected dahlia tubers with pathogenic bacteria and *S. rolfsii* were high comparing to control (un-inoculated) during the two experiments. Free phenols were high also in infected dahlia tubers with *S. rolfsii* comparing to control treatment and inoculated tubers with pathogenic bacteria.

Table (24): Effect of artificial inoculation with tuber rot pathogens on phenols content (mg/g fresh weight) of tubers incubated *in vitro*.

Pathogens	Total phenols	Conjugated phenols	Free phenols
		Experiment I	
***Erwinia carotovora*-4**	14.6	6.1	8.5
***Erwinia carotovora*-12**	20.5	5.4	15.1
Pseudomonas cepacia	21.1	4.4	16.7
Sclerotium rolfsii	29.4	8.4	21.0
Control	18.7	1.8	16.9
		Experiment II	
***Erwinia carotovora*-4**	21.2	5.7	15.5
***Erwinia carotovora*-12**	17.7	6.3	11.4
Pseudomonas cepacia	22.3	6.4	15.9
Sclerotium rolfsii	33.4	10.9	22.5
Control	25.2	4.8	20.4

8. Enzymatic studies:

8.1. In synthetic media

Data presented in **Table (25)** show that the fungus *S. rolfsii* and different tested pathogenic bacteria were not able to produce cellulase enzyme in Czapek's media containing sucrose, pectin and CMC. Meanwhile, these tested isolates which mentioned previously their pathogenic ability on dahlia tubers were able to produce xylanase enzyme in Czapek's media containing sucrose, pectin and CMC. In this respect the highest activity of xylanase was produce in media containing CMC (0.2125 unit/ml) that inoculated with *E. carotovora*-4, followed by media containing sucrose which was inoculated with *E. carotovora* 12 (0.1903 unit/ml). It is clear from these results also, that all pathogenic bacteria were able to produce xylanase

enzyme more than the fungus on media containing sucrose, pectin and CMC. Production of xylanase by *E. carotovora*-4 and -12 in Czapek's media containing pectin was less than in case of sucrose and CMC. Meanwhile, *S. rolfsii* and *Ps. cepacia* were not able to produce xylanase in media containing pectin.

Concerning polygalacturonase (PG) enzyme, the fungus *S. rolfsii* and other tested bacterial pathogens had the ability to produce PG in Czapek's media containing sucrose, pectin and CMC, but with different quantities ranged between 0.008-2.2 u/ml. *S. rolfsii* was the highest PG producer in media containing CMC and pectin as a carbon sources compared with other tested bacterial pathogens. Meanwhile, *Ps. cepacia* produced the highest activity of PG in media containing sucrose more than *E. carotovora* (4&12) and *S. rolfsii* grown at the same conditions.

Table (25): Cell wall degrading enzymes (cellulase, xylanase and PG) produced in synthetic media (unit/ml).

Pathogens	Cell wall degrading enzyme produced in media containing								
	Cellulase			xylanase			PG		
	Sucrose	Pectin	CMC	Sucrose	Pectin	CMC	Sucrose	Pectin	CMC
S. rolfsii	-	-	-	0.057	-	0.009	0.012	0.800	2.200
E. carotovora-4	-	-	-	0.187	0.025	0.213	0.128	0.035	0.008
E. carotovora-12	-	-	-	0.190	0.145	0.160	0.128	0.170	0.500
Ps. cepacia	-	-	-	0.016	-	0.080	1.250	0.016	0.170

8.2. On dahlia tubers

Data in **Table (26)** reveal that the fungus *S. rolfsii* and other tested bacterial pathogens were not able to produce cellulase in infected tissue of dahlia tubers where no activities of this enzyme were detected in this case. On the other hand, xylanase activity was detected in infected tissues of dahlia tubers with *S. rolfsii* and *E. carotovora* (4 & 12 isolates) only. The highest activity of xylanase was recorded in case of *S. rolfsii* infection followed by *E. carotovora*-12. The highest activity of PG enzyme was 15.5 u/ml, followed by 10.5 u/ml in infected tissues of dahlia tubers with *S. rolfsii* and *E. carotovora*-4. *E. carotovora*-12 and *Ps. cepacia* produced also considerable activities in infected dahlia tubers but so little comparing to the other mentioned pathogens.

Table (26): Cell wall degrading enzyme (cellulase, xylanase and PG) enzymes produced on dahlia tubers (unit/ml).

Pathogens	Cell wall degrading enzyme (u/ml) produced in media containing		
	Cellulase	Xylanase	PG
S. rolfsii	-	0.224	15.50
E. carotovora-4	-	0.074	10.50
E. carotovora-12	-	0.198	2.20
Ps. cepacia	-	-	1.25

9. Evaluation of different fungicides against *S. rolfsii*:

9.1. On culture plates (*In vitro*)

In this experiment seven different fungicides belonging to different chemical groups (systemic and non-systemic) (**Table 2**) at five concentration (50, 100, 250, 500, 750 ppm) were tested *in vitro* for their effect on growth of *S. rolfsii* the causal agent of dahlia tuber rot. Data in **Table (27)** show that all tested fungicides had the ability to inhibit or reduce the growth of *S. rolfsii* depending on tested concentrations and time of incubation. In this respect, Vitavax-200 and Topsin-M70 were completely inhibited the growth of *S. rolfsii* at concentrations starting from 100 - 750 ppm, while, visual growth of the fungus was remarkable at concentration 50 ppm only for the two fungicides, where the measured growth was 14-23 mm and 16-32 mm after 2 and 4 days post inoculation with the fungus, respectively. On the other hand, Sanlight, Tachigaren and Rizolex-T50 completely inhibited the growth of *S. rolfsii* at concentrations starting from 250-750 ppm. Meanwhile, concentrations 50 and 100 ppm could not inhibit the growth, where there was a remarkable growth at 2 and 4 days of the fungus. Also, Galben copper and Copper oxychloride inhibited the growth of the fungus only at the high concentration (750 ppm). Although, the fungus grew on some concentrations for all tested fungicides (systemic and non-systemic), this growth was slight and less than control (without fungicide).

Table (27): Effect of different concentrations of some systemic fungicides on linear growth (mm) of *S. rolfsii 'in vitro'*.

Fungicide	Conc. (ppm)	Linear growth (mm) after 2 and 4 days	
		2	4
Tachigaren	50	23	35
	100	13	23
	250	0	0
	500	0	0
	750	0	0
Vitavax-200	50	14	23
	100	0	0
	250	0	0
	500	0	0
	750	0	0
Sanlight	50	12	45
	100	0	17
	250	0	0
	500	0	0
	750	0	0
Topsin-M70	50	16	32
	100	0	0
	250	0	0
	500	0	0
	750	0	0
Rizolex-T50	50	18	39
	100	16	31
	250	0	0
	500	0	0
	750	0	0
Galben copper	50	26	41
	100	24	39
	250	21	31
	500	13	20
	750	0	0
Copper oxychloride	50	32	49
	100	26	39
	250	21	31
	500	18	25
	750	0	0
Control		47	90

9.2. Under greenhouse conditions (*In vivo*):

9.2.1. Soil treatment

Data in **Table (28)** reveal that treating soil with fungicides control effectively *S. rolfsii* infection on dahlia tubers comparing with untreated soil during two seasons. In this respect, treating soil with Rizolex-T50 and Vitavax-200 completely control the infection of *S. rolfsii* during the first season, where the survived dahlia plants were 100%. As well as, the same fungicides were also the highest effective ones during the second season comparing with other fungicides. Copper oxychloride and Galben copper were the least effective fungicides during the two seasons. It is obvious that the effect of Topsin-M70 and Sanlight was raised during the second season more than the first season.

Table (28): Effect of soil application by some fungicides on disease incidence caused by *Sclerotium rolfsii* in dahlia tuber-roots during 2001/2002 and 2002/2003 seasons in greenhouse.

Fungicides (3 g/pot)	2001/2002 season		2002/2003 season	
	Dead Plants (%)	Survived plants (%)	Dead Plants (%)	Survived plants (%)
Tachgaren	50.0	50.0	37.5	62.5
Topsin M70	37.5	62.5	25.0	75.0
Rizolex T50	0.0	100.0	12.5	87.5
Sanlight	37.5	62.5	25.0	75.0
Vitavax 200	0.0	100.0	20.8	79.2
Copper oxycloride	75.0	25.0	62.5	37.5
Galben copper	62.5	37.5	58.3	41.7
Control	75.0	25.0	83.3	16.7

9.2.2. Tuber treatment

The previously tested fungicides *in vitro* were tested again in this experiment as tuber treatment under greenhouse conditions using concentration 750 ppm for their effect on infection development by *S. rolfsii.* Data in **Table (29)** show that dipping dahlia tubers in Rizolex-T50 and Vitavax-200 suspension before sowing gave the best control for *S. rolfsii* infection during the first and second seasons, respectively, where the survived plant for the first fungicide was 83.3% at the first season, while, 79.2% for Vitavax-200 at the second season comparing with control treatment. Also, dipping dahlia tubers in Topsin-M70, Sanlight and Vitavax-200 gave good control with 25% dead plants, whereas it was 75% in control treatment (inoculated with pathogen) during the first season 2001/2002. In addition, treating dahlia tubers with Topsin-M70, Rizolex-T50 and Sanlight gave a good disease control where the dead plants were 25% for the first two fungicides and 37.5% for the last fungicide when compared with control treatment at the second season. On the other hand, treating dahlia tubers with Tachigaren followed by Copper oxychloride and Galben copper were the least effective fungicides in controlling *S. rolfsii* infection during the two seasons.

Table (29): Effect of tuber dipping in some fungicides on disease incidence caused by *Sclerotium rolfsii* in dahlia tuber during 2001/2002 and 2002/2003 seasons under greenhouse condition.

Fungicides (750 ppm)	2001/2002 season		2002/2003 season	
	Dead plants (%)	Survived plants (%)	Dead plants (%)	Survived plants (%)
Tachigaren	50.0	50.0	45.8	54.2
Topsin-M70	25.0	75.0	25.0	75.0
Rizolex-T50	16.7	83.3	25.0	75.0
Sanlight	25.0	75.0	37.5	62.5
Vitavax-200	25.0	75.0	20.8	79.2
Copper oxychloride	54.2	45.8	62.5	37.5
Galben copper	54.2	45.8	50.0	50.0
Control	75.0	25.0	83.3	16.7

10. Evaluation of different bactericides against bacterial pathogens:

10.1. On culture plates (*In vitro*)

In this experiment five antibiotics, i.e, ampicillin, erythromycin, streptomycin, tetracycline and penicillin as well as Galben copper and Copper oxychloride "as bactericides" were tested for their inhibition effect against pathogenic bacteria infecting dahlia tubers *in vitro*. Data in **Table (30)** show that all tested antibiotics and bactericides inhibited the growth of bacterial pathogens depending on antibiotic kind and used concentrations because increasing the concentration of tested materials whether the antibiotics or other bactericides from 25-200 ppm increase gradually the inhibited zone of pathogenic

bacteria. Streptomycin was the first effective antibiotic against growth of tested bacteria where it gave high inhibition zone 33 mm against *Bacillus polymxa*-7 followed by 30.6 mm against *Ps. cepacia*-14. While, penicillin was the second effective antibiotic against *Ps. cepacia* -11 and *E. carotovora*- 4 where the inhibition zones were 30.6 and 29.2 mm, respectively. Meanwhile, erythromycin was also effective against *E. carotovora*-12. Copper oxychloride and Galben copper "as bactericides" were the least effective materials against growth of all tested bacterial pathogens.

Table (30): Effect of different concentrations of some antibiotics and copper compounds on growth of pathogenic bacteria isolates of dahlia tubers *'in vitro'*.

Bactericides	Concn. (ppm)	Pathogenic bacterial isolates									
		E. carotovora isolate No.			*B. polymyxa* isolate No.			*Ps. cepacia* isolate No.			
		4	12	M	7	9	M	10	11	14	M
Ampicillin	25	15	16	15.5	20	12	16.0	10	10	0	6.6
	50	24	19	21.5	21	24	22.5	15	14	12	13.6
	100	26	20	23.0	22	28	25.0	18	20	15	17.6
	150	28	24	26.0	24	30	27.0	19	30	20	23.0
	200	30	25	27.5	33	35	34.0	21	35	24	26.6
	M	24.6	20.8	22.7	24.0	25.8	24.9	16.6	21.8	14.2	17.35
Erythromycin	25	13	25	19.0	0	0	0.0	10	0	17	9.0
	50	16	26	21.0	0	10	5.0	13	11	20	14.6
	100	20	28	24.0	10	15	12.5	18	13	21	17.3
	150	21	30	25.5	15	18	16.5	19	18	25	20.6
	200	25	31	28.0	16	30	23.0	23	19	28	23.3
	M	19.0	28.0	23.5	8.2	14.6	11.4	16.6	12.2	22.2	17.0
Streptomycin	25	17	12	14.5	29	23	26.0	10	18	20	16.0
	50	20	16	18.0	30	25	27.5	15	22	22	19.6
	100	21	17	19.0	32	28	30.0	24	25	30	26.3
	150	25	24	24.5	36	29	32.5	27	28	37	30.6
	200	26	25	25.5	38	30	34.0	37	34	44	38.3
	M	21.8	18.8	20.3	33.0	27.0	30.0	22.6	25.4	30.6	26.2
Tetracycline	25	13	0	6.5	16	0	8.0	13	0	17	10.0
	50	20	10	15.0	22	11	16.5	18	10	20	16.0
	100	22	12	17.0	24	15	19.5	20	13	21	18.0
	150	24	13	18.5	31	18	24.5	21	14	23	19.3
	200	25	15	20.0	33	20	26.5	23	15	25	21.0
	M	20.8	10.0	15.4	25.2	12.8	19.0	19.0	10.4	21.2	16.9
Penicillin	25	17	19	18.0	0	0	0.0	18	20	0	12.6
	50	27	20	23.5	25	0	12.5	25	28	18	23.6
	100	30	22	26.0	28	18	23.0	28	30	20	26.0
	150	35	25	30.0	30	25	27.5	29	35	30	31.3
	200	37	30	33.5	35	33	34.0	30	40	40	36.3
	M	29.2	23.2	26.2	23.6	15.2	19.4	26.0	30.6	21.6	26.1
Galben copper	25	0	0	0.0	0	0	0.0	0	0	0	0.0
	50	0	0	0.0	9	9	9.0	10	0	7	5.6
	100	0	0	0.0	10	10	10.0	11	9	9	9.6
	150	7	10	8.5	11	11	11.0	12	10	10	10.6
	200	11	13	12.0	13	13	13.0	15	13	11	13.0
	M	3.6	4.6	4.1	8.6	8.6	8.6	9.6	6.4	7.4	7.8
Copper oxychloride	25	0	10	5.0	0	0	0.0	0	0	0	0.0
	50	0	16	8.0	10	12	11.0	12	9	13	11.3
	100	10	18	14.0	12	15	13.5	14	10	14	12.6
	150	13	20	16.5	15	18	16.5	15	13	16	14.6
	200	14	33	23.5	17	21	19.0	20	16	17	17.6
	M	7.4	19.4	13.4	10.8	13.2	12.0	14.6	9.6	12.0	12.1

10.2. Under greenhouse conditions (*In vivo*)

Data in **Table (31)** indicated that, dipping dahlia tubers in antibiotic solution at concentration 200 ppm before sowing in pots under greenhouse conditions was effective in controlling tested pathogenic bacteria. In this respect, during the first season, erythromycin followed by ampicillin were the best effective antibiotics in controlling *E. carotovora*-4 & -12 where the survival plants were 79.2 & 75.0 and 66.7 & 70.8%, respectively, comparing to other tested antibiotics and control treatment. Also, streptomycin and tetracycline were the best for controlling *Ps. cepacia* were the survival plants 75 and 70.8% comparing with other tested antibiotics and bactericides as well as control treatment. In the second season, the same trend was recorded for erythromycin and ampicillin as the best effective materials against *E. carotovora*-4 & -12. Meanwhile, tetracycline followed by streptomycin and ampicillin in controlling *Ps. cepacia* infection on dahlia tubers where the dead plants were only 25%, 29.2% and 29.2%, respectively. Tetracycline and penicillin were not effective for controlling *E. carotovora*-4 infection during the two seasons *in vivo*. On the other hand, Galben copper and copper oxychloride, as bactericides, were the least effective in this respect.

Data in **Table (32)** indicate that mixing antibiotics with copper oxychloride improved the efficacy of antibiotics against bacterial pathogens which rotted dahlia tubers during cultivation for two seasons. In this respect, the mixed erythromycin was the best against *E. carotovora*-4 & -12 where the survived plants were 87.5%, followed by streptomycin and mixed tetracycline

against *E. carotovora*-12, meanwhile, mixed ampicillin gave 25.0% dead plants in case of *E. carotovora*-4. On the other hand, mixed ampicillin followed by the mixed antibiotics (tetracycline, streptomycin and penicillin) was the best against *Ps. cepacia* respectively. The same trend was remarkable during the second season where the mixed erythromycin was more effective in controlling *E. carotovora*-4 & -12 than other mixed antibiotics. While, mixed streptomycin and ampicillin were the best for controlling *Ps. cepacia*. It is clear from the results that treating dahlia tubers with all mixed antibiotics improved effectively the survived dahlia plants comparing with un-treated dahlia tubers.

Table (31): Effect of some antibiotics and two copper compounds on growth of dahlia rot pathogen in greenhouse.

Bactericides (200 ppm)	Bacterial isolates					
	E. carotovora-4		*E. carotovora*-12		*Ps. cepacia*	
	Dead plants (%)	Survived plants (%)	Dead plants (%)	Survived plants (%)	Dead plants (%)	Survived plants (%)
Season 2001/2002						
Erythromycin	20.8	79.2	25.0	75.0	50.0	50.0
Penicillin	62.5	37.5	62.5	37.5	62.5	37.5
Streptomycin	50.0	50.0	50.0	50.0	25.0	75.0
Tetracycline	62.5	37.5	54.2	45.8	29.2	70.8
Ampecilline	33.3	66.7	29.2	70.8	37.5	62.5
Copper oxychloride	50.0	50.0	50.0	50.0	37.5	62.5
Galben copper	62.5	37.5	62.5	37.5	54.2	45.8
Control	100.0	0.0	75.0	25.0	87.5	12.5
Season 2002/2003						
Erythromycin	25.0	75.0	25.0	75.0	54.2	45.8
Penicillin	54.2	45.8	50.0	50.0	62.5	37.5
Streptomycin	45.8	54.2	62.5	37.5	29.2	70.8
Tetracycline	66.7	33.3	50.0	50.0	25.0	75.0
Ampecilline	37.5	62.5	25.0	75.0	29.2	70.8
Copper oxychloride	54.2	45.8	50.0	50.0	50.0	50.0
Galben copper	75.0	25.0	54.2	45.8	50.0	50.0
Control	100.0	0.0	79.2	20.8	79.2	20.8

Table (32): Evaluation of mixing antibiotics with copper oxychloride on bacterial pathogens infecting dahlia tubers under greenhouse conditions.

Bactericides	Bacterial isolates					
	E. carotovora-4		*E. carotovora*-12		*Ps. cepacia*	
	Dead plants (%)	Survived plants (%)	Dead plants (%)	Survived plants (%)	Dead plants (%)	Survived plants (%)
Season 2001/2002						
Erythromycin + copper	12.5	87.5	12.5	87.5	37.5	62.5
Penicillin + copper	33.3	66.7	33.3	66.7	12.5	87.5
Streptomycin + copper	37.5	62.5	12.5	87.5	12.5	87.5
Tetracycline + copper	50.0	50.0	20.8	79.2	12.5	87.5
Ampicillin + copper	25.0	75.0	33.3	66.7	0.0	100.0
Control	100.0	0.0	75.0	25.0	87.5	12.5
Season 2002/2003						
Erythromycin + copper	0.0	100.0	12.5	87.5	33.3	66.7
Penicillin + copper	37.5	62.5	37.5	62.5	20.8	79.2
Streptomycin + copper	37.5	62.5	25.0	75.0	16.7	83.3
Tetracycline + copper	37.5	62.5	25.0	75.0	25.0	75.0
Ampicillin + copper	37.5	62.5	33.3	66.7	16.7	83.3
Control	100.0	0.0	79.2	20.8	75.0	25.0