EXPERIMENTAL RESULTS

I. Isolation of the causal organisms

Diseased sunflower seedlings and mature plants were collected from Giza Governorate during 1975. Isolation of the causal organisms of root-rot disease was carried out from the infected roots. Data in Table (2) indicate frequency of fungi isolated from 500 random samples.

Table 2 : Frequency of fungi isolated from diseased sunflower plants grown at Giza area

Fungi	Frequency %
Macrophomina phaseoli (Maubl.) Ashby	60
Rhizoctonia solani kuhn	20
Sclerotium rolfsii Sacc. Gurzi	18
Fusarium fusarioides (Frag.& Cif.) Booth	15
Fusarium spp.	4
Aspergillus sp.	18
Alternaria sp.	13
Curvularia sp.	3

From data presented in Table (2) it was evident that M.phaseoli was the most prevailing fungus in which 60 % of diseased sunflower plants were infected. Rhizoctonia solani and S. rolfsii were found in diseased roots in approximately equal ratios (18-20 %). Different species of Fusarium, Aspergillus, Alternaria and Curvularia were also isolated but with low frequences.

II. Pathogenicity tests

Pathogenicity of the isolated fungi, to Giza-1 sunflower variety, was tested by the soil infestation technique in the greenhouse. Data obtained represent percentages of pre-, post-emergence and diseased plants are tabulate in Table (3).

Table 3 : Pathogenicity and effect of the fungi isolated from roots of diseased plants to Giza-1 sunflower cultivar

Fungi	Emergence:		Diseased plants	
	Pre-	Post-	%	Symptoms
Sclerotium rolfsii	53.6	71.4	100.0	foot rot and wilt
Rhizoctonia solani	33.0	29.2	47.05	stunting
Macrophomina phaseoli	10.3	17.1	51.72	charcoal-rot
Fusarium fusarioides	18.5	9.7	39.28	wilt
Fusarium spp.	12.38	5.72	0.0	--
Control	82.5 % healthy plants			
L.S.D. at 5%	1.46	0.91	0.49	

Data in Table (3) indicated the following results :

1- Sclerotium rolfsii was the most destructive fungus causing the highest percentage of pre- or post-emergence (53.6 and 71.4 % respectively). Sunflower seedlings raised in inoculated pots were foot rotted, then wilted and dried (Figs.1.a,b&c) after 7 weeks from planting.

2- Rhizoctonia solani was also pathogenic during seedling stages however, it was minor disease causal organism if compared with S. rolfsii. Percentages of pre and post damped seedlings were 33.0 and 29.2 % respectively. Disease symptoms on sunflower plants were noticed as plant stunting (Fig. 2).

3- Macrophomina phaseoli came next to S.rolfsii in late stages of plant growth but came after next to R.solani in the post-emergence phase. It was more active in disease incidence after emergence. Low percentages of pre and post emergence damping-off were recorded 10.3 and 17.1% respectively. On old plants charocal rot disease symptom was noticed as dark brown lesions appear on the basal stem, black sclerotia that can be seen through the epidermis of the stalk.

When the stalk is cut lengthwise, the interior appears disintegrated with the fibrovascular strands utterly covered with small black sclerotia. In addition, charcoal-rot disease caused premature death of sunflower plants. (Figs. 3a&b).

4- Fusarium fusarioides gave low percentage of damped seedlings, however, it proved to be pathogenic to 39.28 % of old plants and caused wilt symptoms, (Fig. 4).

The other fungi Fusarium spp. caused unremarkable percentage of damping-off and no symptoms appeared.

III. Physiological studies

1- Detection of oxalic acid produced by S.rolfsii

Sclerotium rolfsii was left to grow on potato plugs for 15 days at 30°C. The filtrate was tested for the presence of oxalic acid. Adding ammonium hydroxide and calcium chloride to the filtrate proved the presence of oxalic acid in which a white precepitate of ammonium chloride was formed. Uninoculated potato plugs however, showed no change.

2- Detection of oxalic acid concentration after 15 days incubation

Activity of 15 days old S.rolfsii filtrate on potato cylinders was compared with different concentrations of pure oxalic acid. The inverse of the time taken for the potato cylinders to loss coherence was estimated. Data are presented in Table (5) and Fig. 6.

Table 5 : Effect of S.rolfsii filtrate and different concentrations of pure oxalic acid on loss coherence of potato cylinders.

Time in hrs.	% Pure oxalic acid concentrations								Filt-rate
	1	2	3	4	5	6	7	8	
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	+	+	+	+	+	–
4	–	–	–	+	+	+	+	++	–
5	–	–	+	+	+	++	++		+
6	–	+	+	++	++				+
7	–	+	++						++
8	+	+							
9	+	++							
10	++								

Control : —

– no change

\+ partial loss of coherence

++ complete loss of coherence

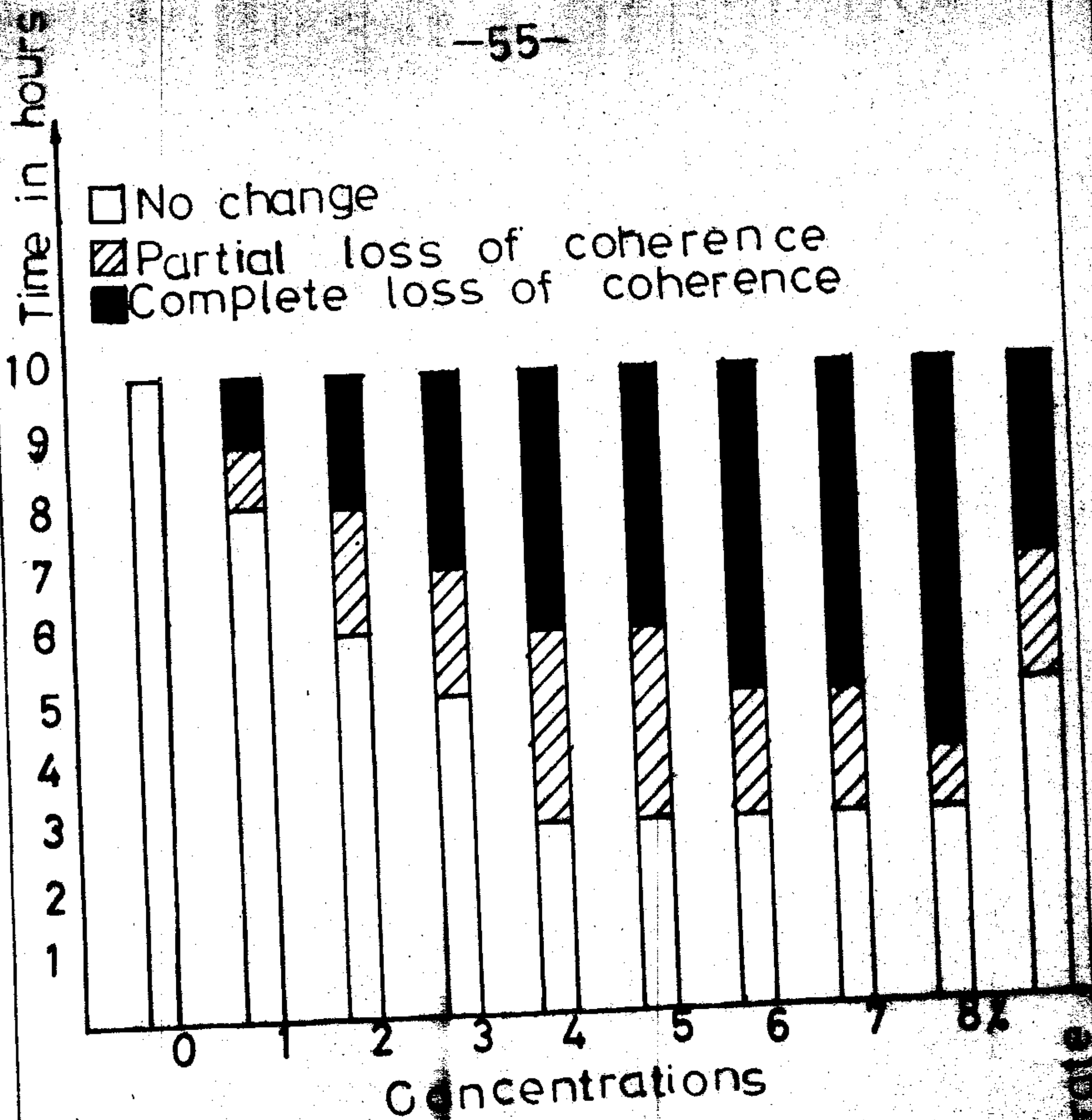


Fig.6: Effect of *S. rolfsii* filtrate and different concentrations of pure oxalic acid on loss coherence of potato cylinders

Data in Table (5) indicated that the activity of prepared oxalic acid increased with the increase of the acid concentration while, the time required for loss of coherence decreased. Partial loss of coherence of potato cylinders was recorded after 3 hours when immersed in 4,5,6,7 and 8% oxalic acid solutions, and after 5 hours when immersed in filtrate of *S.rolfsii* or 3% oxalic acid solution. However, all potato cylinders completely lost their coherence after 7 hours of immersion in fungal filtrate or 3% oxalic acid except those immersed in water (control).

In another experiment the effect of 3 % oxalic acid or filtrate of *S.rolfsii* on sunflower seedlings (Fig.7) was studied.

It is noticed, Fig.7, that the action of both *S.rolfsii* filtrate and the oxalic acid solution were similar in which wilt followed by drying to sunflower seedlings of 15 days old was noticed.

3- Effect of fungal filtrates on seeds and seedlings of different sunflower varieties :

A- Effect of the fungal filtrates on seed germination:

Data in Table (6) shows the percentage of seed germination of 3 sunflower varieties grown on filter papers containing 15 days old *S.rolfsii*, *R.solani*, *M.phaseoli* and *F.fusarioides* filtrates.

Table 6 : Effect of the fungal filtrates on the percentage of seed germination of different sunflower varieties

Fungi	% seed germination ;		
	Giza-1	Miak	import-61
Sclerotium rolfsii	0.0	0.0	0.0
Rhizoctonia solani	80.0	20.0	10.0
Macrophomina phaseoli	70.0	27.5	20.0
Fusarium fusarioides	75.0	37.5	10.0
Control	90.0	77.5	40.0

L.S.D. at 5% for varieties 1.254

L.S.D. at 5% for fungi 0.794

L.S.D. at 5% for interaction 1.374

Data, Table 6, and Fig.8, indicated that filtrates of S.rolfsii. Completely inhibited seed germination of all varieties. Mild reduction in percentage of Giza-1 seed germination was obtained from filtrates of the other tested fungi. The effect of these fungi was greater on import-61 seeds followed by Miak variety.

B- Effect of the fungal filtrates on sunflower seedlings

The effect of immersing 15 days old sunflower seedlings in the filtrates of S.rolfsii, R.solani, M.phaseoli and F.fusarioides was studied.

Table 7 : Effect of the fungal filtrates on percentage of wilted seedling of different sunflower varieties

Fungi	% wilt			
	Giza-1		[illegible]	
	24 hrs.	48 hrs.	24 hrs.	[illegible]
Sclerotium rolfsii	100.0	--	60.0	[illegible]
Rhizoctonia solani	80.0	100.0	40.0	[illegible]
Macrophomina phaseoli	80.0	80.0	40.0	[illegible]
Fusarium fusarioides	60.0	80.0	40.0	[illegible]
Control	0.0	0.0	0.0	[illegible]

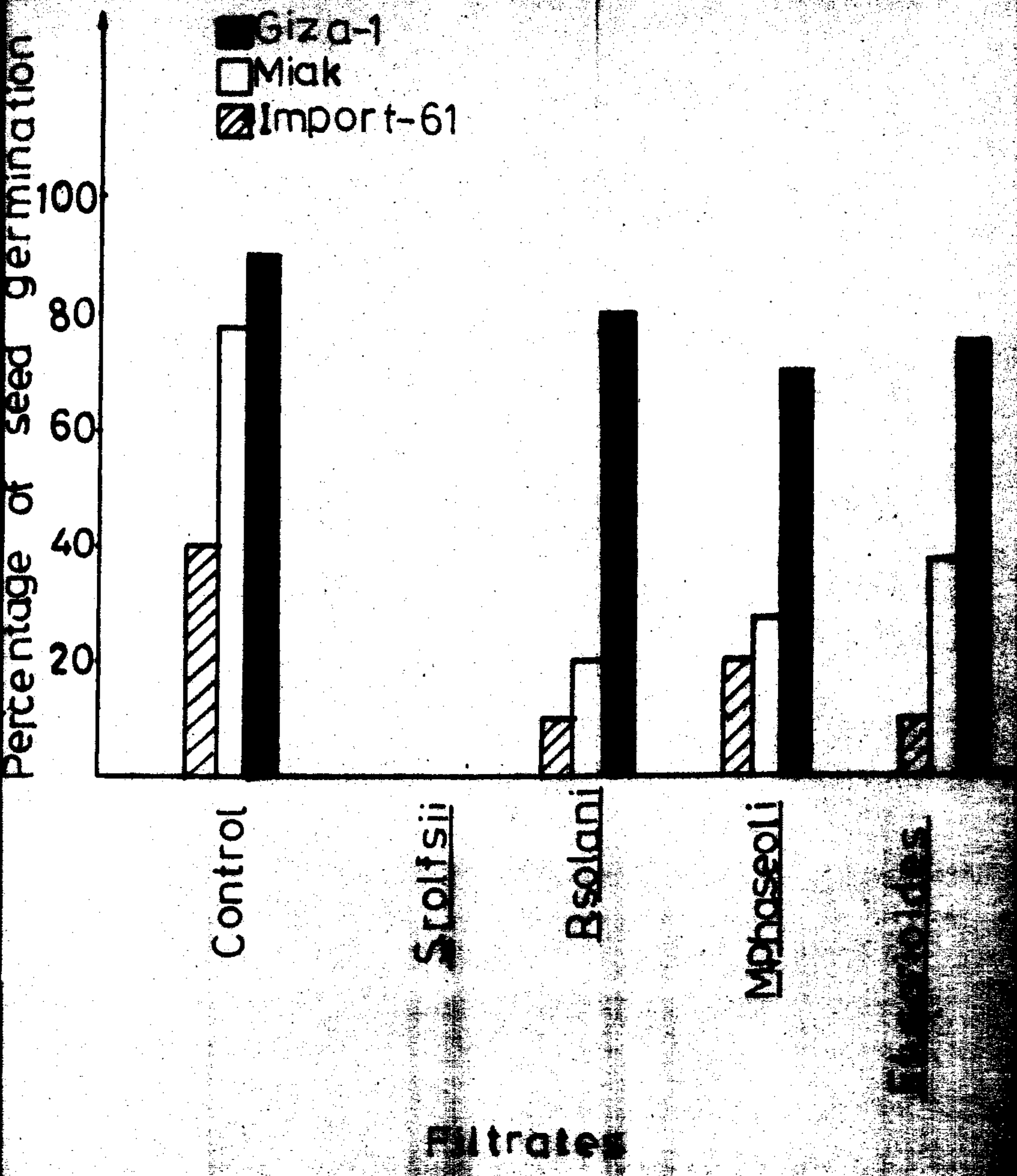


Fig.8:Effect of [illegible] fung[illegible] the perc[illegible] of [illegible]

Data in Table (7) proved that Giza-1 variety was the more affected than Miak variety after 24 hrs. After 48 hrs, most of seedlings (Figs. 9 and 10) in both varieties were wilted and dried as result of fungal filtrates.

Sclerotium rolfsii caused loss of coherence of the base stem and wilt of leaves after 24hrs, Rhizoctonia solani, M. phaseoli and F. fusarioides caused seedlings wilt after 24 hrs.

Generally, filtrates of the four fungi caused, after 48 hrs dry of head seedlings, fire of cotyledons and dry the top stem of seedlings of Giza-1 variety while, seedlings of Miak variety were completely dried.

IV. Pot experiments

1- Effect of different levels of inocula on disease severity

The inoculum of *S. rolfsii*, *R. solani*, *M. phaseoli* and *F. fusarioides* was added to sterilized soil at the rates 1.25, 2.5, 3.75 and 5 % of soil weight. The percentage of pre- and post-emergence damping-off, and diseased sunflower plants at each fungal level was determined are presented in Table (9).

Table 9 : Effect of different levels of inoculum on disease severity

Fungi	Levels of inoculum to soil weight %											
	1.25			2.5			3.75			5.0		
	Pre-	Post-	Dis. plt.	Pre-	Post-	Dis. plt.	Pre-	Post-	Dis. plt.	Pre-	Post-	Dis. plt.
Sclerotium rolfsii	10.0	27.7	76.9	10.0	33.3	83.3	15.0	41.2	100	53.6	71.4	100
Rhizoctonia solani	0.0	0.0	15.0	10.0	5.5	23.5	10.0	16.6	26.6	33.0	29.2	47.1
Macrophomina phaseoli	0.0	0.0	10.0	5.0	0.0	15.8	5.0	0.0	31.6	10.3	17.1	51.7
Fusarium fusarioides	10.0	0.0	0.0	10.0	0.0	5.5	10.0	0.0	22.2	18.5	9.7	39.3
Control	100 % healthy plants											

	Pre-	Post-	Diseased
L.S.D . at 5% for fungi	N.S.	0.434	N.S.
L.S.D. at 5% for level of inoculum	0.514	0.437	0.438
L.S.D. at 5% for interaction	0.718	N.S.	0.876

The previous data, Table 9, proved that the low level of inoculum (1.25 % of soil weight) was not enough to cause pre or post-emergence damping-off in the case of R. solani, M.phaseoli and F. fusarioides. However, this low level of inoculum produced 10% and 27.7% of damped seedlings in S.rolfsii case. Sunflower plants grown at this level of inoculum were affected, where 76.9 % of plants were wilted due to S.rolfsii, 15.0% for R.solani and 10% for M.phaseoli. The number of affected seedlings or plants was found to increase by increasing inoculum level in the soil. However, the moderate level of inoculum 2.50 and 3.75% of soil weight have an equal effect on pre and post emergence stage in the case of R.solani, M. phaseoli and F.fusarioides. The high inoculum level 5% of soil weight showed that S.rolfsii and R.solani were aggressive pathogens during pre and post emergence stages, where, M.phaseoli and F.fusarioides were markedly effective after seedling stage.

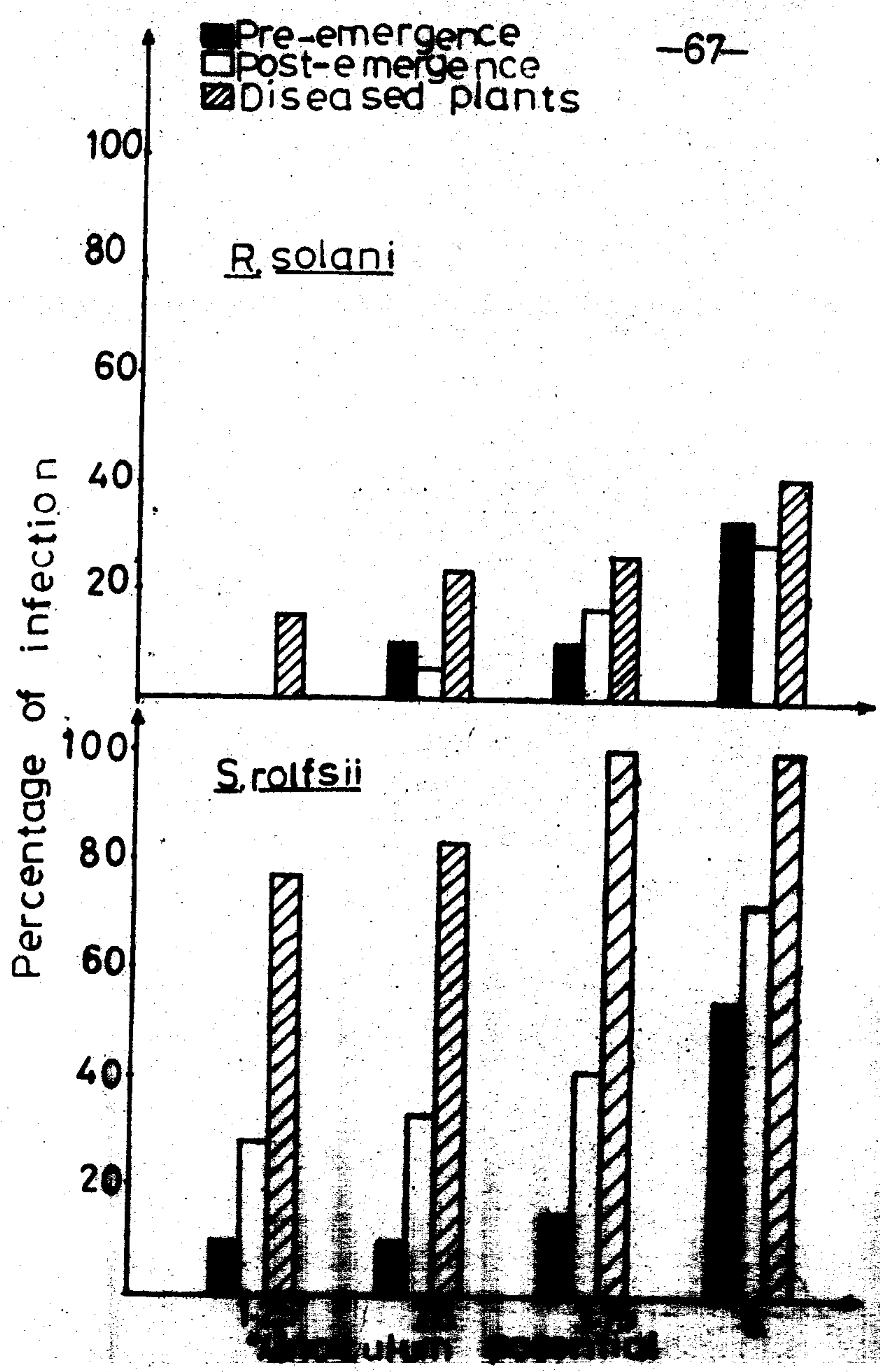


Fig. 11a: Inoculum potential of S. rolfsii and R. solani to infect sunflwer plants

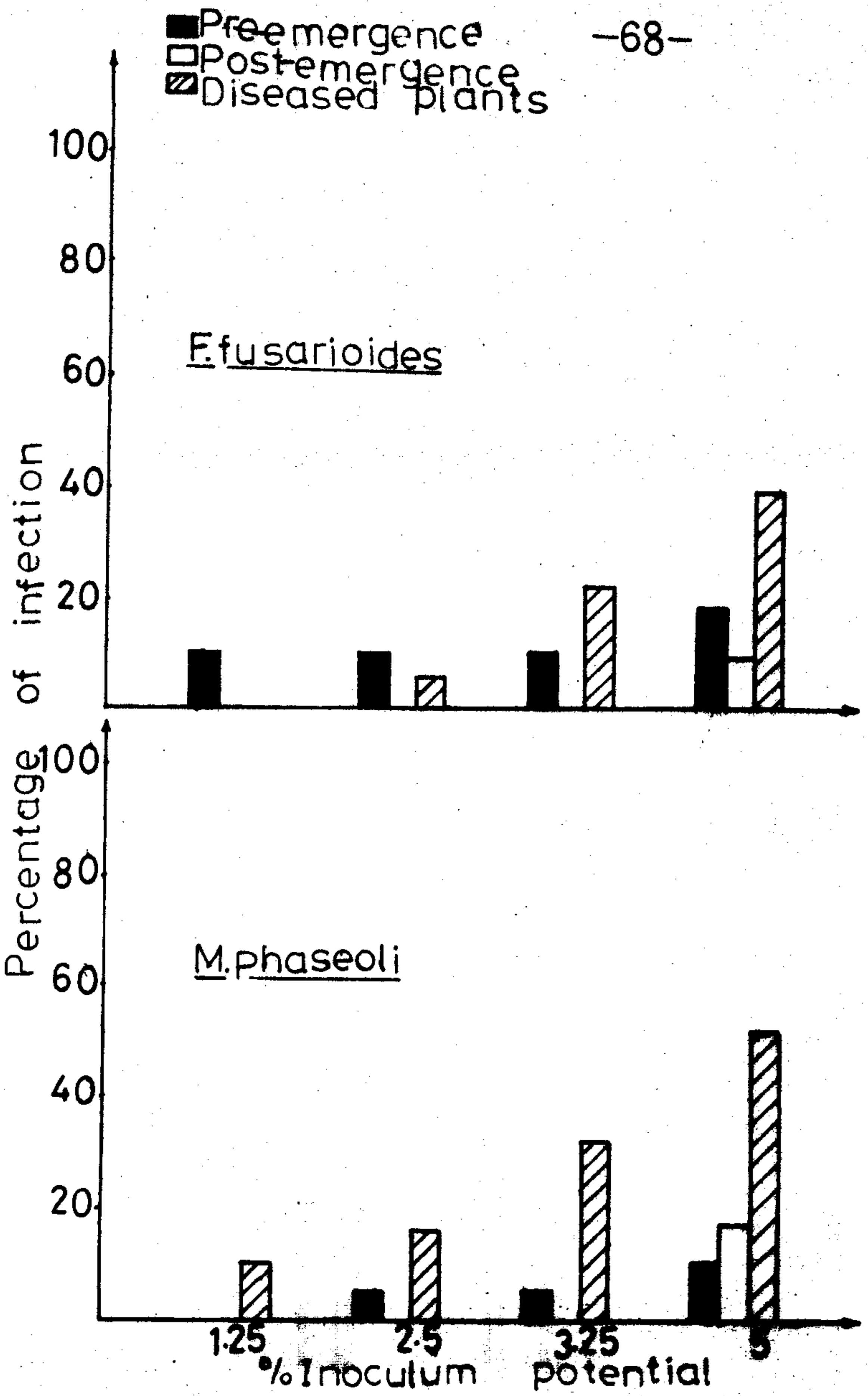


Fig.11b: Inoculum potential of M.Phaseoli and F.fusarioides to infect sunflower plants

2- Disease reaction of different sunflower varieties

The effect of *S.rolfsii*, *R.solani*, *M.phaseoli* and *F.fusarioides* on the infectivity of four sunflower varieties namely; Giza-1, Miak, Import-61 and Import-500 was studied by the soil infestation technique. Percentage of pre-and post emergence damping-off as well as disease plants are shown in Table (10).

Table 10 : Reaction of four sunflower varieties to the diseases

Fungi	Giza-1 Pre-	Post-	Dis. plt.	Miak Pre-	Post-	Dis. plt.	Import-61 Pre-	Post-	Dis. plt.	Import-500 Pre-	Post-	Dis. plt.
Sc[illegible]	53.6	71.4	100	46.9	80.0	100	33.8	55.0	100	26.3	46.2	100
Rh[illegible]ia solani	33.0	29.2	47.1	43.8	58.3	0.0	40.5	68.8	0.0	37.5	40.0	100
Ma[illegible]ina phaseoli	10.3	17.1	51.7	50.0	25.0	50.0	35.4	26.3	21.4	26.3	0.0	15.4
Fu[illegible]oides	[illegible]	[illegible].7	39.3	39.1	20.0	58.3	42.2	20.0	33.3	7.5	5.5	23.5
[illegible]ol	82.5 %			62.5 %			67.5 %			75% health plants		

	Pre	Post	Diseased
[illegible].D. at 5% for varieties	1.1496	N.S.	0.694
[illegible] for [illegible]	1.307	0.735	0.727
[illegible] for [illegible]action	2.614	N.S.	1.455

From the previous data it was evident that all the tested varieties were susceptible to the disease during pre and post emergence stages. As regard to S.rolfsii, the imported 500 was the least affected variety during pre-emergence stage followed by imported 61, Miak and Giza-1. On the other hand, Giza-1 variety was the least susceptible to R.solani and M.phaseoli followed by imported 500, imported 61 and Miak, respectively, with respect to F.fusarioides, imported 500 showed a degree of resistance, however, imported 61 and Miak were highly susceptible.

Table 11 : Competitive saprophytic ability of four fungi under two treatments

% inoculum in inoculum-soil mixtures	% colonization of inoculum-soil discs							
	S.rolfsii		*R.solani*		*M.phaseoli*		*F.fusarioides*	
	a	b	a	b	a	b	a	b
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	33.3	16.66	0.0	0.0	33.3	0.0	58.3	91.66
25	50.0	33.3	0.0	0.0	50.0	8.3	87.0	100.0
50	58.3	50.0	0.0	0.0	58.3	33.3	100.0	100.0
75	80.0	75.0	0.0	0.0	58.3	58.3	100.0	100.0
90	83.0	75.0	0.0	25.0	50.0	66.7	100.0	100.0
100	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

a. Inoculum-soil mixtures were not allowed to interact.

b. Inoculum-soil mixtures were allowed to interact for [illegible] hrs.

From data presented in Table (12.a) it is evident that the four tested fungi continued its normal growth under the two treatments, however, no reduction in colony diameter due to micro-organisms containing soil was noticed. This results denoted that the four tested fungi have a high degree of tolerance to antibiotics produced by soil microorganisms.

Data in Table (12.b)showed that S.rolfsii was the most sensitive fungus to spore-forming bacteria, however, average diameter of formed inhibition zone was 10 mm. when medium was inoculated with the two organisms at the same time, and 19 mm. when inoculation with S.rolfsii was carried out after 24 hrs. Rhizoctonia solani and M.phaseoli showed the same sensitivity to bacterial exudates. Fusarium fuscrioides was the most affected fungus, where, the recorded diameter of inhibition zones were 25 and 30 mm, respectively, Table 12.b and Fig. 13.

Table 12.a : Tolerance to antibiotics produced by soil microorganisms (Wastie cellophane method)

Fungi	% reduction in colony diameter*	
	A	B
Sclerotium rolfsii	0.0	0.0
Rhizoctonia solani	0.0	0.0
Macrophomina phaseoli	0.0	0.0
Fusarium fusarioides	0.0	0.0

* Inoculum of the fungus was placed on soil-inoculated plates : (A) immediately after they were prepared. (B) 24 hrs. later.

Table 12.b : Sensitivity to spore-forming bacteria method.

Fungi	Mean diameter of inhibition zones/mm.*	
	A	B
Sclerotium rolfsii	10.0	19.0
Rhizoctonia solani	19.3	22.3
Macrophomina phaseoli	18.3	23.3
Fusarium fusarioides	25.0	30.0

L.S.D. at 5% for fungi	5.585
L.S.D. at 5% for time	2.610
L.S.D. at 5% for interaction	5.221

* Inoculum of the fungus were placed on both sides of the bacterial streak :

(A) immediately after streaking,

(B) [illegible]

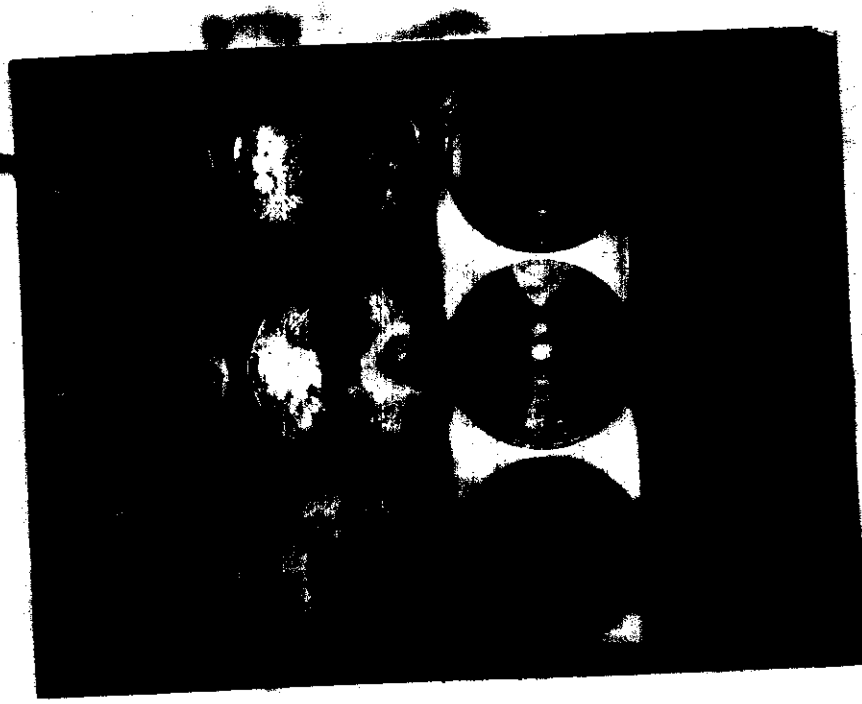


Fig.13 : Sensitivity to spore-forming bacteria:
A- Control B- immediately after streaking, C- 24 hrs. later.
1- F. [illegible]arioides 2- S. rolfsii
3- M. [illegible]aseoli 4- R. solani

VI. Effect of fungicides

1- In vitro

Average linear growth of *S.rolfsii*, *R.solani*, *M.phaseoli* and *F.fusarioides* as affected by adding a series of increasing concentrations of different systemic fungicides is shown in the following Tables 13, 14, 15 and 16.

Table 13 : Effect of different concentrations of four fungicides on the growth of *S.rolfsii*

Fungicides	% Toxicity at conc., p.p.m.							
	1	3	5	50	100	500	1000	[illegible]
Bavistin	0.0	0.0	0.0	5.5	20.3	100		
Topsin M	0.0	0.0	0.0	0.0	0.0	0.0	[illegible]	[illegible]
Vitavax/captan	88.8	94.4	100	[illegible]				
Vitavax/thiram	91.[illegible]	94.1	100					

L.S.D. [illegible]

[illegible]

[illegible]

Data in table 13, indicated that vitavax/captan and vitavax/thiram were the most effective fungicides used, where high toxicity percentage were obtained at low concentration (1-5 p.p.m.). Bavistin was also effective, the toxicity was noticed up to 50 p.p.m. and reached maximum at 500 p.p.m. (Table 13). Topsin M was the least effective fungicides, however, the fungus was sensitive to high fungicide concentration 1000 and 1500 p.p.m.

Table 14: Effect of different concentrations of four fungicides on the growth of R. solani.

Fungicides	% Toxicity at conc., p.p.m.			
	1	3	5	50
Bavistin	11.1	100.0		
Vitavax/captan	61.1	66.7	68.6	100.0
Vitavax/thiram	77.7	80.6	88.8	[illegible]
L.S.D. at 5% for fungicides			2.490	
L.S.D. at 5% for concentrations			1.133	
L.S.D. at 5% for interaction			1.963	
	5	10	15	[illegible]
Topsin M	[illegible]	[illegible]	94.4	[illegible]
L.S.D. at [illegible]			[illegible]	

Data in table 14, show that R.solani was sensitive to low concentration of Bavistin, where, complete toxicity were obtained with 3 p.p.m. Fungus growth was also inhibited with 5-20 p.p.m. Topsin M, 1-50 p.p.m. vitavax/captan or vitavax/thiram.

Table 15: Effect of different concentrations of four fungicides on growth of M.phaseoli

Fungicides	% Toxicity at conc., p.p.m.			
	5	50	100	500
Topsin M	69.4	78.6	100.0	
Vitavax/captan	0.0	0.0	100.0	
vitavax/thiram	5.5	34.7	59.3	100.0
L.S.D. at 5% for fungicides			6.768	
L.S.D. at 5% for concentrations			2.085	
L.S.D. at 5% for interaction			3.612	

	1	3
Bavistin	58.3	100.0
L.S.D. at 5% for concentrations		1.776

As for M.phaseoli, data in table 15, indicated that Bavistin was the best fungicide used, however, 3 p.p.m. concentration were sufficient to inhibit fungal growth. The other tested fungicides were also effective at 100 p.p.m. for vitavax/captan, 100 p.p.m. for Topsin M and 500 p.p.m. for vitavax/thiram.

Table 16 : Effect of different concentrations of four fungicides on the growth of F.fusarioides

Fungicides	% Toxicity at conc., p.p.m.				
	5	50	100	500	1000
Vitavax/captan	5.5	11.1	27.7	77.7	100.0
Vitavax/thiram	0.0	0.0	38.8	88.8	100.0

L.S.D. at 5% for fungicides N.S.
L.S.D. at 5% for concentrations 0.380
L.S.D. at 5% for interaction 0.538

	1	5	10	15	20
Bavistin	0.0	100.0			
Topsin M	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

L.S.D. at 5% for fungicides [illegible]
L.S.D. at 5% for concentrations [illegible]
L.S.D. at 5% for interaction [illegible]

From the foremationed data Table (16) it is evident that *F. fusarioides* was markedly affected with low concentration of Bavistin or Topsin M, where, maximum toxicity was recorded at 5 and 20 p.p.m. respectively. Toxicity was found to began with 5 p.p.m. vitavax/captan and 100 p.p.m. vitavax/thiram and increased with increasing fungicide concentration till 1000 p.p.m. for both fungicides.

2- Distribution of systemic fungicides in the plants

This experiment was carried out to study the distribution of systemic fungicides in sunflower plants.

Table 17 : Remaindence of four systemic fungicides in roots, stems and leaves of sunflower plants at different ages using *Aspergillus niger* as test organism.

Fungicides	Mean diameter of inhibition zones (m.m.)											
	Roots				Stems				Leaves			
	Plant age / weeks											
	1	2	3	4	1	2	3	4	1	2	3	4
Bavistin	11.63	11.56	11.19	0.0	5.25	6.06	4.94	0.0	0.0	2.81	1.25	0.0
Topsin M	12.88	12.13	10.75	0.0	1.31	5.19	2.75	0.0	0.0	2.31	0.75	0.0
Vitavax/captan	13.0	9.88	8.94	0.0	2.0	5.19	6.13	0.0	0.0	0.0	0.94	0.0
Vitavax/thiram	12.5	8.88	8.19	0.0	1.0	3.88	5.63	0.0	0.0	0.0	0.31	0.0
L.S.D.at 5% for fungicides			0.90			0.88				0.47		
L.S.D.at 5% for plantage			0.94			1.20				0.52		
L.S.D.at 5% for interaction			1.89			2.41				1.05		

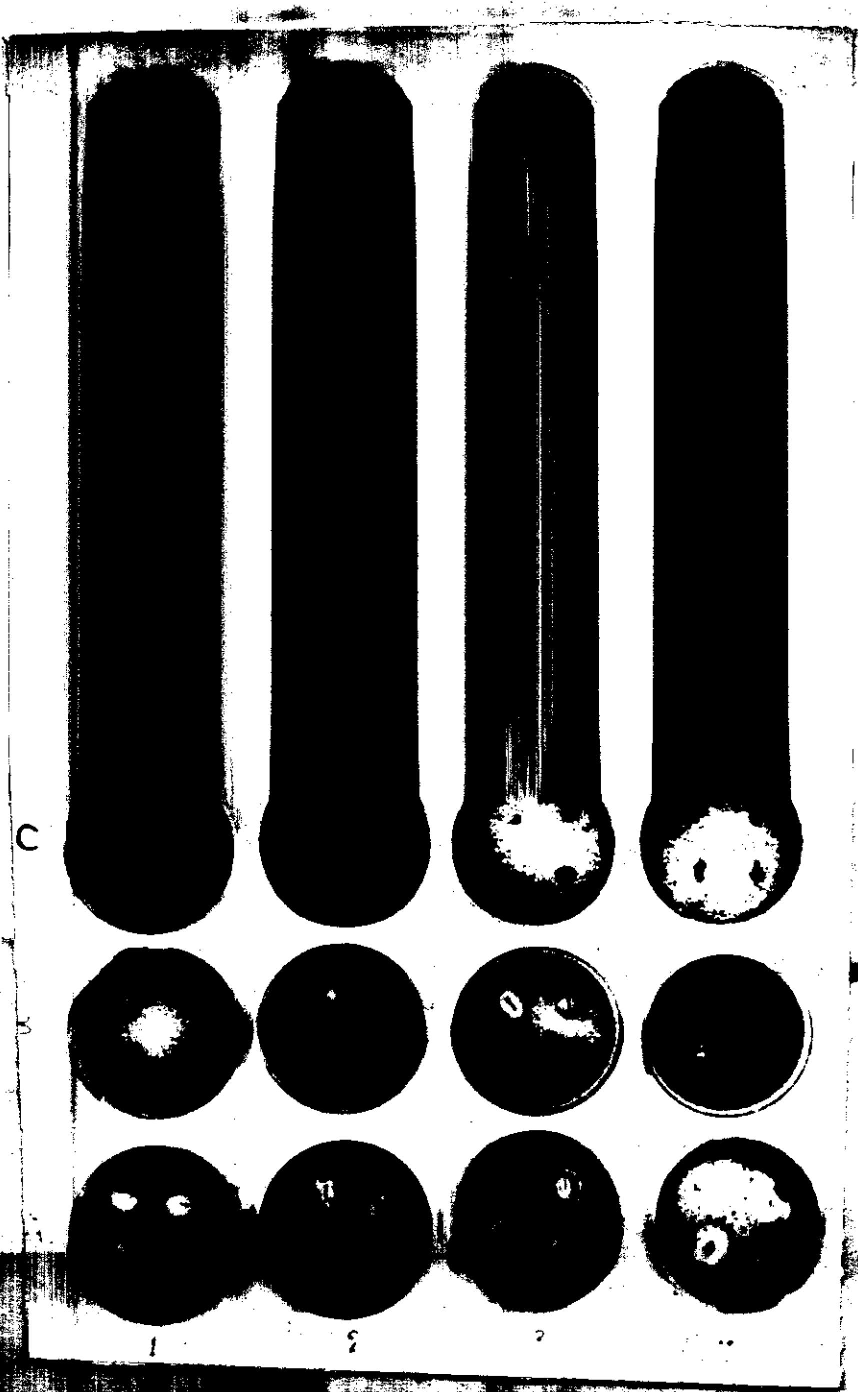
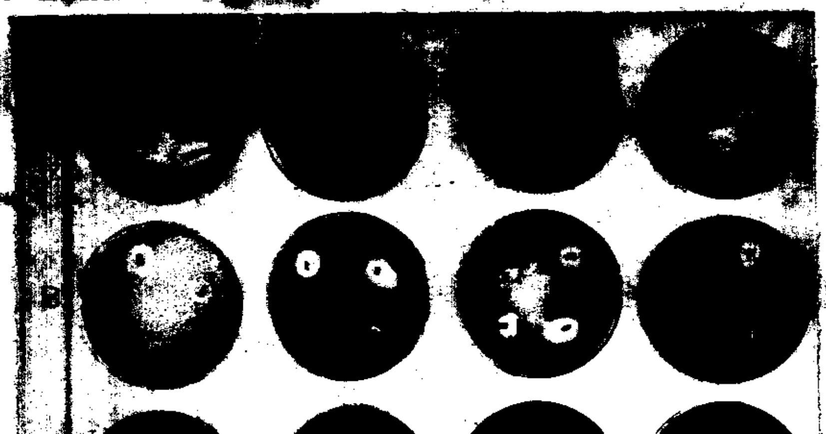


Fig. 14a:



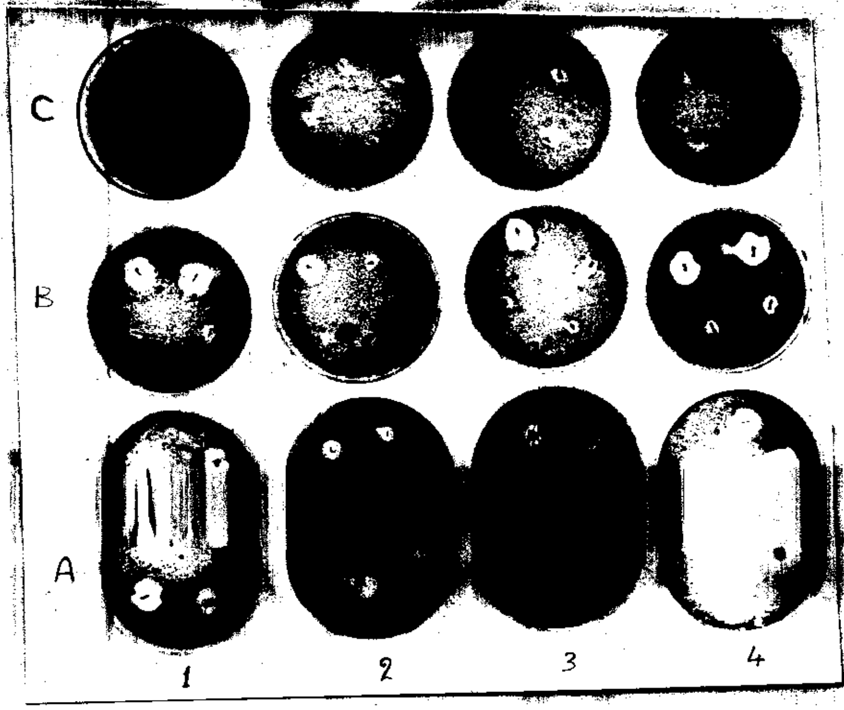


Fig.14c:

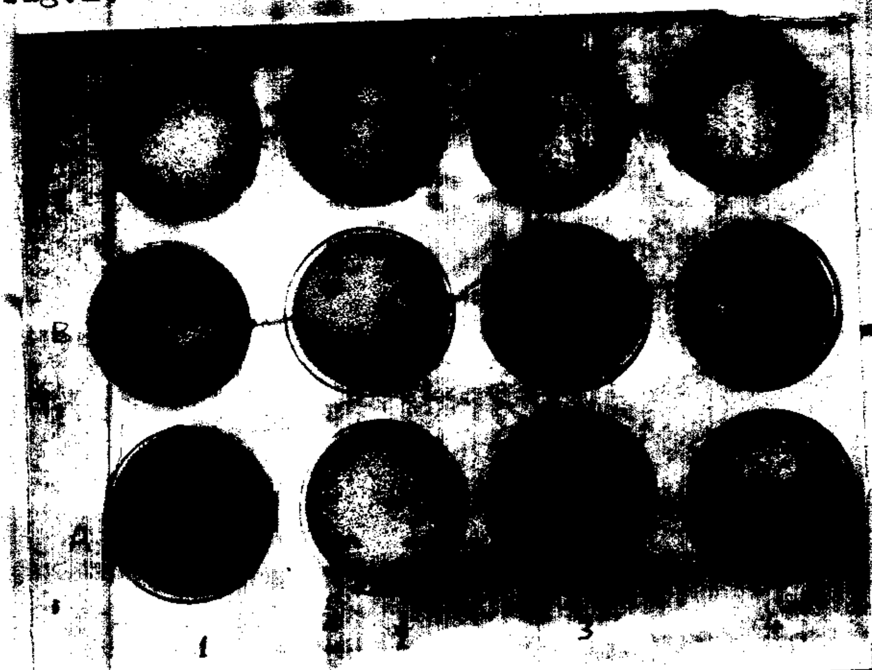


Fig.14d: Distribution of systemic fungicides in sunflower plant parts; A=Roots, B=stems and C=leaves.
1=Bavistin, 2=Topsin M, 3=vitavax captan and 4=vitavax thiram.
(c=3 and d=4 weeks of age).

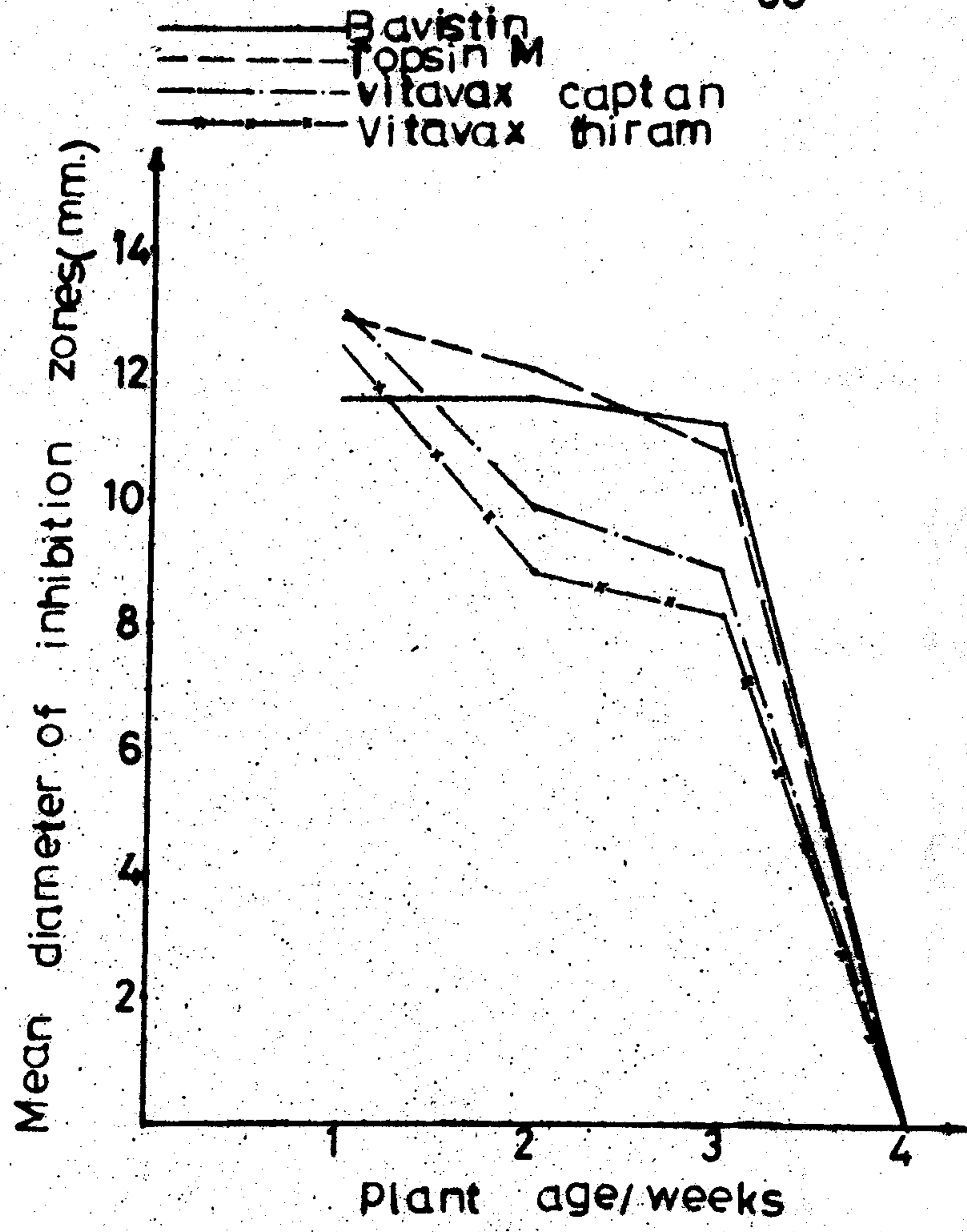


Fig.15-a: Distribution of four systemic fungicides in roots of Giza-1 sunflower plants at different ages

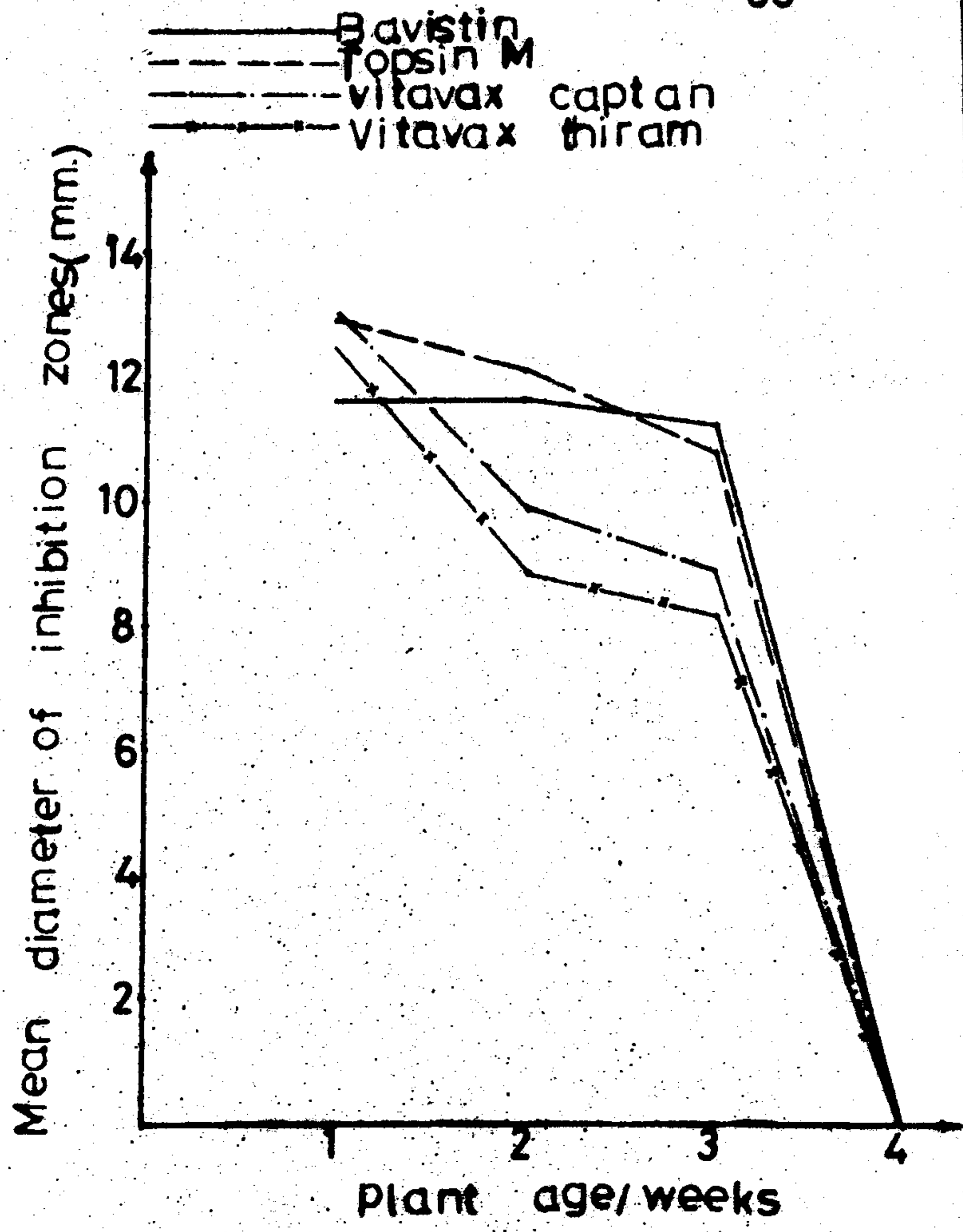


Fig.15-a: Distribution of four systemic fungicides in roots of giza 1 sunflower plants at different ages

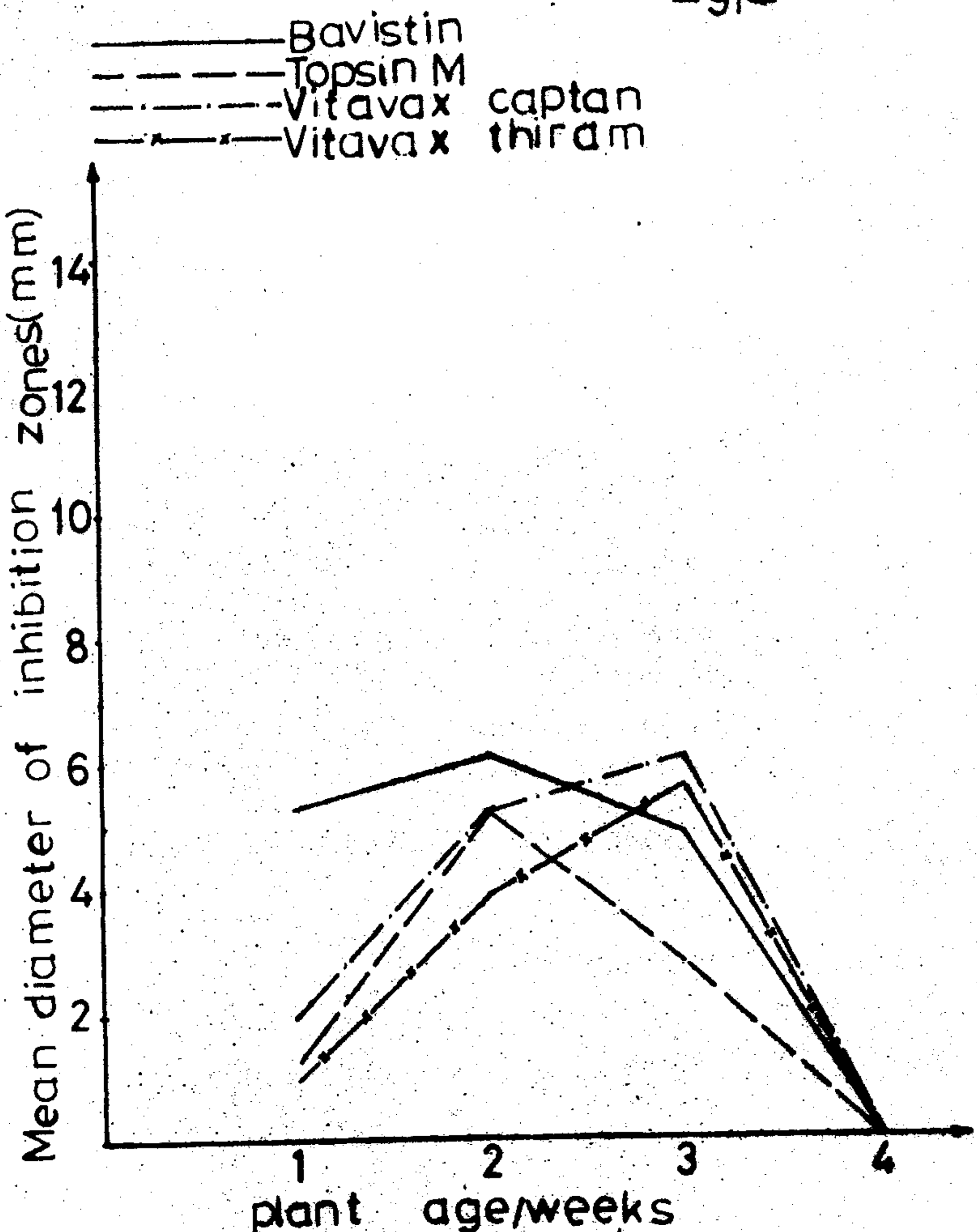


Fig15b: Distribution of four systemic fungicides in stems of Giza-1 sunflower plants at different ages

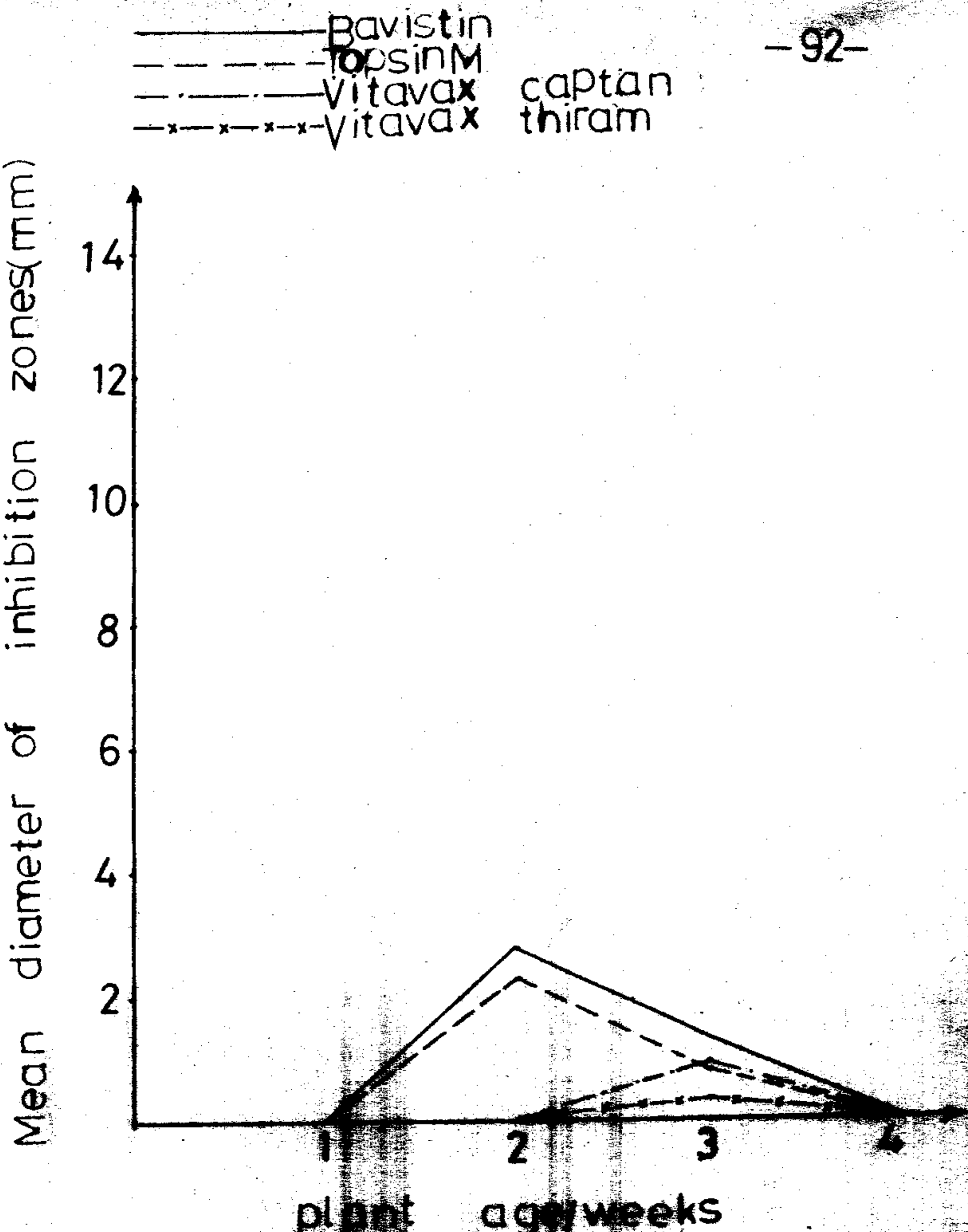


Fig.15-C: Distribution of four systemic fungicides in leaves of Giza sunflower plants at different ages

3- Effect of seed dressing fungicides on disease severity

The effect of seed treatment with different fungicides on the percentage of pre-, post-emergence damping-off and diseased plants of sunflower was studied. Data presented in Table 18, indicate that :

a. In case of Sclerotium rolfsii the lowest percentages of pre-emergence damping-off were obtained, when seeds were treated with 0.2% vitavax thiram or Topsin M and 0.1% vitavax/captan or Bavistin. As regards post-emergence phase, vitavax/captan or Topsin M at 0.2% and Bavistin at 0.4% and vitavax/thiram at 0.[illegible]% showed the lowest percentage of post-emergence seedlings. Most of mature plants were died, however, Bavistin at 0.4% gave the highest number of healthy plants.

b. Bavistin, Topsin M or vitavax/captan at 0.1% or vitavax/thiram at 0.2% reduced pre-emergence damping-off caused by Rhizoctonia solani to minimum percentage. The best control of post-emergence damping-off was obtained with Bavistin and vitavax/captan at 0.1% or Topsin M at 0.4% or vitavax/thiram at 0.2%. The minimum percentage of diseased plants were also obtained when seeds were dressed with 0.2% Bavistin.

Table 18 : Effect of different rates of seed dressing fungicides on percentage of pre-,post-emergence damping-off and diseased plants.

Treatment	Rate	S.rolfsii Pre-	S.rolfsii Post-	S.rolfsii Dis.	R.solani Pre-	R.solani Post-	R.solani Dis.	M.phaseoli Pre-	M.phaseoli Post-	M.phaseoli Dis.	F.fusarioides Pre-	F.fusarioides Post-	F.fusarioides Dis.
Bavistin	0.1	38.0	18.18	100	1.8	0.0	6.94	0.8	0.0	19.34	1.2"	0.43	16.33
	0.2	48.0	16.6	75.0	4.5	0.57	0.0	1.2	0.0	20.06	0.0	0.0	17.50
	0.4	46.0	0.0	57.1	2.7	0.0	12.75	0.4	0.0	14.94	0.3	0.77	15.12
[illegible]	0.1	18.0	9.52	76.5	2.7	2.12	27.05	1.2	0.28	33.33	0.6	0.0	43.74
	0.2	16.0	2.27	71.4	6.3	1.86	19.33	4.4	0.0	25.65	0.6	0.79	35.89
	0.4	22.0	7.89	81.3	6.3	1.24	25.92	2.0	0.31	27.08	0.9	0.82	34.10
Vitavax captan	0.1	10.0	4.0	91.30	3.6	0.0	5.98	2.8	0.0	9.42	2.1	0.48	31.82
	0.2	22.0	0.0	68.42	4.5	0.0	9.33	2.4	0.0	11.28	1.8	0.93	31.82
	0.4	12.0	0.0	83.3	4.5	0.0	15.55	1.6	0.89	14.13	1.5	1.33	31.82
Vitavax thiram	0.1	22.0	2.63	66.66	7.2	2.60	21.6	3.2	0.70	16.25	2.7	1.06	18.42
	0.2	6.0	3.70	84.0	2.7	0.53	11.96	3.6	0.73	14.25	1.8	0.0	19.44
	0.4	14.0	2.17	68.18	11.7	6.72	9.28	4.8	0.43	6.37	2.4	0.0	7.95
Control	0.0	60.0	50.0	100	27.0	14.28	38.88	12.0	7.69	54.16	9.0	11.1	58.33
L.S.D.at 5% for fungicides		0.93	N.S	0.51	0.98	0.71	0.32	1.27	N.S	0.32	0.95	N.S.	0.34
L.S.D.at 5% for rates		1.27	0.66	0.71	0.40	0.35	0.47	0.91	0.40	0.42	0.39	N.S.	0.36
L.S.D.at 5% for interaction.		2.54	N.S	1.42	1.82	0.70	0.93	1.82	N.S.	0.83	0.79	N.S.	0.72

c. The four tested fungicides were highly effective against the disease caused by M.phaseoli. Seed treatment with 0.1% Topsin M. or vitavax/thiram or 0.4% Bavistin or vitavax/captan gave the lowest percentage of pre-emergence damping-off. All tested fungicides with the three rates of application controlled the post emergence stage. The highest numbers of healthy plants were also raised with 0.2% vitavax/captan or 0.4% vitavax/thiram or Bavistin.

d. Data also showe that the four fungicides with their levels were highly effective against pre- and post-emergence damping-off caused by F.fusarioides, however, percentage of diseased seedling was ranged from 0.0 to 2.7 % compared with 9.0% in control treatment. Bavistin was the superior fungicide in controlling the disease after seedling stage followed by vitavax/thiram.

D I S C U S S I O N

Macrophomina phaseoli, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium fusarioides* and other species of *Aspergillus*, *Fusarium*, *Alternaria* and *Curvularia* were isolated, with prevailing of *M. phaseoli*, from diseased sunflower plants collected from Giza governorate. The first three organisms were reported by other investigators as the incitent of sunflower root disease (Weber, (1931); Briton-Jones and Baker, (1933); Simmonds, (1956); Marras, (1963); Pastorino, (1965); El-Helaly *et al*, (1966); Bouhot, and Luciano and Davreux, (1967); Hulea *et al*, (1973); and Elzarka, (1976)). *Fusarium fusarioides* was not isolated before from diseased sunflowers, while it was isolated from ground nut roots in Egypt (Apou-Talib, 1970) and from different hosts in other countries i.e., cotton (Ray and McLaughlin, 1942), tomato (Linnasalmi, 1952), and Sorghum (Siddiqui and Khan, 1973). Therefore, *F. fusarioides* is reported for the first time in this investigation as one of causal organisms of sunflower root disease.

Pathogenicity experiments proved that *S. rolfsii* was the most destructive fungus causing high percentage of pre- or post- emergence, foot rot, wilt and drying. Previous reports also mentioned that S.rolfsii caused root rot, crown or base and stem rots of many host plants (Simmonds, 1956; Sackston, 1957; Middleton, 1971; and Elzarka, 1976). Rhizoctonia solani, came next to S.rolfsii, causing stunting of the sunflower plants, Macrophomina phaseoli caused dark brown lesions appear on the basal of sunflower stalks while black sclerotia can be seen through the epidermis and resulting premature death of the plants. These results were coincided with the results of Hoffmaster et al.(1943) who stated that M.phaseoli was chiefly injurious to seedlings and immature plants devitalized by environmental extremes, wounds, or infection by other organisms. Bekesi (1970) also reported that M.phaseoli caused sudden wilt of plants after pollination. On the other hand, necrotic leaf spots were attributed to a toxin produced by M.phaseoli in vitro and in vivo and the fungus penetrated hypocotyls by forming appressoria on the epidermis of axenic sunflower seedlings within 13 hrs. and adult plants in 18 hrs. at 30°C, these may help both mechanical and chemical penetration. (Chan and Sackston 1967, 1969 and 1973). Fusarium fusarioides caused wilt to the mature plants. In 1942 Marengo stated that F. solani var. minus caused wilt and damping-off to sunflower plants. Generally, the tested fungi could be arranged according to their virulence as follows; S. rolfsii, R. solani, M. phaseoli and F. fusarioides, while isolation ratios of these fungi from diseased sunflower plants grown at Giza region were 18, 20, 60 and 15%, respectively.

Double fungal inoculations revealed that all combinations included S. rolfsii showed very high percentage of infection, this result was confirmed by Sabet and Khan (1969 b) who found that in cotton root-infecting fungi the competitive pathogenic ability of a particular fungus was dependent upon interacting combinations, host variety and soil conditions. Many combinations decreased disease severity; many others caused an increase in disease severity and a third group of combinations completely suppressed disease development.

The production of oxalic acid by S. rolfsii was proved when the fungal filtrate of 15 days old was tested chemically. This result was found in agreement with Higginis (19[illegible]), Bateman and Beer (1965), Maxwell and Bateman (1968), and Abd El-Al (1969). Husain (1958) reported that *S.rolfsii* was a common facultative parasite on various crops. The rotting tissues of the affected plants was due to pectic enzymes. Propectinase and depolymerase played an important role in the maceration of host cells after infection. In another *in vitro* test, potato cylinders lost their turgidity and coherence when immersed in *S.rolfsii* filtrate or different oxalic acid concentrations. On the other hand, the action of both *S.rolfsii* filtrate and 3% oxalic acid solution on sunflower seedlings were similar, as wilt followed by drying. Thus, *S. rolfsii* foot rot of sunflower plants, may be due to oxalic acid secretion and not to the presence of the fungus inter host tissues.

Filtrate of *S.rolfsii* prevented germination of seeds. This result was confirmed by El-Bigawi (1969) who mentioned that filtrate of *S.rolfsii* caused a reduction in seed germination of rice. *Rhizoctonia solani*, *M.phaseoli* and *F.fusarioides* filtrates reduced percentage of seed germination of import-61 and Miak sunflower varieties. In this respect, Ashour and Gamal El-Deen (196[illegible]) found that filtrate of *R.solani* increased seed germination of cineraria, pansy and antirrhinum plants. Disease symptoms may be due principally to the action of fungal filtrates. This fact was proved when sunflower seedlings of 15 days old were immersed for 24 and 48 hrs. in fungal filtrates obtained from 15 days old potato water cultures. Generally, filtrates of the four fungi caused dry of sunflower seedlings after 48 hrs., fire of cotyledons and drying of seedlings shoots of Giza-1 variety, while dried all seedlings of Miak variety. These results were in agreement with El-Bigawi (1969) who found that the filtrate of S.rolfsii causing death to rice seedlings. In this respect, Stino (1959) found that the toxins of R.solani induced wilt on watermelon seedlings, Bateman (1963) indicated that water extracts of diseased bean hypocotyls, which were grown in R.solani infested soil, contained pectinpoly-galacturonase and a mixture of pectin-methyl-estrase, Zayed (1967) mentioned that filtrate of R.solani had a toxin effect on ground-nut seedlings, Abd El-Momem (1969) recorded that R. solani secreted toxic substances which had toxin effect on broad-bean seedlings, Mathur (1968) mentioned that filtrate of M. phaseoli cultures induced symptoms on cut sunflower shoots similar to those on inoculated plants, Waggoner and Dimond (1955) stated that PME and

PG were not excreted by Fusarium sp. in glucose media. Both enzymes were produced by the fungus in pectin medium and PG disappeared from the media before PME.

As soil inoculum increased from season to another according to the prevailing environmental factors. It was found essential to study the effect of different levels of inoculum on the severity of the disease. The obtained results indicated that disease severity represented by pre-, post- and diseased plants increased as inoculum level increased in the soil. Sclerotium rolfsii showed disease symptoms at low level of inoculum (1.25% to soil weight) however, the other tested fungi R.solani, M. phaseoli and F.fusarioides showed inability. The moderate levels of inoculum (2.50, 3.75% to soil weight) gave similar ratios of disease severity, however, 5% inoculum showed that S.rolfsii and R. solani were aggressive pathogens during pre- and post- emergence stages, the other fungi M. phaseoli and F.fusarioides showed high activity after seedling stage. Similar results were obtained by (Linford, (1931); Garrett, (1956); Gooding and Lucas, (1959); Rao and Rao, (1963); Mansour, (1969) and Mousa, (1969).

In order to control the disease by means of resistant varieties; different local and imported sunflower varieties were screened and subjected to 5% of fungal inoculum. The results showed that all sunflower varieties were susceptible to the disease, however, imported 500 showed a degree of resistant against S.rolfsii during pre-mergence stage on the other hand, Giza-1 was the least affected variety with R.solani and M.phaseoli. Regarding F.fusarioides it was found that imported 500 showed little resistant if compared with the other tested varieties. Generally, there was no source of resistance among the tested sunflower varieties.

From inoculum potential experiments it was found that disease severity was affected greatly with inoculum level in the soil. Therefore, it was found important to study the competitive saprophytic ability of the fungi causing the disease. The agar plate modification of the "Cambridge method" (Rao 19[illegible] and Wastie 1961) was adopted. The obtained results showed that F.fusarioides was the only fungus which showed highest colonisation ratings under all levels of inoculum. While R.solani was a poor coloniser of the agar plates. On the other hand, colonisation ratings for the four fungi increased gradually with the increase in the amount of inoculum. The four fungi were also tolerant to antibiotics secreted by soil microorganisms as no reduction in colony diameter or inhibition zones appeared when examined by Wasties cellophane method (Wastie 1961) or tested against streaks of spore-forming bacteria (Wastie 1961), respectively. This result was recommended by Rao (1959) who conducted a comparative study of competitive saprophytic ability in 12 root infecting fungi by the agar plate method. He classified a number of fusaria and M.phaseoli as vigorous saprophytes and R.solani and others were classified as low saprophytes. On the other hand, Sabet and Khan (1969 a) reported that the competitive saprophytic colonization of the agar plates of S.bataticola or F.oxysporum increased with the increase of inoculum level.

Laboratory screening, of various fungicides for the control of the four sunflower pathogenic fungi, showed that the four systemic fungicides tested gave partial or complete inhibition to the fungal growth. The inhbition power varied among the fungicides and the concentrations used, as this power increased with the increase in fungicide concentration. Generally, low concentration of Bavistin (3-5 p.p.m.) were enough to inhibit *R. solani*, *M. phaseoli* and *F. fusarioides* growth, however, *S.rolfsii* was not sensitive even to these level of fungicide. *Sclerotium rolfsii* growth was found to affect by vitavax/thiram, it was noticed that 1-3 p.p.m. of the fungicide induced high growth toxicity. Similar trend of results was reported by El-Kazzaz *et al* (1977).

Studying the translocation of systemic fungicides from seeds to different plant parts indicated that the 4 fungicides used persisted active in the plants until 3 weeks and localized in the root tissues more than in stems or leaves as gradually decreased upwards. This result was in agreement with Kirk *et al* (1969) who mentioned that both chloroneb and vitavax were translocated upwards in cotton seedling. On the other hand, thiophanatemethyl moved upward in sunflower plants and persisted in roots and stems for 70 days while it moved downward and persisted in stem tissues 10 and 20 days after application (Sesha *et al* 1975).

In vitro experiments were confirmed by *in vivo* experiment however, the best seed treatments control were obtained by, vitavax/captan at 0.2% or Bavistin at 0.4% for S.rolfsii, Bavistin at 0.1% for R.solani, Bavistin or vitavax/captan at 0.1% for M. phaseoli, and Bavistin at 0.2% for F.fusarioides, These results were confirmed by, El-Kazzaz et al (1977) who mentioned that vitavax/captan and Bavistin increased significantly the percentage of healthy seedlings of rice grown in artificially inoculated soil with Rhizoctonia sp. Fadl and Hessien (1977) also mentioned that vitavax/captan gave the best control for the diseases of; S.rolfsii, R.solani, M.phaseoli, F.oxysporum and F.solani on Soybean in Egypt.

SUMMARY

The present investigation was planned to study the causal organisms of sunflower root diseases, the effect of some physiological factors, saprophytic behaviour and their control. The findings can be summarized as follows :

1- Isolation of the causal organisms of sunflower root disease was carried out from diseased roots collected from Giza Governorate during 1975. Sclerotium rolfsii, R.solani, M.phaseoli, F. fusarioides and other species of Aspergillus, Fusarium, Alternaria and Curvularia were isolated with prevailing of M.phaseoli.

2- Pathogenicity tests using Giza-1 sunflower variety indicated that S.rolfsii caused foot root, wilt and blight; R.solani caused stunting; M.phaseoli caused charcoal rot and F.fusarioides caused stunting, then wilt. In addition, the four fungi caused pre- or post-emergence damping-off at different ratios. Sclerotium rolfsii was found to be the most aggressive pathogen than the other isolated fungi.

3- The interaction between the different pathogens in vivo showed that both mixed inocula of all fungi and

S.rolfsii + R.solani increased the infection than each of them alone.

4- Sclerotium rolfsii secreted oxalic acid, in cultures. Potato cylinders were soft rotted when immersed in S.rolfsii filtrate or different concentrations of oxalic acid. Thus, the loss of coherence was fast in high concentrations of the acid.

5- Filtrate of S.rolfsii caused no germination to sunflower seeds. Filtrates of the other fungi however, caused drying of seedlings shoots of Giza-1 variety after 48 hrs., while dried all seedlings of Miak variety.

6- Percentage of diseased plants gradually increased with the increase in amount of inoculum.

7- All the tested sunflower varieties were found to be susceptible to the disease. Giza-1 variety gave moderate resistance to all fungi except S.rolfsii.

8- Fusarium fusarioides showed only the highest colonization ratings. While, R.solani was a poor colonizer of the agar plates. Colonization ratings increased gradually with the increase in the amount of inoculum. The four fungi showed high degree of tolerance to antibiotics.

9- Mycelial growth of the four fungi decreased with increasing the concentrations of fungicides when incorporated in P.D.A. medium. Moreover, fungicides differed in the concentrations at which fungal growth was inhibited. Bavistin and Topsin M. were the best fungicides to R.solani, M.phaseoli and F.fusarioides as they inhibited growth at lower concentrations, while vitavax/captan and vitavax/thiram were the most effective fungicides against S.rolfsii.

10- The four systemic fungicides used, remained active in the plants for about 3 weeks. These fungicides translocated from the seeds and localized in the root tissues more than in stems or leaves and gradually decreased upwards.

11- Seed treatment with each of vitavax/captan at 0.2% or Bavistin at 0.4% was the best fungicides for controlling the disease.