

## RESULTS

### Effect of fungicides on soil microflora:

#### BACTERIA:

##### A- Effect of Vitavax Captan on total count:

Data in Table (1) show that the application of Vitavax Captan as seed dressing, gradually inhibited the rhizosphere microflora of cotton plants at early stage of plant growth. Minimal counts were obtained after 15 days with the recommended dose, and after 30 days, from application, with the 10 fold normal dose. Then microorganisms increased gradually thereafter. At the end of the experiment, after 90 days from sowing, the counts of both treatments (normal dose and 10 fold normal dose) were approximately equal to the control. In case of the non-cultivated soil, the total counts with both fungicide treatments followed the same trend of the cultivated soil. However, the total count did not reach that of control even after 90 days from application.

##### B- Effect of Topsin M 70% on total count:

In cultivated soil, the normal field application dose

Table (1): Total counts of bacteria in the soil and rhizosphere of cotton plants as influenced by Vitavax Captan.

(Number in mil./g dry weight)

Time in days after Vitavax - Captan  application	Rate of field application					
	Cultivated soil			non-cultivated soil		
	Control	N	10 N	Control	N	10 N
0-time	122.5	153.75	130.6	125	146.25	130.25
3-days	108.8	99.3	94.03	112	105.6	60.0
7-days	120.0	44.7	33.194	127	71.75	35.0
15-days	125.0	44.8	40.0	129	56.1	22.0
30-days	116.0	68.4	36.6	111	83.1	34.4
60-days	156.0	133.6	95.5	133	83.8	62.2
90-days	138.8	134.4	129.4	132	101.1	97.77

Table (2): Total counts of bacteria in the soil and rhizosphere of cotton plants as influenced by topsin M 70

(Number in mil/g dry weight)

Time in days after Topsin M application	Rate of field application					
	Cultivated soil			non-cultivated soil		
	Control	N	10 N	Control	N	10 N
0-time	122.5	170.6	87.0	125	158.0	102.5
3-days	108.8	151.11	53.23	112	113.0	63.1
7-days	120.0	104.22	30.0	127	55.0	33.3
15-days	125.0	97.33	48.8	129	68.8	22.2
30-days	116.0	146.1	69.40	111	108.0	41.1
60-days	156.0	176.6	78.0	133	112.2	53.3
90-days	138.8	167.1	88.3	132	120.5	96.6

reduced the total bacterial counts during the periods 7 and 15 days from sowing, then bacterial counts increased and surpassed that of control after 30 days from sowing and thereafter. The 10 fold normal dose treatment showed more drastic effect on the total count, than normal dose and total count failed to level that of control till the end of the experiment. In case of non-cultivated soil, the effect of the fungicide Topsin M 70%, on total count showed the same trend as in cultivated soil. However, the effect of the fungicide was more drastic in the uncultivated soil, and counts with both rates of application could not reach that of control even after 90 days from commencement.

#### SPORE-FORMER BACTERIA:

##### A- Effect of Vitavax Captan on spore-former bacteria:

Data in Table (3) show that in cultivated soil, normal field application rate of Vitavax Captan reduced the density of spore-former bacteria where minimal counts were obtained after 15 days from sowing. Then spore-former counts started to increase till it surpassed that of control after 90 days from sowing. The 10 fold application rate showed the same trend of the normal application rate, but the effect on the

Table (3): Counts of spore-former bacteria in soil and rhizosphere of cotton plants as influenced by Vitavax Captan.

(Number in mil/g dry weight)

Time in days after Vitavax - Captan application	Rate of field application					
	cultivated soil			non-cultivated soil		
	Control			Control		
		N	10 N		N	10 N
0-time	29.375	10.75	38.75	17.5	23.1	13.75
3-days	40.0	8.8	12.46	10.0	4.83	0.50
7-days	37.0	2.22	0.97	10.6	1.22	0.832
15-days	32.2	0.713	0.366	31.77	0.783	0.372
30-days	35.0	2.22	2.33	24.44	3.055	1.088
60-days	33.33	13.22	2.77	17.77	3.33	2.33
90-days	33.33	36.66	5.77	17.22	1.605	1.311

Table (4): Counts of spore-former bacteria in soil and rhizosphere of cotton plants as influenced by Topsin M 70.

(Number in mil/g dry weight)

Time in days after topsin M application	Rates of field application					
	cultivated soil			non-cultivated soil		
	Control			Control		
		N	10 N		N	10 N
0-time	29.375	23.75	16.25	17.5	20.0	22.5
3-days	40.0	12.22	7.7	10.0	3.3	7.0
7-days	37.0	4.444	0.862	10.6	1.6	0.944
15-days	32.2	2.094	0.602	31.77	1.263	1.206
30-days	35.0	4.188	1.5	24.44	2.66	2.972
60-days	33.33	3.10	3.866	17.77	3.22	3.333
90-days	33.33	1.716	2.33	17.22	2.22	1.88

spore-former counts was more harmful, where counts remained lower than the control until the end of the experiment after 90 days from sowing. The effect of Vitavax Captan was more obvious in the uncultivated soil than cultivated soil in both concentrations used. Cultivation of the soil reduced the harmful effect of the fungicide on spore-former counts. However, it seems that the poisonous effect of the fungicide when applied at normal field rate disappeared greatly from the cultivated soil after 90 days from commencement.

B. Effect of Topsin M 70% on spore-former bacteria:

Data in Table (4) show that the application of Topsin M 70% decreased greatly the density of spore-formers in both concentrations used. The severe harmful effect of the fungicide on spore-formers at both concentrations in cultivated and uncultivated soil lasted until the end of the experiment.

ACTINOMYCETES:

A. Effect of Vitavax Captan on actinomycetes count:

Data in Table (5) show that Vitavax Captan reduced actinomycetes counts. The extent of inhibition was in line with the rate of application. However, at the end of the

Table (5): Actinomycetes counts in the soil and rhizosphere of cotton plants as influenced by vitavax captan.

(Number in mil/g dry weight)

Time in days after Vitavax - Captan application	Rate of field application					
	Control	cultivated soil		Control	non-cultivated soil	
		N	10 N		N	10 N
0-time	50.6	46.87	26.25	53.75	98.75	47.75
3-days	47.77	44.0	18.33	78.8	64.4	26.6
7-days	53.3	33.33	5.5	55.5	46.6	25.7
15-days	42.2	27.08	17.33	49.16	24.4	21.8
30-days	37.7	21.1	21.1	46.6	28.8	13.3
60-days	33.3	27.77	22.2	44.4	38.7	31.1
90-days	38.8	49.0	38.3	33.3	53.3	32.2

Table (6): Actinomycetes counts in the soil and rhizosphere of cotton plants as influenced by topsin M 70.

(Number in mil/g dry weight)

Time in days after topsin M application	Rate of field application					
	Control	cultivated soil		Control	non-cultivated soil	
		N	10 N		N	10 N
0-time	50.6	93.75	31.25	53.75	44.6	33.7
3-days	47.77	57.7	22.0	78.8	36.0	11.0
7-days	53.3	37.77	7.77	55.5	34.4	9.0
15-days	42.2	23.75	31.77	49.16	37.2	5.0
30-days	37.7	36.66	28.8	46.6	22.7	11.1
60-days	33.3	62.22	27.03	44.4	44.4	26.6
90-days	38.8	28.8	28.88	33.3	48.3	33.3

experiment, actinomycetes counts surpassed that of control at the normal application rate, and hardly levelled the control counts with the 10 fold normal rate treatment. This trend was found in both cultivated and uncultivated soils.

B. Effect of Topsin M 70% on Actinomycetes counts:

Table (6) indicates that the effect of the fungicide Topsin M 70% on actinomycetes counts almost resembled that of Vitavax Captan on this group of microorganisms.

Fungi:

A. Effect of Vitavax Captan on fungi count:

Data in Table (7) show that the application of Vitavax Captan as seed-dressing highly decreased the density of the fungi in the rhizosphere of cotton plants till the end of experiment (after 90 days from sowing) in both normal dose and 10 fold normal dose treatments. However, the effect was more drastic with the higher dose. The effect of Vitavax Captan on fungi in the uncultivated soil showed the same trend found in the cultivated soil. In all treatments, the fungi counts were far lower than that of control. The fungicide Vitavax Captan showed a special harmful effect on fungi.

Table (7): Fungi counts in the soil and rhizosphere of cotton plants as influenced by vitavax captan.

(Number in thousands/g dry weight)

Time in days after Vitavax - Captan application	Rate of field application					
	Control	cultivated soil		Control	non-cultivated soil	
		N	10 N		N	10 N
0-time	142.5	137.5	142.6	135.67	48.25	58.3
3-days	138.88	36.663	29.330	121.666	25.555	13.333
7-days	126.64	67.776	30.555	148.333	19.75	14.444
15-days	81.110	63.887	27.330	140.000	37.777	13.333
30-days	143.333	35.555	28.888	129.444	28.44	24.444
60-days	155.550	33.333	27.777	116.666	22.777	33.0
90-days	133.333	41.111	21.666	140.000	45.555	31.111

Table (8): Fungi counts in the soil and rhizosphere of cotton plants as influenced by topsin M 70.

(Number in thousands/g dry weight)

Time in days after topsin M 70 application	Rate of field application					
	Control	cultivated soil		Control	non-cultivated soil	
		N	10 N		N	10 N
0-time	142.5	172	164	135.67	42.45	55.5
3-days	138.88	155	14.66	121.666	20.555	20.0
7-days	126.64	84.444	10.69	148.333	56.60	45.0
15-days	81.110	32.75	14.88	140.00	45.00	40.0
30-days	143.333	27.7	31.111	129.444	39.00	28.0
60-days	155.55	25.555	34.444	116.666	40.00	35.5
90-days	133.33	25.555	37.777	140.000	75.777	36.111



B. Effect of Topsin M 70% on fungi count:

Data in Table (8) show that the application of Topsin M 70% as seed-dressing greatly decreased the fungi counts in the rhizosphere of cotton plants than control. The higher dose (10 fold the normal dose) was more harmful to fungi than the lower one (normal dose). The toxic effect of the fungicide on fungi lasted till the end of the experiment. In the uncultivated soil, the effect of Topsin M 70% on fungi showed the same trend found with the cultivated soil.

Anaerobic nitrogen fixers:

A. Effect of Vitavax Captan on anaerobic nitrogen fixers count:

Table (9) indicates that Vitavax Captan application showed a harmful effect on the anaerobic nitrogen fixers in the cultivated and uncultivated soil at both concentrations used. In case of the normal field application, anaerobic nitrogen fixers count decreased till 7 days then started to increase where counts surpassed that of control after 30 days from sowing and thereafter. The higher dose of the fungicide (10 fold normal dose) adversely affected the anaerobic nitrogen fixers allover the experimental period, and counts

Table (9): Anaerobic nitrogen fixers count in the soil and rhizosphere of cotton plants as influenced by vitavax captan.

(Number in thousands/g. dry weight)

Time in days after Vitavax - Captan application	Rate of field application					
	cultivated soil			non-cultivated soil		
	Control			Control		
		N	10 N		N	10 N
0-time	260	430	208.0	170	95	140
3-days	280	204	138.6	147	17	45
7-days	216	34	50.0	126	17	14
15-days	212	130	9.600	117	14	2.1
30-days	170	200	35.0	141	35	92
60-days	180	390	56.0	120	72	150
90-days	160	370	154.0	162	160	180

Table (10): Anaerobic nitrogen fixers count in the soil and rhizosphere of cotton plants as influenced by topsin M 70.

(Number in thousands/g. dry weight)

Time in days after topsin M application	Rate of field application					
	cultivated soil			non-cultivated soil		
	Control			Control		
		N	10 N		N	10 N
0-time	260	200	280	170	98	164
3-days	280	56	25	147	24	12
7-days	216	14	12	126	56	46
15-days	212	21	8	117	17	14
30-days	170	35	14	141	28	64
60-days	180	28	28	120	43	160
90-days	160	64	92	162	92	210

hardly approximated that of control after 90 days from sowing. In the uncultivated soil, the normal field application rate of the fungicide decreased the anaerobic nitrogen fixers upto 15 days from commencement then counts increased to hardly level that of the control at the end of the experiment. The 10 fold application rate treatment showed the same trend, but anaerobic nitrogen fixers counts increased than that of control after 60 days from commencement and thereafter.

B. Effect of Topsin M 70% on anaerobic nitrogen fixers counts:

Data in Table (10) show that the fungicide Topsin M70% reduced the anaerobic nitrogen fixers in the rhizosphere of cotton plants. Anaerobic nitrogen fixers decreased by the effect of the fungicide up till 7-15 days, from application, then started to increase; but could not level control counts till the end of the experiment.

In case of non-cultivated soil, the same trend found in the cultivated soil was observed, but counts of anaerobic nitrogen fixers increased than that of control in 10 fold application rate at the end of the experiment. Survivors of anaerobic nitrogen fixers may have used the metabolites of the chemical as a source for nutrition.

### Aerobic nitrogen fixers:

#### A. Effect of Vitavax Captan on aerobic nitrogen fixers counts:

Table (11) shows that the fungicide Vitavax Captan, reduced the Azotobacter counts in cultivated and non-cultivated soil specially after 7 days from application. However, at the normal rate of application Azotobacter restored to or surpassed, the control counts at the end of the experimental period. On the other hand, the high concentration of the chemical reduced Azotobacter counts allover the experimental period.

#### B. Effect of Topsin M70% on aerobic nitrogen fixers counts:

Data in Table (12) show the effect of the fungicide Topsin M 70% on Azotobacter counts in cultivated and non-cultivated soil. Topsin M 70 adversely affected Azotobacter counts in manner approximately similar to that found with Vitavax Captan. Both the fungicides showed approximately the same trend.

### NITRIFYING BACTERIA:

#### A. Effect of Vitavax Captan on nitrifying bacteria count:

Table (13) indicated the drastic effect of the fungicide Vitavax Captan on nitrifiers at both concentrations

Table (11): Aerobic nitrogen fixers counts in the soil and rhizosphere of cotton plants as influenced by vitavax captan.

(Number in thousands/g. dry weight)

Time in days after Vitavax - Captan application	Rate of field application					
	Control	cultivated soil		Control	non-cultivated soil	
		N	10 N		N	10 N
0-time	220	139	280	120	140	170
3-days	280	62.70	1.848	138	120	2.80
7-days	56.000	22.20	0.975	145	3.3	12.
15-days	48.000	28.000	6.4000	180	140	17.
30-days	160.000	160.000	16.	240	160	35.
60-days	130.000	180.000	21.000	140	150	39.
90-days	180.000	120.000	47.000	280	920	9.5

Table (12): Aerobic nitrogen fixers counts in the soil and rhizosphere of cotton plants as influenced by topsin M 70.

(Number in thousands/g. dry weight)

Time in days after Topsin M 70 application	Rate of field application					
	Control	cultivated soil		Control	non-cultivated soil	
		N	10 N		N	10 N
0-time	220	140	140	120	140	110
3-days	280	24.00	13.86	138	170	1.7
7-days	56.	48.	3.000	148	78.	5.400
15-days	48.	44.	3.000	180	17.	20.
30-days	160	220	16.0	540	92	6.4
60-days	130	260	92.0	140	92	17.
90-days	180	380	100	280	240	10.

Table (13): Nitrifiers counts in the soil and rhizosphere of cotton plants as influenced by vitavax captan.

(Number in thousands/g. dry weight)

Time in days after Vitavax - Captan application	Rate of field application					
	Control	cultivated soil		Control	non-cultivated soil	
		N	10 N		N	10 N
0-time	350	130	158	160	330	170
3-days	350	7.25	5.41	140	2.260	7.700
7-days	280	1.08	1.97	120	2.10	2.210
15-days	280	2.75	2 16	35	1.11	5.540
30-days	160	1.70	3.90	11.	3.5	1.160
60-days	22.0	2.70	3.30	11	3.33	1.640
90-days	35.0	5.4	7.90	17	4.00	1.770

Table (14): Nitrifiers counts in the soil and rhizosphere of cotton plants as influenced by Topsin M 70.

(Number in thousands/g dry weight)

Time in days after Topsin M 70 application	Rate of field application					
	Control	cultivated soil		Control	non-cultivated soil	
		N	10 N		N	10 N
0-time	350	280	79.00	160	330.0	240.00
3-days	350	7.8	4.22	140	110.0	12.00
7-days	280	2.88	2.30	120	64.0	11.00
15-days	280	4.00	1.40	35	35.00	16.00
30-days	160	6.100	4.50	11	2.800	2.000
60-days	22	3.310	1.40	11	6.400	5.400
90-days	35	5.540	7.90	17	16.000	9.200

used in cultivated and uncultivated soils. The harmful effect of the chemical on nitrifiers lasted till the end of the experiment (after 90 days from commencement).

B. Effect of Topsin M 70% on nitrifying bacteria count:

Data in Table (14) show that Topsin M 70% adversely affected nitrifiers counts in the rhizosphere of cotton plants at both concentrations used. Such effect lasted till the end of the experiment.

Effect of the fungicides Vitavax Captan and Topsin M 70% on pathogenic fungi in vitro:

A. Effect of Vitavax Captan on pathogenic fungi:

Data in Table (15) show that Vitavax Captan completely inhibited the growth of Sclerotium rolfsii at very low concentration (5 ppm), but Rhizoctonia solani required 100 ppm of the chemical for its complete inhibition. In case of Fusarium oxysporum, 500 ppm of Vitavax Captan were required for its complete inhibition.

This result indicates that Sclerotium rolfsii was very sensitive to Vitavax Captan. Rhizoctonia solani was completely inhibited by moderate dose (100 ppm), but Fusarium oxysporum

Table (15): Effect of the fungicides vitavax captan and Topsin M 70 on the growth of the pathogenic fungi R. solani, P. oxysporum and Sci. rolfsii.

1- Vitavax captan

Fungi	Rhizoctonia solani				Fusarium oxysporum				Sclerotium rolfsii			
	Inhibition index at intervals				Inhibition index at intervals				Inhibition index at intervals			
	(hours from commencement)				(hours from commencement)				(hours from commencement)			
Concentration	48 hr.	72 hr.	96 hr.	120 hr.	48 hr.	72 hr.	96 hr.	120 hr.	48 hr.	72 hr.	96 hr.	120 hr.
ppm												
5 ppm	56.36	71.81	81.1		35.41	27.39	12.22	14.28	100	100	100	100
50 ppm	60.	78.18	85.5		54.16	45.	14.	21.45	100	100	100	100
100 ppm	100	100	100		100	100	86.66	81.42	100	100	100	100
200 ppm	100	100	100		100	100	100	83.57	100	100	100	100
300 ppm	100	100	100		100	100	100	88.5	100	100	100	100
400 ppm	100	100	100		100	100	100	88.5	100	100	100	100
500 ppm	100	100	100		100	100	100	100	100	100	100	100
1000 ppm	100	100	100		100	100	100	100	100	100	100	100
5000 ppm	100	100	100		100	100	100	100	100	100	100	100
10,000 ppm	100	100	100		100	100	100	100	100	100	100	100

2- Topsin M 70

5 ppm	16.36	16.36	42.2		16.66	21.91	23.3	35.7	00.00	00.00	00.00	00.00
50 ppm	47.27	59.09	67.77		54.	72.6	76.66	85.	20	42.8	42.9	44.44
100 ppm	56.36	67.27	75.55		100	100	100	100	28	47.6	47.96	48.8
200 ppm	60.	70.	77.77		100	100	100	100	36	52.38	52.52	52.99
300 ppm	63.63	74.5	82.22		100	100	100	100	44	54.76	56.81	57.5
400 ppm	67.27	78.18	83.33		100	100	100	100	50	57.14	62.28	62.28
500 ppm	70.9	81.81	85.5		100	100	100	100	54	61.90	68.18	78.9
1000 ppm	100	100	100		100	100	100	100	100	71.42	74.24	75.
5000 ppm	100	100	100		100	100	100	100	100	78.57	78.78	78.9
10,000 ppm	100	100	100		100	100	100	100	100	81.5	82.	86.66



tolerated higher doses, and 500 ppm of the fungicide were required for the complete inhibition of the fungus.

B. Effect of Topsin M 70% on pathogenic fungi:

Data in Table (15) indicate that Fusarium oxysporum was sensitive to Topsin M 70%, where 100 ppm of the fungicide completely inhibited the fungus. Rhizoctonia solani could tolerate high doses of the chemical and was completely inhibited by the addition of 1000 ppm of the fungicide. Sclerotium rolfsii tolerated very high concentrations of the fungicide, since the highest dose used in this investigation (10,000 ppm) did not cause complete inhibition of the fungus.

Effect of the fungicides Vitavax Captan and Topsin M70% on reducing the damping-off in cotton plants sown in sterilized soil infested with R.solani, F.oxysporum and Scl.rolfsii:

Data in Table (16) indicate that the investigated fungi proved to be pathogenic to cotton seedlings. These pathogenic fungi reduced the percentages of germination and survival plants and increased the pre-emergence and almost

increased the post-emergence damping-off as compared to their respective control. Concerning the virulence of the pathogens, Rhizoctonia solani was the most virulent followed by Sclerotium rolfsii then Fusarium oxysporum. However, the infestation with the three pathogens gave the most severe effect than when any of the pathogens was inoculated solely.

Fungicides application increased the percentages of germination and survival plants and almost decreased the pre- and post emergence damping-off as compared to their respective percentages in the infested soil with any of the pathogens. This was true for both fungicides Vitavax Captan and Topsin M 70% applied at normal field application rate against the pathogens R.solani, Scl.rolfsii and F.oxysporum. It seems that Vitavax Captan was more effective against R.solani and Scl.rolfsii since it gave higher survival plants 72.5% and 87.5% as compared to those of Topsin M 70% which were 70% and 67.5%, respectively. On the other hand, Topsin M 70% seemed to be more effective against F.oxysporum since the percentage of survival plants was 82.5% as compared to that obtained with Vitavax Captan (77.5%).

Table (16): Effect of the fungicides on the percentages of germination pre- and post- emergence damping-off and survival plants of cotton in sterilized soil infested with R.solani, F.oxysporum and Scl.rolfsii.

Treatment	% germination	% damping-off		% Survival plants
		pre - emergence	post -	
Control	95	5	2.5	92.5
<u>Rhizoctonia solani</u>	67.5	32.5	10	57.5
Vitavax captan	75	25	2.5	72.5
Topsin M 70	77.5	22.5	7.5	70.
<u>Fusarium oxysporum</u>	75	25	5	70
Vitavax captan	80	20	2.5	77.5
Topsin M70	85	15	2.5	82.5
<u>Sclerotium rolfsii</u>	70	30	2.5	67.5
Vitavax captan	87.5	12.5	..	87.5
Topsin M70	75	25	7.5	67.5
Mixture of the 3-pathogens.....	62.5	37.5	12.5	50.
Vitavax captan	77.5	22.5	7.5	70.
Topsin M70	75	25.	7.5	67.5

Effect of fungicides on fungal counts in soil and rhizosphere of cotton plants sown in sterile soil infested with the pathogens R.solani and Scl.rolfsii:

Fungicides application decreased the fungal counts in sterilized soil infested with the pathogens Rhizoctonia solani or Sclerotium rolfsii. This was observed in the rhizosphere of cultivated soil and in the uncultivated soil. However, the effect was more drastic in the uncultivated soil. It seems that root exudates supply the pathogens with nutrient compounds which increase their tolerance to the toxic effect of such chemicals. Data also show that the higher the application dose the more effect on fungal counts. This logic result was observed with both fungicides in cultivated and uncultivated soils infested with Rhizoctonia solani and Sclerotium rolfsii.

The toxic effect of the fungicides on the pathogens was obvious after 7 days from application and the severe effect lasted at least up to 30 days from application after which fungal counts either continued to decrease or remained nearly constant or started to increase slowly. However,

fungus counts; in all treatments; were far lower than that of control till the end of the experiment after 90 days from fungicide application. This indicates the persistence of the fungicides in the sterilized soil in the absence of soil microflora which play an important role in the degradation of these compounds.

The data also indicate that the fungicide Vitavax Captan seemed to be more toxic than Topsin M 70% to the pathogens Rhizoctonia solani and Sclerotium rolfsii. This result is in agreement with the earlier results obtained in vitro.

The fungicides proved their drastic effect on the pathogenic fungi Rhizoctonia solani and Sclerotium rolfsii in sterilized infested soil which is a step forward condition nearly resembling the natural conditions.

Table (17): Effect of fungicides on fungal counts in soil and rhizosphere of cotton plants sown in sterile soil infested with the pathogens R.solanii and Sol.rolfsii.  
( $\times 10^5$ /g dry weight)

Time of sampling (days)	Cultivated sterilized soil				Uncultivated sterilized soil			
	Control with inoculum	Vitavax captan	Topsin M		Control with inoculum	Vitavax captan	Topsin M	
			N	10 N			N	10 N
infested with <u>Rhizoctonia solani</u>								
0-time	10.2	11.	6.5	8.66	4.5	4.22	5.05	5.44
7-days	18.4	5.62	1.0	6.6	4.7	1.677	0.22	2.88
15-days	35.5	2.57	0.99	5.33	5.9	1.16	0.17	1.33
30-days	33.6	1.68	1.08	5.33	4.4	1.15	0.722	1.27
60-days	20.7	4.37	3.12	12.4	5.5	1.44	0.511	1.37
90-days	39.7	16.25	12.	23.3	6.5	1.44	0.14	2.34
infested with <u>Sclerotium rolfsii</u>								
0-time	7.3	4.11	4.11	5.66	5.5	3.02	4.14	5.77
7-days	10.1	1.77	0.77	3.78	5.5	1.18	0.16	2.60
15-days	18.3	1.20	0.32	2.11	5.9	0.14	0.13	0.46
30-days	22.2	1.83	0.66	2.87	6.5	0.45	0.50	0.28
60-days	34.4	3.11	1.66	3.22	3.6	0.611	0.160	0.94
90-days	52.5	4.1	4.6	8.8	4.5	1.11	0.10	2.00
infested with Mixture from <u>R.solani</u> and <u>Scl.rolfsii</u>								
0-time	7.1	4.44	7.22	5.77	4.8	3.55	7.22	2.22
7-days	9.5	2.44	0.57	3.65	1.37	0.58	0.711	1.33
15-days	12.2	0.24	0.51	2.37	0.46	0.324	0.18	1.11
30-days	22.6	1.44	0.7	1.55	0.60	0.351	0.322	0.58
60-days	24.9	2.77	0.27	2.41	1.13	0.315	0.522	1.32
90-days	32.5	6.2	4.88	8.88	4.5	1.11	1.0	1.52

Effect of infestation with the pathogens and application  
of fungicides on microbial counts:

1- Effect of soil infestation with R.solani and application  
of fungicides on total bacterial count:

Data in Table (18) show that total bacterial count in control I (uninfested) increased in the rhizosphere of cultivated soil with the increase of plant age, being always higher than the uncultivated soil (control I of the uncultivated soil). The highest counts were obtained from rhizosphere samples taken 60-90 days after sowing. This period represents the active vegetative growth of cotton plants where root exudates are abundant.

Infestation with R.solani increased the total bacterial counts in rhizosphere samples but showed no obvious trend in the uncultivated soil.

Table(18) show that the application of the fungicides Vitavax Captan and Topsin M 70 reduced the bacterial counts in the infested, cultivated and uncultivated, soils during the earlier stages of the experiment. However, at the later stages, bacterial counts levelled or even surpassed their respective control (control II, soil infested with R.solani).

Effect of soil infestation with R.solani and fungicides  
application on actinomycetes counts:

Table (18) indicates that actinomycetes counts increased in the rhizosphere (control I) at the latter stages of the experiment (60-90 days after sowing). This may be due to the abundance of root exudates at this stage of growth.

Infestation of the soil with R.solani increased actinomycetes counts in the rhizosphere of cotton plants after 30 days from sowing and thereafter. However, the infestation with R.solani in the uncultivated soil slightly increased the actinomycetes counts in all investigated samples.

Fungicides application reduced the actinomycetes counts in cultivated and uncultivated soils as compared to their respective control (control II). This was true for both fungicides Vitavax Captan and Topsin M 70%.

Effect of soil infestation with R.solani and fungicides  
application on fungal count:

Data in Table (18) show that fungal counts in control I increased in the rhizosphere of cotton plants, being always



higher than that of the uncultivated. The increase of fungal counts in the rhizosphere with the progress of plant age may be due to the increase in root exudates.

Infestation of the soil with R.solani increased the fungal counts in the cultivated and uncultivated soils. However, the increase in fungal counts due to infestation was more pronounced in the cultivated soil.

The application of the fungicides Vitavax Captan and Topsin M 70% decreased the fungal counts in soil infested with R.solani. The higher the application rate, the greater the reduction in fungal counts. The fungicides adversely affected the fungal counts, and the most drastic effect was observed after 7-15 days from fungicide application. Then, fungal counts increased progressively till the end of the experiment where it remained far lower than the respective control.

Effect of soil infestation with Sclerotium rolfsii and fungicides application on microbial counts:

Soil infestation with Scl.rolfsii increased the total bacterial, actinomycetes and fungal counts in the rhizosphere

Table (18): Count of microflora in cultivated and uncultivated soil, infested with R. solani and with fungicides treatment

Time of sampling	Cultivated soil				Non-cultivated soil			
	Control		Control		Control		Control	
	C I		C II		C I		C II	
	Uninoculated	inoculated	Vitavax captan	Topsin M 70	Uninoculated	inoculated	Vitavax captan	Topsin M 70
	N	10 N	10 N	N	N	10 N	10 N	10 N
Total count (x 10 <sup>5</sup> /g. dry weight)								
0-time	118	149.5	217	148	122.2	83	93.	91.5
7-days	183	234	129	133	64	93	81.	52.3
15-days	132	412	168	136	58	129	72.	61.1
30-days	165	515	168	141.5	116	140	164.	67.4
60-days	340	480	360	359	280	189	155.	211
90-days	312	490	473	239.13	421.7	193	149.	223
Actinomyceetes (x 10 <sup>5</sup> /g. dry weight)								
0-time	77	55	36.3	35	39.2	44.	32.4	41.1
7-days	71	62	19.0	20.4	22.	26.6	32.5	40.2
15-days	59	89	26	17	51	53.7	29.1	38.4
30-days	23	148	57	97.8	130	65.7	27.9	36.9
60-days	164	390	210	236	280	228	27.3	35.1
90-days	364	680	736	757	690	693	29.9	35.0
Fungi (x 10 <sup>3</sup> /g. dry weight)								
0-time	63.2	85.4	105	75	103.7	97.5	53.3	78
7-days	83.2	99.5	75	30.12	81.0	47.	42.2	101
15-days	81.	120	92	20.	104	39.	40.	112
30-days	104	245	112.	95	168	119	44.	98
60-days	110	511	265	235	291	225	47.	96
90-days	166	810	462	300	514	500	53.	74
Topsin M 70								
0-time								
7-days								
15-days								
30-days								
60-days								
90-days								
Vitavax captan								
0-time								
7-days								
15-days								
30-days								
60-days								
90-days								
Topsin M 70								
0-time								
7-days								
15-days								
30-days								
60-days								
90-days								

Control I ( C I ) = Uninfested soil  
Control II( C II ) = Soil infested with R. solani

of cotton plants.

In case of the uncultivated soil, the fungi count increased with the Scl.rolfsii inoculation. Scl.rolfsii inoculated in the uncultivated soil, increased the bacterial counts in the early stages; but in the later stages bacterial counts were greatly reduced. Soil infestation with Scl.rolfsii slightly increased the actinomycetes counts in the uncultivated soil.

The fungicides application decreased the bacterial actinomycetes, fungal counts in soil (uncultivated) and rhizosphere of cotton plants. The higher the rate of application the greater the reduction in microbial counts. This was observed with both fungicides in the cultivated and non cultivated soils.

Effect of infestation with R.solani and Scl.rolfsii and fungicides application on microbial counts:

Infestation of the soil with both pathogens increased the microbial counts (bacteria, fungi and actinomycetes) in cultivated soil, and also increased fungi, and actinomycetes counts in the uncultivated soil. However, total bacterial

Table (19): Count of microflora in cultivated and non-cultivated soil infested with Scl. rolfsii and with fungicides treatments

Time of sampling	Control				Cultivated soil				Non-cultivated soil					
	without		with	inoculation	without		with	inoculation	without		with	inoculation		
	inoculation	N	N		Vitavax captan	Topsin M 70	N		Vitavax captan	Topsin M 70	N		Vitavax captan	Topsin M 70
Total count (x 10 <sup>6</sup> /g. dry weight)														
O-time	118	132.9	215	135.2	125	188	83	177.3	210	196	183	118		
7-days	183	201.4	140.9	38	44.5	37.5	93	101.4	43.	44.1	66.4	28.2		
15-days	132	289.3	135.5	105	71.2	51.01	129	98.9	81.1	58.8	34.09	42.04		
30-days	165	366.6	152.5	94	101.3	142.1	140	92.9	69.6	52.8	65.1	25.8		
60-days	340	419.3	154	175	98.4	194.3	189	88.8	45.5	87.	114	41.		
90-days	312	512.9	242.1	315	304.3	295	193	84.5	169	102.4	123	119.5		
Actinomycetes (x 10 <sup>5</sup> /g. dry weight)														
O-time	77	66.6	64.	45.2	44.4	55	32.4	42.4	29.9	34	31	35		
7-days	71	76.4	34.	16.3	20.9	11	32.5	43.9	10.39	8.39	12.3	4.7		
15-days	59	111.1	25.	43.1	32.42	14.71	29.1	40.3	14.29	4.89	12.9	4.94		
30-days	23	249.9	60.9	30.9	44.2	33.7	27.9	39.2	11.1	11.1	18.08	1.34		
60-days	164	414.3	168	55.7	128.26	59.7	27.3	30.1	22	17.8	18.33	3.64		
90-days	394	498.5	420	300	367.3	330.4	29.9	32.3	25	22.5	17.02	21.27		
Fungi (x 10 <sup>3</sup> /g. dry weight)														
O-time	63.2	89.2	66.25	75	66.25	50	53.3	94.3	87.5	87.4	68.4	73.75		
7-days	83.3	112.9	6.125	6.75	16.25	5.0	43.2	114.2	20.0	13.37	16.25	17.87		
15-days	81.	149.3	5.25	3.75	14.0	3.5	40.	131.9	15.25	12.75	18.25	16.0		
30-days	104.	188.9	98.75	62.5	63.75	14.75	44	101.4	33.75	10.5	70.0	11.0		
60-days	110	212.9	150	137.5	156.25	77.5	47	98.8	112.5	75	162	23.75		
90-days	166	354.2	287.5	225	262.5	250.	53	99.3	137.5	112	125	125		

Control I (C I)= Uninfested soil.  
Control II (C II) = Soil infested with Scl. rolfsii

count in the uncultivated soil increased in the initial stages and decreased in the later stages due to the infestation with both fungi.

The fungicides application reduced the microbial counts in the cultivated and uncultivated soils infested with both of the two pathogens. This trend was observed with both fungicides, specially at the earlier stages before the degradation of these chemicals.

During plate counting, microbial isolates were obtained from the rhizosphere of, seed dressed with fungicides, cotton plants sown in soil infested with the pathogens. The obtained isolates (675 fungal isolates, 722 bacterial isolates and 627 actinomycetes isolates) were investigated for antagonizing the root-rot pathogens, in vitro. This was carried out to answer on a question whether the fungicide application destroy the natural antagonists, or natural antagonists still exist inspite of fungicides application.

Table (26): Counts of microflora in cultivated and uncultivated soil infested with mixture of (*R. solani* and *Scl. rolfii*) and treated with fungicides.

Time of sampling	Control		Cultivated soil						Control						Non-cultivated soil					
	C I without Inoculation	C II with Inoculation	Viteavax captan				Topasin M 70		C I without Inoculation	C II with Inoculation	Viteavax captan				Topasin M 70					
			N	10 N	N	10 N	N	10 N			N	10 N								
Total counts ( $\times 10^6/\text{g. dry weight}$ )																				
0-time	118	172.1	122.7	205.6	167.7	165.8	83	102	125.5	132.5	165.8	156.4								
7-days	183	199.4	62.5	126	118.8	118.8	93	144	54.6	46.5	54.7	94.								
15-days	132	261.3	103.3	44	94.3	83	129	120	78.8	44.4	163.6	31.8								
30-days	165	414.2	118.2	104.3	202.2	123.5	140	101.5	42.6	55.	86.5	26.9								
60-days	340	512.9	329	412	247	330	189	93.2	266	55.	110	52.								
90-days	312	684.1	473.6	431.5	489.1	473.9	193	71.9	271.7	108	251.	117								
Actinomycoetes ( $\times 10^5/\text{g. dry weight}$ )																				
0-time	77	75.5	80	90	83	112.2	32.4	88.1	64.	81	94.2	94.2								
7-days	71	99.9	14.7	5.1	11.1	10.1	32.5	81.9	3.48	10.4	31.7	21.7								
15-days	59	139.2	41.9	10.32	56	50.5	29.1	72.4	3.59	1.46	22.4	33.7								
30-days	23	313.9	64.9	20.6	58	58.9	27.9	71.1	13.3	21.1	15.6	31.25								
60-days	164	545	427	105	65	127.17	27.3	81.1	43.4	184.7	122.2	159.5								
90-days	364	674.	705	721	540	740	29.9	74.4	217	184	257	140.4								
Fungi ( $\times 10^3/\text{g. dry weight}$ )																				
0-time	63.2	85.2	62.	102.5	61.25	120	53.3	94.3	90.0	71.25	85	78.75								
7-days	83.3	94.8	7.75	10.0	15.0	15.0	43.2	101.6	10.0	12.5	18.75	7.55								
15-days	81.	109.3	9.75	6.75	16.37	9.62	40.	98.3	11.62	9.5	25.0	2.70								
30-days	104.	194.3	15.25	37.5	16.75	8.0	44	88.1	30.	18.37	40.0	15.12								
60-days	110	249.1	50.0	65.	37.5	50.	47	84.9	82.	106	85.0	103.75								
90-days	166	315.9	287.	250	150	125	53	83.2	125	112	200	225								

Control I (C I) = Uninfested soil

Control II (C II) = Soil infested with *R. solani* + *Scl. rolfii*

Antagonistic efficiency of microbial isolates from the rhizosphere of seed dressed with fungicides, cotton plants against root pathogens R. solani and Scl. rolfsii.

A. Antagonistic Fungi:

1- Fungal antagonists against R. solani:

Data in Table (21) show the efficiency of the fungal isolates from the rhizosphere of, seed dressed with fungicides, cotton plants against R. solani.

The data indicate that out of 675 fungal isolates, 170 isolates antagonized R. solani. These antagonists consisted of 38 weak isolates, 50 moderate and 82 potent antagonists against R. solani.

Concerning the potent antagonists it was found that all the light brown Aspergillus isolates (30 isolates) and the Trichoderma (16 isolates) were potent antagonists and 80% of Penicillium (olive, 25 isolates) were potent antagonists against R. solani.

2- Fungal antagonists against Scl. rolfsii:

Data in Table (22) present the antagonistic fungi against Scl. rolfsii. The data show that out of 675 fungal isolates investigated, 150 isolates showed antagonism against Scl. rolfsii. Out of these antagonists, 35 isolates were found

Table (21) : Antagonistic fungal isolates, from the rhizosphere of cotton plants, against Rhizoctonia solani , on soil extract agar medium.

Fungi	Number of tested isolates	Number of antagonists	Number of antagonists which were:			Percentage of antagonists			Percentage of total antagonists (%)
			weak	moderate	high	weak	moderate	high	
<u>Aspergillus</u> spp.	170	42	-	-	30	-	-	100	24.7
Light brown	30	30	-	-	-	-	-	-	
Green.	40	12	10	2	-	83.3	16.7	-	
Black.	100	-	-	-	-	-	-	-	
<u>Penicillium</u> spp.	129	73	-	11	5	-	68.75	31.25	56.5
Brown.	34	16	-	5	20	-	20.00	80.00	
Olive green.	25	25	-	19	11	6.25	59.30	34.45	
Miscellaneous.	70	32	2	-	-	-	-	-	
<u>Rhizopus</u> spp.	139	-	-	-	-	-	-	-	
<u>Alternaria</u> spp.	84	8	6	2	-	75.00	25.00	-	9.4
<u>Fusarium</u> spp.	73	4	4	-	-	100.00	-	-	5.4
<u>Cladosporium</u> spp	22	12	4	8	-	33.30	66.60	-	54.5
<u>Myrothesium</u> spp.	24	-	-	-	-	-	-	-	-
<u>Epicoicum</u> spp.	18	15	12	3	-	80.00	20.00	-	83.3
<u>Trichoderma</u> spp.	16	16	-	-	16	-	-	100.00	100.0
Total	675	170	38	50	82	22.30	29.40	48.20	25.1



Table ( 22 ) : Antagonistic fungal isolates from the rhizosphere of cotton plants, against Sclerotium rolfsii, on soil extract agar medium.

Fungi	Number of tested isolates	Number of antagonists	Number of antagonists which were:			Percentage of antagonists which were:			Percentage of total antagonists
			weak	moderate	high	weak	moderate	high	
<u>Aspergillus</u> spp	170	42	-	-	30	- %	- %	100 %	24.7 %
Light brown	30	30	-	-	30	- %	- %	100 %	100 %
Green	40	12	12	-	-	100 %	- %	- %	30 %
Black	100	-	-	-	-	- %	- %	- %	- %
<u>Penicillium</u> spp	129	68	-	-	-	-	-	-	-
Brown	34	20	4	13	3	20 %	65 %	58.8%	52.7 %
Olive green	25	25	-	-	25	-	-	100%	58.8 %
Miscellaneous	70	23	3	11	9	13 %	47.8 %	39.1%	100 %
35.8 %									
<u>Rhizopus</u> spp	139								
84									
<u>Alternaria</u> spp	84	4	3	1	-	75 %	25 %	- %	4.78 %
<u>Fusarium</u> spp	73	3	3	-	-	100 %	- %	- %	
<u>Cladosporium</u> spp	22	9	4	5	-	44.4 %	55.5%	-	40.9 %
<u>Myrothecium</u> spp	24	-	-	-	-	-	-	-	
<u>Epicoecum</u> spp	18	8	6	2	-	75 %	25 %	-	44.4 %
<u>Trichoderma</u> spp.	16	16	-	-	16	-	-	100 %	100 %
<b>Total</b>	<b>675</b>	<b>150</b>	<b>35</b>	<b>32</b>	<b>83</b>	<b>23.3%</b>	<b>21.3</b>	<b>55.3 %</b>	<b>22.5 %</b>

to be weak, 32 isolates moderate and 83 isolates high antagonists. All the investigated isolates of the light brown Aspergillus, olive Penicillium and Trichoderma proved to be potent antagonists against Scl.rolfsii. These results may facilitate the detection of the potent antagonists against the root-rot pathogens.

#### B. Antagonistic Bacteria:

##### 1- Bacterial antagonists against R.solani:

Data in Table (23) indicate that out of 722 bacterial isolates investigated, 160 isolates antagonized R. solani. Regarding the efficiency of the antagonists, against R.solani, 83 isolates were found to be weak antagonists 43 moderate isolates and 34 isolates were high antagonists.

Most of the potent bacterial antagonists against R.solani were found to belong to genera Pseudomonas and Bacillus.

It is observed from the data that more antagonists were found in the sample of the later stages of plant growth than that of early stages. This is a logic result, since toxicity of the fungicides diminish at least partially in

Table (23): Antagonistic bacterial isolates from the rhizosphere of cotton plants, against Rhizoctonia solani on soil extract agar medium.

Stage of isolation	Number of tested isolates	Number of antagonists	Number of antagonists which were : -----			Percentages of antagonists which were : (%) -----			Percentage of total antagonists (%)
			weak	moderate	high	weak	moderate	high	
0 time	97	12	6	4	2	50	33.3	16.7	12.5
7 days	50	4	1	3	-	25	75.0	-	8.0
15 days	83	17	7	6	4	41	35.29	23.52	20.48
30 days	152	23	11	6	6	48	26.0	26.0	44.2
60 days	160	50	29	8	13	58	16.0	26.0	31.2
90 days	180	54	29	16	9	53	29.6	16.6	30.0
Total	722	160	83	43	34	51.8	26.8	21.2	22.1

the later stages. However, potent antagonists were found in the soil samples taken after 15 days from fungicide application, a period of maximal toxicity of the fungicides on microorganisms (from earlier experiments). This indicates the persistence of the potent bacterial antagonists to fungicides.

## 2- Bacterial antagonists against *Scl.rolfsii*:

Data in Table (24) indicate that out of 722 bacterial isolates investigated, 171 isolates antagonized *Scl.rolfsii*. Out of these antagonists, 66 isolates were weak, 51 isolates moderate and 54 isolates proved to be efficient antagonists. The preliminary identification showed that most of the potent antagonists belonged to genera *Pseudomonas* and *Bacillus*. It is of interest to find a significant number of potent bacterial antagonists in samples taken from periods where the effect of the fungicides was supposed to be severe. This may indicate the tolerance of bacterial antagonists to the effect of these chemicals in soil.

Table ( 24 ) : Antagonistic bacterial isolates from the rhizosphere of cotton plants, against Sclerotium rolfsii, on soil extract agar medium.

Stage of isolation	number of tested isolates	No. of antagonists	Number of antagonists which were:			percentage of antagonists which were :			percentage of total antagonists
			weak	moderate	high	weak	moderate	high	
0 Time	97	13	13	-	-	100 %	- %	-	13.4 %
7 days	50	2	1	1	-	50 %	50 %	-	4 %
15 days	83	42	21	11	10	50 %	26 %	24 %	50.6 %
30 days	152	52	14	20	18	26.9%	38.4 %	34.7%	34.2 %
60 days	160	22	5	3	14	22.7%	13.6 %	63.6%	13.75%
90 days	180	40	12	16	12	30 %	40 %	30 %	22.2 %
Total	722	171	66	51	54	38.8%	29.8%	31.5%	23.6 %

### C. Antagonistic actinomycetes:

#### 1- Actinomycetes antagonists against R. solani:

Data in Table (25) indicate that out of 627 actinomycetes isolates investigated, 281 isolates showed antagonism against R. solani. The actinomycetes antagonists consisted of 81 weak isolates, 78 moderate and 122 isolates proved to be high antagonists.

In case of the non-coloured actinomycetes, the percentage of isolates which showed antagonism against R. solani was 40%, but coloured actinomycetes showed higher percentages of antagonists.

Concerning the efficiency of the antagonists, 25.5% of the non-coloured antagonists proved to be potent antagonists. In case of the coloured actinomycetes the percentages of potent antagonists almost were higher than that of the non-coloured.

The majority of, the violet antagonists (86.96%), yellow antagonists (80%) and orange antagonists (66.67%) were potent antagonists. On the other hand, the poorest coloured antagonists were the blue and dark brown isolates.

Grouping actinomycetes on the basis of their colours was done in order to facilitate the detection and isolation

Table (25): Antagonistic actinomycetes isolates, from the rhizosphere of cotton plants, against Rhizoctonia solani, on soil extract agar medium.

Colour of isolates (pigment)	Number of tested isolates	Number of tested antagonists	Number of antagonists which were:-----			Percentages of antagonists which were:-----			Percentage of total antagonists
			weak	moderate	high	weak	moderate	high	(%)
Non-pigmented.	235	94	38	32	24	40	34.5	25.5	40
Pigmented.									
Dirty	41	27	8	6	13	25.6	22.2	48.2	65.8
Rose	24	12	6	4	2	50.0	33.3	16.7	50.0
Blue	5	1	1	-	-	100.0	-	-	20.0
Violet	121	46	-	6	40	-	13.1	86.9	38.0
Brown	50	30	8	8	14	26.6	26.6	46.8	60.0
Dark brown	8	2	2	-	-	100.0	-	-	25.0
Orange	14	6	2	-	4	33.3	-	66.7	45.8
Yellow	40	20	-	4	16	-	20.0	80.0	50.0
Black	42	20	8	4	8	40.0	20.0	40.0	47.6
Grey	26	12	6	6	-	50.0	50.0	-	46.1
Miscellaneous	21	11	2	8	1	18.1	72.7	9.09	52.3
Total	627	281	81	78	122	28.8	43.5	43.5	44.8

of the potent coloured antagonists.

## 2- Actinomycetes antagonists against Scl.rolfsii:

Data in Table (26) indicate that out of 627 actinomycetes isolates investigated, 279 isolates antagonized Scl.rolfsii. The antagonists were grouped to 87 weak , 93 moderate and 99 potent antagonistic isolates.

In case of the non-coloured actinomycetes, the percentage of isolates which antagonized Scl.rolfsii was 41.7%, but some coloured actinomycetes showed higher percentages of antagonists. However, the percentages of antagonists were 80% of the yellow, 64% of the brown and 53.8% of the grey actinomycetes. The other coloured isolates gave approximately equal or lower percentages of antagonists.

Concerning the efficiency of the antagonists against Scl.rolfsii, it was found that 32.70% of the non-coloured antagonists were potent in antagonizing Scl.rolfsii. However, some coloured actinomycetes showed higher percentages of the potent antagonists among their respective groups. It was found that 57.3% of the grey antagonists, 52% of the violet, 43.5% of the brown antagonists were potent in antagonizing



Table (26): Antagonistic actinomycetes isolates, from the rhizosphere of cotton plants, against Sclerotium rolfsii, on soil extract agar medium.

Colour of isolates (pigment)	Number of tested isolates	Number of antagonists	Number of antagonists which were:			Percentage of antagonists which were: (%)			Percentage of total antagonists (%)
			weak	moderate	high	weak	moderate	high	
Non-pigmented	235	98	28	38	32	28.5	38.8	32.7	41.7
Pigmented:									
Dirty	41	18	10	4	4	56.0	22.0	22.0	43.9
Rose	24	6	4	-	2	66.6	-	33.4	25.0
Blue	5	1	-	1	-	-	100.0	-	20.0
Violet	121	46	11	11	24	24.0	24.0	52.0	38.0
Brown	50	32	10	8	14	31.5	25.0	43.5	64.0
Dark brown	8	3	-	2	1	-	66.6	33.4	37.5
Orange	14	6	2	4	-	33.4	66.6	-	42.8
Yellow	40	32	14	8	10	43.7	25.0	31.3	80.0
Black	42	12	4	4	4	33.3	33.3	33.3	28.5
Grey	26	14	2	4	8	14.2	28.5	57.3	53.8
Miscellaneous.	21	11	2	9	-	18.1	81.9	-	52.3
Total	627	279	87	93	99	33.1	33.3	35.6	44.4

Scl.rolfsii. On the other hand, the blue and orange actinomyces antagonists did not give potent isolate against Scl.rolfsii.

Effect of the fungicides Vitavax Captan and Topsin M 70% on the most potent antagonistic fungi, Aspergillus sp., Penicillium sp. and Trichoderma sp. isolates, in vitro:

A- Effect of Vitavax Captan on the most potent fungal antagonists:

Data in Table (27) show that Vitavax Captan deleteriously affected the growth of the potent antagonist, Aspergillus sp. isolate at low concentration (50 ppm) and completely inhibited the growth of the fungus at 100 ppm. The growth of Penicillium sp. isolate was greatly affected (Inhibition index 82.41%) at a concentration of 200 ppm and the growth of the fungus was completely inhibited at 300 ppm. In case of Trichoderma sp. isolate, the fungicide Vitavax Captan greatly inhibited the growth at 400 ppm and completely inhibited the growth at 500 ppm.

These results indicate that the potent antagonistic fungi differed in their tolerance to Vitavax Captan.

B. Effect of Topsin M 70% on the most potent antagonistic fungi:

Data in Table (27) show that Topsin M 70 showed slight toxicity to the potent antagonist, Aspergillus sp. isolate which tolerated 1000 ppm of Topsin M 70, and 5000 ppm were required for the complete inhibition of this fungus.

The fungicide Topsin M 70 was more effective on the growth of Penicillium sp. and Trichoderma sp. isolates. In case of Penicillium sp. isolate, 100 ppm of the chemical were sufficient for its complete inhibition. The growth of Trichoderma sp. isolate was completely inhibited at 200 ppm of Topsin M 70.

These results indicate that Aspergillus sp. isolate was very sensitive to Vitavax Captan, but can tolerate high concentrations of Topsin M 70. The potent antagonist Penicillium sp. isolate was sensitive to Topsin M 70 than Vitavax Captan. The efficient antagonist, Trichoderma sp. isolate was sensitive to Topsin M70, but could tolerate moderate concentrations of Vitavax Captan.

**antagonistic strand (in vitro).**

## Footnotes

10,000 ppa

**Topix in 70%**

10:00 p.m.

10:00 p.m.

Effect of the fungicides Vitavax Captan and Topsin M70% on  
the potent antagonistic bacteria, *Pseudomonas* sp. and  
*Bacillus* sp. isolates, in vitro:

A. Effect of Vitavax Captan on most potent antagonistic  
bacteria:

Data in Table (28) show that Vitavax Captan had a drastic effect on the counts of the antagonistic bacterial isolates number 1,2 and 5. These isolates were very sensitive to Vitavax Captan and 5 ppm of the chemical caused complete inhibition. The isolate number (4) (*Pseudomonas* sp. tolerated 5 ppm of the chemical and 50 ppm caused complete inhibition of this isolate. The bacterial isolate number 3 (*Bacillus* sp. tolerated 10,000 ppm Vitavax Captan.

B. Effect of Topsin M 70 on the most potent antagonistic  
bacteria :

Data in Table (28) indicate that Topsin M 70 % showed low toxicity to the potent antagonistic bacterial isolates. The isolate number 1 (*Bacillus* sp.) tolerated 5000 ppm of the fungicide Topsin M 70 % and 10,000 ppm of this chemical were required for the complete inhibition of this isolate.

Table (28): Effect of the fungicides Vitavax Captan and Topsin M70 on the most potent antagonistic (Pseudomonas sp. and Bacillus sp.) isolates.

Treatment	Inhibition Index				
	Bacillus	Pseudomonas	Bacillus	Pseudomonas	Bacillus
	sp.	sp.	sp.	sp.	sp.
	(1)	(2)	(3)	(4)	(5)
Vitavax Captan					
5 ppm	100	100	62.73	00.00	100
50 ppm	100	100	68.32	100	100
100 ppm	100	100	69.87	100	100
200 ppm	100	100	78.26	100	100
300 ppm	100	100	78.88	100	100
400 ppm	100	100	80.74	100	100
500 ppm	100	100	81.98	100	100
1000 ppm	100	100	82.29	100	100
5000 ppm	100	100	84.78	100	100
10,000 ppm	100	100	90.99	100	100
Topsin M 70					
5 ppm	32.55	42.10	50.62	7.14	30.33
50 "	44.18	56.14	72.5	9.99	80.42
100 "	48.83	64.91	67.5	10.28	80.82
200 "	53.48	70.17	70.	25.55	100
300 "	56.97	78.94	71.25	29.41	100
400 "	63.95	91.22	72.99	34.87	100
500 "	69.76	100	100	44.44	100
1000 "	74.41	100	100	81.48	100
5000 "	84.42	100	100	100	100
10,000 "	100	100	100	100	100

The isolate number (2) was more sensitive than number(4). The isolate number 4 (Pseudomonas sp.) tolerated 1000 ppm. of Topsin M 70 and 5000 ppm caused complete inhibition. The isolate number 5 (Bacillus sp.) was more sensitive to Topsin M 70 %, it could tolerate 100 ppm and the complete inhibition occurred at 200 ppm of the fungicides.

Effect of the fungicides Vitavax Captan and Topsin M 70 on the most potent antagonistic actinomycetes, in Vitro.

Data in Table (29) show that Vitavax Captan deleteriously affected the growth of the potent antagonistic actinomycetes. The highest concentration which could be tolerated was 200 ppm. It's worthy to mention that different isolates showing the same pigment (colour) differed in their tolerance to Vitavax Captan.

Effect of Topsin M 70 on the most potent antagonistic actinomycetes :

Data in Table (29) show that Topsin M 70% could be tolerated by most of the potent actinomycetes antagonists. Data show that 3 out of 4 potent antagonistic isolates (dirty black colour) could tolerate up to 5000 ppm of the fungicide Topsin M 70%.

Table (29): Effect of the fungicides Vitavax Captain and Topsin M 70% on the most potent antagonistic Ascomycetes.

Treatment	Inhibition Index of the potent antagonistic coloured ascomycetes isolates														
	Violet					Dirty					Yellow				
	1	2	3	4	1	2	3	4	1	2	1	2	1	1	1
Vitavax Captain															
5 ppm	100	11.00	15.00	20.00	100	12.22	9.12	10.00	100	21.25	100	100	14.7	100	100
50 "	100	25.0	100	100	100	40.5	100	100	100	61.29	100	100	100	100	100
100 "	100	92.5	100	100	100	80.4	100	100	100	80.00	100	100	100	100	100
200 "	100	100	100	100	100	99.2	100	100	100	100	100	100	100	100	100
300 "	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
400 "	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
500 "	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1000 "	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
5000 "	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
10,000 "	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Topsin M 70%															
5 ppm	79.	00.0	00.00	0.0	100	13.33	6.66	4.00	47.72	3.00	25.34	86.93	00.00	EL.96	
50 "	84.	00.0	00.00	0.0	100	20.	14.44	20.00	62.	35.48	66.6	100	16.66	100	
100 "	86.	00.00	0.0	0.0	100	33.33	18.8	28.8	69	36.7	95.6	100	50.55	100	
200 "	89.	00.00	0.0	0.0	100	66.66	22.2	40.4	79	37.8	100	100	52.22	100	
300 "	93.	00.00	0.0	0.0	100	80.	33.3	52.3	86	39.9	100	100	66.66	100	
400 "	99.5	00.00	0.0	0.0	100	86.6	41.4	60.9	92	41.93	100	100	68.88	100	
500 "	100	00.00	0.0	0.0	100	88.8	49.9	62.7	100	49.35	100	100	75.8	100	
1000 "	100	25.	0.0	50.	100	90.0	57.7	76.8	100	56.45	100	100	83.33	100	
5000 "	100	75	33.3	66.	100	93.3	66.6	84.4	100	88.7	100	100	96.66	100	
10,000 "	100	100	72.2	100	100	100	93.3	100	100	100	100	100	100	100	



The data show that the investigated potent actinomycetes antagonists tolerated higher concentrations of Topsin M 70% than Vitavax Captan.

Biological control as compared to chemical control of damping-off in cotton (Giza 70) sown in soil infested with R.solani, F.oxysporum and Scl.rolfsii:

The effect of seed inoculation with potent antagonists namely Aspergillus sp. and Penicillium sp. isolates on the percentages of germination, pre- and post-emergence damping-off and survival plants in cotton (Giza 70) sown in natural soil infested with the pathogens R.solani, F.oxysporum and Scl.rolfsii was presented in Table (30). The effect of seed dressing with the recommended dose of the fungicides Vitavax Captan and Topsin M 70% on reducing damping-off in cotton was also presented in Table (30) for comparison.

Data in Table (30) show the following:

Seed inoculation with the potent antagonist, Aspergillus sp. isolate, increased the percentages of germination and survival plants and decreased the damping-off percentages as compared to control (II).

The effect of seed-inoculation with the potent antagonist Penicillium sp. isolate on reducing the damping-off in cotton was not as great as that obtained with the Aspergillus sp. isolate inoculation. However, seed inoculation with the two potent antagonistic isolates (Aspergillus sp. isolate + Penicillium sp. isolate) showed the greatest effect than when any of the antagonists were inoculated solely. Inoculation with both the two antagonists (Aspergillus + Penicillium) increased the germination percentage from 55% (control II) to 80% and survival plants percentage from 37.5% (control II) to 80%, and decreased the pre-emergence damping-off from 45% (control II) to 20% and post-emergence damping-off from 17.5% to Zero%.

Table (30) also show the effect of seed dressing with the fungicides Vitavax Captan and Topsin M 70% on reducing the damping-off in cotton.

The results recorded in Table (30) show that biological control nearly levelled chemical control of damping-off in cotton. This indicates that biological control could substitute chemical control to avoid the problem of environmental pollution and the side effect of such chemicals on

Table (30): Effect of seed inoculation with the potent antagonists (Aspergillus sp. and Penicillium sp. and seed-dressing with Vitavax Captan and Topsin M 70 on the percentages of germination and pre- and post-emergence damping-off in cotton.

Treatments	% germination	% damping-off		% survival plants
		pre - emergence	post - emergence	
Control I <sup>*</sup>	95	5	2.5	92.5
Control II <sup>**</sup>	55	45	17.5	37.5
<u>Aspergillus</u> spp.	75	25	2.5	72.5
<u>Penicillium</u> spp.	70	30	5	65
Mixture (Asp. + Penic)	80	20	0.0	80
Vitavax Captan	82.5	18.5	2.5	80
Topsin M 70	75	25	5	70

\* Control I: Untreated cotton seeds sown in natural soil.

\*\* Control II: Untreated cotton seeds sown in natural soil infested with the three pathogens (R.solani + F.oxysporum + Scl.rolfsii).

saprophytic microflora which are of great importance to soil fertility.

Effect of biological and chemical control of damping-off  
on the growth of survival plants of cotton (Giza 70) sown  
in soil infested with R.solani, F.oxysporum and Scl.rolfsii:

The effect of seed inoculation with the potent antagonists, Aspergillus sp. isolate, Penicillium sp. isolate on the growth of survival plants of cotton (Giza 70) sown in natural soil infested with the three pathogens (R.solani + F.oxysporum + Scl.rolfsii) was recorded in Table (31).

Data in Table (31) show the following results:

1) Infestation of the soil with the three pathogens (R.solani + F.oxysporum + Scl.rolfsii) decreased the length of the shoot system and the dry weight of the root system of survival plants.

2) Seed inoculation with the potent antagonists Aspergillus sp. and Penicillium sp. isolates almost increased the length, fresh and dry weight of the root and shoot systems of survival plants. However, seed

inoculation with a mixture of both of the two antagonists (Aspergillus sp. + Penicillium sp.) almost increased the plant growth than when any of the antagonists was inoculated solely.

The aforementioned results are in agreement with results of earlier investigators including Ali (1967), Sirry et al. (1970) and Neweigy et al. (1982).

Seed dressing with fungicides then sown in soil infested with the pathogens showed a trend of increasing the foliage growth. This result is in agreement with Eisa and Barakat (1978) and Habib (1979).

The aforementioned results show that biological control and chemical control almost increase the growth of survival plants.

Table (31): Effect of seed inoculation with the potent antagonists, Aspergillus sp., and Penicillium sp. and treatment with Vitavax Captain and Topsin M 70 on growth of cotton plants sown in soil infested with pathogenic fungi.

Treatment	Germination %	Shoot system			Root system		
		Length (cm)	Fresh weight (gm)	Dry weight (gm)	Length (cm)	Fresh weight (gm)	Dry weight (gm)
Control I*	95	11.76	1.44	0.328	9.147	0.250	0.098
Control II**	55	10.91	1.4	0.35	11.23	0.295	0.075
<u>Aspergillus</u> spp	75	14.87	1.512	0.350	15.31	0.271	0.110
<u>Penicillium</u> sp.	70	13.6	5.25	0.949	14.4	0.578	0.215
Mixture (Asp. + Penic)	80	16.	4.5	0.943	21.16	0.592	0.233
Vitavax Captain	82.5	14.44	2.37	0.447	12.3	0.241	0.063
Topsin M 70	75	16.33	4.933	1.080	9.33	0.73	0.361

\* Control I : Untreated cotton seeds sown in natural soil.

\*\* Control II: Untreated cotton seeds sown in natural soil infested with the three pathogens (R. solani + P. oxysporum + Sol. rolfii).

## DISCUSSION

### 1- The effect of fungicides on total count:

The effect of both the two fungicides Vitavax Captan and Topsin M 70 on total count, is presented in Tables (1 and 2), which show that fungicides adversely affected the total microbial counts. The higher the concentration of the fungicide, the greater the harmful effect on total microbial count. This result is in harmony with those of Halleck and Cochrane, 1950; Domsch, 1959; Chandra and Bollen, 1960; Mahmoud et al., 1972; Van Faassen, 1974 and Abd-El-Nasser, et al. 1979.

The toxic effect of both fungicides was observed after 3 days from application. Total count continued to decrease up to 15 days with the normal field application dose, but the inhibition with the 10 fold normal dose continued up to 30 days from application. After the inhibition period total counts increased progressively till the end of the experiment where at normal dose, counts hardly reached that of control with Vitavax Captan or surpassed control counts with Topsin M, in the rhizosphere of cotton plants. However, at the 10 fold dose total count failed to restore to control.

In the uncultivated soil total count failed to level that of control with both fungicides.

During the period of microbial inhibition, it seems that fungicides changed or degraded to more toxic compounds. After this period, fungicides or their toxic effect may have diminished gradually resulting in increasing the total microbial counts. In addition, survivors to the effect of fungicides may increased progressively due to the absence of competition and antagonism of other microflora which had been killed by the fungicides. Also, the decomposition of dead microorganisms supply survivors with nutrients for their multiplication.

## 2- Effect of fungicides on spore-formers count:

The effect of Vitavax Captan on spore-formers counts showed the same trend observed with the effect of the fungicides on total count. In case of Topsin M, it seems that this fungicide, at both concentrations used, was more harmful since its toxic effect lasted till the end of the experiment (90 days from application). Topsin M may affected spore-formers count either by interfering adversely with spore-germination or sporangium multiplication. Consequently, the counts of survivors, to the effect of the chemical ,



remained lower than control allover the experimental period.

### 3- Effect of fungicides on actinomycete counts:

Vitavax Captan and Topsin M showed a moderate effect on actinomycetes count, at the normal field application, but more harmful effect was observed at the 10 fold dose. The general trend of the effect of the fungicides on total count was observed with actinomycetes. At the normal field application rate, actinomycetes counts, in all treatments, almost surpassed that of control at the end of the experimental period. This result is in agreement with Domsch, 1959. The increase in actinomycetes counts at the end of the experimental period may be due to that actinomycetes are well known group active in decomposing such chemicals and may be capable of utilizing the products of decomposition.

### 4- Effect of the fungicides on fungi counts:

The fungicides Vitavax Captan and Topsin M showed a drastic effect on the fungi counts in the rhizosphere of cotton plants. At normal field application dose, Vitavax Captan reduced greatly the fungal counts in an early stage (3 days from application) indicating the harmful effect of

the fungicide itself on rhizosphere fungi. On the other hand, the toxicity of Topsin M started later (after 7 days from application) indicating a lag period required for changing or degrading the compound to more toxic compound on fungi than the original compound. In this respect, Clemons and Sisler (1969); Selling et al. (1970) and Vonk and Kaars-Sijpesteijn (1970) reported that Topsin 70%; Thiophanate methyl (TPM) is hydrolyzed to more toxic derivative, methyl 2-benzimidazole carbamide (MBC). In all treatments, the fungi counts were far lower than that of control, indicating the broad spectrum of both investigated fungicides on the different members consisting the fungi group .

#### 5- Effect of the fungicides on anaerobic nitrogen fixers:

Vitavax Captan, at the normal field application, reduced anaerobic nitrogen fixers, in the rhizosphere of cotton plants where minimal counts were obtained (34000) after 7 days as compared to control (216000/g dry weight). Then anaerobic nitrogen nitrogen-fixers count increased and surpassed that of control after 30 days from application and thereafter . This result is in accordance with the findings of Mahmoud et al. (1972). The increase in the count of this group at

the later stages might be due to the utilization of the nutrients released from the decomposition of microorganisms killed by the fungicide. In addition, products of the fungicide decomposition may be utilized by this group of microorganisms. However, the 10 fold application rate reduced the anaerobic nitrogen fixers in the rhizosphere of cotton plants all over the experimental period.

Topsin M showed harmful effect on anaerobic nitrogen fixers in the rhizosphere of cotton plants which lasted till the end of the experiment (90 days from application). This may be due to sensitivity of anaerobic nitrogen fixers to Topsin M or its compounds of degradation of residues.

#### Effect of fungicides on aerobic nitrogen fixers:

The density of aerobic nitrogen fixers in the rhizosphere of cotton plants, at the normal field application rate was reduced in the early stages. However, at the later stages aerobic nitrogen fixers increased till it reached or even surpassed that of control. At the 10 fold dose, aerobic nitrogen fixers decreased than their respective controls all over the experimental period. This result is in agreement

The effect of the fungicides Vitavax Captan and Topsin M on the different groups of microorganisms in the uncultivated soil, almost showed the same trend found in the rhizosphere of cotton plants.

The above discussion indicated that the degradation products of the fungicides may be more toxic to the investigated groups of microorganisms than the fungicides themselves. Most of the investigated groups of microorganisms were affected greatly within the period 7-15 days from application of the normal field rate. However, the drastic effect was extended to 30 days from application with the 10 fold normal dose.

The results of this investigation through the light on the harmful effect of these compounds on saprophytic microorganisms responsible for soil fertility. So, it is recommended to search for fungicides having selective toxicity to soil-borne pathogens.

EFFECT OF FUNGICIDES ON PATHOGENIC FUNGIA. Effect of the fungicides Vitavax Captan and Topsin M 70% on pathogenic fungi in vitro:

Sclerotium rolfsii was very sensitive to Vitavax Captan (5ppm). Rhizoctonia solani was completely inhibited by moderate dose (100 ppm), but Fusarium oxysporum tolerated higher doses and 500 ppm of the fungicide were required for the complete inhibition of the fungus.

Concerning the effect of Topsin M70% on pathogenic fungi Fusarium oxysporum was sensitive to Topsin M 70, where 100 ppm of the fungicide completely inhibited the fungus. Rhizoctonia solani could tolerate high doses of the chemical and 1000 ppm of the fungicide were required for the complete inhibition of the fungus. Sclerotium rolfsii tolerated very high concentrations of the fungicide, and the highest dose used in the investigation (10,000 ppm) did not caused complete inhibition of the fungus.

It seems that sensitivity of the pathogen to certain fungicide is governed, at least partially, by the speed of penetration of the chemical to the fungus. Mathre (1968) suggested that the fungicide (carboxin) (DMOC) or its product

of degradation oxycarboxin (DCMOD) were fungistatic against R.solani and Ustilago mydis. Although he found a rapid uptake of  $C^{14}$  DMOC (carboxin) and  $C^{14}$  DCMOD (oxycarboxin) by the above fungi, the resistant fungus Fusarium oxysporum f. sp. lycopersici absorbed very little of the fungicide from solution.

When Topsin M (70% thiophanate methyl) was used against the pathogens R.solani, F.solani and Scl.rolfsii, it was found that lower concentrations caused partial inhibition. At these lower concentrations, the effect of thiophanate methyl (TPM), on the growth of the fungi, increased with time. This may be due to hydrolysis of the compound to more toxic derivative. The in vitro conversion of thiophanate methyl (TPM) to carbendazin (MBC) was recorded by earlier investigators. Clemons and Sisler (1969), reported that benomyl is readily hydrolyzed in aqueous solution or agar media to methyl 2-benzimidazole carbamate (MBC). Selling et al. (1970) reported that when TPM was shaken in tap-water, for 5 days, MBC was detected. Vonk and Kaars-Sijpesteijn (1970) reported that the conversion of TPM to MBC increased the fungitoxic effect of TPM.

They also suggested that benzimidazole nucleus carries the actual fungistatic activity and it might interfere with cell energy production and nucleic acid synthesis.

B. Effect of the fungicides Vitavax Captan and Topsin M 70  
on reducing the damping-off in cotton plants in sterilized  
soil infested with R.solani, F.oxysporum and Scl.rolfsii:

The investigated fungi proved to be pathogenic to cotton plants. They reduced the percentages of germination and survival plants and increased the pre-emergence and almost increased the post-emergence damping-off as compared to control. Concerning the virulence, Rhizoctonia solani was the most virulent followed by Sclerotium rolfsii then Fusarium oxysporum. However, the infestation with the three pathogens gave the most severe effect than when any of the pathogens was inoculated solely. It seems that the pathogens intensified the virulence of each other. Fungicides application increased the percentages of germination and survival plants and almost decreased the pre- and post-emergence damping-off as compared to their respective

percentages in the control (infested soil, without fungicide application). This was true for both fungicides Vitavax Captan and Topsin M 70 applied at normal field application rate against the pathogens R.solani, Scl.rolfsii and F.oxysporum. It seems that Vitavax Captan was more effective against R.solani and Scl.rolfsii since it gave higher survival plants 72.5% and 87.5% as compared to those of Topsin M 70 which were 70% and 67.5%, respectively. On the other hand, Topsin M 70 seemed to be more effective against F.oxysporum since the percentage of survival plants was 82.5% as compared to that obtained with Vitavax Captan (77.5%). These results are in line with the results obtained in vitro, concerned with the sensitivity of these pathogens to the investigated fungicides.

C. Effect of fungicides on fungal counts in soil and rhizosphere of cotton plants sown in sterile soil infested with the pathogens R.solani and Scl.rolfsii:

Fungicides application decreased the fungal counts in sterilized soil infested with the pathogens Rhizoctonia solani or Sclerotium rolfsii. This was observed in the rhizosphere of



cultivated soil and in the uncultivated soils. However, the effect was more drastic in the uncultivated soil. It seems that root exudates supply the pathogens with nutrient compounds which increased their tolerance to the toxic effect of such chemicals. It was found that the higher the application dose the more effect on fungal counts. This logic result was observed with both fungicides in cultivated and uncultivated soils infested with Rhizoctonia solani and Sclerotium rolfsii.

Effect of infestation with the pathogens and application  
of fungicides on microbial counts:

1-Rhizoctonia solani

A. Effect of soil infestation with R.solani and application  
of fungicides on total bacterial count:

Total bacterial count increased in the rhizosphere of cultivated soil with the increase of plant age, being always higher than that of the uncultivated soil. The highest counts were obtained from rhizosphere samples taken 60-90 days after sowing. This period represents the active vegetative growth

of cotton plants where root exudates are abundant.

Infestation with R.solani increased the total bacterial counts in rhizosphere samples.

It seems that the development of disease, due to the infestation with the pathogen, resulted in seeping of nutrients from the roots, which enhanced higher microbial densities as compared to control.

Root-rot pathogens produced cell wall degrading enzymes for the maceration of root tissues (Garibaldi and Bateman, 1971; Goel and Mehrotra, 1974) resulted in the existence of high amounts of macerated tissues, thus encouraged the growth and proliferation of soil microflora.

The application of the fungicides Vitavax Captan and Topsin M 70 reduced the bacterial counts in the infested, cultivated and uncultivated soils during the earlier stages of the experiment. However, at the later stages, bacterial counts levelled or even surpassed their respective control.

The decrease in the microbial counts is due to the toxic effect of the fungicides on soil microflora, in the early stages before fungicides degradation. The increase

in microbial counts in the later stages may be due to the proliferation of survivors on nutrients from the decomposed dead microorganisms and the degradation products of the fungicides.

B. Effect of soil infestation with R.solani and fungicides application on actinomycetes counts:

Actinomycetes counts increased in the rhizosphere of cotton plants at the later stages of the experiment (60-90 days after sowing). This may be due to the abundance of root exudates at this stage of growth. This result is in agreement with the findings of Lugauskas (1963) and Parkinson and Thomas (1969).

Infestation of the soil with R.solani decreased actinomycetes in the earlier stages, then actinomycetes counts in the rhizosphere of cotton plants were increased after 30 days from sowing and thereafter. However, the infestation with R.solani in the uncultivated soil slightly increased the actinomycetes counts in all investigated samples.

The decrease in actinomycetes counts in the earlier stages is in agreement with the results of Mahmoud et al. (1980).

This may be due to the competition between the added pathogen and rhizosphere actinomycetes. The increase in actinomycetes counts in samples taken 15 days, from sowing, and thereafter may be due to seeping of nutrients from infected roots or decomposed root debris. Also, the counts of actinomycetes may increased due to sporulation (conidia formation).

Fungicides application reduced the actinomycetes counts in the infested cultivated and uncultivated soils in the early stages. However, at the later stages, actinomycetes counts levelled or surpassed that of control. This was true for both fungicides Vitavax Captan and Topsin M 70.

C. Effect of soil infestation with *R.solani* and fungicides application on fungal count:

Fungal counts increased in the rhizosphere of cotton plants with the increase of plant age. This may be due to the increase in root exudates. Fungi counts in the rhizosphere were always higher than that of the uncultivated soil.

Infestation of the soil with R.solani increased the fungal counts in the cultivated and uncultivated soils. However, the increase in fungal counts due to infestation was more pronounced in the cultivated soil. The increase in fungal counts in the rhizosphere due to R.solani is in agreement with Mahmoud et al. (1980).

The application of both fungicides Vitavax Captan and Topsin M 70 decreased the fungi counts in the cultivated and uncultivated soils infested with R.solani. The higher the application rate, the greater the reduction in fungal counts. The fungicides adversely affected the fungal counts, and the most drastic effect was observed after 15 days from fungicide application. This may be due to the degradation of the fungicides to more toxic compounds. Then, fungal counts increased progressively till the end of the experiment where it remained far lower than the respective control.

## 2- Effect of soil infestation with Sclerotium rolfsii and fungicides application on microbial counts:

Soil infestation with Scl.rolfsii increased the total bacterial, actinomycetes and fungal counts in the rhizosphere of cotton plants.

This may be due to the seeping of the nutrients from injured roots due to infection. Dicknson and Coly Smith (1970) found the same result with the total bacterial count. In vitro, they found the stimulation of bacteria on agar plates was due to the leakage of substances from Sclerotia.

The fungicides application decreased the bacterial actinomycetes, and fungal counts in soil and rhizosphere of cotton plants. The higher the rate of application the greater the reduction in microbial counts. This was observed with both fungicides in the cultivated and non-cultivated soils.

### 3- Effect of infestation with *R.solani* and *Scl.rolfsii* and fungicides application on microbial counts:

Infestation of the soil with both pathogens increased the microbial counts (bacteria, fungi, and actinomycetes) in cultivated soil, and also increased fungi, and actinomycetes counts in the uncultivated soil. However, total bacterial count in the uncultivated soil increased in the intial stages and decreased in the later stages due to the infestation with both fungi.

The increase in microbial counts in the rhizosphere

of cotton plants due to infestation with the pathogens may be due to leakage of nutrients from infected roots. In the uncultivated soil, fungi increased due to infestation and the increase in actinomycetes count in the infested soil may be due to sporulation.

The fungicides application reduced the microbial counts in the cultivated and uncultivated soils infested with both the two pathogens. This trend was observed with both fungicides.

Antagonistic efficiency of microbial isolates from the rhizosphere of seed dressed with fungicides, cotton plants against root pathogens R.solani and Sc1.rolfsii.

A. Antagonistic Fungi:

1- Fungal antagonists against R.solani:

Out of 675 fungal isolates investigated, 170 isolates antagonized R.solani. These antagonists consisted of 38 weak isolates, 50 moderate and 82 potent antagonists against R.solani.

Concerning the potent antagonists it was found that all the light brown Aspergillus isolates (30 isolates) and

the Trichoderma (16 isolates) were potent antagonists and 80% of Penicillium (Olive green, 25 isolates) were potent antagonists against R.solani.

## 2- Fungal antagonists against Scl.rolfsii:

Out of 675 fungal isolates investigated, 150 isolates showed antagonism against Scl.rolfsii. Out of these antagonists, 35 isolates were found to be weak, 32 isolates moderate and 83 isolates high antagonists. All the investigated isolates, of the light brown Aspergillus, olive green Penicillium and Trichoderma proved to be potent antagonists against Scl.rolfsii. These results may facilitate the detection of the potent antagonists against the root-rot pathogens.

The findings obtained in this investigation are in agreement with many earlier investigators. Mitchell and Alexander (1962), Huber et al. (1966); Orazov and Sizova (1966) ; Sirry et al. (1981); and Neweigy et al. (1982) found that Aspergillus sp. isolates, from soil, antagonized the root-rot pathogens. Penicillium sp. isolates from soil were found to antagonize the root-rot pathogens by many investigators including Sirry et al. (1981). Trichoderma sp. isolates were found to antagonize the root-rot pathogens



by many investigators including, Seiketov and Nikitina (1962); Kelley and Rodriguez-Kabona (1976); Chet et al. (1978); Henis et al. (1979); Sirry et al. (1981) ; and Newelgy et al. (1982). Horsfall and Diamond (1960) reported that Trichoderma sp. isolates produce gliotoxin or viridin which may be responsible for the inhibition of the root-rot pathogens.

#### B. Antagonistic Bacteria:

##### 1- Bacterial antagonists against R.solani:

The study indicated that out of 722 bacterial isolates investigated, 160 isolates antagonized R.solani. Regarding the efficiency of the antagonists, against R.solani, 83 isolates were found to be weak antagonist, 43 isolates moderate and 34 isolates were high antagonists.

Most of the potent bacterial antagonists against R. solani were found to belong to genera Pseudomonas and Bacillus.

##### Bacterial antagonists against Scl.rolfsii:

Concerning Scl.rolfsii, it was found that out of 722 bacterial isolates investigated, 171 isolates antagonized

Sci. rolfsii out of these antagonists , 66 isolates were weak, 51 isolates ~~moderate~~ and 54 isolates proved to be efficient antagonists. The preliminary identification showed that most of the potent antagonists belonged to genera Pseudomonas and Bacillus.

The production of antibiotic substances by members of the genus Pseudomonas was demonstrated by Naim and Husein (1958); Teliz-Ortiz and Brukholder (1960); Mikhaleva et al. (1965), Roa and Roa (1966); and Shklyar and Mansurova (1968) and El-Said (1976).

Naim and Husein (1958); Roa and Roa (1966); Broadbent and Mansurova (1968); Vlachov (1968); Broadbent and Baker (1969); Broadbent and Water worth(1971); Aly (1974); Mahmoud et al.(1980); Sirry et al.(1981) and Neweigy et al. (1982), found that Bacillus sp. isolates, from soil, antagonized the root-rot pathogens. The effect of Bacillus sp. isolates may be due to the production of antibiotics by these isolates. Olsen (1965) and Roa and Roa (1968) reported that members of the genus Bacillus produce antibiotics in soil. So, Bacillus isolates may inhibited the pathogens in vitro by antibiotics production.

C. Antagonistic actinomycetes:

1- Actinomycetes antagonists against R.solani:

The study showed that out of 627 actinomycetes isolates investigated, 281 isolates showed antagonism against R.solani. Th actinomycetes antagonists consisted of 81 weak isolates, 78 moderate and 122 isolates proved to be high antagonists.

In case of the non-coloured actinomycetes, the percentage of isolates which showed antagonism against R.solani was 40%, but coloured actinomycetes showed higher percentages of antagonists.

Concerning the efficiency of the antagonists, 25.5% of the non-coloured antagonists proved to be potent antagonists. In case of the coloured actinomycetes the percentages of potent antagonists almost were higher than that of the non-coloured.

The majority of the violet antagonists (86.9%), yellow antagonists (80%) and orange antagonists (66.7%) were potent antagonists. On the other hand, the poorest coloured antagonists were the blue and dark brown isolates.

Grouping the actinomycetes on the basis of their colours was done in order to facilitate the detection and isolation of the potent coloured antagonists.

2- Actinomycetes antagonists against Scl.rolfsii:

Concerning Scl.rolfsii, the study indicated that out of 627 actinomycetes isolates investigated, 279 isolates antagonized Scl.rolfsii. The antagonists were grouped into 87 weak, 93 moderate and 99 potent antagonistic isolates.

In case of the non-coloured actinomycetes, the percentage of isolates which antagonized Scl.rolfsii was 41.7%. However, the percentages of antagonists in the potent coloured groups were 80% for the yellow, 64% for the brown and 53.8% for the grey actinomycetes. The other coloured isolates gave approximately equal or lower percentages of antagonists.

Concerning the efficiency of the antagonists against Scl.rolfsii, it was found that 32.70% of the non-coloured antagonists were potent in antagonizing Scl.rolfsii. However, some coloured actinomycetes showed higher percentages of the potent antagonists, among the antagonists of their respective groups. It was found that 57.3% of the grey antagonists, 52% of the violet, 43.5% of the brown antagonists

were potent in antagonizing Scl.rolfsii. On the other hand, the blue and orange actinomycetes antagonists did not give any potent isolate against Scl.rolfsii.

The study showed high percentage of antagonistic actinomycetes (from the tested actinomycetes isolates) as compared to bacteria and fungi. This may be due to that actinomycetes are well known group as antibiotic producer (Waksman, 1957). Kurylowicz (1972) reported that more than 50% of the known antibiotics are produced by the actinomycetes. The preliminary identification of the potent actinomycetes antagonists against R.solani and Scl.rolfsii showed that they belonged to genus Streptomyces. This result is in accordance with Kurylowicz (1972) who reported that more than 98% of the antibiotics of the actinomycetes are produced by of the genus Streptomyces. Moreover, Streptomyces isolates were found to antagonize the root-rot pathogens by many investigators including; Shklyar and Mansurova (1968), Broadbent and Baker (1969); Broadbent and Waterworth (1971); Sirry et al. (1981); and Neweigy et al. (1982).

The study of antagonism was carried out using microbial isolates from the rhizosphere of seed dressed

with fungicides, cotton plants sown in soil infested with the root-rot pathogens. These isolates obtained from the seed-bed where the fungicide and the pathogen were added. These isolates were studied for their efficiency in antagonizing the root-rot pathogens in vitro.

The results of the antagonism study showed that inspite of using fungicides, as seed dressing against the root-rot pathogens, the rhizosphere still contain a great number of antagonists. In other words, applying the chemical control of the root-rot pathogens did not abolish the biological control.

Effect of fungicides Vitavax Captan and Topsin M 70 on the potent microbial antagonists against the root-rot pathogens:

1- Effect of fungicides on the potent fungal antagonists:

Vitavax Captan completely inhibited the potent antagonists Aspergillus sp. , Penicillium sp. and Trichoderma sp. at the concentrations 100 ppm, 300 ppm and 500 ppm, respectively. Topsin M 70 completely inhibited Aspergillus sp. , Penicillium sp. . and Trichoderma sp. isolates at 5000 ppm, 100 ppm and 200 ppm, respectively. These results

show that fungal antagonists differed in their tolerance to the tested fungicides. An antagonist may be sensitive to certain fungicide but could tolerate high concentrations of another fungicide.

2- Effect of fungicides on the potent bacterial antagonists:

The study showed that out of the 5 bacterial antagonists, 3 isolates were very sensitive to Vitavax Captan and were completely inhibited at 5 ppm. of the chemical. The fourth isolate was inhibited at 50 ppm, while the fifth isolate (Bacillus sp.) highly tolerated the toxicant and 10,000 ppm could not cause complete inhibition.

On the other hand, Topsin M, showed lower toxicity to the tested antagonists. The study showed that one isolate was sensitive, 2 isolates were moderate and 2 isolates highly tolerated Topsin M 70. It is worthy to mention that the strain which tolerated high concentrations of Vitavax Captan, tolerated only moderate concentrations of Topsin M 70. The other strains which were very sensitive to Vitavax Captan tolerated moderate or high contrations of Topsin M 70.

It seems that the effect of the fungicide against certain microorganism is correlated with the chemical

composition of the fungicide and the sensitivity of the organism may be correlated with the rate of uptake of the fungicide by the microorganism. The higher the rate of the fungicide uptake the higher the sensitivity of the microorganism (Muthre, 1968).

3- Effect of fungicides on the potent actinomycetes antagonists:

Vitavax Captan was more harmful than Topsin M on the growth of antagonistic actinomycetes. Actinomycete isolates giving the same pigment, differed in their sensitivity to each of the investigated fungicides.

In this study, the investigator gave only a touch on the sensitivity of the potent antagonists to different fungicides. This was carried out to through the light on this important topic which is concerned with the effect of chemical control on the antagonists responsible for biological control.



Biological control as compared to chemical control of  
damping-off in cotton (Giza 70) sown in soil infested  
with R.solani, F.oxysporum and Scl.rolfsii:

The infestation of the soil with the three pathogens (R.solani + F.oxysporum + Scl.rolfsii) greatly decreased the percentages of germination and survival plants and greatly increased the pre and post-emergence damping-off. It seems that pathogens intensified the virulence of each other and thus caused severe symptoms of the disease.

Seed inoculation with the potent antagonist Aspergillus sp. isolate increased the percentages of germination and survival plants, while the pre- and post-emergence damping-off were reduced, as compared to their respective control. Aspergillus isolates were found to antagonize the root-rot pathogens by many investigators including Huber et al. (1966); Orazov and Sizova (1966); Sirry et al. (1981); and Neweigy et al. (1982).

The effect of seed inoculation with the potent antagonist; Penicillium sp. isolate on reducing the damping-off in cotton was not as great that obtained with Aspergillus sp. isolate inoculation.

However, seed inoculation with the two potent antagonistic isolates (Aspergillus sp. isolate + Penicillium sp. isolate) showed the greatest effect than when any of the antagonists was inoculated solely. Seed inoculation with multiple antagonists, showed that antagonists intensified the antagonistic activity of each other.

The results showed that biological control nearly levelled the chemical control of damping-off in cotton plants. This indicates that biological control could substitute chemical control to avoid the problem of enviromental pollution and the side effect of such chemicals and saprophytic microflora which are of great importance to soil fertility.