EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

1. SURVEY

Data in Table (3) and illustrated in Fig. (1) represent the average percent of infection with pink root rot disease in fifteen localities, four localities in each of Giza; Fayoum and Ismailia governorates, two localities in Beni-Suef governorate and one locality in Assiut governorate through two successive seasons 1985/1986 and 1986/1987. Data were recorded through the period extended from the first of November to the end of December in both 1985 and 1986 in Upper Egypt governorates as well as from December to February and March seasons 1985/1986 and 1986/1987 in Ismailia governorate.

It is very clear from data that the infection of onion pink root rot was very severe in Ismailia localities (Fayed; El-Tell El-Kibier; Abou-Souer and Sarabiom) as the average percentages of diseased plants were 24.94, 27.25%; 23.25, 28.00%; 24.00, 26.25% and 23.63, 25.63% through the both seasons 1985/1986 and 1986/1987, respectively. Also data belonging to Fayoum localities (Quota; Koum-Oshem; El-Hamoli and Ibshwai) showed a considerable spread of the disease through onion plants as the average percentages reached 18.06, 21.00%; 20.38, 17.25%; 16.50, 19.38% and 17.50, 13.00% through 1985/1986 and 1986/1987. Average percentage occurred in Assiut governorate (Koum-Seeda locality) was relatively low and ranged from 8.94% to 6.38% in 1985/1986 and 1986/1987, respectively.

Table (3): Average percentage of infection with onion pink root disease in 5 different governorates (including 15 localities) through the period extended from November to December in both 1985 and 1986 in Upper Egypt and from December to February and March seasons 1985/1986 and 1986/1987 in Ismailia governorate.

Governorate	Center	Locality	Average percentage of infection	
			1985/86	1986/87
1. Giza	El-Badrashine	El-Hwamidia	3.94%	4.38%
Giza	El-Saff	Mazghona	2.75	3.13
Giza	Giza	El-Saff	3.88	3.00
Giza	Giza	Giza	3.88	3.38
2. Beni-Suef	Beni-Suef	Mokobil	5.81	6.25
Beni-Suef	El-Wastta	Sanhour	3.50	4.38
3. Assiut	El-Badary	Koum-Seeda	8.94	6.38
4. Fayoum	Ibshwai	Ibshwai	17.50	13.00
Fayoum	Ibshwai	El-Hamoli	16.50	19.38
Fayoum	Koum-Oshem	Koum-Oshem	20.38	17.25
Fayoum	Ibshwai	Quota	18.06	21.00
5. Ismailia	Abou-Souer	Abo-Souer	24.00	26.25
Ismailia	Sarabiom	Sarabiom	23.63	25.63
Ismialia	El-Tell-El-Kibier	El-Tell-El-Kibier	23.25	28.00
Ismailia	Fayed	Fayed	24.94	27.25
L.S.D.; 0.05			4.03	7.09

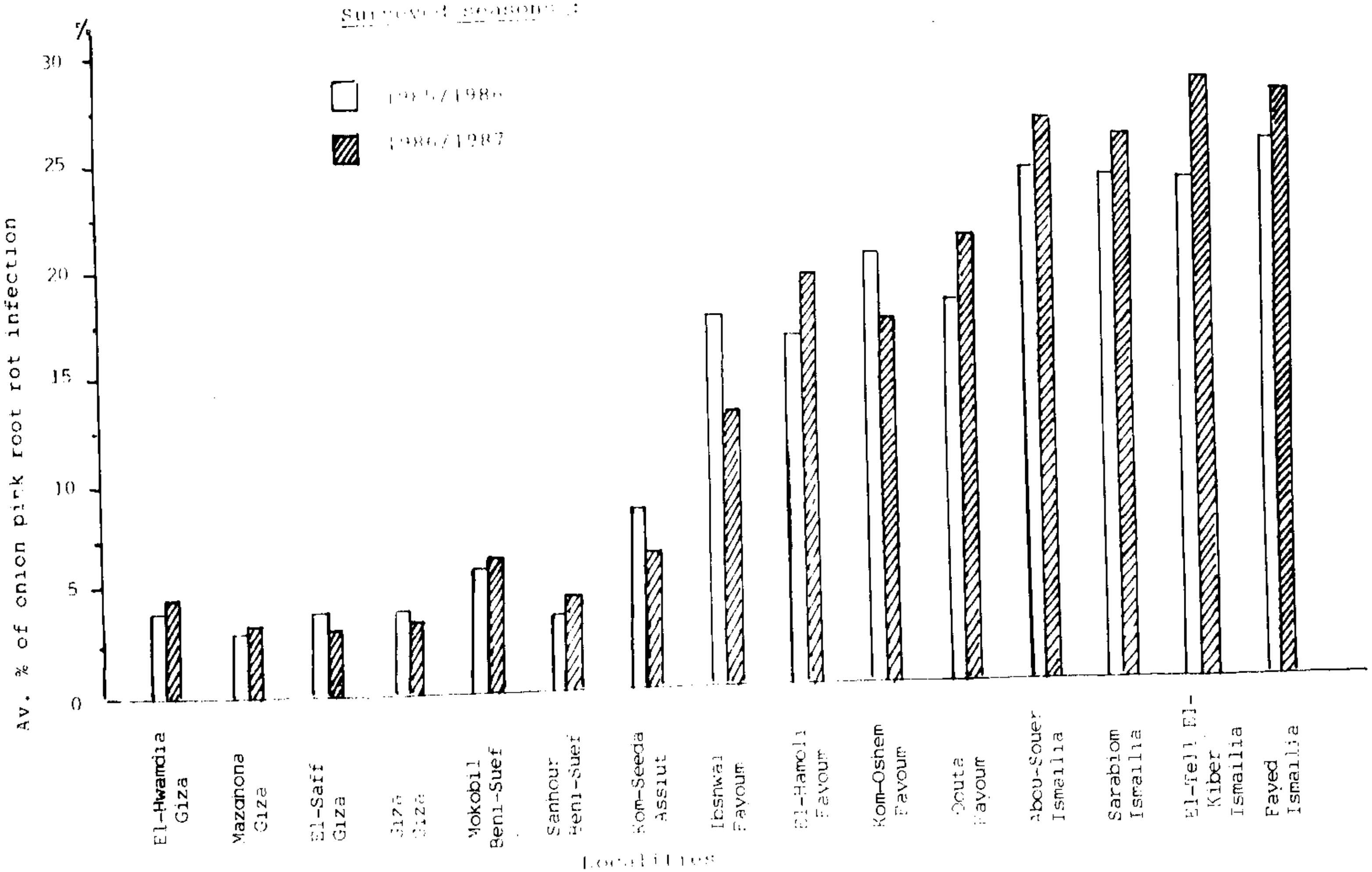

Fig. (1): Average percent infection of onion pink root rot disease in two successive seasons 1985/1986 and 1986/1987 in 15 localities.

The disease infection in Beni-Suef localities (Sanhour and Mokobil) was less than those occurred in the former nine localities which belonging to Ismilia; Fayoum and Assiut governorates and only reached 3.50, 4.38% and 5.81, 6.25% respectively. Meanwhile the survey study indicated that percentage of disease infection was significantly less in Giza localities (Mazghona; El-Saff; Giza and El-Hwamdia) as the average percentages were very limited and reached only 2.75, 3.13%; 3.88, 3.00%; 3.88, 3.38% and 3.94, 4.38% through surveyed seasons 1985/1986 and 1986/1987 respectively. In general data shown in Table (4) and illustrated in Fig. (2) show that the average percentage of infection with pink root rot disease was significantly high in both Ismailia and Fayoum governorates as the average percent of infection ranged from 23.96, 18.11% to 26.28, 17.66% through seasons 1985/1986 and 1986/1987 respectively. As regard to the average percentage of natural infected roots in Fayoum governorate, it was lower than those of Ismailia governorate as the infection reached 18.11, 17.66% in the growing season of 1985/1986 and 23.96, 26.28% in the successive season 1986/1987. Data also indicated that noticeable natural infected plants with pink root rot disease was recorded in Assiut governorate (El-Badary Center- Koum Seeda locality) as the infection reached 8.94% and 6.38% through 1985/1986 and 1986/1987 respectively. Average percentage records in Beni-Suef governorate ranged from 4.66% in 1985/1986 season to 5.32% in 1986/1987. Meanwhile the survey study showed average percentages of infection significantly lower in Giza governorate as the infection was very

Table (4): Average percentage of infection with onion pink root rot disease in 5 different governorates through the period extended from November to December in both 1985; 1986 in Upper Egypt and from December to February and March, seasons 1985/1986 and 1986/1987 in Ismailia governorate.

Governorates	Average percentage of infection:	
	1985/1986	1986/1987
Giza	3.61	3.47
Beni-Suef	4.66	5.32
Assiut	8.94	6.38
Fayoum	18.11	17.66
Ismailia	23.96	26.28
L.S.D.; 0.05	1.76	4.38

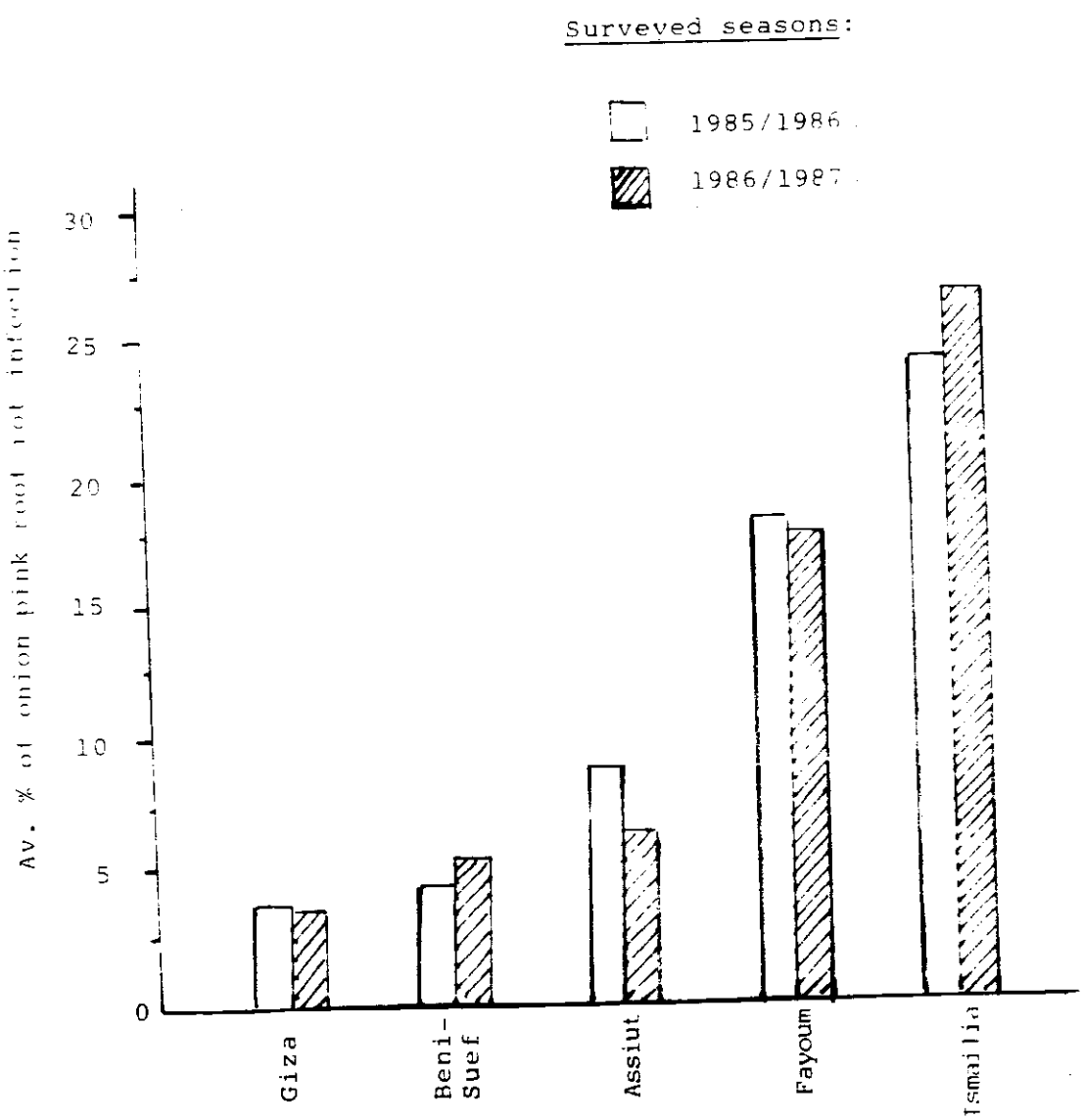

Fig. (2): Average percent infection of onion pink root rot disease in two successive seasons 1985/1986 and 1986/1987 in 5 governorates.

limited and reached only 3.61% and 3.47% through 1985/1986 and 1986/1987 respectively.

Table (5) represents the meterological elements i.e., the maximum; minimum and mean of temperature; percentage of relative humidity and the total rain fall recorded through the period extended from November to December in both 1985; 1986 in Upper Egypt governorates and from December to February and March seasons 1985/1986 and 1986/1987 in Ismailia governorate. Also Table (5) indicates the average percentages of pink root rot disease which recorded throughout the two surveyed seasons.

Maximum; minimum and mean of temperature-recorded during surveyed period - were relatively similar, however the average percentages of onion pink root rot disease in 1985; 1986 were greatly differed as the infection was relatively high in Ismailia (23.96%) ;moderate in Fayoum (18.11%) and low in Assiut; Beni-Suef and Giza governorates (8.94, 4.66 and 3.61% respectively). Similar results were also recorded in the season (1986/1987), as the disease infections were 26.28; 17.66; 6.38; 5.32 and 3.47% in Ismailia; Fayoum; Assiut; Beni-Suef and Giza respectively. So, it is evident that there is no accurate correlation between the temperature degrees and the onion pink root rot incidence. Concerning the relative humidity it is also very clear that although the infection was relatively high in Ismailia governorate in the both seasons and was very low in Giza governorate, the relative humidity was relatively higher in the season (1985/1986) than those recorded in the season (1986/1987)

Table (5): Correlation between different meteorological elements recorded during surveyed periods extended from November to March in both two successive seasons 1985/86; 1986/87 and the average percent infection of pink root disease.

	Governorates	Temperatures (°C.)															% of R.H.					Rain fall					Average percent of pink root rot disease
		Nov.			Dec.			Jan.			Feb.			Mar.														
		Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Nov.	Dec.	Jan.	Feb.	Mar.	Nov.	Dec.	Jan.	Feb.	Mar.	
1985/1986	Ismailia	26.9	14.6	20.8	21.2	10.0	15.6	23.2	7.9	15.6	22.4	9.7	16.1	24.5	11.8	18.1	66.0	72.3	60.2	64.0	62.7	0.2	4.2	0.0	0.0	0.0	23.96** %
	Giza	26.0	14.8	20.4	20.6	10.2	15.4	21.0	7.1	14.1	22.6	10.2	16.4	24.3	12.0	18.1	68.7	75.0	57.3	64.0	64.0	1.6	3.8	0.3	0.2	3.1	3.61**
	Beni-Suef	26.2	14.5	20.4	21.3	9.3	15.3	22.0	6.6	14.3	23.4	9.3	16.4	25.4	11.7	18.6	61.0	67.0	54.8	56.2	55.7	0.6	1.4	0.1	0.1	1.2	4.66*
	Fayoum	26.2	14.5	20.4	21.3	9.3	15.3	22.0	6.6	14.3	23.4	9.3	16.4	25.4	11.7	18.6	61.0	67.0	54.8	56.2	55.7	0.9	2.2	0.2	0.1	1.8	18.11**
	Assiut	26.4	11.5	19.0	22.1	8.4	15.2	23.0	6.1	14.5	24.2	8.4	16.3	26.4	11.3	18.8	53.3	58.0	52.3	48.3	47.3	0.0	0.2	0.0	0.0	0.0	8.94++
	LSD at level 5%																										1.76
1986/1987	Ismailia	23.5	12.0	17.8	20.3	8.1	14.2	22.4	7.7	15.1	22.9	9.9	16.4	20.9	9.3	15.1	68.0	69.7	59.7	64.0	55.7	0.5	5.6	0.0	1.0	3.3	26.28%**
	Giza	23.2	12.7	18.0	19.8	8.4	14.1	21.9	7.3	14.6	22.6	9.6	16.1	21.2	12.0	16.6	65.3	62.3	54.7	58.3	55.7	2.8	5.4	0.0	0.3	4.6	3.47**
	Beni-Suef	23.3	11.5	17.4	19.6	6.6	13.1	22.0	6.6	14.3	22.9	9.0	15.7	22.0	10.2	16.2	57.0	53.1	52.9	50.7	48.2	1.1	2.0	0.0	0.1	1.7	5.32*
	Fayoum	23.3	11.5	17.4	19.6	6.6	13.1	22.0	6.6	14.3	22.9	9.0	15.7	22.0	10.2	16.2	57.0	58.1	52.9	50.7	48.2	1.6	3.1	0.0	0.2	2.7	17.66**
	Assiut	23.5	10.3	16.9	19.4	4.8	12.1	22.4	5.3	13.8	23.3	7.3	15.3	23.9	8.4	16.1	48.7	54.0	51.0	43.0	40.7	0.2	0.5	0.0	0.0	0.0	6.38++
	LSD at level 5%																										4.38

++ Average percent infection of onion pink root disease in one locality.
* Average percent infection of onion pink root disease in two localities.
** Average percent infection of onion pink root disease in four localities.

in Ismailia, whereas the relative humidity in Giza, was also rather high in the season (1985/1986) than those of (1986/1987) and also rather high than those recorded in Ismailia governorate. According to rain fall, it is very clear that there was no relation between the average rain fall and the percentage of infection. So, another evidence that no correlation could be obtained between the relative humidity; rain fall and the incidence of pink root rot disease.

2.1 ISOLATION; PURIFICATION AND IDENTIFICATION OF THE CAUSAL ORGANISM

1. Isolation and Purification :

The causal organism of onion pink root rot disease which was isolated from diseased transplants which showing typical symptoms, Fig. (3) could be differentiated in two distinguished groups. Fifteen pure cultures of the first group and seven pure cultures of the second group were obtained using single spore technique (Richer and Ricker, 1936) for the first and single pycnidio-spore and pycnidium technique for the second. The fifteen isolates of the first group produced mycelium with white to violet pigment; micro conidia; macro-conidia and Chlamydospores. The second group of the causal organism which included seven isolates, produced white to grey mycelium with pycnidio spores; conidia and dark pycnidia. Table (6) indicates the two groups of the causal organism and the localities which these isolates obtained from. Data obtained show that the first causal organism was isolated from all surveyed localities and

Fig. (3): Natural infection of onion pink root rot disease on Giza-20 onion cultivar after 30; 60 and 90 days from transplantation.

H: Healthy plant.

D: 30; 60 and 90 day old infected onion plants.

Table (6): Fungi isolated from fifteen different ıring survey study through two successive seasons 1985/1986 and 1

Governorate	Center	Locality	Isolates	
			t group	The second group
1. Giza	El-Badrashine	El- Hwamidia	+	-
Giza	El-Saff	Mazghona	+	-
Giza	El-Saff	El-Saff	+	-
Giza	Giza	Giza	+	-
2. Beni-Suef	Beni-Suef	Mokobil	+	-
Beni-Suef	El-Wassta	Sanhour	+	-
3. Assiut	El-Badary	Koum-Seeda	+	+
4. Fayoum	Ibshwai	Ibshwai	+	+
Fayoum	Ibshwai	El-Hamoli	+	-
Fayoum	Koum-Oshem	Koum-Oshem	+	+
Fayoum	Ibshwai	Qouta	+	+
5. Ismailia	Abo-Sour	Abo-Sour	+	+
Ismailia	Sarabiom	Sarabiom	+	+
Ismailia	El-Tell El-Kibier	El-Tell-El-Kibier	+	-
Ismailia	Fayed	Fayed	+	+

the second causal organism was isolated from some localities through the same collected samples. The second group of the causal organism was collected from the following localities:-

Ismailia governorate (Abo-Sour center) Abo-Sour locality
Ismailia governorate (Sarabiom center) Sarabiom locality
Ismailia governorate (Fayed center) Fayed locality
Fayoum governorate (Ibshwai center) Ibshwai locality
Fayoum governorate (Koum-Oshem center) Koum Oshem locality
Fayoum governorate (Ibshwai center) Quota locality
Assiut governorate (El-Badary center) Koum-Seeda locality

Fifteen pure isolates of the first group of the causal organism produced abundant floccuse mycelia; violet pigment. Macroconidia usually not numerous; light curved; and typically 3-septate and occasionally 4 or 5 septate. Microconida are abundant and usually consist of one cell. Clamydospores intercalary or in terminal set. These cultural and morphological characteristics of the fifteen obtained isolates were in complete agreement with Booth (1971) and identified as *Fusarium oxysporum* f. sp. *cepae* (Hanz.), Snyder and Hansen.

Seven isolates of the second group of the causal organism showed the following characteristics: All isolates have white to grey mycelium with dark pycnidia. Pycnidia ostiolate; nearly globose; erumpent with a few simple bristles (setae); especially near the ostiole. Conidiophore simple or branched. Conidia small; 1-celled; hyaline; ovate to elongate. These cultural and morphological characteristics of these seven isolates were

completely agreed with Barneet (1960) and identified as: *Pyrenochaeta terrestris* (Hansen); Gorenz; Walker and Larson. All pure cultures of Fusaria and *Pyrenochaeta terrestris* were mentained at 5°C, on PDA and corn-meal agar media, respectively to the forcoming studies.

2. Frequency of *Fusarium oxysporum* f. sp. *cepae* and *Pyrenochaeta terrestris* isolated from onion pink rooted roots collected from 15 localities :

Data summarized in Table (7) indicate the percentage of frequency of the pathogens isolated from onion transplants showing pink root rot typical symptoms and collected from 15 localities belonging to five governorates namely Giza; Beni-Suef; Assiut; Fayoum and Ismailia. *Fusarium oxysporum* f. sp. *cepae* was singly isolated from all pink rooted roots of onion transplants collected from all localities belonging to Giza and Beni-Suef governorates; one locality (El-Hamoli-Ibshwai center) Fayoum governorate and one locality (El-Tell El-Kibier) Ismailia governorate. So, it is very clear that the percentage of frequency of *F.oxysporum* f. sp. *cepae*, isolated from all onion transplants collected from mentioned localities was 100%.

Data also indicate that both *F. oxysporum* f. sp. *cepae* and *Pyrenochaeta terrestris* were isolated from onion transplants— suffering from pink root rot disease— collected from one locality (Koum-Seeda) El-Badary center, Assiut governorate; three localities (Ibshwai; Koum-Oshem and Qouta) - Ibshwai; Koum-Oshem and Ibshwai centers respectively; Fayoum governorate and

Table (7): Percentage frequency of *Fusarium oxysporum* f. sp. *cepae* and *Pyrenochaeta terrestris* isolated from onion transplants infected with pink root rot disease at 15 localities belonging to five different governorates.

Governorate	Center	Locality	Percentage frequency of :				
			F.oxysporum f.sp.*cepae*	*Pyreno-chaeta terrestris*	*F.oxys.*f.sp. *cepae* + *Pyrenochaeta terrestris*	Total count of *F.ox.*f.sp. *cepae*	Total count of *Pyreno-chaeta terrestris*
1. Giza	El-Badrashin	El- Hwamidia	100.0	000.0	000.0	100.0	000.0
Giza	El-Saff	Mazghona	100.0	000.0	000.0	100.0	000.0
Giza	El-Saff	El-Saff	100.0	000.0	000.0	100.0	000.0
Giza	Giza	Giza	100.0	000.0	000.0	100.0	000.0
2. Beni-Suef	Beni-Suef	Mokobil	100.0	000.0	000.0	100.0	000.0
Beni-Suef	El-Wassta	Sanhour	100.0	000.0	000.0	100.0	000.0
3. Assiut	El-Badary	Koum-Seeda	87.5	9.4	3.1	90.6	12.5
4. Fayoum	Ibshwai	Ibshwai	74.3	18.6	7.1	81.4	25.7
Fayoum	Ibshwai	El-Hamoli	100.0	000.0	000.0	100.0	000.0
Fayoum	Koum-Oshem	Koum-Oshem	74.1	23.5	2.5	76.6	26.0
Fayoum	Ibshwai	Qouta	73.1	22.4	4.5	77.6	26.9
5. Ismailia	Abou-Sour	Abou-Sour	77.6	19.4	3.1	80.7	22.5
Ismailia	Sarabiom	Sarabiom	90.5	9.5	000.0	90.5	9.5
Ismailia	El-Tell El-Kibier	El-Tell El-Kibier	100.0	000.0	000.0	100.0	000.0
Ismailia	Fayed	Fayed	74.8	24.1	1.1	75.9	25.2

three localities Abo-Sour; Sarabiom and Fayed - Ismailia governorate, as data show that the percentage frequency of only *F. oxysporum* f. sp. *cepae* ranged from 73.1 - 90.5% whilst the percent frequency of *Pyrenochaeta terrestris* ranged from 9.4 to 24.1% only, also the percentage of onion sigments infected with both mentioned pathogens ranged from 1.1 to 7.1%. So, the final percent frequency of *F. oxysporum* f. sp. *cepae* , isolated from Assiut; Fayoum and Ismailia governorates, ranged from 75.9-90.6% whereas those of *Pyrenochaeta terrestris* ranged from 9.5-26.9%.

3. PATHOGENICITY TESTS

Data in Table (8) and illustrated in Figs. (4) and (5a & b) indicate that isolate No. 3 collected from Ismailia governorate Abo-Sour locality was the most virulent causing pink root rot infection to 61.94% on Giza-20 onion transplants under artificial inoculation and greenhouse conditions at 27°C. ± 2. It is also clear that this isolate was the most virulent, as the severity of the disease incidence reached 41.91% according to infected plants and 33.79% according to tested plants. Isolates collected from Fayoum governorate (Qouta and Kom-Oshem localities) and Assiut governorate (El-Badary locality) were moderate in their virulence causing average percent of infection 44.17 % ; 42.51% and 40.71% of the tested transplants respectively, meanwhile the average percent of the disease severity ranged from 30.26% to 27.97% according to infected plants and ranged from 11.93% to 8.97% according to tested plants. Moreover, isolates No. 2 and 5 collected from Ismailia governorate (Sarabiom

Table (8): Average percent of onion pink root rot disease on Giza-20 cultivar incited by different isolates of *Pyrenochaeta terrestris* collected from seven different localities belonging to 3 governorates after 70 days from transplantation.

No.	Governorate	Locality	Av. % of : Infection	Severity according to: Infected plants	Severity according to: Tested plants
1	Ismailia	Fayed	26.40%	19.10%	3.49%
2	Ismailia	Sarabiom	33.80	26.70	6.34
3	Ismailia	Abo-Sour	61.94	41.91	33.79
4	Fayoum	Kom-Oshem	42.51	30.26	11.61
5	Fayoum	Ibshwai	28.40	23.07	3.76
6	Fayoum	Qouta	44.17	29.34	11.93
7	Assiut	El-Badary	40.71	27.97	8.97
8	Control		0.00	0.00	0.00
L.S.D. at level 5%			9.92	4.73	5.79

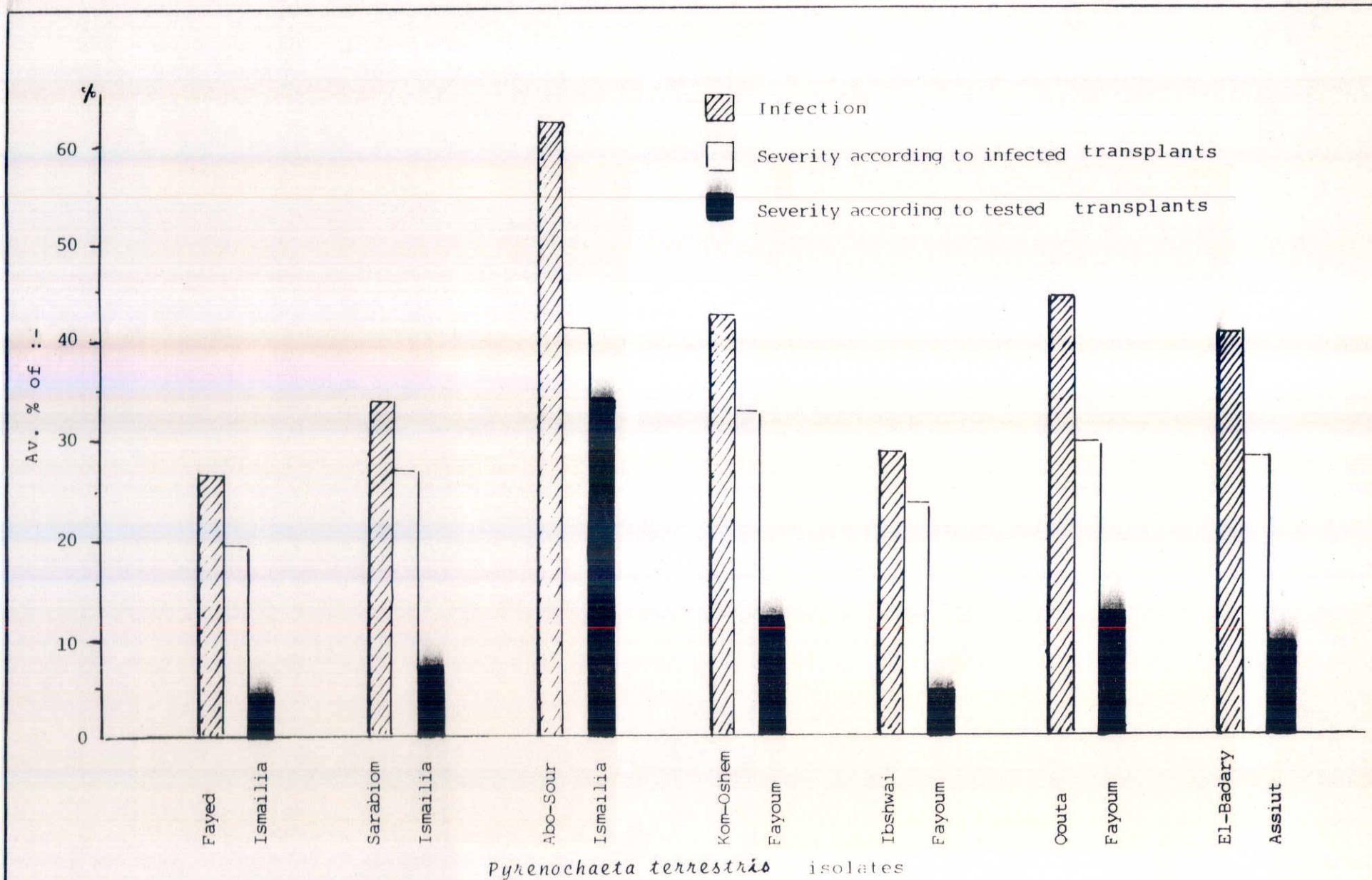

Fig. (4): Average percent infection and disease severity according to infected and tested plants under artificial inoculation with seven isolates of *Pyrenochaeta terrestris* after 70 days from transplanting under greenhouse conditions at 27°C ± 2.

Fig. (5 a)

locality) and Fayoum governorate (Ibshawi locality) showed the lowest virulence in either percentage of infection or disease severity as the percent of infection was 33.80% and 28.40% whereas the disease severity was 26.70% and 23.07% according to infected plants and ranged from 6.34% to 3.76% according to tested plants respectively. It is also clear that isolate No.1 collected from Ismailia governorate (Fayed locality) was the least pathogenic one, causing average percent of disease infection not more than 26.40% and disease severity 19.10% according to infected plants and 3.49% according to tested plants. It is also very clear that there is a correlation between average percent of onion pink root rot infection and severity of the disease incidence incited by the mentioned isolates of *Pyrenochaeta terrestris*.

Concerning to the high virulence of isolate No. (3), collected from Ismailia governorate (Abo-Sour locality), this isolate will be used in all following researches.

As for pathogenicity test for ten isolates of *Fusarium oxysporum* f. sp. *cepae*, data in Table (9) indicate that all tested isolates were pathogenic with non-significant difference. Typical symptoms of basal rot disease for all tested Fusaria isolates, recorded as percentage of infection were ranged from 7.6 to 19.0% at harvesting time, 3 months from transplantation.

Table (9): Average percentages of onion basal rot disease on Giza-20 onion cultivar incited by different isolates of *Fusarium oxysporum* f. sp. *cepae* isolated from ten different localities belonging to 5 governorates after 90 days from transplantation.

Sources of isolates :				Average percent of basal rot infection
No.	Governorate	Center	Locality	
1	Giza	El-Badrashin	El-Hawamdia	7.6%
2	Giza	El-Saff	Mazghona	7.6
3	Beni-Suef	El-Wassta	Sanhour	11.4
4	Assiut	El-Badary	Koum-Seeda	11.4
5	Fayoum	Ibshwai	El-Hamoli	7.6
6	Fayoum	Koum-Oshem	Koum-Oshem	11.4
7	Fayoum	Ibshwai	Qouta	15.2
8	Ismailia	Abou-Sour	Abou-Sour	19.0
9	Ismailia	Sarabiom	Sarabiom	19.0
10	Ismailia	Fayed	Fayed	15.2
11	Control			00.0
L.S.D.; 0.05				N.S.

4. EVALUATION OF SOME ONION CULTIVARS FOR RESISTANCE AND/OR SUSCEPTIBILITY TO ONION PINK ROOT ROT DISEASE :

Varietal reaction of the three onion cultivars, namely, Giza-6; Giza-20 and Shandaweel-1, shown in Table (10) and illustrated in Fig. (6), indicated clearly that Giza-20 showed relatively high susceptibility to pink root rot disease which artificially induced by the most virulent isolate of *Pyrenochaeta terrestris* (isolate No. 3), as the fungal infection was more than 79% of the tested (inoculated) plants after 90 days from transplantation; whereas Giza-6 was moderately affected, as the percentage of infection reached 53.84% moreover Shandaweel-"1" showed high resistance to the disease, as the diseased plants reached 36.68% after 90 days from transplantation. According to the disease severity, it was noticed that the disease was most severe on Giza-20 concerning both infected and tested (inoculated) plants, as the disease severity was 56.8% and 50.5% respectively, whereas the disease severity of both Giza-6 and Shandaweel-1 was the same and significantly showed less values than that of Giza-20 particularly after 90 days from transplantation. Finally, the most noticeable observation that, both the percentage of infection and the disease severity — according to both infected and tested plants — were increased as the plant age increased.

Table (10): Evaluation of three onion cultivars under artificial inoculation with the most virulent isolate of *Pyrenochaeta terrestris* (Ismailia isolate) and greenhouse conditions at 27°C. ± 2 after 30;60 and 90 days from transplantation.

Onion cultivars	Average percent of :-								
	Pink root rot infection after :			Severity according to infected plants after:			Severity according to tested plants after :		
	30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
1. Shandaweel-1 (R)	15.96	29.12	36.68	6.7	19.8	22.6	1.3	4.8	8.0
2. Giza-6 (M)	26.32	36.48	53.84	18.3	25.7	20.6	5.8	10.0	14.5
3. Giza-20 (S)	34.16	46.16	79.36	31.2	40.0	56.8	11.3	24.0	50.5
L.S.D.;0.05	(C.)	15.82		(C.)	11.12		(C.)	15.5	
	(P.)	15.82		(P.)	11.12		(P.)	15.5	
	(C.) X (P.)	N.S.		(C.) X(P.)	19.25		(C.) X (P.)	26.9	

R.: resistant Cv.
M.: moderately resistant Cv.
S.: Susceptible Cv.

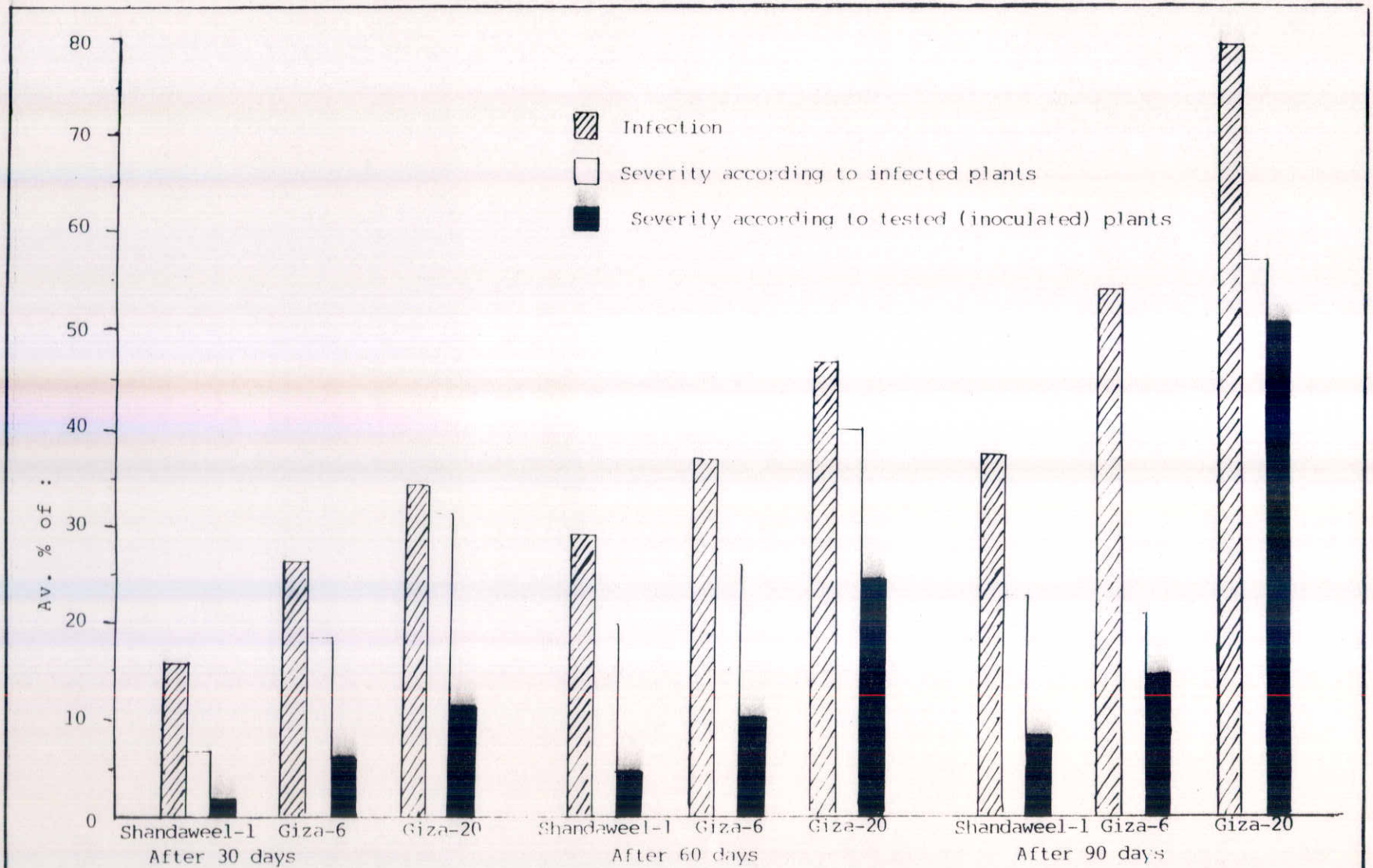

Fig. (6): Varietal reaction of three onion cultivars after 30, 60 and 90 days from transplantation under artificial inoculation with the most virulent isolate (No. 3) of *Pyrenochaeta terrestris* and greenhouse conditions at 27°C. ± 2.

5. MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES :

Variability of *Pyrenochaeta terrestris* isolates :

1. Morphological and cultural characteristics:

Data summarized in Table (11) and illustrated in Fig. (7) show the linear growth; dry weight of mycelial mass; colour of culture and the nature of growth of seven isolates of *Pyrenochaeta terrestris* . The average number of Pycnidia; pycnidiospores and conidia and their measurements are tabulated in Table (12) and their illustration shown in Figs. (8: a; b; c; d...g).

A- Linear growth :

The isolates grown on PDA medium for 9 days at 27°C. varied in their linear growth. Isolates No.3;4 and 6 significantly grew faster than isolates No. 2; 5 and 7. However isolate No. 1 significantly showed the least linear growth.

B- Dry weight of mycelium :

The isolates also varied in their dry weight of mycelial mass when grown on normal Czapek's liquid medium for 15 days at 27°C. Isolates No. 1;3;4 and 7 significantly produced the highest dry weight of mycelial mass (over 0.3994 mgs.) than isolate No. 5 which yielded 0.3729 mgs. However isolates No.2 and 6 significantly produced the least mycelial mass which respectively yielded 0.2975 and 0.2915 mgs.

C- Colour of culture :

The isolates varied in their mycelial colour as the colour of isolate No. 1 colonies was very dark black accompined with many

Table (11): Linear growth in cm.; dry weight of mycelial mass in mgs., colour of culture and nature of growth of seven isolates of *Pyrenochaeta terrestris* after incubation at 27°C. for 9 days.

Isolate	Linear growth (in cm.)	Dry weight (in mg.)	Colour of culture	Nature of growth
1. El-Badary (Assiut)	8.12	0.4384	Very dark black and many sectors with whitish colour at the colony's margin.	The mycelium is compact with witish sectors at the colony's margin.
2. Ibshwai (Fayoum)	8.32	0.2975	Grey	The mycelium is aerial and erect on the cultural surface and has concentric rings.
3. Abo-Souer (Ismailia)	8.64	0.4500	whitish- grey	
4. Kom-Oshem (Fayoum)	8.68	0.3994	Black with whitish grey margins.	
5. Fayed (Ismailia)	8.36	0.3729	Grey	
6. Qouta (Fayoum)	8.57	0.2915	Whitish - grey	
7. Sarabiom (Ismailia)	8.22	0.4328	Whitish-grey	
L.S.D. at level 1%	0.275	0.0754		

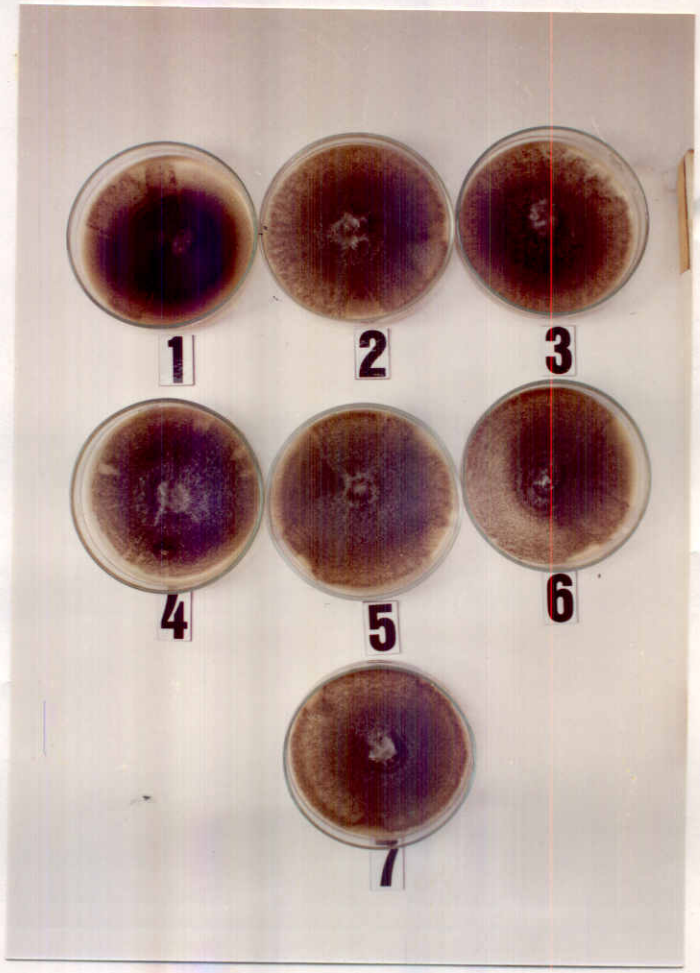

Fig. (7): The growth of seven isolates of *Pyrenochaeta terrestris* after 9 days from inoculating PDA medium at 27°C.

Note : The great similarity of morphology

sectors with whitish colour at the colony's margin, meanwhile isolate No. 4 produced black colonies with whitish grey margins. However, isolates No. 2;3;5;6 and 7 produced colonies differed in their colours from gray to witish grey.

D- Nature of growth :

The tested isolates were relatively varied in their nature of growth, where isolate No. 1 produced compact mycelium with whitish sectors at the colony's margin. On the other hand there were not apparently differences in the morphological characteristics of the rest tested isolates, which produced abundant aerial and erect mycelium, on the cultural surface and had concentric rings according to the pycnidial formation.

E- Pycnidial; pycnidio and conido-spores formations:

Concerning the average number of pycnidia in one square cm.; the number of pycnidio and conidio spores in million/one cc. of the spore suspension, Table (12) show clearly that all isolates significantly produced the same number of Pycnidia and pycnidio spores. Also, all isolates significantly produced the same number of conidiospores, except for isolate No. 1 which significantly produced less number of such spores.

F- Pycnidial; pycnidio and conidio spores shape :

Pycnidial shape of *Pyrenochaeta terrestris* isolates varied from isolate to another, Figs. (8a, b,c,d, ..g) showed that glomeration shape is the general character for the all tested isolates. Pycnidia are varing from globose to subglobose and generally ostiolate and papillate. Bristles (setae)

Table (12): Average number of pycnidia in one cm^2; pycnidio and conidio-spores in million/1 cc.; pycnidial; bristles and pycnidio and conidio-spores measurements in microns of seven isolates of *Pyrenochaeta terrestris* after 9 days from incubation on PDA medium at $27^{o}C$.

Isolates	Average number of :-			(Measurements of :-)																	
				Pycnidia						Bristles			Pycnidiospores						Conidiospores					
				Length			Width						Length			Width			Length			Width		
	Pycnidia in cm^2	Pycnidio-spores in million/1 cc	Conidio-spores in million/1 cc	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
1. El-Badary (Assiut)	885	2.95	0.625	265.0	79.5	170.7	185.5	42.4	124.6	328.6	68.9	164.4	10.4	3.9	9.00	6.5	2.6	4.8	2.9	0.7	2.2	2.2	0.7	1.6
2. Ibshwai (Fayoum)	828	2.79	1.125	275.6	116.6	179.4	185.5	68.9	108.5	424.0	137.8	245.1	13.0	10.4	11.5	7.8	2.6	5.5	2.5	1.4	1.8	2.5	1.4	1.6
3. Abou-Souer (Ismailia)	853	2.31	1.000	164.3	63.6	116.6	127.2	42.4	81.9	174.9	31.8	80.9	11.7	5.2	8.5	5.2	2.6	4.3	2.9	1.4	2.0	2.9	1.4	1.8
4. Koum-Oshem (Fayoum)	718	2.11	0.938	217.3	68.9	141.8	169.6	47.7	102.0	227.9	58.3	112.6	11.7	6.5	9.1	6.5	3.9	5.1	2.9	1.1	2.0	2.5	1.1	1.6
5. Fayed (Ismailia)	678	2.08	0.859	286.2	84.8	165.1	206.7	68.9	123.4	222.6	58.3	134.4	11.7	6.5	9.9	6.5	3.9	5.2	2.9	1.4	2.0	2.5	1.4	1.6
6. Qouta (Fayoum)	713	2.11	0.922	217.6	82.9	149.6	150.4	62.2	108.4	176.1	31.1	78.0	10.0	5.7	8.8	7.2	3.6	4.8	2.9	1.1	2.0	3.6	0.7	1.6
7. Sarabiom (Ismailia)	814	2.57	1.050	191.7	98.4	137.4	165.8	57.0	94.1	165.8	46.6	106.3	11.4	5.7	8.6	6.5	3.6	5.0	3.6	2.2	2.6	2.9	1.4	1.9
L.S.D.; 0.05	N.S.	N.S.	0.388																					

Max.: Maximum length and/or width of pycnidia and its bristles, pycnidospores and conidiospores.
Min.: Minimum length and/or width of pycnidia and its bristles, pycnidiospores and conidiospores.
Mean.: Mean of length and/or width of fifty pycnidia and its bristles, pycnidiospores and conidiospores.

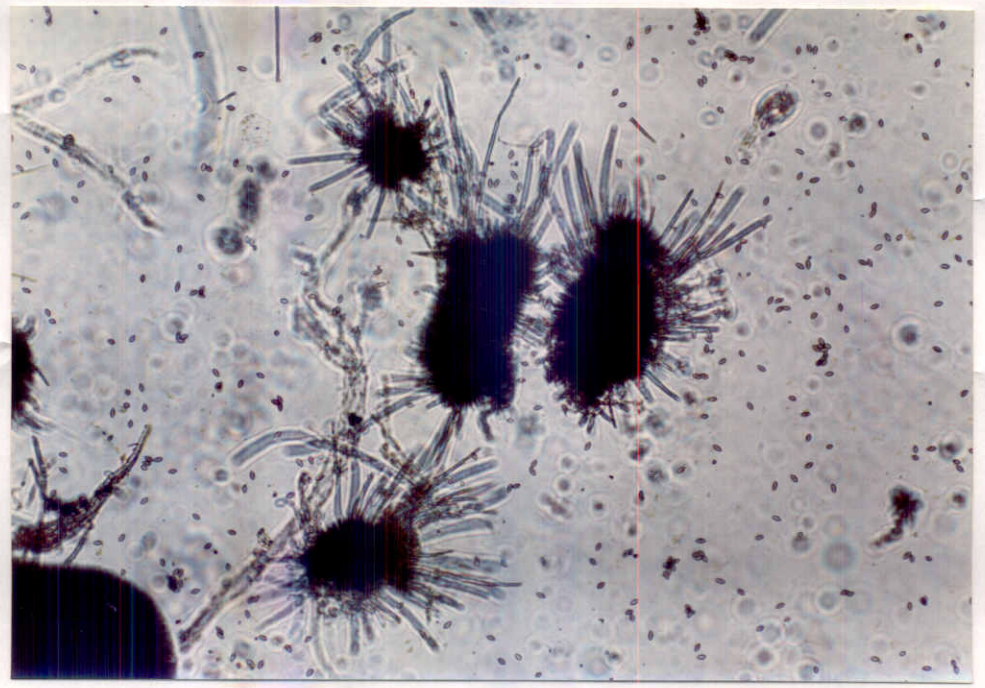

Fig. (8 a): Pycnidial stage of isolate No "1"
Note: the well developed pycnidia with its bristles and the huge number of oblonged brown pycnidiospores (100 X)

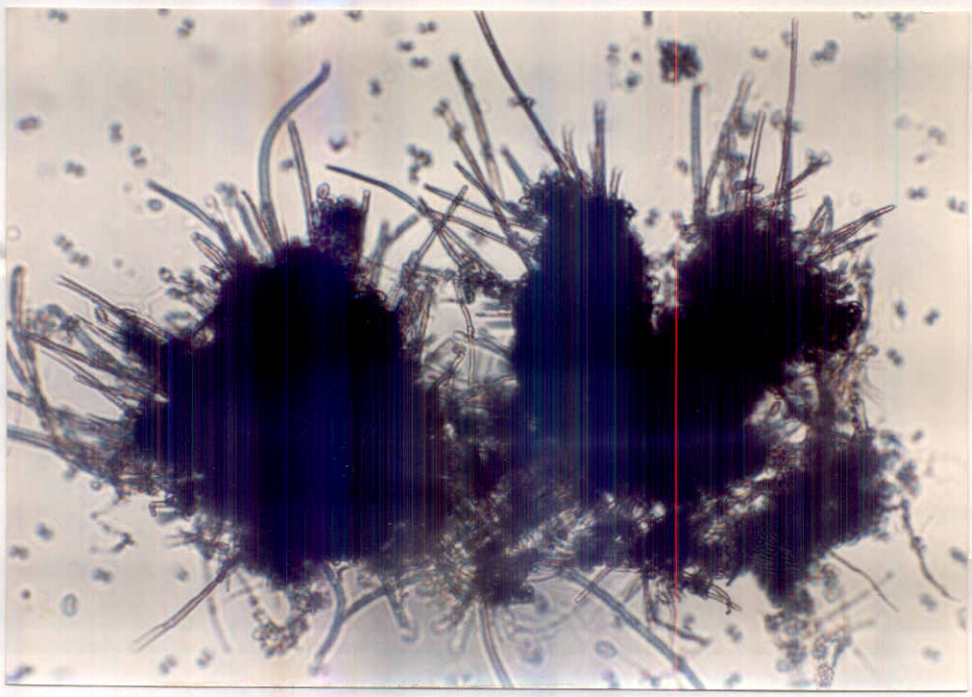

Fig. (b): Group of pycnidia of isolate No. "2", dark; ostiolate; nearly globose.
Note: bristles (setae) especially near ostoile (A)
Pycnidiospores varying from brown to dark brown (B) (200 X)

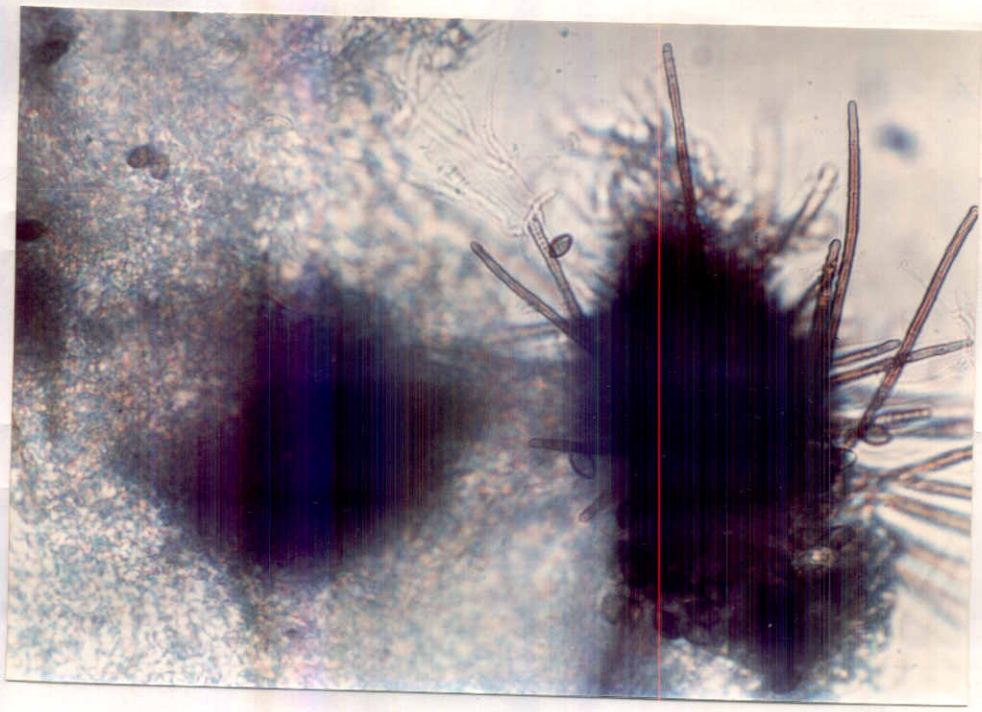

Fig. (c): Pycnidia of isolate No. "3"
Note: the pear shaped developed pycnidium with septate bristles near the ostiole. Mechanical opening of pycnidium and the dispersal of pycnidiospores. (200 X).

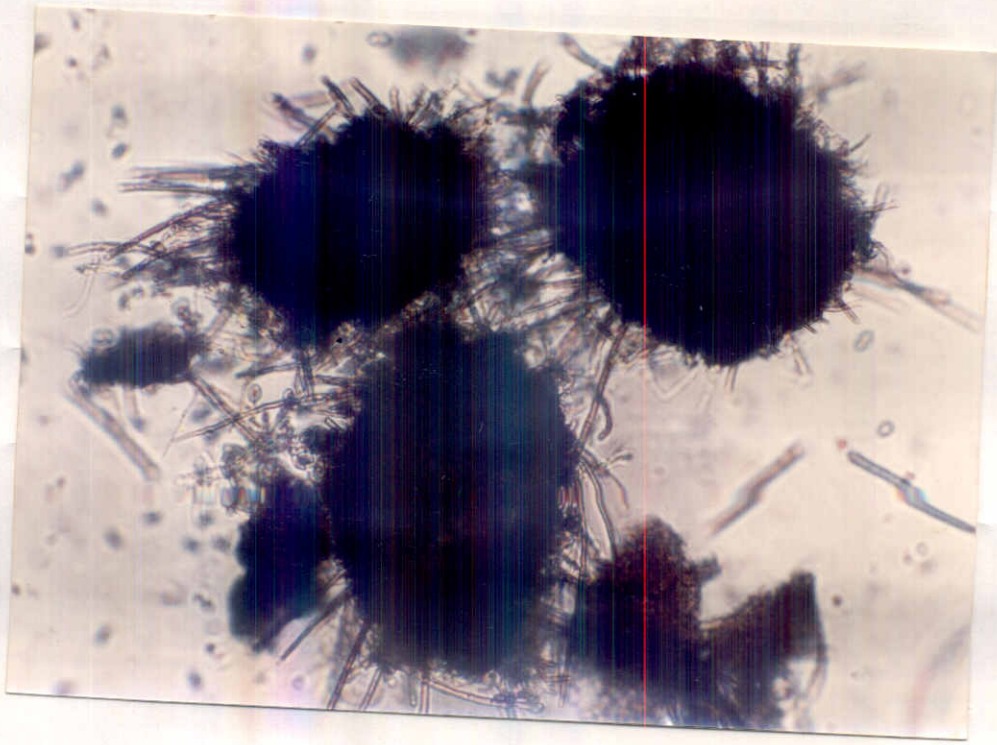

Fig. (d): Mature pycnidia of isolate No. "4"
Note: the sphaerical dark pycnidia with simple bristles (setae) and the distingushed pycnidiospores (200 X).

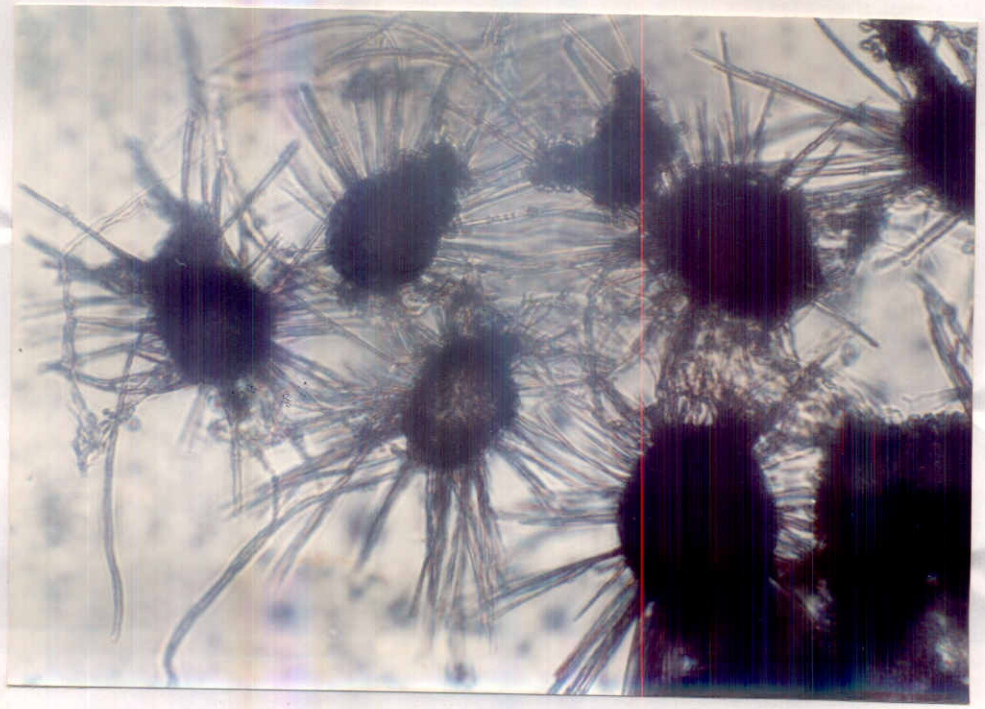

Fig. (e): Group of mature pycnidia of isolate No. "5" Note: the typical shape of pycnidia with well developed bristles which abundantly formed near the ostiole (200 X).

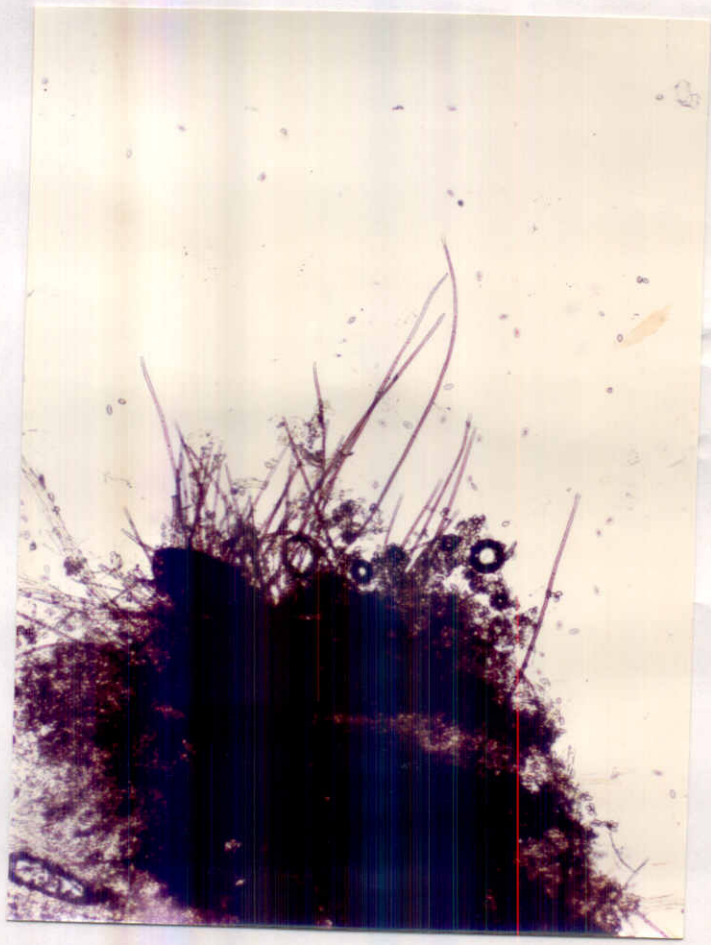

Fig. (f): Pycnidia of isolate No. "6"
Note: the long bristles which mounded especially near the ostiole and the dispersed well developed pycnidiospores. (100 X).

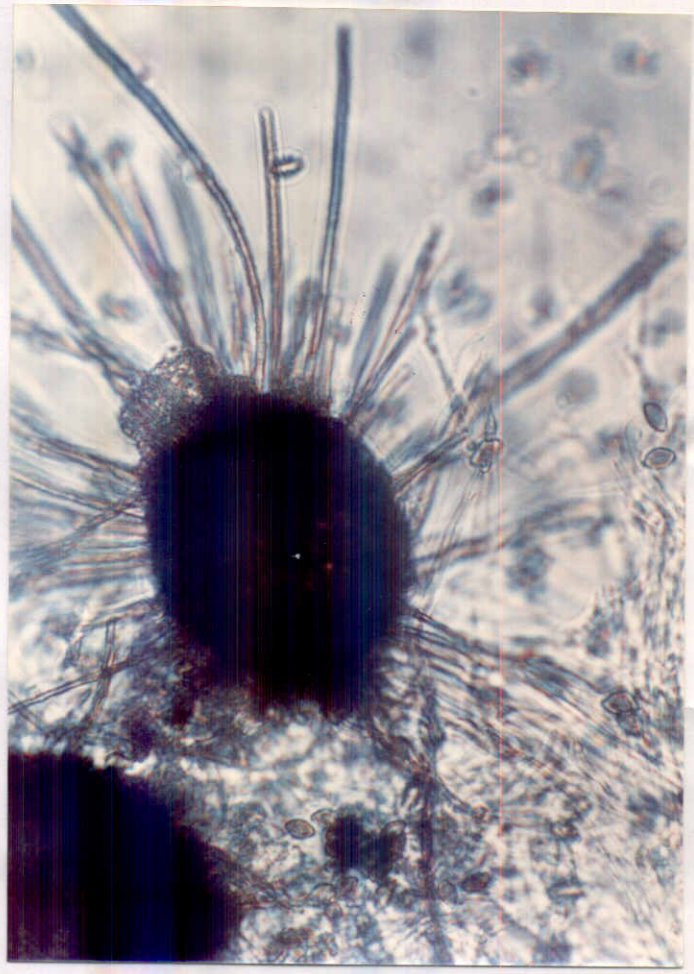

Fig. (9): Mature pycnidia of isolate No. 7
Note: the abundant bristles and opend ostiole (400 X).

are being formed all over the surface of pycnidium but the longest ones are being formed near the ostiole. Pycnidiospores varied from light to dark brown and 1-celled. Conidiospores are small; hyaline ; oblong to ovoid and 1-celled. Mycelium is hyaline; septate; and carries conidia on simple or branched conidiophores.

G- Pycnidial, pycnidio and conido-spores measurements:

As shown in Table (12) pycnidia measurements ranged from 63.6 to 286.2 µ in length through all tested isolates, while the mean length of fifty pycnidia ranged from 116.6 to 179.4 µ. The pycnidial width ranged from 42.4 to 206.7 µ, and its mean ranged from 81.9 to 124.6 µ. The bristle's length ranged from 31.1 to 424.0 µ (78.0 to 245.1 µ in mean). Pycnidial spore length ranged from 3.9 to 13.0 µ (8.5 to 11.5 µ in mean) and from 2.6 to 7.8 µ (4.3 to 5.5 µ in mean) in width. Conidial spore length ranged from 0.7 to 3.6 µ (1.8 to 2.6 µ in mean) and from 0.7 to 3.6 µ (1.6 to 1.9 µ in mean) in width.

2. Effect of different complicated carbon sources on linear growth; pycnidia; pycnidio and conidio-spores formation and weight growth of different isolates of *Pyrenochaeta terrestris* :

A- On linear growth; pycnidia; pycnidio and conidio-spores formation :

The effect of different complicated carbon sources on the linear growth; number of pycnidia; pycnidio spores and conidia

produced by six isolates of *Pyrenochaeta terrestris* recorded in Table (13) and illustrated by Fig. (9). It is very clear that isolate No. 3 grew best at all modified Czapek's media which, starch; cellulose or pectine substituted for sucrose as its general mean of growth was 8.17 cm. however, isolates 1;2; 4;5 and 6 showed significantly less linear growth, which ranged from 3.95 to 4.85 cm. only. On the other hand, starch and cellulose carbon sources were more suitable than pectine for the growth of all isolates.

According to the effect of such different carbon sources on the production of pycnidia, it is very clear that growing the isolates on starch containing medium developed more number of pycnidia rather than those obtained when the isolates were grown on other carbon sources. Moderate numbers of pycnidia for each isolate was obtained when the isolates were grown on cellulose containing medium whereas the lowest number of pycnidia for all isolates were obtained when these isolate had been grown on pectine containing one. However all isolates were significantly the same in calculating the general mean of pycnidia.

Similar behaviours could be observed according to the production of both pycnidio and conidio-spores, as starch containing medium encouraged the production of such spores, followed by cellulose, meanwhile the pectine containing medium had the least effect. Also, it is very clear that isolate No. 3 produced the highest number of such spores in consideration with

Table (13): Effect of different complicated carbon sources on linear growth; number of pycnidia; pycnidio and conidio-spores of six isolates of *Pyrenochaeta terrestris* on modified Czapek's medium after 8 days from incubation at 27°C.

Isolate	Average linear growth (in cm)				Average number of :- Pynidia/microscopic field				Pycnidiospores (in million /1 cc)				Conidiospores (in million /1 cc)			
	Starch	Cellulose	Pectine	General Mean	Starch	Cellulose	Pectine	General Mean	Starch	Cellulose	Pectine	General Mean	Starch	Cellulose	Pectine	General Mean
1. El-Badary	6.15	5.10	1.35	4.20	20.00	17.00	5.0	14.00	0.213	0.088	0.025	0.100	0.313	0.238	0.088	0.213
2. Ibshwai	6.10	5.36	3.03	4.83	37.00	23.00	4.2	21.40	0.500	0.150	0.050	0.233	0.363	0.175	0.063	0.200
3. Abo-Souer	8.30	7.90	8.30	8.17	39.60	29.80	6.8	25.40	0.700	0.450	0.100	0.417	1.150	0.438	0.263	0.617
4. Koum-Oshem	7.63	5.28	1.65	4.85	32.40	26.80	6.2	21.80	0.350	0.425	0.025	0.267	0.363	0.188	0.038	0.196
5. Fayed	5.91	5.81	1.78	4.50	16.40	15.20	3.2	11.60	0.225	0.313	0.025	0.188	0.338	0.163	0.025	0.175
6. Qouta	5.98	5.04	0.84	3.95	23.20	19.40	3.8	15.47	0.500	0.213	0.025	0.246	0.288	0.200	0.000	0.163
General Mean	6.68	5.75	2.82		28.10	21.87	4.87		0.415	0.273	0.042		0.469	0.233	0.079	

L.S.D. at level 1%

Isolates :	0.64	14.65	0.122	0.129
Carbon sources :	1.11	25.50	0.213	0.224
Interaction :	0.87	22.30	0.166	0.175

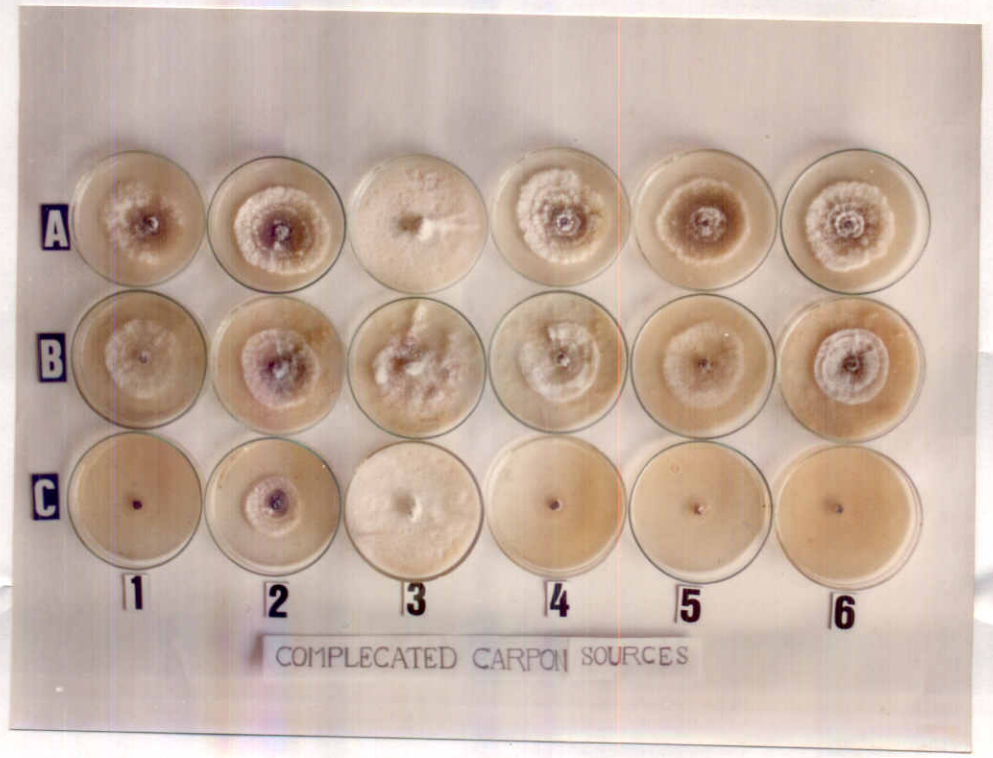

Fig. (9): Effect of different complecated carbon sources as starch; cellulose and pectin on linear growth and morphological characteristics of six isolates of *Pyrenochaeta terrestris* on modified Czapek's medium after 8 days from incubation at 27°C.

Isolates :

1. El-Badary
2. Ibshwai
3. Abo-Souer
4. Koum-Oshem
5. Fayed
6. Quota

Complicated carbon sources :

A. Starch.
B. Cellulose.
C. Pectine.

C-7

the general mean of spores, whilest the rest isolates produced significantly less number of such spores.

B- On the weight growth :

The complemental data on the production of mycelial mass of six isolates of *Pyrenochaeta terrestris* on different complicated carbon sources were shown in Table (14). It is very clear that all isolates, with non-significant difference ; grew best and produced considerable weight of mycelial mass when they were grown on modified Czapek's media, which starch or cellulose substituted for sucrose.

Also the weight of the mycelial mass of all isolates were very low on the pectine containing medium. One of the most valuable observation of the reaction using complicated carbon sources, is that the effect of starch or cellulose or both in the encouragement of the mycelial production was decrease once any of them or both complicated carbon sources were mixed with pectine to reach the standard level of carbon in the media.

3. Some factors affecting, morphological (linear growth; number of pycnidia; pycnidio and conidio-spores) and cultural characteristics of the most virulent isolate of *Pyrenochaeta terrestris* (Isolate No. 3).

A- Effect of different nutrient media :

Data written in Table (15) and illustrated in Fig. (10) indicate the effect of six different media i.e. synthetic media [Ranker; Czapek and Sucrose ammonium nitrate (SAN)] and natural

Table (14): Effect of different complicated carbon sources on the dry weight of mycelium mass of six isolates of *Pyrenochaeta terrestris* on modified C'zapek's medium after 15 days from incubation at 27°C.

Isolates:	Dry weight of mycelium mass in mg.							Gemeral mean
	Starch	Cellulose	Pectine	Starch + Cellulose	Starch + Pectine	Cellulose + Pectine	Starch + Cellulose + Pectine	
1. El-Badary	0.1395	0.1252	0.0492	0.1228	0.0381	0.0189	0.0242	0.0740
2. Ibshwai	0.2943	0.1306	0.0238	0.1516	0.0126	0.0470	0.0255	0.0979
3. Abo-Souer	0.0756	0.0619	0.0630	0.3175	0.0760	0.0481	0.0981	0.1057
4. Koum-Oshem	0.1470	0.1153	0.0361	0.1578	0.0357	0.0207	0.0414	0.0791
5. Fayed	0.1407	0.1454	0.0329	0.1489	0.0188	0.0433	0.0239	0.0791
6. Qouta	0.1569	0.1317	0.0108	0.1979	0.0416	0.0202	0.0168	0.0823
General Mean	0.1590	0.1184	0.0360	0.1827	0.0371	0.0330	0.0383	
L.S.D. at level 1%				0.1234				

Table (15): Effect of different nutrient media on the morphological (linear growth; number of pycnidia; pycnidio and conidio-spores), and cultural characteristics of the most virulent isolate of *Pyrenochaeta terrestris* after 9 days from incubation at 27°C.

Media	Morphological characteristics				Cultural characteristics
	Av. number of :-				
	Linear growth (in cm)	Pycnidia per microscobic field	Pycnidiospores (in million/ 1 cc	Conidiospores (in million/ 1 cc	
Synthetic media					
1. Ranker	3.90	9.20	0.038	0.094	The mycelium was abnormal, whitish, compact and had an irrigular margin.
2. Czapek	8.26	24.20	0.204	0.163	The mycelium of the colonies was cottony, witish lemon surrounded by a ring of cottony white.
3. Sucrose ammonium nitrate.	8.30	37.75	0.350	0.738	
Natural Media					
1. Onion-dextrose agar. (ODA)	7.75	36.00	3.388	0.788	The growth was normal, vigorous, had no any pigments and contained a huge number of pycnidia.
2. Potato-dextrose agar. (pDA)	8.30	37.20	3.663	0.838	
3. Corn-meal agar(CMA)	8.30	48.80	4.300	0.938	
L.S.D.; 01	0.89	11.54	1.023	0.227	

Fig. (10): Effect of different nutrient media on the linear growth and cultural characteristics of the most virulent isolate of *Pyrenochaeta terrestris* after 9 days from incubation at 27°C.

1. Ranker.
2. Czapek.
3. Sucrose ammonium nitrate (SAN)
4. Onion dextrose agar (ODA)
5. Potato dextrose agar (PDA)
6. Corn meal agar (CMA)

media [onion dextrose agar (ODA), potato dextrose agar (PDA) and corn meal agar (CMA) on the linear growth; pycnidia production; sporulation and the morphological characteristics of the most pathogenic isolate of *Pyrenochaeta terrestris* after 9 days from incubation at 27°C. It is very clear that the fungus grew best on all natural media and also in both Czapek and (SAN) synthetic media, as the diameter of growth was significantly the same and ranged from (7.75-8.30 cm). Synthetic Ranker's medium showed less significant effect as the fungus grew to a distance reached not more than 3.90 cm. of the growth diameter. It is also very clear that all natural media and only (SAN) synthetic medium were more suitable media for the production of the conidial stage, as the number of conidia formed ranged from 0.738-0.938 million spores/ 1 cc. According to the formation of pycnidiospores, data indicated clearly that only natural media gave excellent results for the production of such spores (from 3.338 - 4.300 million/ 1 cc.) if compared with the production on the synthetic media (from 0.038 - 0.350 million/ 1 cc.).

Concerning the formation of pycnidia, only (CMA) natural medium was the most suitable for pycnidia production.

According to the cultural description of the mentioned synthetic and natural media, the mycelium on Ranker's medium was abnormal; whitish; compact and had an irrigular margin. On both Czapek and (SAN) media the mycelium of the colonies was cottony; whitish lemon surrounded by a ring of cottony white mycelium.

On the other hand, the growth on the natural media was normal; vigorous; had no any pigments and contained a huge number of pycnidia.

B- Effect of different carbon; nitrogen sources and C/N ratios:

a- Carbon sources :

Data recorded in Table (16) and illustrated in Fig. (11) show the effect of nine different sugars used as carbon sources and substituted for sucrose in the modified Czapek's medium on the linear growth; pycnidial formation; pycnidio and conidiospores production of *Pyrenochaeta terrestris* isolate No. 3. Also the effect of these different carbon sources on the cultural characteristics was also recorded. It is very clear that all reduced sugars namely: glycerol; mannitol; mannose; glucose and dextrose ; non-reduced sugar (sucrose) and reduced plus non-reduced sugars (sucrose plus glucose) encouraged the fungal linear growth whereas both maltose and lactose indicated less importance in the requirement for the vegetative growth. Concerning the effect of the mentioned carbon sources on the pycnidial formation, it is enough clear that the original carbon source (sucrose) and (sucrose plus glucose) gave considerable possitive variation followed by dextrose; glucose and mannose. In the respect of pycnidiospores production, it is very clear that only glucose was the best carbon source for increasing the number of such spores followed significantly by dextrose; sucrose and (sucrose plus glucose). Data also indicate clearly that all

Table (16): Effect of different carbon sources on morphological (linear growth; number of pycnidia; pycnidiospores and conidia) and cultural characteristics of the most virulent isolate of *Pyrenochaeta terrestris* after 9 days from incubation at 27°C.

Carbon sources:	Morphological characteristics				Cultural characteristics
	Linear growth (in cm.)	Average number of :			
		Pycnidia per micro-scopic field	Pycnidio spores (in million) per 1 cc.	Conidio spores (in million) per 1 cc.	
Reducing sugars					
Glycerol	7.53	1.2	0.000	0.000	The growth of the fungal colonies was very weak and had very irrigular margins.
Mannitol	8.11	1.4	0.138	0.100	
Mannose	8.30	9.2	0.400	0.288	The colour of the colonies yellowish lemon arround main inoculum.
Glucose	8.30	11.8	1.025	0.425	The colour of the fungal growth colonies white greyish and surrounded by yellow zones.
Dextrose	8.01	16.8	0.925	0.400	
Non-reducing sugars					
Maltose	3.48	4.6	0.150	0.225	The colonies colour was white yellowish.
Sucrose	8.30	25.6	0.488	0.313	The growth was vigorous and produced some yellow pigment arround the main inocula.
Lactose	5.68	2.2	0.044	0.100	The growth was very weak, greatly look alike the growth on the plane agar and had an irrigular margin.
Red. & non-red. sugars					
Sucrose plus Glucose	7.80	23.0	0.544	0.388	The growth was normal with no any cultural changes.
L.S.D.; 0.01	1.03	5.84	0.229	0.257	

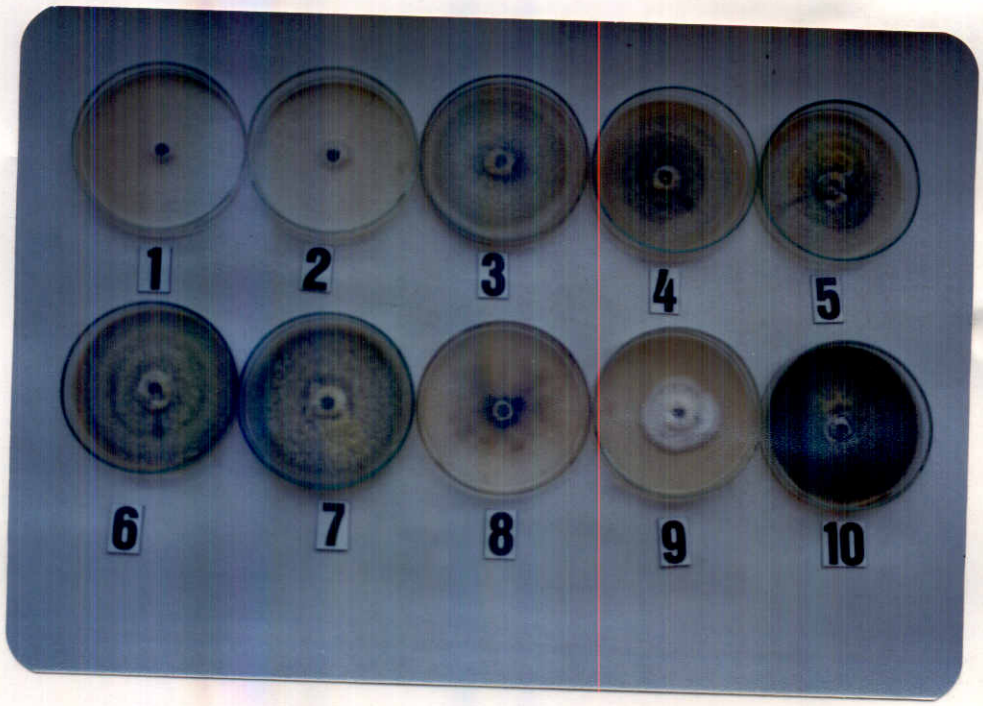

Fig. (11): Effect of different carbon sources on the linear growth and cultural characteristics of the most virulent isolate of *Pyrenochaeta terrestris* after 9 days from incubation on modified Cźapek's medium at 27°C.

1. Glycerol
2. Manitol
3. Mannose
4. Glucose
5. Dextrose
6. Sucrose
7. Sucrose plus Glucose
8. Lactose
9. Maltose
10. PDA for check

sugars could be considered good sources for conidiospores production with the exception for glycerol; mannitol and lactose. According to the cultural characteristics for the colonies of the most virulent isolate which was grown on modified Czapek's media provided with different sugars- as carbon sources - it was observed that the growth of the fungal colonies was very weak and had very irrigular margins on the media containing either glycerol or mannitol. On the medium contained mannose, the colour of the growing colonies changed into yellowish lemon arround the main inoculum. In both glucose and dextrose containing media, the colour of the fungal growth colonies changed into white greyish and surrounded by yellow zones. According to the three different non-reduced sugars maltose; sucrose and lactose, it was noticed that the cultural changes of the fungal colonies were greatly differed as the colony's colour was white yellowish on maltose containing medium, while the growth was vigorous and produced some yellow pigment arround the main inocula on the normal Czapek's medium which contained the sucrose as carbon source, meanwhile on the lactose containing medium the growth was very weak; greatly looked alike the growth occurred on the plane agar and had an irrigular margin. On the medium containing sucrose plus glucose - as carbon source- the growth was normal with no any cultural changes and was similar to that occurred on the normal Czapek's medium.

b- Nitrogen sources :

Data written in Table (17) and illustrated in Fig. (12) indicate the effect of ten different nitrogen sources on

Table (17): Effect of different nitrogen sources on the morphological (linear growth; number of pycnidia; pycnidiospores and conidia), and cultural characteristics of the most virulent isolate of *Pyrenochaeta terrestris* after 9 days from incubation at 27°C.

Nitrogen sources :	Morphological characteristics				Cultural characteristics
	Linear growth (in cm.)	Average number of :			
		Pycnidia per microscopic field	Pycnidiospores (in million) per 1 cc.	Conidiospores (in million) per 1 cc.	
Amino acids :					
Glycine	6.00	6.8	0.100	0.175	The fungal growth was very poor looking alike the growth on the plane agar.
L-serine	6.51	2.4	0.113	0.100	
L-Arginine	8.16	22.8	0.588	0.325	The growth was strong, vigorous and producing yellowish pigment far from the main inocula.
L-Methionine	3.51	0.0	0.000	0.000	The nature of growth was very poor, bright, viscus and had an irrigular margin.
DL-Treptophan	5.18	1.6	0.163	0.138	The growth was aerial, relatively vigorous greyish and had a regular margin.
Nitrogenous salts :					
Sodium nitrate	8.26	29.2	0.238	0.338	The growth was very strong, grey yellowish, had regular margins and contained great number of pycnidia.
Potassium nitrate	8.24	33.4	0.138	0.200	
Ammonium nitrate	8.24	50.4	1.988	0.625	
Ammonium tartarate	2.91	0.0	0.000	0.000	The growth was limited, flattened, completely connected with the surface medium and had an irrigular margin.
Nitrogenous organic compounds :					
Urea	1.10	0.0	0.000	0.000	The growth was abnormal.
L.S.D.; 0.01	0.74	6.32	0.317	0.165	

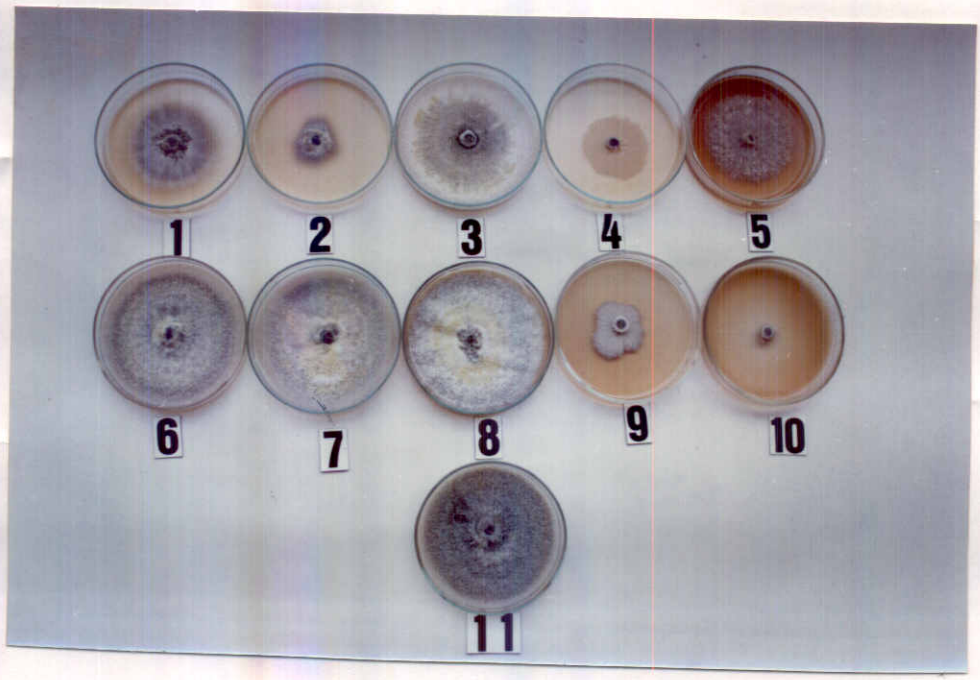

Fig. (12): Effect of different nitrogen sources on the linear growth and cultural characteristics of the most virulent isolate of *Pyrenochaeta terrestris* after 9 days from incubation on modified Czapek's medium at 27°C.

1. L-serine.
2. Ammonium tartarate
3. L-Glycine
4. L-Methionine
5. L-Arginine
6. Sodium nitrate
7. Potassium nitrate
8. Ammonium nitrate
9. DL-Treptophan
10. Urea
11. PDA for check

Pyrenochaeta terrestris linear growth; pycnidial formation and sporulation after 9 days growing in modified Cźapek's medium at 27°C. It is enough clear that only the nitrogen source ammonium nitrate gave satisfactory results in increasing the linear growth; the production of pycnidia; pycnidio and conidio-spores, followed significantly by sodium and potassium nitrate and arginine sources. According to the cultural characteristics in the ten media which differed in nitrogen sources, it was observed that the fungal growth was very poor looking alike the growth happened on the plane agar in both glycine and serine containing media. On arginine containing medium the growth was strong producing yellowish pigment several cms. far from the main inoculum, it was clear that the growth in arginine medium was more vigorous than that grown in media containing any of the other amino acids. On the methionine containing medium, the nature of growth was greatly changed. It was very poor; bright; viscus and had an irrigular margin. On treptophan containing medium, the growth was aerial relatively vigours; greyish in colour and had regular margins. Potassium; sodium and ammonium nitrate containing media, distinguished with very strong growth; very yellowish in colour; had regular margins and contained relatively great number of pycnidia. On ammonium tartarate containing medium, the growth was limited; flattened; completely connected with the surface medium; had an irrigular margin and was free from pycnidia or conidiospores as well as the colour of growth was whitish grey. The fungal growth on urea containing medium was completely abnormal.

c- C/N ratios :

Data recorded in Table (18) and illustrated in Fig. (13) indicate the effect of different carbon/nitrogen ratios (C/N ratios) on the linear growth; pycnidia formation; pycnidio and conidio-spores production of the most virulent isolate *Pyrenochaeta terrestris* (No. 3) after 8 days from incubation at 27°C. on Cźapek's medium. It is very clear that the C/N ratios (50/3) and (40/3) were the best for giving the greatest linear growth (7.54 and 7.13 cm., respectively); producing the largest number of pycnidia (33.2 and 29.8 pycnidia per microscopic field respectively); pycnidiospores (0.175 and 0.125 million/ 1 cc, respectively) and conidiospores (0.263 and 0.188 million/1 cc, respectively). Also the C/N ratio (30/10) gave non-significantly good results except for in the case of pycnidiospore production. However, the C/N ratios (30/5) and (30/15) were good for the fungal growth, but were greatly differed according to the spore production as the first C/N ratio was more suitable for conidiospores production and the second was for pycnidia formation. Also it is very clear that the growth was very hyaline in the C/N ratios (0/3) and (30/0) with a complete disappearance of pycnidia; pycnidio and conidio-spores.

C- Effect of different temperature degrees and pH values:

a- Temperature degrees :

Data shown in Table (19) and illustrated in Fig. (14) indicate the effect of different temperature degrees in °C. on the linear growth; pycnidia formation; pycnidio and conidio-spores

Table (18): Effect of different Carbon/Nitrogen Ratios on the linear growth, number of pycnidia; pycnidio and condio-spores of the most virulent isolate of *Pyrenochaeta terrestris* after 8 days from incubation at 27°C, on Czapek's medium.

C/N ratios	Linear growth (in cm.)	Average number of :		
		Pycnidia per microscopic field	Pycnidiospores (in million)/ 1 cc.	Conidiospores (in million)/ 1 cc.
50/3	7.54	33.20	0.175	0.263
40/3	7.13	29.80	0.125	0.188
30/3*	6.09	25.80	0.100	0.175
20/3	6.54	19.80	0.025	0.188
10/3	5.55	11.20	0.013	0.200
0/3	6.93	0.00	0.000	0.000
30/0	4.50	0.00	0.000	0.000
30/5	6.80	24.20	0.088	0.200
30/10	7.23	26.20	0.050	0.150
30/15	6.90	26.80	0.025	0.125
L.S.D., 0.01	0.89	8.40	0.060	0.133

* Normal ratio in C'zapek's medium.

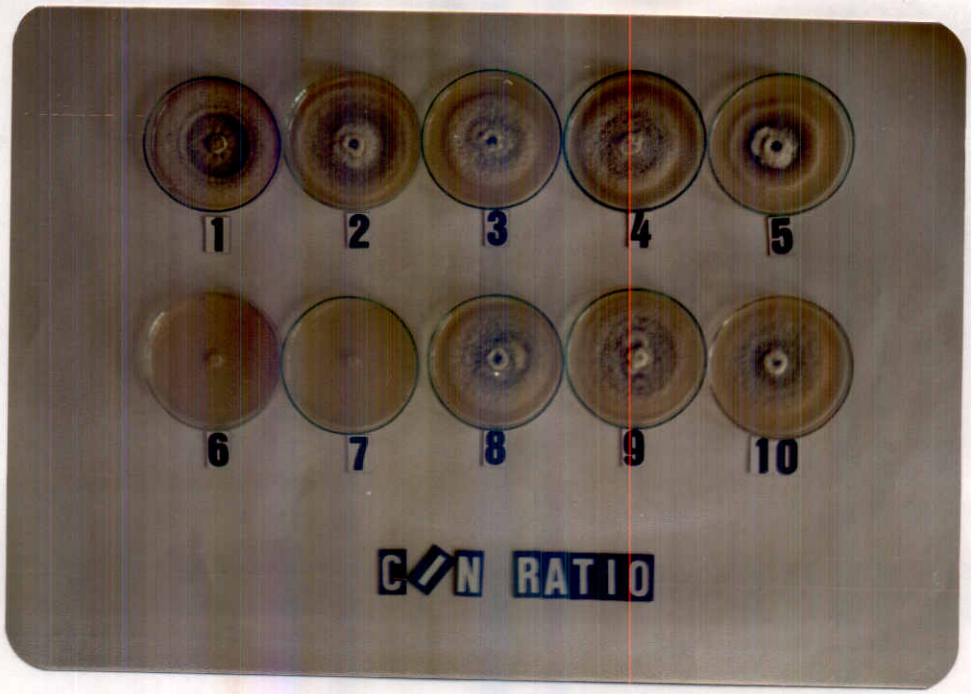

Fig. (13): Effect of different Carbon/Nitrogen ratios on the linear growth and cultural characteristics of the most virulent isolate of *Pyrenochaeta terrestris* after 8 days from growing on Czapek's medium at 27°C.

1. 50/3
2. 40/3
3. 30/3
4. 20/3
5. 10/3
6. 0/3
7. 30/0
8. 30/5
9. 30/10
10. 30/15

Table (19): Effect of different temperature degrees on the linear growth; number of pycnidia; pecnidio and conidio-spores of the most virulent isolate of *Pyrenochaeta terrestris* after 9 days from incubation on PDA medium.

Temperature degrees (C^o)	Linear growth (in cm.)	Average number of :-		
		Pycnidia per microscopic field	Pycniciospores (in million) 1 cc.	Conidiospores (in million) 1 cc.
10	0.00	0.00	0.000	0.000
15	3.46	9.20	0.150	0.125
20	3.79	19.40	0.325	0.350
25	8.08	34.80	0.975	0.500
30	6.58	39.40	0.350	0.325
35	4.15	11.40	0.025	0.025
40	1.31	0.00	0.000	0.000
L.S.D.; 0.01	1.90	7.93	0.066	0.072

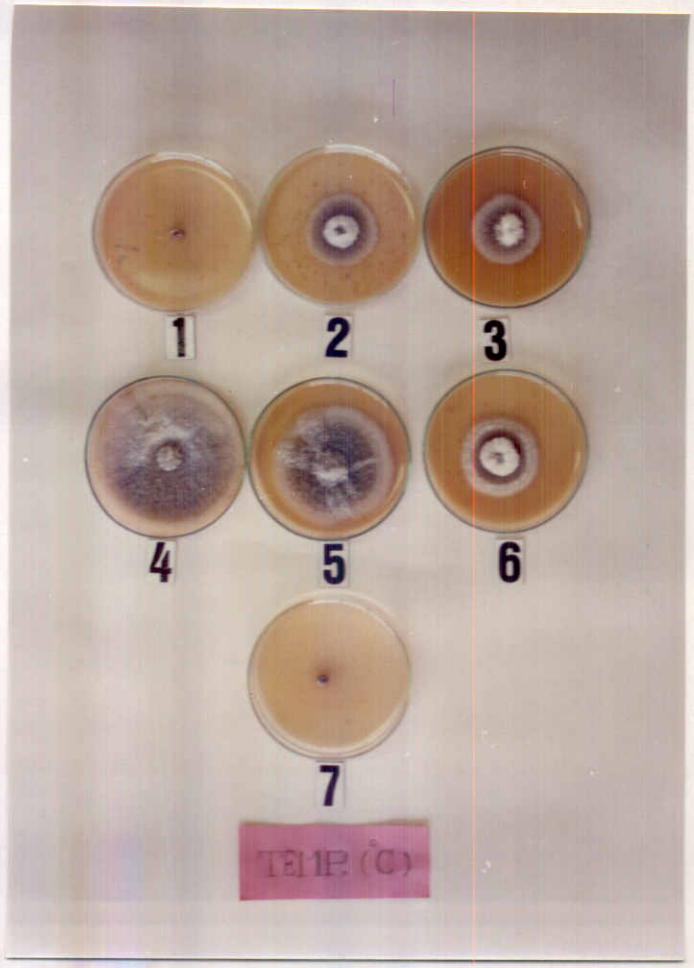

Fig. (14): Effect of different temperature degrees on the linear growth and cultural characteristics of the most virulent isolate of *Pyrenochaeta terrestris* after 9 days from incubation on PDA medium.

1. 10°C.
2. 15°C.
3. 20°C.
4. 25°C.
5. 30°C.
6. 35°C.
7. 40°C.

production of the most virulent isolate of *Pyrenochaeta terrestris* No. 3) after 9 days from incubation on PDA medium. It is very clear that the optimum temperature for obtaining the best growth and the maximum number of pycnidia; pycnidio; and conidio-spores produced by the fungus was 25°C. Also it is very clear that decreasing or increasing the temperature degrees down or above 25°C. resulted a sharp reduction in both the fungal growth and the production of pycnidia; pycnidio and conidio-spores. So it is clear from data that, the more decreasing or increasing in the incubation temperature down or above the optimum temperature (25°C), the more reduction of growth; pycnidia production and sporulation could be produced, however the complete inhibition of both mycelial growth and spore production occurred at 10 and 40°C.

b- pH values :

Data recorded in Table (20) and illustrated in Fig. (15) show the effect of different pH values on the linear growth; pycnidia formation; pycnidio and conidio-spores production of the most virulent isolate (No. 3) of *Pyrenochaeta terrestris* after 9 days from incubation at 27°C. on Richard's medium.

It is very clear that the fungus grew well and produced the maximum number of pycnidia; pycnidio and conidio-spores at pH values ranged from 6.9 to 7.7 particularly at pH 7.3. However, the growth of the fungus did not affected when the pH decreased to very acidic conditions (from pH 5.9 to 3.2), meanwhile the growth was significantly decreased as pH value increased to

Table (20): Effect of different pH values in the linear growth; number of pycnidia; pycnidiospores and conidia of the most virulent isolate of *Pyrenochaeta terrestris* after 9 days from incubation at 27°C. on Richard's medium.

pH values	Linear growth (in cm.)	Average number of :		
		Pycnidia per microscopic field	Pycnidiospores (in million)/ 1 cc.	Conidiospores (in million)/ 1 cc.
3.2	8.10	6.2	0.025	0.013
4.0	8.30	13.4	0.025	0.038
4.5	8.30	17.2	0.050	0.088
5.9	8.30	23.2	0.125	0.175
6.9	8.30	33.2	0.175	0.200
7.3	8.30	32.8	0.188	0.250
7.7	8.06	29.6	0.138	0.225
8.0	7.54	24.8	0.038	0.163
9.5	7.24	11.2	0.000	0.025
10.0	6.23	7.8	0.000	0.013
L.S.D., 0.01	0.35	7.88	0.072	0.092

Fig. (15): Effect of different pH values on the linear growth and cultural characteristics of the most virulent isolate of *Pyrenochaeta terrestris* after 9 days growing on Richard's medium at 27°C.

1. pH 3.2.
2. pH 4.0.
3. pH 4.5.
4. pH 5.9.
5. pH 6.9.
6. pH 7.3.
7. pH 7.7.
8. pH 8.0.
9. pH 9.5.
10. pH 10.0.

very alkaloide conditions (from pH 8.0 to 10.0). It is also clear that the more increasing or decreasing of pH values above or down the optimum rang (6.9-7.7), the more reduction of growth; pycnidia; pycnidio and conidio-spores production could be occurred.

6. ENZYMOLOGY STUDIES :

1. Production of pectinolytic, cellulolytic and oxidative enzymes by *Pyrenochaeta terrestris* isolates in relation to onion pink root rot infection :

A- *In vivo* :

Data summarized in Tables (21 & 22) and illustrated in Figs. (16 & 17), indicate the activities of pectinolytic; cellulolytic and oxidative enzymes of seven different isolates of *Pyrenochaeta terrestris*, which determined in both the root and the bulb tissues of diseased and healthy transplants of Giza-20 onion cultivar after 70 days from transplantation. Concerning pectinolytic enzymes, Poly-Galacturonase (PG) and Pectin-Methyl-Estrase (PME), it is enough clear that the most pathogenic isolate No. 3- collected from Ismailia - infected more than 61 % of the tested plants and caused the most severe infection after 70 days, showed the highest activity either in the root or in the bulb tissues of infected transplants if compared with the activities of such enzymes in healthy ones. The moderate pathogenic isolates (No. 1; 4 and 6) produced moderate quantities of the mentioned enzymes and so moderate activities of both enzymes were assayed. Also, it is clear that the least pathogenic isolates (No. 2;5 and 7), induced the least quantities of

Table (21): Pectinolytic and cellulolytic enzyme activities in healthy and diseased onion Giza-20 cultivar with seven isolates of *Pyrenochaeta terrestris* after 70 days from transplantation under greenhouse conditions at 27°C. ± 2.

Isolates		Hydrolytic enzyme activities						Average percent of :		
		PME 0.1% NaOH cc.		% Reduction in viscosity:				Infection	Severity according to :	
				PG		CX			Infected plants	Tested plants
		Bulbs	Roots	Bulbs	Roots	Bulbs	Roots			
1. El-Badary	(Assiut)	6.5	9.2	16.67	33.33	19.04	94.48	40.71%	27.97%	8.97%
2. Ibshwai	(Fayoum)	4.8	7.5	23.08	28.57	17.58	95.25	28.40	23.07	3.76
3. Abo-Sour	(Ismailia)	10.2	23.5	23.53	60.71	42.13	94.29	61.94	41.91	33.79
4. Koum-Oshem	(Fayoum)	7.8	8.8	16.67	50.00	27.21	98.18	42.51	30.26	11.61
5. Fayed	(Ismailia)	5.2	6.8	17.39	23.08	17.76	97.05	26.40	19.10	3.49
6. Qouta	(Fayoum)	7.4	10.2	28.57	38.46	23.76	97.35	44.17	29.34	11.93
7. Sarabiom	(Ismailia)	5.8	8.0	16.67	30.77	18.75	94.69	33.80	26.70	6.34
8. Control		3.6	5.2	9.09	6.67	7.14	20.00	0	0	0
L.S.D.; 0.05								9.92	4.73	5.79

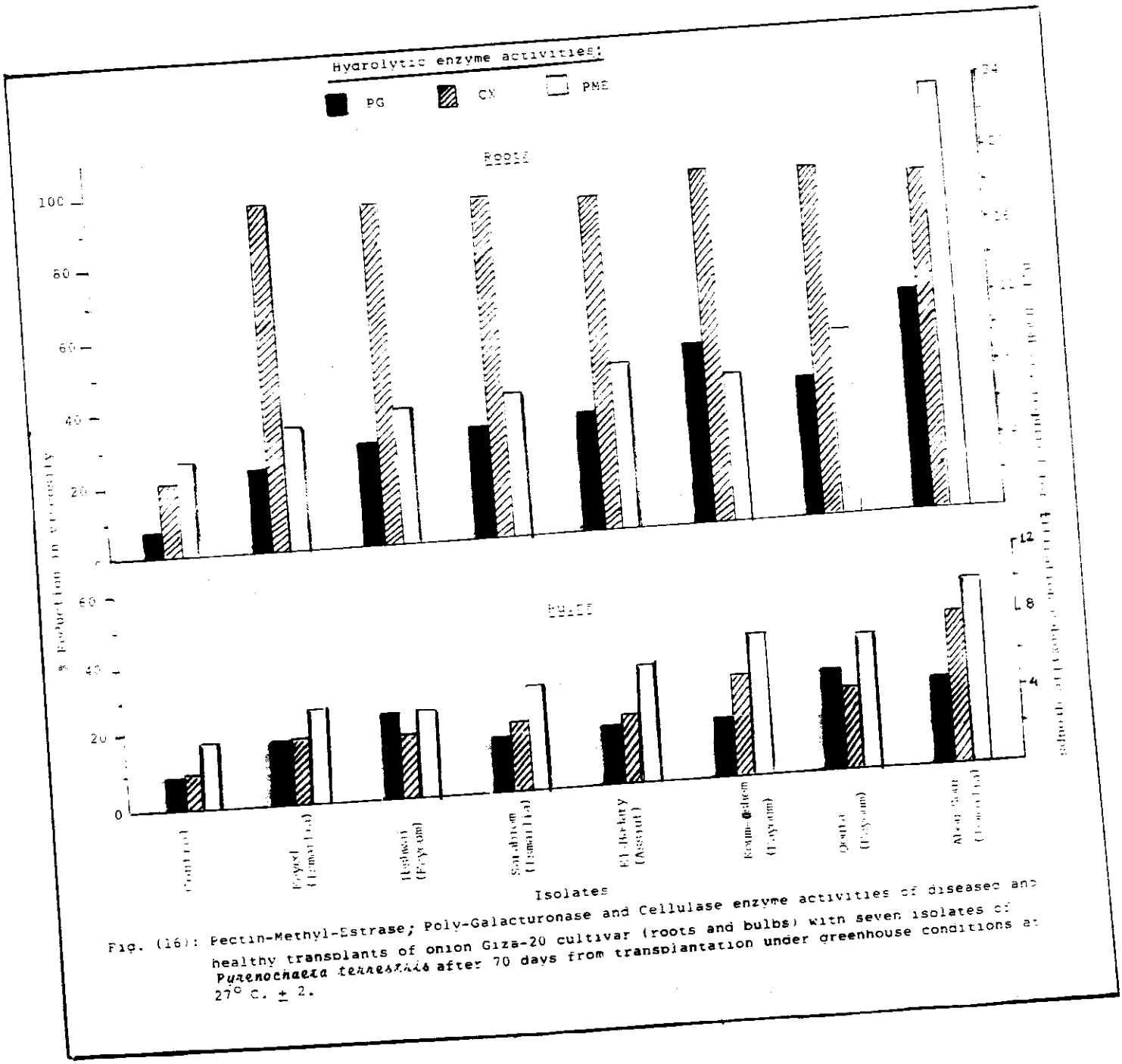

Fig. (16): Pectin-Methyl-Estrase; Poly-Galacturonase and Cellulase enzyme activities of diseased and healthy transplants of onion Giza-20 cultivar (roots and bulbs) with seven isolates of *Pyrenochaeta terrestris* after 70 days from transplantation under greenhouse conditions at 27° C. ± 2.

Table (22): Oxidative enzyme activities in healthy and diseased onion Giza-20 cultivar with seven isolates of *Pyrenochaeta terrestris* after 70 days from transplantation under greenhouse conditions at 27°C. ± 2.

Isolates	Oxidative enzyme activities as optical density												Average percent of:		
	Peroxidase						Polyphenoloxidase						Infection	Severity according to:	
	Bulbs			Roots			Bulbs			Roots				Infected plants	Tested plants
	0.5*	2.5**	5.0***	0.5*	2.5**	5.0***	0.5*	2.5**	5.0***	0.5*	2.5**	5.0***			
1. El-Badary (Assiut)	0.191	0.886	1.201	0.255	1.285	1.735	0.534	0.541	0.546	0.252	0.297	0.318	40.71%	27.97%	8.97%
2. Ibshwai (Fayoum)	0.075	0.380	0.610	0.269	1.177	1.260	0.437	0.440	0.446	0.225	0.263	0.283	28.40	23.07	3.76
3. Abo--Sour(Ismailia)	0.203	0.565	0.887	0.241	0.580	0.661	0.621	0.634	0.637	0.369	0.463	0.514	61.94	41.91	33.79
4. Koum-Oshem (Fayoum)	0.291	1.120	1.462	0.343	1.142	1.328	0.582	0.592	0.594	0.308	0.369	0.413	42.51	30.26	11.61
5. Fayed (Ismailia)	0.181	0.790	1.223	0.171	0.850	0.970	0.465	0.468	0.473	0.232	0.265	0.290	26.40	19.10	3.49
6. Qouta (Fayoum)	0.136	0.738	1.170	0.295	1.295	1.378	0.566	0.572	0.576	0.278	0.328	0.396	44.17	29.34	11.93
7. Sarabiom (Ismailia)	0.158	0.710	1.105	1.529	1.640	1.681	0.541	0.544	0.549	0.264	0.310	0.340	33.80	26.70	6.34
8. Control	0.107	0.283	0.345	0.051	0.460	0.712	0.201	0.204	0.206	0.146	0.168	0.186	0	0	0
L.S.D.; 0.05													9.92	4.73	5.79

* After 0.5 minute from the activity initiation.
** After 2.5 minute from the activity initiation.
*** After 5.0 minutes from the activity initiation.

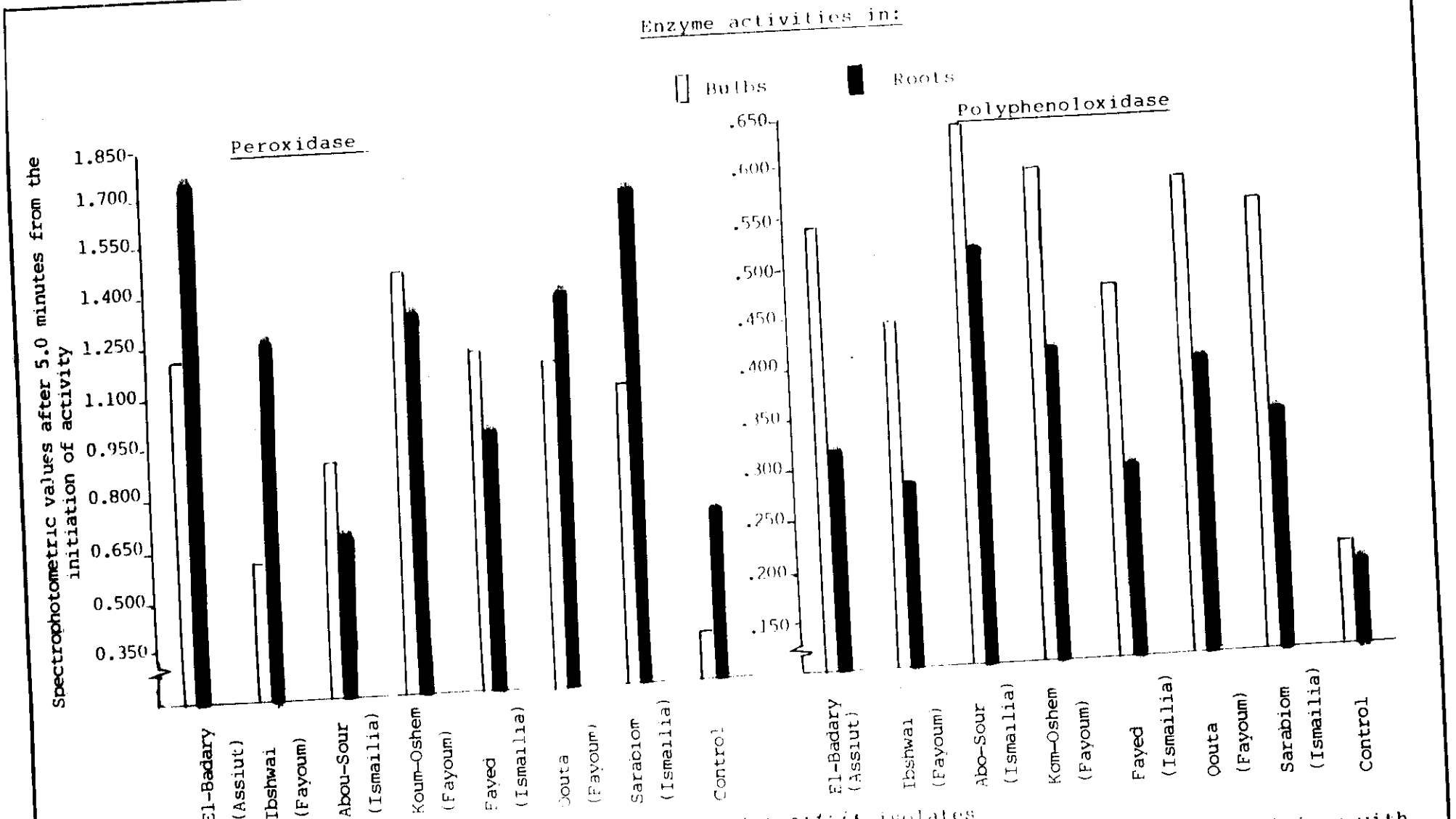

Fig. (17): Oxidative enzyme activities of healthy and diseased transplants of Giza-20 cultivar with seven isolates of *Pyrenochaeta terrestris* after 70 days from transplantation under green-house conditions at 27°C. ± 2.

pectinolytic enzymes and so the least activities of such enzymes could be determined. So, data revealed an evidence that the more virulence of the isolate, the higher pectinolytic enzyme activities could be obtained.

Also, one of the most noticeable observation that the enzyme activities of pectinolytic enzymes were rather high in the roots of infected transplants than those determined in the bulb tissues. This finding was noticed all over the isolates used. According to the Cellulase activity, it was observed that all isolates approximately produced this enzyme at equal level, as the reduction in viscosity in CMC medium ranged from 94.29 to 97.35% in the root tissues of the diseased transplants. Meanwhile the activity of this enzyme was relatively low in the bulbs of the diseased transplants and ranged from 17.58 to 27.21% however, isolate No. 3 (the most virulent isolate) caused higher Cellulase activity which reached 42.13% and 94.29% in bulb and root tissues respectively. According to oxidative enzymes, data in Table (22) and illustrated in Fig. (17) indicate that pink root rot infection, obviously increased both Peroxidase and Polyphenoloxidase activities than those of healthy transplants. The increase in activities of both enzymes were observed in both root and bulb tissues of diseased transplants. Also, data indicate clearly that the activity of Peroxidase was generally higher in the root tissues of diseased transplants rather than those of its bulbs, as the activity of this enzyme was higher in the diseased roots infected with isolates No. 1;2;6 and 7 rather than its bulb tissues, while the reverse was true

in the case of isolates No. 3; 4 and 5. On the other hand, Polyphenoloxidase activity was higher in the bulb tissues of the transplants infected with any of the tested isolates than the activity determined in the infected root tissues. Also, one of the most noticeable observation that there was a direct correlation between the virulence of the causal organism isolates and the increasing in Polyphenoloxidase activity after infection. It is very clear that, the greater in the isolate agressiveness, the more activity of Polyphenoloxidase could be incident. On the other hand, no correlation could be observed between the virulence of *Pyrenochaeta terrestris* isolates and the increasing in Peroxidase activity which followed the disease infection.

B- *In vitro* :

It is very clear from data written in Table (23) and illustrated in Figs. (18 and 19) that all isolates of *Pyrenochaeta terrestris* , which grown on modified Czapek's medium for 15 days, greatly differed in producing Poly-Galacturonase (PG). Isolate No. 3- isolated from Ismailia governorate Abo Sour locality- produced the largest quantity of this enzyme, reducting 71.43% of the medial viscosity. Isolates No. 1; 2; 4; 6 and 7 were approximately the same in secreting moderate quantities of this enzyme and the reduction of the medial viscosity caused by these isolates ranged from 21.43 to 33.33%, however Isolate No. 5 secreted the least PG quantity and the reduction of the medial viscosity was not more than 8.33%. Concerning Pectin-Methyl-Estrase (PME) activity, it was noticed that

Table (23): Pectinolytic; cellulolytic and oxidative enzyme activities produced by different isolates of *Pyrenochaeta terrestris* after 15 days incubation in a modified Czapek's medium at 27°C.

Isolates :	Hydrolytic and oxidative enzyme activities								
	PME	PG	CX	Peroxidase as optical density			Polyphenol oxidase as optical density		
	0.1 % NaOH cc	% Reduction in viscosity of 1% pectin	% Reduction in viscosity of 1% C.M.C.	0.5*	2.5**	5.0***	0.5*	2.5**	5.0***
1. El-Badary (Assiut)	6.3	25.00%	78.41%	0.353	0.361	0.367	0.649	0.652	0.657
2. Ibshwai (Fayoum)	5.0	21.43	82.51	0.393	0.400	0.409	0.618	0.623	0.628
3. Abo-Sour (Ismailia)	4.7	71.43	81.88	0.288	0.297	0.304	0.546	0.551	0.555
4. Koum-Oshem (Fayoum)	3.1	33.33	79.41	0.338	0.342	0.347	0.622	0.626	0.631
5. Fayed (Ismailia)	4.6	8.33	78.82	0.375	0.381	0.389	0.539	0.542	0.549
6. Qouta (Fayoum)	4.5	27.27	78.82	0.345	0.352	0.357	0.621	0.625	0.629
7. Sarabioum (Ismailia)	4.0	25.00	80.56	0.363	0.369	0.378	0.523	0.528	0.532

* After 0.5 minute from the activity initiation.

** After 2.5 minute from the activity initiation.

*** After 5.0 minutes from the activity initiation

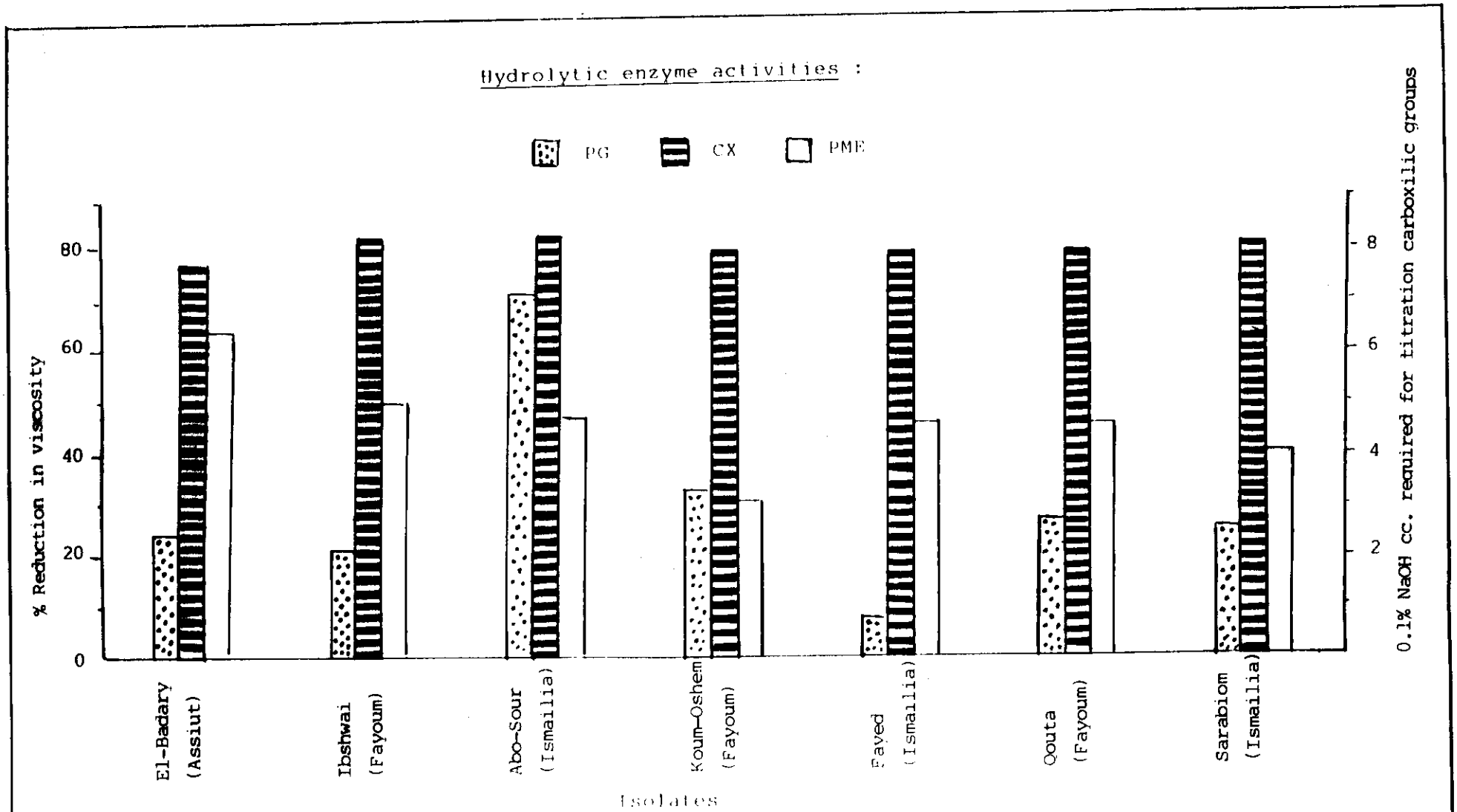

Fig. (18): Pectin-Methyl-Estrase; Poly-Galacturonase and Cellulose activities produced by different isolates of *Pyrenochaeta terrestris* after 15 days incubation in modified Czapek's medium at 27°C.

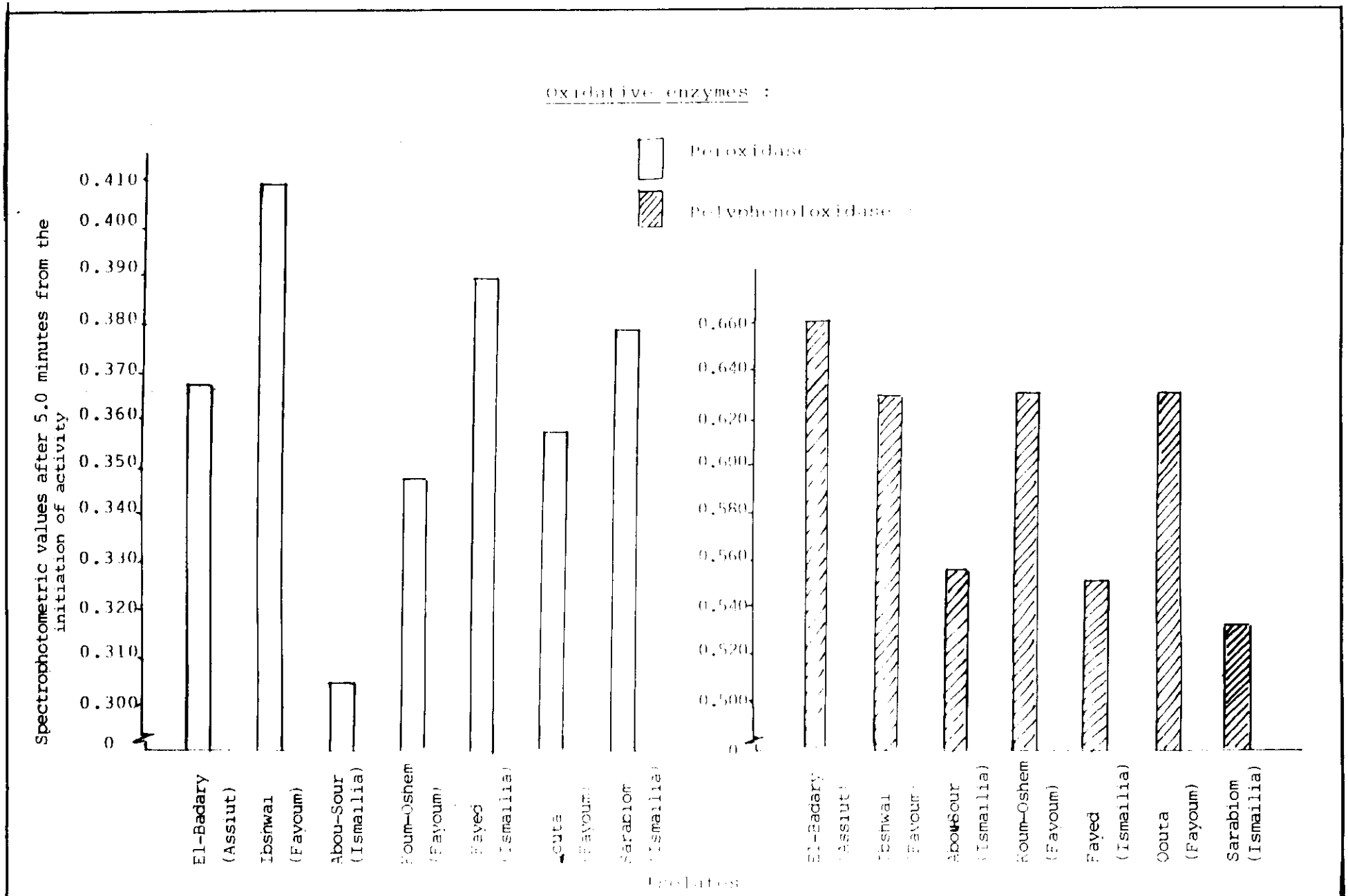

Fig. (19): Oxidative enzyme activities, produced by different seven isolates of *Pyrenochaeta terrestris in vitro*.

isolates No. 3; 4; 5 ; 6 and 7 relatively produced the same quantities of such enzyme, as the 0.1% NaOH solution required for titration ranged from 3.1 to 4.7 cc. Isolates No. 1 and 2 showed higher PME activity, as the 0.1% NaOH solution was 6.3 and 5.0 cc., respectively.

Also, it is very clear that all isolates secreted Cellulase enzyme (CX) in the experimental modified medium. Quantities of this enzyme approximately showed the same level as the reduction in viscosity of the 1% CMC added to the medium, ranged from 78.41 to 82.51%.

According to oxidative enzymes, it is very clear that the activities of Peroxidase and Polyphenoloxidase were very limited. The increasing of Peroxidase activity ranged from 0.009 to 0.016 during the experimental period of activity (0.5 to 5.0 minutes), whereas the increasing of Polyphenoloxidase activity ranged from 0.008 to 0.010 during the same experimental period.

2. Enzyme activities in relation to resistance and/or susceptibility of some onion cultivars to pink root rot disease :

The relation between hydrolytic (pectinolytic; cellulolytic enzymes) and oxidative enzymes and the resistance and/or susceptibility of three onion cultivars namely Giza-20 (susceptible); Giza-6 (moderately resistant) and Shandaweel-1 (resistant) to onion pink root rot disease was carefully investigated under artificial inoculation with the most pathogenic isolate

(Isolate No. 3). Data were obtained after 30; 60 and 90 days from transplantation in both bulb and root tissues of diseased and healthy transplants. In the same time the average percentage of infection and the average percentage of disease severity according to both infected and tested plants were also recorded.

Concerning pectinolytic and cellulolytic enzymes, it is very clear as shown in Table (24) and illustrated in Figs. (20; 21 and 22) that the activities of Poly-Galacturonase (PG); Pectin-Methyl-Estrase (PME) and Cellulase (CX) were obviously increased in both the root and the bulb tissues of the diseased onion transplants than those of healthy ones. Also, it is worthy to mention that the activities of such enzymes, showed gradual increasing in both root and bulb tissues of diseased transplants during the development of the disease, and reached the maximum after 90 days from transplantation in the infested soil. On the other hand, data indicate that the rate of increasing of such hydrolytic enzymes in the healthy transplants was limited either in the root or in the bulb tissues. Also, it is very clear that Pectin-Methyl-Estrase (PME) activity was rather high in the root than in the bulb tissues of diseased and healthy transplants of the three tested Cvs. Different reactions were observed according to Poly-Galacturonase (PG) activity, The activity of this enzyme was rather high in the root tissues than that recorded in bulbs of infected transplants in both susceptible Giza-20 and moderately resistant Giza-6 Cvs., whilest the reverse was true in the inoculated resistant Shandaweel-1 Cv.

Table (24): Pectinolytic and cellulolytic enzymes activities of diseased and healthy transplants of some onion cultivars after 30, 60 and 90 days from transplantation in infested soil with the most virulent isolate of *Pyrenochaeta terrestris* under greenhouse conditions at 27°C. ± 2.

Onion cultivars	Enzyme activities																Average % of :								
	PME activity (0.1% NaOH cc) after : -						% Reduction in viscosity of :										Infection after :			Severity according to :					
							1% pectin (PG) after:						1% CMC (CX) after:							Infected plants			Tested plants		
	30 days		60 days		90 days		30 days		60 days		90 days		30 days		60 days		90 days									
	B	R	B	R	B	R	B	R	B	R	B	R	B	R	B	R	B	R	30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
Shandaweel-1(D)	6.50	9.80	5.20	13.30	7.80	15.10	22.86	10.00	22.50	23.81	28.26	25.71	18.73	73.93	25.08	67.71	23.88	80.29	15.96	23.12	36.68	6.70	19.80	22.60	1.30	4.80	8.00
Shandaweel-1(H)	5.45	8.30	4.40	7.90	6.40	9.90	2.27	2.17	3.77	2.38	3.85	4.53	9.77	7.72	13.62	8.51	16.00	11.92									
Giza - 6 (D)	5.90	15.20	6.50	19.50	8.50	26.00	15.29	12.94	17.78	31.25	25.00	42.50	23.71	93.94	25.26	94.10	27.05	93.97	26.32	36.48	53.84	18.30	25.70	20.60	5.80	10.00	14.50
Giza - 6 (H)	4.45	7.10	4.00	7.50	5.90	8.60	3.16	2.78	3.85	3.06	4.41	3.92	11.08	7.91	12.96	9.18	14.20	10.55									
Giza - 20 (D)	8.00	18.00	10.00	25.00	14.00	29.00	20.00	36.11	26.19	42.50	27.78	57.78	25.99	93.36	29.51	94.72	40.26	97.55	34.16	46.16	79.36	31.20	40.00	56.80	11.30	24.00	50.50
Giza - 20 (H)	6.00	7.90	4.70	8.15	7.20	10.30	3.41	3.85	4.55	4.08	5.26	4.88	11.72	9.31	13.03	10.88	14.79	13.52									
L.S.D.; 0.05 :-							for Cultivars (C.) or Periods (P.)												15.82			11.12			15.50		
							for Interaction (C.) X (P.)												0.3			19.25			26.90		

(D): Diseased transplants. (H): Healthy transplants (B): Bulbs (R): Roots

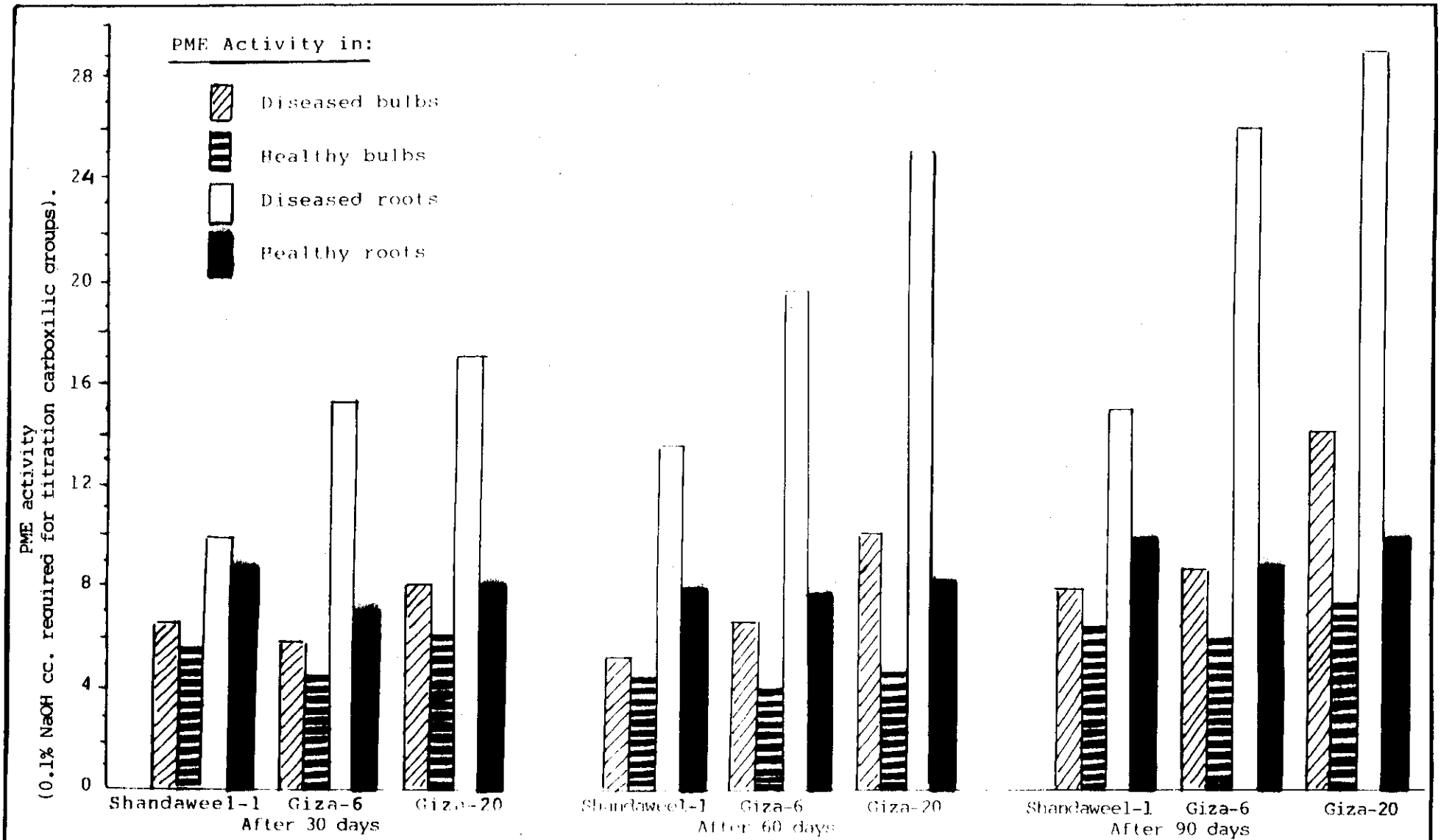

Fig. (20): Pectin-Methyl-Estrase activity of diseased and healthy three onion cultivars after 30;60 and 90 days from transplantation in infested soil with the most virulent isolate of *Pyrenochaeta terrestris* under greenhouse conditions at 27°C. ± 2.

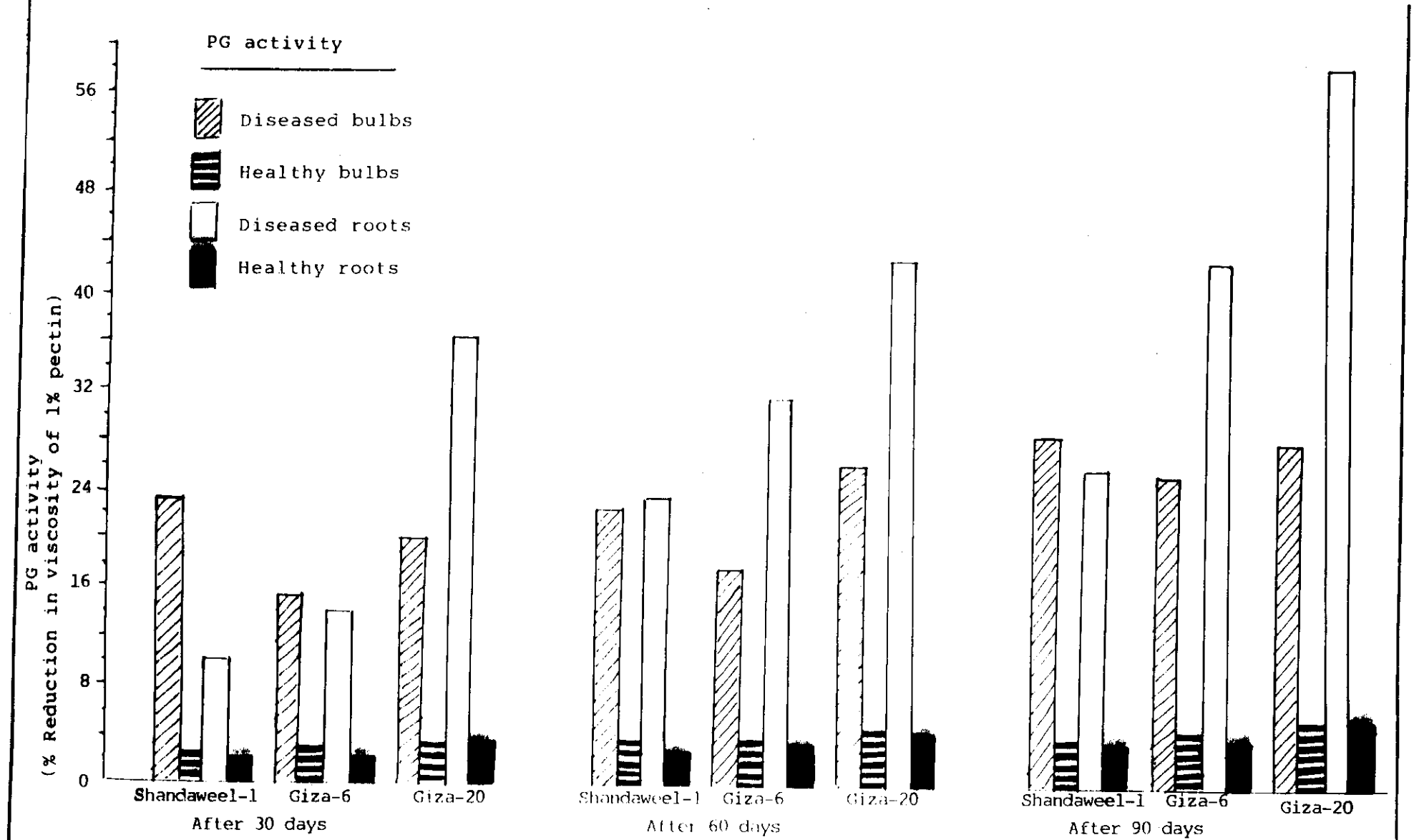

Fig. (21): Poly-Galacturonase activity of diseased and healthy three onion cultivars after 30, 60 and 90 days from transplantation in infested soil with the most virulent isolate of *Pyrenochaeta terrestris* under greenhouse conditions at 27°C ±2.

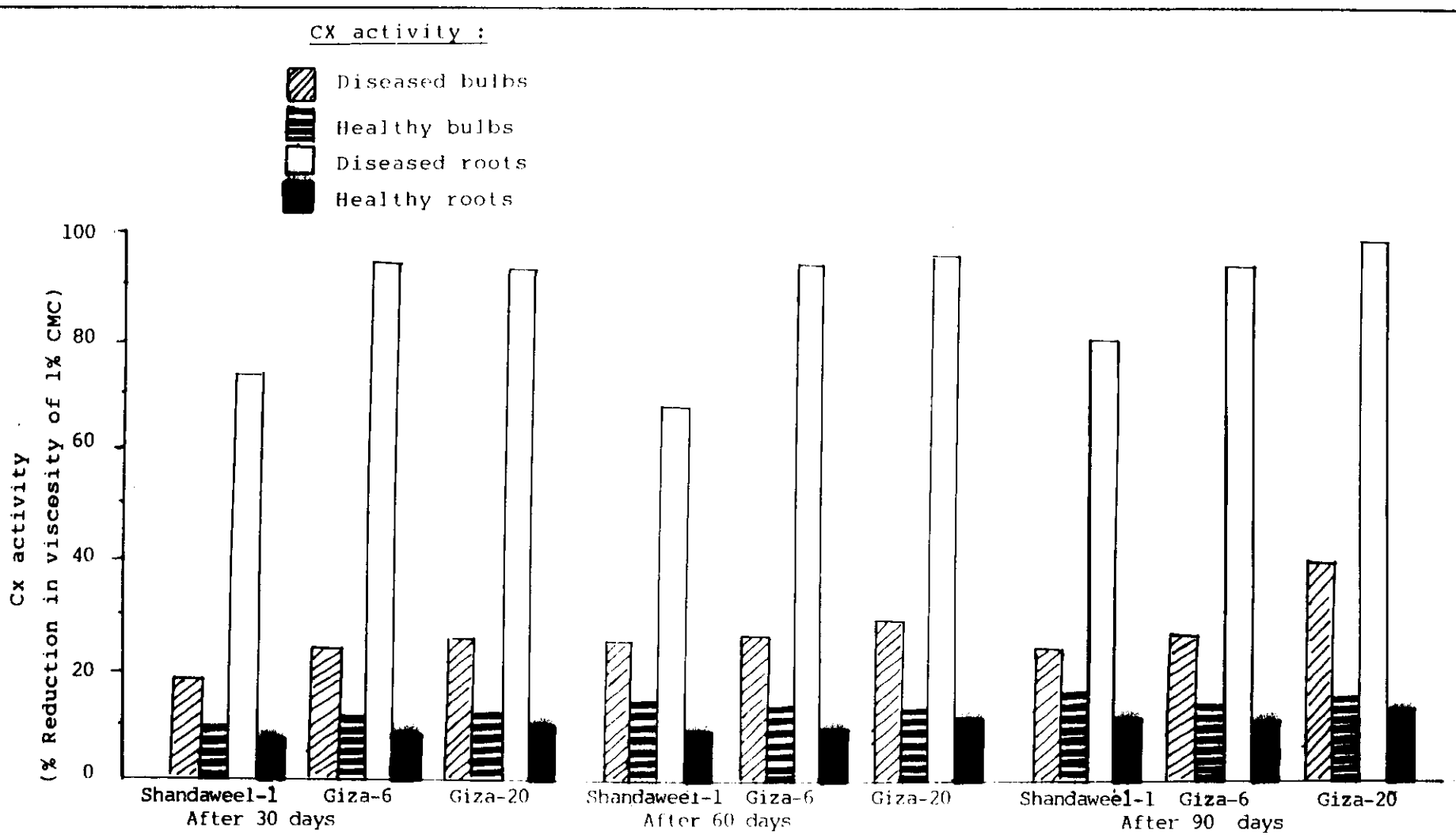

Fig. (22): Cellulase activity of diseased and healthy three onion cultivars after 30;60 and 90 days from transplantation in infested soil with the most virulent isolate of *Pyrenochaeta terrestris* under greenhouse conditions at $27^{o}C. \pm 2$.

However PG activity in the bulb tissues of healthy transplants was greater than those of its root tissues. Cellulase activity (CX) was higher in the root tissues than those occurred in the bulb tissues of diseased transplants of the three tested Cvs., whereas the reverse was enough true according to the healthy transplants of all tested Cvs. According to Polyphenoloxidase activity, data written in Table (25) and illustrated in Fig. (23) show that infection of onion pink root rot disease caused relatively increase in Polyphenoloxidase, particularly in the root tissues of diseased transplants of the resistant Cv. Shandaweel-1 after 90 days from transplantation. The bulb tissues of either diseased or healthy transplants of the three Cvs. exhibited - in general - higher activity of Polyphenol-oxidase, rather than those determined in its roots. This result was observed after 30; 60 and 90 days from transplantation in the infested soil. Data also indicate a valuable observation that Polyphenoloxidase activity was decreased after 60 days then re-increased after 90 days in the root tissues of both diseased and healthy transplants, meanwhile the activity of such enzyme showed continual increase in the bulb tissues of diseased and healthy transplants, except for the healthy bulbs of the resis-tant and susceptible Cvs. as the activity of this enzyme inc-reased clearly after 60 days then showed a slight decrease after 90 days from transplantation. According to Peroxidase enzyme, Table (26) and illustrated in Fig. (24) indicates clearly that pink root rot infection increased the content of such enzyme in both root and bulb tissues than those of healthy transplants.

Table (25): Polyphenoloxidase activity of diseased and healthy transplants of some onion cultivars after 30, 60 and 90 days from transplantation in infested soil with the most virulent isolate of *Pyrenochaeta terrestris* under greenhouse conditions at 27°C. ± 2 (Enzyme activity was expressed as spectrophotometric values after 0.5; 2.5 and 5 minutes from activity initiation).

Onion cultivars	Polyphenoloxidase activities after : -																Average percentage of :-								
	30 days						60 days						90 days				Infection after:			Severity according to infected plants after:			Severity according to infected plants after:		
	Bulbs			Roots			Bulbs			Roots			Bulbs			Roots									
	0.5	2.5	5.0	0.5	2.5	5.0	0.5	2.5	0.5	0.5	2.5	5.0	0.5	2.5	5.0	0.5	2.5	5.0	30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
Shandaweel-1 (D)	.582	.610	.616	.631	.680	.725	.697	.716	.737	.519	.540	.558	.710	.802	.852	.602	.668	.730	15.96	29.12	36.68	6.70	19.80	22.60	1.30	4.80	8.00
Shandaweel-1 (H)	.485	.535	.585	.455	.543	.613	.667	.676	.681	.431	.457	.473	.621	.643	.662	.546	.601	.645									
Giza - 6 (D)	.401	.413	.417	.403	.503	.613	.429	.454	.469	.302	.350	.385	.538	.623	.683	.436	.525	.576	26.32	36.48	53.84	18.30	25.70	20.60	5.80	10.00	14.50
Giza - 6 (H)	.425	.442	.461	.398	.484	.517	.464	.485	.506	.367	.384	.397	.584	.648	.712	.429	.475	.503									
Giza - 20 (D)	.524	.551	.563	440	.486	.495	.591	.604	.612	.345	.434	.495	.582	.606	.613	.549	2634	.673	34.16	46.16	79.36	31.20	40.00	56.80	11.30	24.00	50.50
Giza - 20 (H)	.391	.406	.413	.330	.387	.419	.532	.550	.567	.304	.323	.334	.494	.510	.518	.506	.553	.597									
L.S.D.; 0.05 :-													for Cultivar (C.) or Periods (P.)						15.82			11.12			15.50		
													for Interaction (C.) X (P.)						N.S.			19.25			26.90		

(D) Diseased transplants (H) Healthy transplants

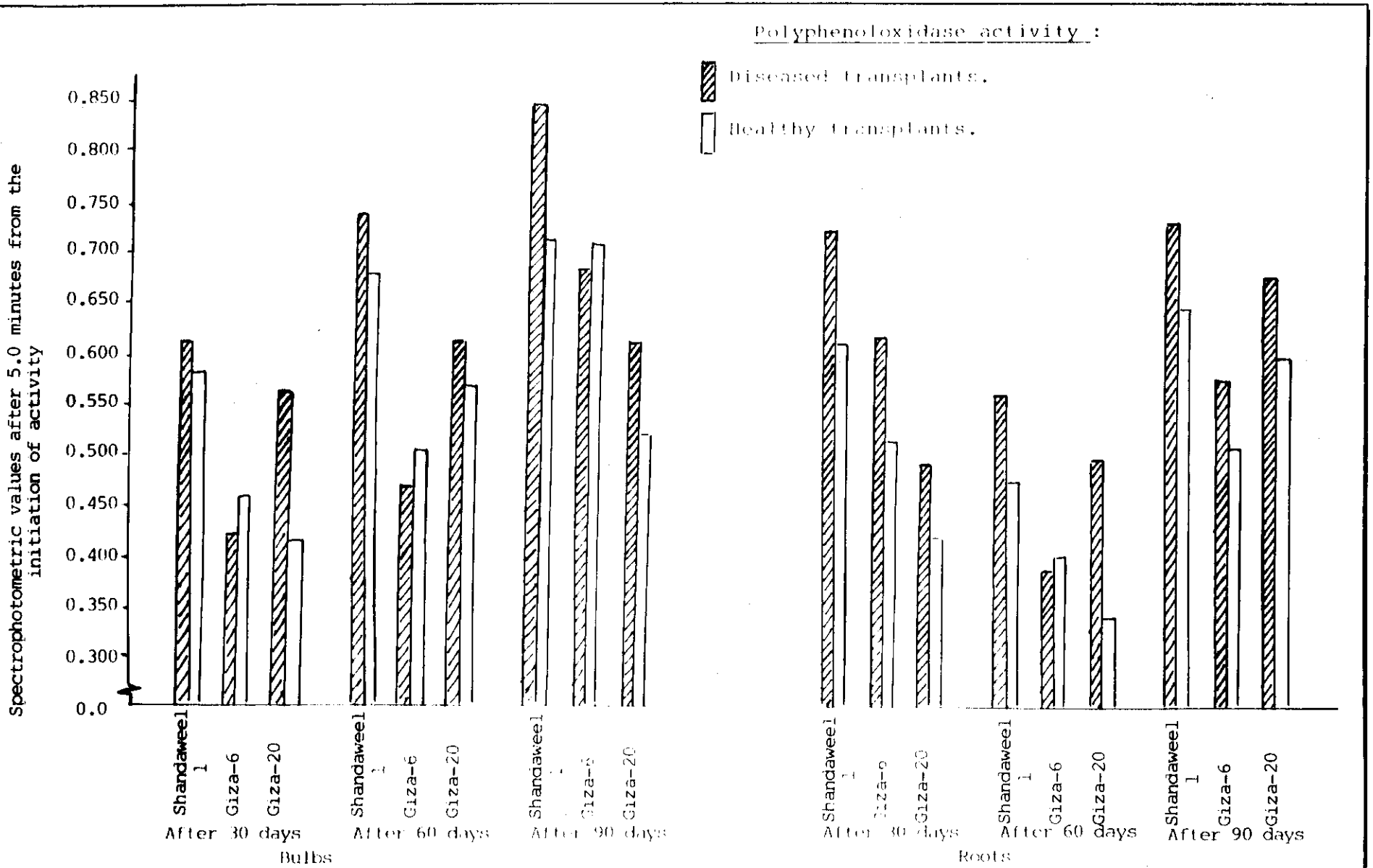

Fig. (23): Polyphenoloxidase activity of disease and healthy three onion cultivars after 30, 60 and 90 days from transplantation in infested soil with the most virulent isolate of *Pyrenochaeta terrestris* under greenhouse conditions at 27° C. ± 2.

Table (26): Peroxidase activity of healthy and diseased transplants of some onion cultivars after 30, 60 and 90 days from transplantation in infested soil with the most virulent isolate of *Pyrenochaeta terrestris* under greenhouse conditions at 27°C ± 2. (Enzyme activity was expressed as spectrophotometric values after 0.5; 2.5 and 5.0 minutes from activity initiation).

Onion cultivars	Peroxidase activities after : -																Average percent of :-								
	30 days						60 days						90 days				Infection after:			Severity according to infected plants			Severity according to tested plants		
	Bulbs			Roots			Bulbs			Roots			Bulbs			Roots									
	0.5	2.5	5.0	0.5	2.5	5.0	0.5	2.5	5.0	0.5	2.5	5.0	0.5	2.5	5.0	0.5	2.5	5.0	30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
Shandaweel-1 (D)	.345	.565	.747	1.028	1.440	1.547	.184	.401	.559	1.120	1.843	1.873	.292	.590	.915	.537	1.028	1.630	15.96	29.12	36.68	6.70	19.80	22.60	1.30	4.80	8.00
Shandaweel-1 (H)	.300	.486	.707	.855	1.200	1.300	.202	.326	.464	.833	1.473	1.630	.250	.549	.784	.351	.977	1.206									
Giza - 6 (D)	.218	.510	.734	.979	1.698	1.852	.170	.370	.560	1.337	1.685	1.899	.176	.374	.556	.619	1.091	1.726	26.32	36.48	53.84	18.30	25.70	20.60	5.80	10.00	14.50
Giza - 6 (H)	.122	.247	.430	.598	.913	1.231	.160	.337	.425	.684	1.358	1.569	.154	.337	.454	.340	.737	1.217									
Giza - 20 (D)	.411	.720	1.193	1.165	1.592	1.654	.275	.432	.685	1.244	1.807	1.890	.347	.593	.783	.613	1.209	1.758	34.16	46.16	79.36	31.20	40.00	56.80	11.30	24.00	50.50
Giza - 20 (H)	.255	.525	.785	.841	1.206	1.330	.087	.230	.360	1.137	1.435	1.670	.113	.240	.306	.427	.814	1.050									
L.S.D.; 0.05													for cultivars (C.) or Periods (P.)							15.82			11.12			15.50	
													for Interaction (C.) X (P.)							N.S.			19.25			26.90	

(D) Diseased transplants (H) Healthy transplants

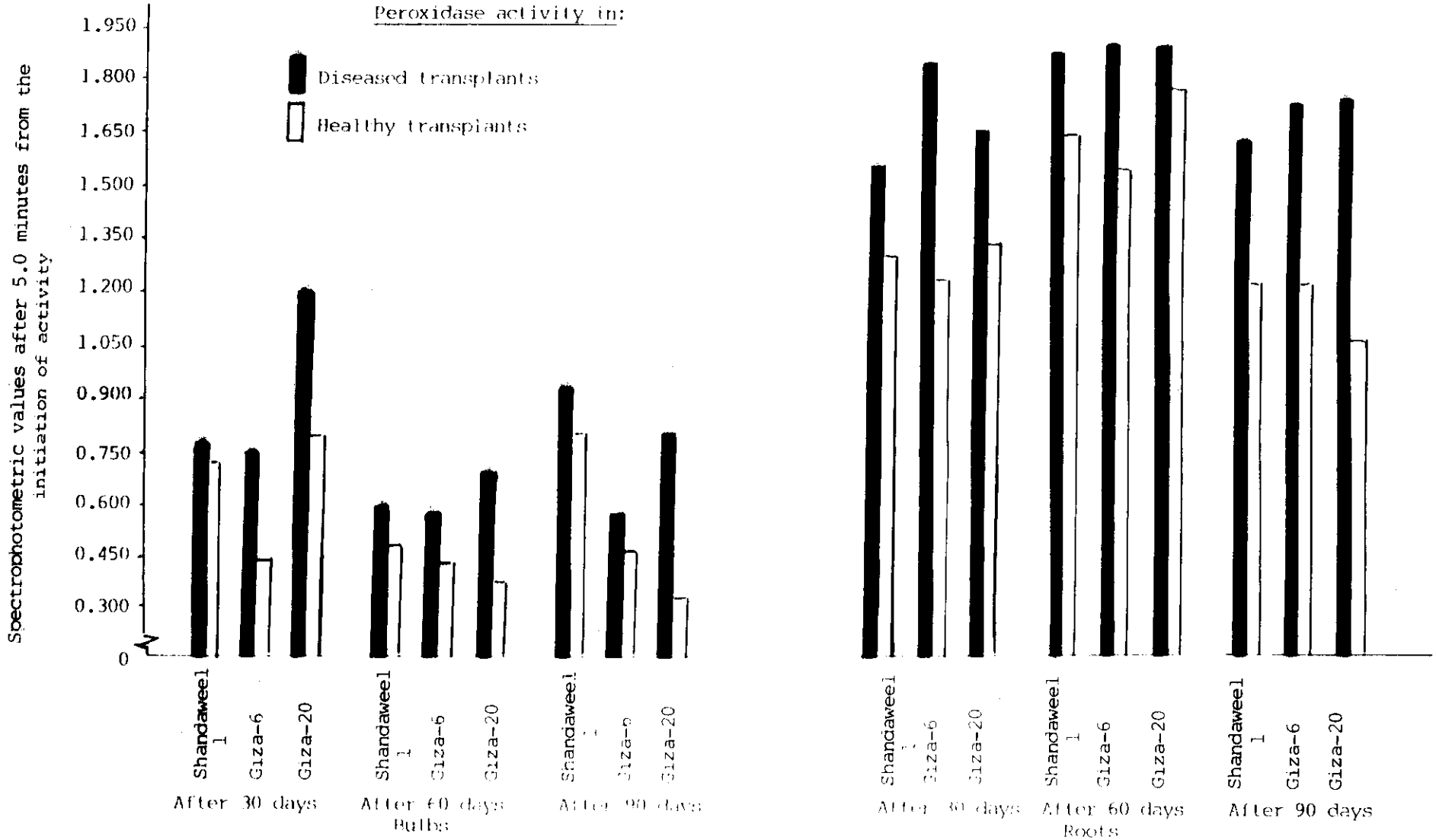

Fig. (24): Peroxidase activity of diseased and healthy three onion cultivars after 30;60 and 90 days from transplantation in infested soil with the most virulent isolate of *Pyrenochaeta terrestris* under greenhouse conditions at 27°C. ± 2.

The rate of Peroxidase activity increase was higher in the root tissues than those occurred in bulb tissues, either after 30; 60 or 90 days from transplantation. This reaction was observed in the three tested Cvs. The activity of Peroxidase was generally increased in both root and bulb tissues of diseased and healthy transplants after 60 days rather than the activities assayed after 30 days, then such activities decreased after 90 days from transplantation. Reverse reaction was observed in the bulbs of both diseased and healthy transplants as most of these activities decreased after 60 days from transplantation then re-increased after 90 days. All mentioned observations were noticed after 0.5; 2.5 and 5.0 minutes from starting the experimental period of the enzyme activity.

3. Effect of different fungicides on the enzyme activities of Giza-20 onion cultivar :

Data in Table (27) and illustrated in Fig. (25) indicate that the activities of hydrolytic enzymes, Pectin-Methyl-Estrase (PME); Poly-Galacturonase (PG) and Cellulase (CX) certainly decrease in the root tissues of onion transplants treated with any of the tested fungicides and transplanted to infested soil rather than those of the un-treated transplants which transplanted to infested soil. Also, it is clear that treated transplants with the most effective fungicides namely, Sumisclex (50%) W.P.; Sumisclex (50%) D.Fl,; Folicure (25%) EC. and Ronilan (50%) W.P. showed lower activities of such enzymes than those determined in the root tissues of transplants treated

Table (27): Effect of different fungicides on the pectinolytic and cellulolytic enzyme activities of healthy and diseased onion cultivar Giza-20 with the most virulent isolate of *Pyrenochaeta terrestris* after 70 days from transplantation (treatments application) under greenhouse conditions at 27°C. ± 2.

Fungicides	Hydrolytic enzyme activities						Average percent of:		
	PME activity 0.1% NaOH cc		% Reduction in viscosity :				Infection	Severity according to infected plants	Severity according to tested plants
			PG activity		CX activity				
	Bulbs	Roots	Bulbs	Roots	Bulbs	Roots			
1. Sumisclex (50%) W.P.	8.10	8.50	20.00	12.50	29.08	25.19	7.60	4.09	1.90
2. Sumisclex (50%) D.Fl.	6.70	8.60	14.29	25.00	23.15	22.52	11.40	13.24	5.70
3. Folicure (25%) EC. and two sprays after 6 and 12 weeks from transplantation.	6.80	14.25	18.75	16.67	35.48	24.92	11.40	10.79	4.51
4. Ronilan (50%) W.P.	6.50	12.25	15.79	27.27	21.13	32.80	11.40	14.04	5.97
5. Ronilan (5%) dust	7.25	19.05	14.29	30.00	36.67	42.36	17.00	20.11	9.61
6. KZ (50%) 120 W.P.	7.00	14.60	21.43	44.44	24.41	54.78	20.80	25.96	11.93
Control (a)	9.80	26.00	37.50	57.14	44.56	83.69	60.14	38.06	30.56
Control (b)	3.90	7.40	8.00	9.43	9.65	18.25	00.00	00.00	00.00
L.S.D.; 0.05							12.26	13.60	7.24

(a) The un-treated transplants were transplanted in infested soil.

(b) The un-treated transplants were transplanted in non-infested soil.

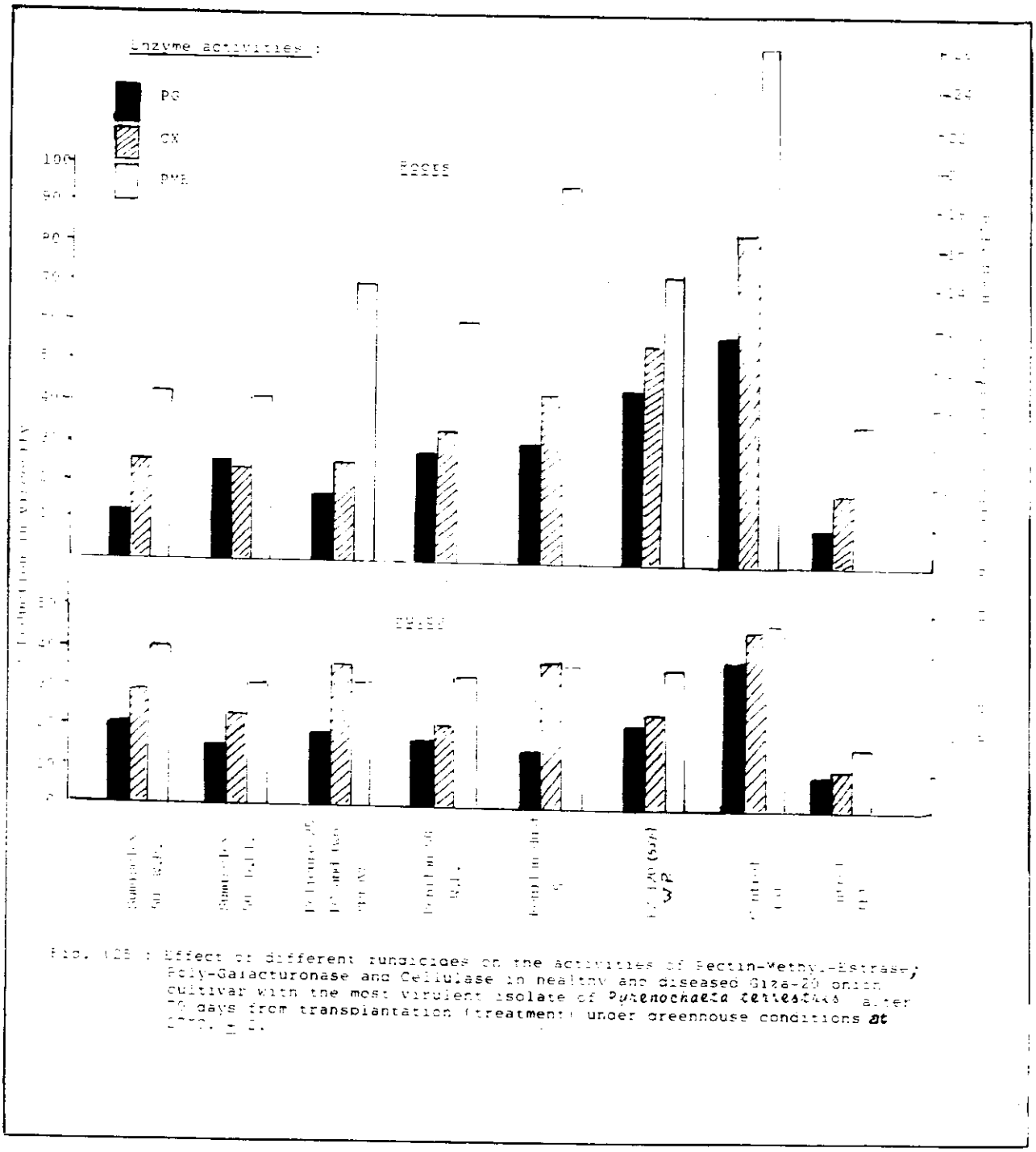

Fig. 12B : Effect of different fungicides on the activities of Pectin-Methyl-Estrase, Poly-Galacturonase and Cellulase in healthy and diseased Giza-20 onion cultivar with the most virulent isolate of *Pyrenochaeta terrestris* after 30 days from transplantation (treatment) under greenhouse conditions at [illegible]

with the less effective fungicides, Ronilan dust (5%) and KZ (50%) 120 W.P. It is also noticed that the more effectiveness of the fungicides used, the lower activities of the three mentioned enzymes may be induced. Also, general valuable observation could be noticed, that the activities of the three hydrolytic enzymes were higher in the root tissues than those in the bulb tissues of transplants treated with the less effective fungicides, Ronilan (50%) W.P.; Ronilan dust (5%) and KZ (50%) 120 W.P., whereas the reverse was true particularly in the cases of PG and CX activities, when the Sumisclex (50%) W.P.; Sumisclex (50 %) D.Fl. and Folicure (25%) EC. were used.

On the other hand, concerning oxidative enzymes, (Table 28 and Fig. 26) it is enough clear that pink root rot infection increased the activities of either Peroxidase or Polyphenoloxidase. Further activities were recorded in the root and bulb tissues of treated transplants with any of the fungicides tested and transplanted to infested soil. Data also indicated clearly that the activities of both oxidative enzymes were generally higher in the root tissues than those determined in the bulb ones.

7. BIOASSAY OF MYCOTOXIN PRODUCED BY *Pyrenochaeta terrestris*:

Data in Table (29) show that all tested isolates of *Pyrenochaeta terrestris* possessed the ability to produce mycotoxin that inhibited the growth of the bioassay agent *Bacillus megaterium* in different degrees.

Table (28): Effect of different fungicides on the oxidative enzyme activities of healthy and diseased onion cultivar Giza-20 with the most virulent isolate of *Pyrenochaeta terrestris* after 70 days from transplantation (treatments application) under greenhouse conditions at 27°C. ± 2 (*In vivo*).

Fungicides	Oxidative enzyme activities as optical density												Average percent of:		
	Peroxidase						Polyphenoloxidase								
	Bulbs			Roots			Bulbs			Roots					
	0.5*	2.5**	5.0***	0.5	2.5	5.0	0.5	2.5	0.5	0.5	2.5	5.0	Infection	Severity according to infected plants	Severity according to tested plants
1. Sumisclex (50%) W.P.	0.098	0.315	0.410	0.345	1.095	1.290	0.397	0.509	0.591	0.669	0.828	0.935	7.60	4.09	1.90
2. Sumisclex (50%) D.Fl.	0.136	0.431	0.595	0.391	0.989	1.398	0.401	0.535	0.627	0.718	0.817	0.944	11.40	13.24	5.70
3. Folicure (25%) EC. and two sprays after 6 and 12 weeks from transplantation.	0.057	0.197	0.289	0.290	0.915	1.111	0.557	0.712	0.845	0.627	0.875	1.041	11.40	10.79	4.51
4. Ronilan (50%)W.P.	0.115	0.224	0.335	0.315	1.335	1.517	0.329	0.412	0.517	0.567	0.753	0.858	11.40	14.04	5.97
5. Ronilan (5%) dust	0.115	0.258	0.345	0.630	1.436	1.632	0.287	0.389	0.471	0.439	0.556	0.675	17.00	20.11	9.61
6. KZ (50%) 120 W.P.	0.023	0.167	0.253	0.069	0.891	1.130	0.290	0.392	0.489	0.513	0.687	0.804	20.80	25.96	11.93
Control (a)	0.052	0.193	0.283	0.245	0.861	1.210	0.245	0.287	0.330	0.389	0.489	0.585	60.14	38.06	30.56
Control (b)	0.035	0.139	0.233	0.121	0.561	0.791	0.123	0.156	0.169	0.239	0.300	0.322	00.00	00.00	00.00
L.S.D.; 0.05													12.26	13.60	7.24

(a) The Un-treated transplants were transplanted in infested soil.
(b) The un-treated transplants were transplanted in non-infested soil sterilized soil.

* After 0.5 minute from the activity initiation.
** After 2.5 minute from the activity initiation.
*** After 5.0 minutes from the activity initiation.

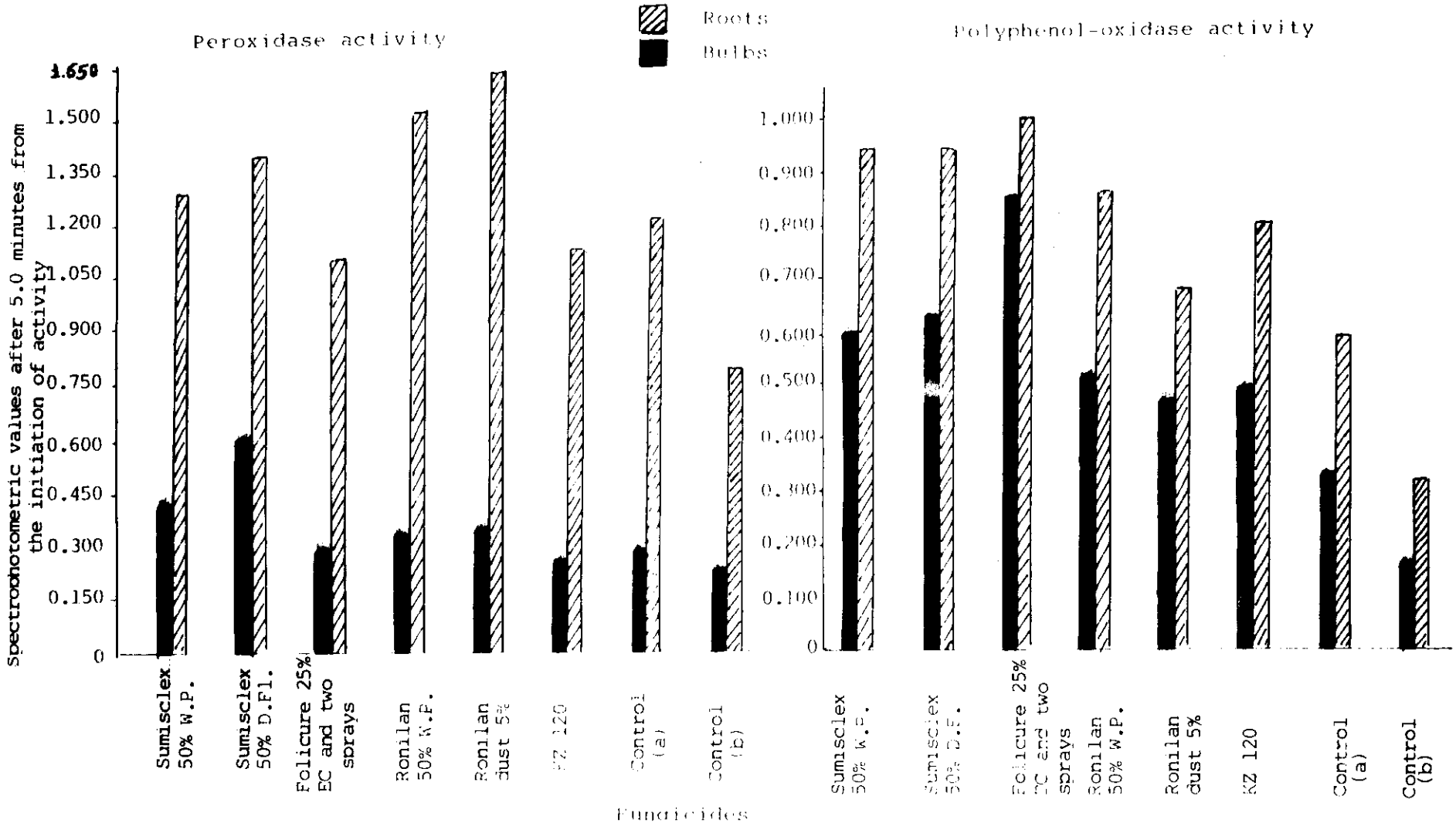

Fig. (26): Effect of different fungicides on the activities of Pyroxidase and Polyphenoloxidase in healthy and diseaaed Giza-20 onion cultivar with the most virulent isolate of *Pyrenochaeta terrestris* after 70 days from transplantation (treatments application) under greenhouse conditions at 27° C. + 2.

Table (29): Inhibition zones of mycotoxin produced by seven isolates of *Pyrenochaeta terrestris* against *Bacillus megaterium* and Av.% of infection and severity of pink root rot disease according to both infected and tested plants.

No.	Isolates		Zones of mycotoxin inhibition (in mm)	Av. % of : -		
				Infection	Severity according to	
					Infected plants	Tested plants
1	El-Badary	(Assiut)	12.0	40.71%	27.97%	8.97%
2.	Ibshwai	(Fayoum)	13.0	28.40	23.07	3.76
3.	Abo-Sour	(Ismailia)	16.0	61.94	41.91	33.79
4.	Kom-Oshem	(Fayoum)	14.0	42.51	30.26	11.61
5.	Fayed	(Ismailia)	10.0	26.40	19.10	3.49
6.	Qouta	(Fayoum)	14.0	44.17	29.34	11.93
7	Sarabiom	(Ismailia)	13.0	33.80	26.70	6.34
8	Control		00.0	00.00	00.00	00.00
L.S.D. at 5%				9.92	4.73	5.79

It is very clear that the most pathogenic isolate (No. 3) produced the highest quantity of such mycotoxin determined as 16 mm. in diameter inhibition zone whereas the lowest pathogenic one (No. 5) gave the least inhibition zone (10 mm), meanwhile the moderate virulent isolates (7; 1; 2; 4 and 6) produced intermediate mycotoxin quantities which resulted moderate inhibition zones ranged from (12-14 mm). So, data suggested that there was a possitive correlation between the virulence of *P. terrestris* isolates and their capability for mycotoxin production.

8. CONTROL STUDIES

A. CHEMICAL CONTROL

Effect of some different systemic fungicides :

1. On fungal growth (*In vitro*)

Data presented in Tables (30 a; b; c and d) and illustrated by Figs.(27 & 28) indicate the effect of twelve different systemic disinfectant fungicides on the linear growth and production of pycnidia; pycnidio and conidio-spores of the most virulent isolate of *Pyrenochaeta terrestris* at 10 ppm concentrations. It is clear from data recorded in Table (30 a) and illustrated in Figs. (27 and 28) that all fungicides significantly inhibited the linear growth of the fungus after 9 days from incubation at 27°C. than the growth of the check.

The fungicides significantly differed in their effects. Bavistin (50%); Benlate (50%) and Folicure (25%) EC were the best and represented category (a), followed by Topsin-M (70%) and

Table (30-a): Effect of twelve different systemic disinfectant fungicides at 10 ppm concentrations on mycelial growth of *Pyrenochaeta terrestris* after 9 days from incubation at 27°C. on poisoned PDA medium. (Average diameter of growth in cm. of two dimentions at right angles).

Fungicides	Concentrations in ppm										
	0.5	1	3	5	10	20	50	100	300	500	General mean
1. Vitavax (75%)	7.13	6.93	5.99	3.28	2.33	1.60	0.00	0.00	0.00	0.00	2.726
2. Vitavax/thiram (75%)	6.38	6.20	6.05	5.90	3.60	3.23	1.56	0.00	0.00	0.00	3.292
3. Vitavax/captan (75%)	6.48	6.21	6.20	5.71	4.38	1.65	0.06	0.00	0.00	0.00	3.069
4. Benlate (50%)	3.18	0.71	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.448
5. Topsin-M (70%)	6.40	6.26	2.89	2.63	2.00	0.15	0.00	0.00	0.00	0.00	2.033
6. Bavistin (50%)	1.33	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.161
7. Sumisclex (50%) W.P.	8.30	7.76	7.43	6.25	5.63	5.53	5.15	2.38	2.20	2.10	5.273
8. Sumisclex (50%) D.Fl.	8.10	7.60	7.00	6.90	6.86	6.46	5.50	2.85	2.63	2.00	5.590
9. Folicure (25 %) EC.	4.28	3.03	1.63	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.940
10. Ronilan (50%) W.P.	7.55	7.09	5.25	4.66	3.33	1.86	1.50	0.73	0.00	0.00	3.197
11. Ronilan (5 %) dust	7.86	6.90	5.31	4.00	1.74	1.69	1.44	0.93	0.28	0.00	3.015
12. KZ (50%) 120 W.P.	7.50	7.25	5.53	4.83	4.60	4.54	3.78	3.14	2.10	1.59	4.486
13. Check	8.30	8.30	8.30	8.30	8.30	8.30	8.30	8.30	8.30	8.30	8.300
General mean	6.37	5.73	4.78	4.07	3.29	2.69	2.10	1.41	1.19	1.08	

L.S.D. at level 1% Fungicides (F.) 1.35 Concentrations (C) 1.43 Fung. (F.) X Conc. (C.) 4.52

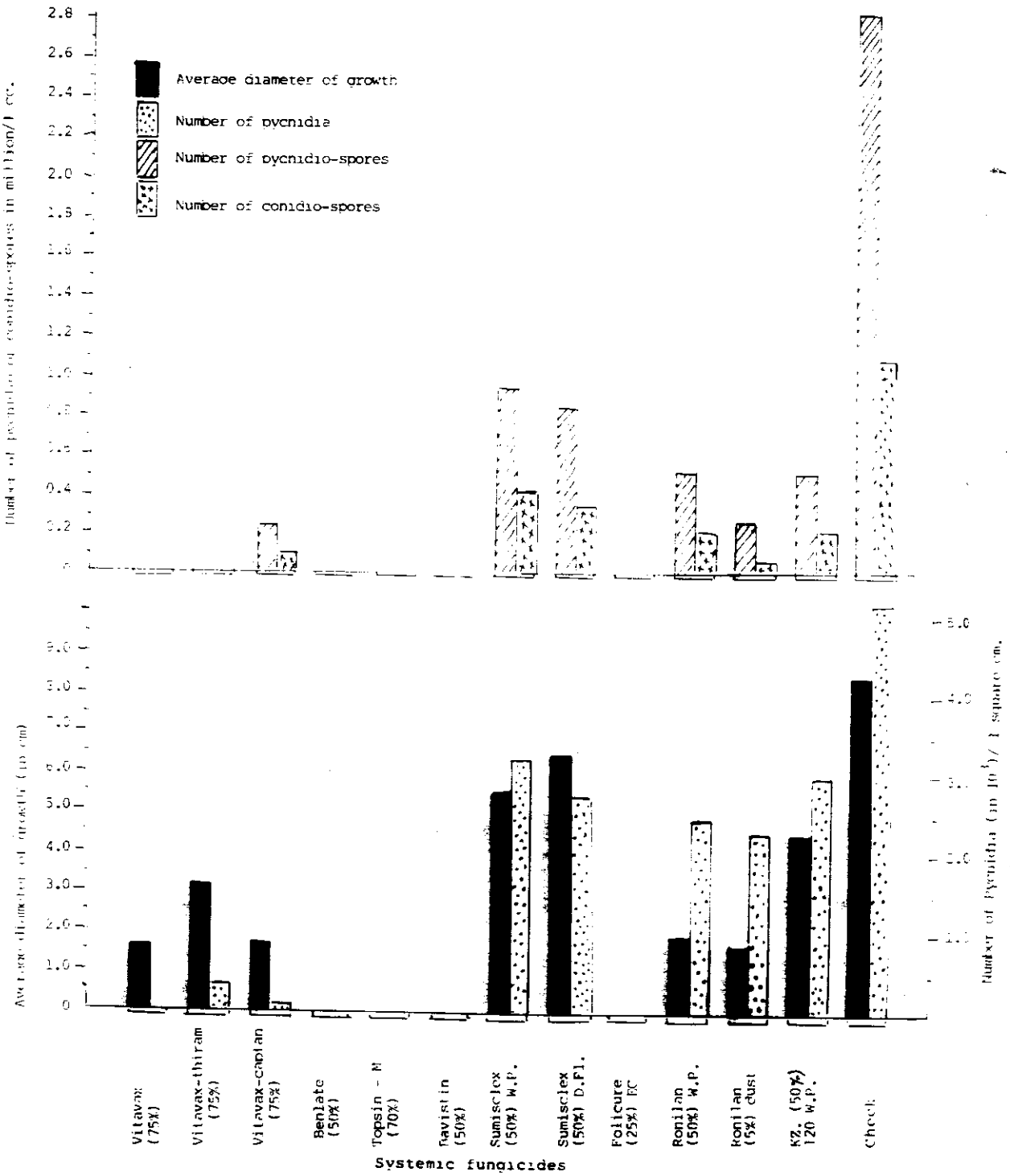

Fig. (28): Effect of twelve different systemic fungicides at 20 ppm concentration on the mycelial growth; pycnidia production; pycnidio and conidio-spores production of the most virulent isolate of *Pyrenochaeta terrestris* after 9 days from incubation at 27°C on poisoned PDA medium.

Vitavax (75%) which represented category (b), meanwhile Ronilan dust (5%); Vitavax/captan (75%); Ronilan (50%) W.P. and Vitavax/thirum (75%) represented category (c). The least effective fungicides which represented category (d) in inhibiting the linear growth were KZ (50%) 120 W.P.; Sumisclex (50%) W.P. and Sumisclex (50%) D.Fl. It is very clear that the toxic effect became more weakness as the concentrations of any of the tested fungicides were lowered. The concentrations 500; 300; 100 and 50 ppm. were significantly the most effective ones followed by 20 and 10 ppm. concs. Less effect was obtained when the fungicides were used at 5 and 3 ppm. concs. Moreover the least toxic effect by all tested fungicides was obtained at the concs. 1 and 0.5 ppm. The fungicides were differed in the ppm concs. which completely inhibited the fungal growth. This concentration was 3 ppm for Bavistin (50%); 5 ppm for Benlate (50%); 10 ppm for Folicure (25%) EC; 50 ppm for Topsin-M (70%) and Vitavax (75%); 100 ppm for Vitavax/captan (75%) and Vitavax/thirum (75%); 300 ppm for Ronilan (50%) W.P. and 500 ppm for Ronilan (5%) dust. However, the fungicides Sumisclex (50%) W.P.; Sumisclex (50%). D.Fl. and KZ (50%) 120 W.P. were non-effective even at 500 ppm conc.

On the other hand data indicate clearly that at 1 and 0.5 ppm concs. only Bavistin (50%); Benlate (50%) and Folicure (25%) EC were the best in inhibiting the linear growth. At 3 ppm conc. Bavistin (50%); Benlate (50%); Folicure (25%) EC. and Topsin M- (70%) showed the best results. At 5 ppm conc. Bavistin

(50%); Benlate (50%); Folicure (25%) EC; Topsin-M (70%); Vitavax (75%) and Ronilan (5%) dust were better than Vitavax/captan (75%); Vitavax/thiram (75%); Sumisclex (50%) W.P. and Sumisclex (50 %) D.Fl. At 10 and 20 ppm concs., all fungicides inhibited the fungal growth more than Sumisclex (50%) W.P.; Sumisclex (50%) D.Fl. and KZ (50%) 120 W.P. Also all fungicides gave good inhibition at the 50 ppm con. except for both Sumisclex (50%) W.P. and Sumisclex (50%) D.Fl. Moreover, it was noticed that no significant difference could be observed between the ten tested fungicides at 100; 300 and 500 ppm concs.

Concerning the effect of the mentioned tested fungicides on inhibiting the production of pycnidia, data tabulated in Table (30 b) and illustrated in Fig. (28) indicate that all fungicides inhibited such structures if compared with those formed in the check culture. The fungicides showed different effects on the production of pycnidia. Bavistin (50%); Benlate (50%); Folicure (25%) E C.; Vitavax (75%); Topsin-M (70%) and Vitavax/thiram (75%) were the most effective fungicides. Moderate toxic effect was obtained by Vitavax/captan (75%); Ronilan (50%) W.P. and Ronilan (5%) dust, whereas the least effective ones were Sumisclex (50%) D.Fl.; KZ (50%) 120 W.P. and Sumisclex (50%) W.P. The best concs. for pycnidia inhibition were 500; 300; 100; 50; 20 and 10 ppm, followed significantly by 5; 3 and 1 ppm, meanwhile the conc. 0.5 ppm showed the least toxicity against the pycnidia production.

All fungicides were highly effective at 500; 300 and 100 ppm. At 50; 20 and 10 ppm concs., Sumisclex (50%) D.Fl.;

Table (30-b): Effect of twelve different systemic disinfectant fungicides at 10 ppm concentrations on pycnidia production of *Pyrenochaeta terrestris* after 9 days from incubation at 27°C on poisoned PDA medium. (Average number of pycnidia in 10^3/one square centemeter).

Fungicides	Concentrations in ppm										
	0.5	1	3	5	10	20	50	100	300	500	General mean
1. Vitavax (75%)	3.49	1.98	0.27	0.25	0.25	0.00	0.00	0.00	0.00	0.00	0.624
2. Vitavax/thiram (75%)	3.47	1.97	1.97	1.59	0.87	0.32	0.03	0.00	0.00	0.00	1.022
3. Vitavax/captan (75%)	3.39	2.98	2.70	2.24	2.06	0.07	0.00	0.00	0.00	0.00	1.344
4. Benlate (50%)	0.50	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.078
5. Topsin-M (70%)	2.89	2.24	0.71	0.56	0.11	0.00	0.00	0.00	0.00	0.00	0.651
6. Bavistin (50%)	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.052
7. Sumisclex (50%) W.P.	4.08	3.97	3.89	3.87	3.26	3.17	2.82	2.56	2.32	2.03	3.197
8. Sumisclex (50%) D.Fl.	5.11	4.33	4.34	3.24	3.12	2.71	2.70	0.60	0.63	0.21	2.699
9. Folicure (25%) EC.	2.12	0.82	0.33	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.347
10. Ronilan (50%) W.P.	3.84	2.98	2.82	2.73	2.12	2.47	1.23	0.31	0.16	0.08	1.874
11. Ronilan (5%) dust	3.70	2.79	2.70	2.54	2.33	2.27	2.07	1.89	0.67	0.64	2.160
12. KZ (50%) 120 W.P.	4.25	4.22	4.14	3.47	3.05	2.96	2.68	1.63	0.56	0.44	2.740
13. Check	5.18	5.18	5.18	5.18	5.18	5.18	5.18	5.18	5.18	5.18	5.180
General mean	3.272	2.748	2.235	1.990	1.719	1.473	1.285	0.895	0.732	0.660	

L.S.D. at level 1% Fungicides (F.) 1.07 Concentrations (C.) 1.14 Fungi (F.) X Conc. (C) 2.67

Sumisclex (50%) W.P. and KZ (50%) 120 W.P. showed less effectiveness, whereas the rest fungicides were more effective. Bavistin (50%); Benlate (50%); Folicure (25%) E.C.; Topsin-M (70%); Vitavax (75%); Vitavax/thiram (75%) and Vitavax/captan (75%) indicated higher toxic effect at 3 ppm conc. than those of Sumisclex (50%) D.Fl.; Sumisclex (50%) W.P.; KZ (50%) 120 W.P.; Ronilan (50%) W.P. and Ronilan (5%) dust. At low concentrations 1 and 0.5 ppm it could be concluded that Bavistin (50%); Benlate (50%); Folicure (25%) E.C. and Topsin-M (70%) were the most effective fungicides for the inhibition pycnidia production.

Concerning the production of pycnidio and conidio-spores in the presence of any of the tested fungicides, similar results could be observed. Data summarized in Tables (30 c and d) and illustrated by Fig. (28) show that Bavistin (50%); Benlate (50%) and Vitavax (75%) gave the excellent results in the inhibition of such spores, followed by Vitavax/thiram (75%); Vitavax/captan (75%); Folicure (25%) E.C. and Topsin-M (70%). On the other hand, the fungicides namely Sumisclex (50%) W.P.; Ronilan (50%) W.P.; Sumisclex (50%) D.Fl.; KZ (50%) 120 W.P. and Ronilan (5%) dust showed less effect.

In general, it could be concluded that both Bavistin (50%) and Benlate (50%) gave satisfactory results in inhibiting the linear growth; production of pycnidia; pycnidiospores and conidia.

Moreover, high effectiveness was observed in inhibiting the linear growth by Folicure (25%) E.C.; the formation of

Table (30-c): Effect of twelve different systemic disinfectant fungicides at 10 ppm concentrations on pycnidiospores sporulation of *Pyrenochaeta terrestris* after 9 days from incubation at 27°C. on poisoned PDA medium (Average number of pycnidiospores in million/one cc.).

Fungicides	Concentrations in ppm										
	0.5	1	3	5	10	20	50	100	300	500	General mean
1. Vitavax (75%)	0.610	0.467	0.256	0.194	0.133	0.000	0.000	0.000	0.000	0.000	0.166
2. Vitavax/thiram (75%)	0.938	0.925	0.669	0.358	0.200	0.000	0.000	0.000	0.000	0.000	0.309
3. Vitavax/captan (75%)	1.075	0.794	0.423	0.307	0.382	0.228	0.000	0.000	0.000	0.000	0.321
4. Benlate (50%)	0.611	0.050	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071
5. Topsin-M (70%)	1.928	0.825	0.569	0.363	0.013	0.000	0.000	0.000	0.000	0.000	0.370
6. Bavistin (50%)	0.106	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013
7. Sumisclex (50%) W.P.	2.300	1.369	1.163	1.131	0.850	0.931	0.638	0.531	0.469	0.200	0.958
8. Sumisclex (50%) D.Fl.	1.819	1.631	1.550	1.003	0.931	0.831	0.731	0.631	0.350	0.094	0.957
9. Folicure (25%) EC.	1.319	0.881	0.169	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.237
10. Ronilan (50%) W.P.	2.025	1.881	1.350	1.066	0.981	0.506	0.116	0.000	0.000	0.000	0.793
11. Ronilan (5%) dust	1.369	0.969	0.531	0.400	0.369	0.250	0.066	0.035	0.000	0.000	0.399
12. KZ (50%) 120 W.P.	1.750	1.581	1.231	0.800	0.600	0.500	0.363	0.300	0.231	0.050	0.741
13. Check	2.806	2.806	2.806	2.806	2.806	2.806	2.806	2.806	2.806	2.806	2.806
General mean	1.435	1.092	0.828	0.648	0.559	0.466	0.363	0.331	0.297	0.242	

L.S.D. at level 1% For Fungicides (F.) 0.376 Concentration (C.) 0.400 Fun. (F.) X Conc. (C.) 0.744

Table (30-d): Effect of twelve different systemic disinfectant fungicides at 10 ppm concentrations on conidiospores sporulaiton of *Pyrenochaeta terrestris* after 9 days from incubation at 27°C. on poisoned PDA medium (Average number of conidiospores in million/one cc.).

Fungicides	Concentrations in ppm										
	0.5	1	3	5	10	20	50	100	300	500	General mean
1. Vitavax (75%)	0.144	0.140	0.075	0.050	0.046	0.000	0.000	0.000	0.000	0.000	0.046
2. Vitavax/thiram (75%)	0.356	0.241	0.238	0.150	0.069	0.000	0.000	0.000	0.000	0.000	0.100
3. Vitavax/captan (75%)	0.331	0.269	0.204	0.156	0.131	0.094	0.000	0.000	0.000	0.000	0.119
4. Benlate (50%)	0.124	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012
5. Topsin-M (70%)	0.518	0.325	0.113	0.094	0.025	0.000	0.000	0.000	0.000	0.000	0.107
6. Bavistin (50%)	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004
7. Sumisclex (50%) W.P.	1.025	0.600	0.531	0.525	0.416	0.400	0.366	0.338	0.300	0.081	0.621
8. Sumisclex (50%) D.Fl.	0.831	0.681	0.675	0.550	0.400	0.331	0.328	0.250	0.150	0.000	0.420
9. Folicure (25%) EC.	0.550	0.400	0.200	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.115
10. Ronilan (50%) W.P.	0.931	0.700	0.521	0.400	0.316	0.200	0.088	0.000	0.000	0.000	0.317
11. Ronilan (5%) dust	0.769	0.481	0.231	0.150	0.100	0.050	0.035	0.025	0.000	0.000	0.184
12. KZ (50%) 120 W.P.	0.950	0.569	0.569	0.319	0.269	0.200	0.131	0.131	0.116	0.081	0.334
13. Check	1.069	1.069	1.069	1.069	1.069	1.069	1.069	1.069	1.069	1.069	1.069
General mean	0.587	0.421	0.341	0.266	0.219	0.180	0.155	0.139	0.126	0.095	

L.S.D. at level 1% for Fungicides 0.268 Concen. (C.) 0.285 Fung. (F.) X Conc. (C.) N.S.

Pycnidia by Folicure (25%) E. C. and Topsin-M (70%); the formation of conidio and pycnidio-spores by Vitavax (75%).

2. On pink root rot disease control (*in vivo*)

A- Under greenhouse conditions :

In complemental study on the fungicidal activity against pink root rot *in vivo* , six different fungicides were used by the specific application belonging each, as shown in Table (31) and illustrated in Fig. (29). It is very clear that Sumisclex (50%) W.P.; Sumisclex (50%) D.Fl., as dip treatment; Folicure (25%) EC as dip treatment and two sprays after 6 and 12 weeks from transplantation and Ronilan (50%) W.P. as also dip treatment gave good control against the disease with non-significant difference and reduced the average percent of disease infection from 60.14% in the control transplants to percent of infection ranged from 7.60 to 11.4%. The same result could be easily observed on the pink root rot severity according to either infected or tested plants, as in the former scale the severity was reduced from 38.06% to values ranged from 4.09 to 14.04%, whereas in the latter one, the reduction in the disease severity was relatively limited and the severity was reduced from 30.56% in the control to percent of severity ranged from 1.09 to 5.97%. Ronilan (5%) dust showed less activity whereas KZ (50%) 120 W.P. was the least effective fungicide in controlling pink root rot disease concerning the percent of infection or disease severity *in vivo* under artificial inoculation.

Table (31): The effect of six different systemic fungicides on the average percent of pink root rot disease on Giza-20 onion cultivar after 70 days from transplantation under greenhouse conditions at 27°C. ± 2.

Fungicides	Average percent of :-		
	Infection	Severity according to infected plants	Severity according to tested plants
1. Sumisclex (50%) W.P.	7.60%	4.09%	1.90%
2. Sumisclex (50%) D.Fl.	11.40	13.24	5.70
3. Folicure (25%) EC. and two sprays after 6 and 12 weeks from transplantation.	11.40	10.79	4.51
4. Ronilan (50%) W.P.	11.40	14.04	5.97
5. Ronilan (5%) dust	17.00	20.11	9.61
6. KZ (50%) 120 W.P.	20.80	25.96	11.93
Control (a)	60.14	38.06	30.56
Control (b)	00.00	00.00	00.00
L.S.D. at level 5%	12.26	13.60	7.24

(a) Un-treated onion transplants were transplanted in infeste soil.

(b) Un-treated onion transplants were transplanted in non-infested (sterilized) soil.

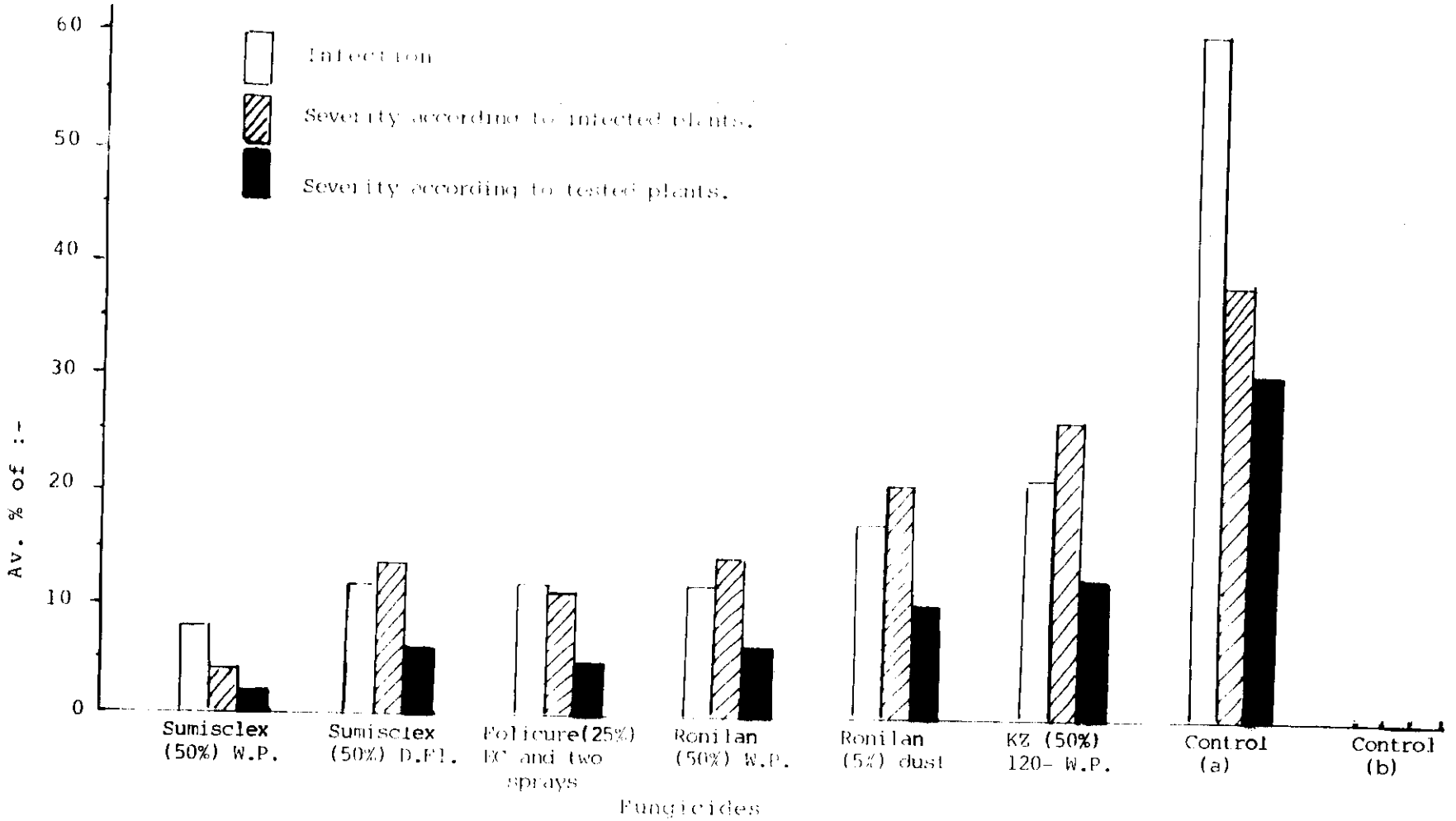

Fig. (29): Effect of six different systemic fungicides on the average percent pink root rot disease of Giza-20 onion cultivar after 70 days from transplantation under green-house conditions at 27^{o}C. $\pm$ 2.

B- Under field conditions :

Data recorded in Table (32) indicate the effect of six different systemic fungicides applied just before transplanting in natural heavily infested soil either at Ismailia Research Station (season 1988/1989 and 1989/1990) or at Moshtohor, Agricultural Research Center, Fac. of Agric. Zagazig Univ., Benha branch, Kalubia governorate in season 1989/1990 . It is very clear that Folicure (25%) EC; Sumisclex (50%) D.Fl. and Sumisclex (50%) W.P. were significantly the most effective fungicides which reduced the infection of the pink root rot disease incidence to the minimum possible level and gave the highest yield. This trend was observed through the three periods of the experiment. Ronilan (50%) W.P. showed less effectiveness, whereas both Ronilan (5%) dust and KZ (50%) 120 W.P. were the least effective fungicides. It is worth to mention that, the higher activity of Folicure (25%) EC.also due to the addetional two sprays which applied after 6 and 12 weeks from transplanting.

B. EFFECT OF DIFFERENT LEVELS OF 'NPK' FERTILIZERS ON THE DISEASE INCIDENCE :

1. Under greenhouse conditions :

Data tabulated in Table (33) indicate the differences of pink root rot disease infection on Giza-20 onion plants treated with eight different NPK levels. It is clear that all treatments decreased the infection from 90% in the non-nutrient transplants to (49.3 - 63.4%). It is also very clear that no significant differences were observed among all treatments used,

Table (32): Effect of six different systemic fungicides on average percent of pink root rot infection and onion yield after 130 days from transplanting in natural heavily infested soil at Ismailia governorate (seasons 1988/1989 and 1989/1990) and at Kalubia governorate (season 1989/1990) on Giza-20 onion Cv.

Fungicides	Governorates								
	Ismailia 1988/1989			Ismailia 1989/1990			Kalubia 1989/1990		
	Av. % of infection	Yield as ton/feddan	% of fungicidal activity	Av. % of infection	Yield as ton/feddan	% of fungicidal activity	Av.% of infection	Yield as ton/feddan	% of fungicidal activity
1. Sumisclex (50%) W.P.	40.33%	2.59	38.7%	33.50%	4.75	39.2%	17.39%	9.73	49.6%
2. Sumisclex (50%) D.Fl.	29.58	2.24	55.0	26.07	4.63	52.7	16.27	10.10	52.8
3. Ronilan (50%) W.P.	33.35	2.19	49.3	38.57	4.05	30.0	22.89	8.90	33.7
4. KZ (50%) 120 W.P.	40.05	2.26	39.1	42.54	3.73	22.8	29.23	8.20	15.3
5. Folicure (25%) EC.	40.05	2.15	39.1	22.48	5.63	59.2	16.21	10.45	53.1
6. Ronilan (5%) dust	39.15	2.44	40.5	40.90	4.20	25.8	26.20	9.55	24.1
7. Control	65.75	1.96		55.13	3.10		34.50	5.90	
L.S.D. at 5%	11.60	N.S.		8.34	1.15		7.42	N.S.	

Table (33): Effect of different levels of NPK on the average percent of infection and severity of pink root rot disease on Giza-20 onion cultivar after 75 days from transplantation under greenhouse conditions at 27°C. ± 2.

Treatments	Average percent of : -		
	Infection	Severity according to infected plants	Severity according to tested plants
N_1PK	49.3	32.9	18.5
NPK*	54.4	42.8	28.0
N_3PK	63.4	41.1	29.1
NP_1K	52.7	42.3	26.9
NP_3K	52.7	38.1	23.9
NPk_1	49.3	40.9	23.3
NPK_3	49.3	42.4	24.1
$N_0P_0K_0$**	90.0	50.7	50.7
L.S.D.;0.05	9.4	7.8	7.5

* Normal fertilization as control.

** No fertilization as control too.

Note: the black letter refers to the normal recommended level of each nutrient source.

except for exceeding nitrogen level as N_3 with other normal rate of P and K. Data also indicate that when the nitrogen decreased to the half recommended rate the severity of infection decreased. Also, the same result could be obtained when the phosphorus was increased to a level equal the double of recommended rate. However no differences in severity according to infected plants were observed when the rate of potassium either increased or decreased over or down the normal rate.

2. Under field conditions :

Table (34) indicates the effect of 'NPK' fertilized combinations on the onion pink root rot disease incidence at Ismailia and Kalubia governorates in the seasons 1988/1989 and 1989/1990. It is very clear that only the increasing nitrogen level over the recommended rate increased the disease infection and surely decreased the onion yield. On the other hand, increasing or decreasing either phosphorus or porassium in combinations with the nitrogenous recommended rate — resulted opposite and satisfactory results as the disease incidence was clearly decreased and the yield either increased or at least remained constant with the exception in the onion yield, yielded in the case of NP_1K treatment of Ismailia in 1989/1990. However, highest infection and lowest yield occurred in un-fertilized treatment.

Table (34): Effect of different NPK fertilized levels on average percent of pink root rot infection and onion yield after 130 days from transplanting in natural heavily infested soil at Ismailia governorate (seasons 1988/1989 and 1989/1990) and at Kalubia governorate (season 1989/1990) on Giza-20 onion cultivar.

Fertilizers in combination and their levels	Governorate					
	Ismailia 1988/1989		Ismailia 1989/1990		Kalubia 1989/1990	
	Av.% of infection	Yield as ton/feddan	Av.% of infection	Yield as ton/feddan	Av.% of infection	Yield as ton/feddan
1. N_1PK	28.50	2.72	30.69	2.90	23.93	7.15
2. NPK*	41.31	2.79	46.69	3.15	28.36	6.10
3. N_3PK	52.61	2.00	49.52	2.70	32.10	5.50
4. NP_1K	30.22	2.54	43.69	2.70	28.93	5.20
5. NP_3K	28.29	2.71	29.92	3.20	24.44	6.10
6. NPK_1	25.94	2.63	40.13	2.95	28.23	5.15
7. NPK_3	25.66	2.16	32.94	3.40	27.84	6.25
8. $N_0P_0K_0$**	53.15	1.53	60.80	1.85	37.60	3.95
L.S.D. at 5%	15.18	0.63	10.15	0.71	7.16	N.S.

* Normal fertilization as control.
** No fertilization as control too.
Note: the black letter refers to the normal recommended level of each nutrient source.

C. EFFECT OF TIMES OF PLANTING AND ONION CULTIVARS ON THE DISEASE INCIDENCE :

The effect of times of planting on the incidence of pink root rot disease was investigated in two successive seasons 1988/1989 and 1989/1990 at Ismailia Agricultural Research Station under natural infection as shown in Table (35 a). Data indicate clearly that 10th of January was the best for minimizing the disease occurrance and gaining the highest yield, whereas the first of December was optimum for the disease incidence and so lower yield could be obtained. Planting the onion transplants in 20th of December resulted intermediate disease occurrance and onion yield with the exception in the onion yield, yielded in the season 1988/1989.

The effect of three conventional onion Cvs. on the incidence of pink root rot disease was studied in two successive seasons 1988/1989 and 1989/1990 at Ismailia Agricultural Research Station and in season 1989/1990 at Moshtohor, Agricultural Research Center, Faculty of Agriculture, Zagazig University, Benha branch, Kalubia governorate as shown in Table (35 b). All experiments were conducted at the first of December under optimum environmental conditions for infection. Data indicate that Shandaweel-1 was relatively resistant and gave the highest yield except in 1988/1989 at Ismailia governorate. Giza-20 was the highest susceptible Cv., yielding the lowest yield meanwhile, Giza-6 showed intermediate reaction.

Table (35-a): Effect of three different times of planting on average percent of pink root rot infection and onion yield of Giza-20 Cv. in naturally heavily infested soil at Ismailia governorate (seasons 88/1989 and 89/1990).

Time of planting	Ismailia 88/1989		Ismailia 89/1990	
	Av.% of infection	Yield as ton/ feddan	Av. % of infection	Yield as ton/ feddan
1st of December	30.53	3.41	46.91	3.45
20th of December	28.48	2.41	43.56	3.65
10th of January	17.53	4.66	39.09	3.90
L.S.D. at level 5%	7.78	1.32	N.S.	N.S.

Table (35-b): Effect of three different conventional onion Cvs. cultivated at the 1st of December on average percent of pink root rot infection and yield in natural heavily infested soil at Ismailia governorate (seasons 88/1989 and 89/1990) and at Kalubia governorate (season 89/1990).

Cultivars	Governorates					
	Ismailia 88/1989		Ismailia 89/1990		Kalubia 89/1990	
	Av. % of infection	Yield as ton/feddan	Av. % of infection	Yield as ton/feddan	Av. % of infection	Yield as ton/feddan
Shandaweel-1	25.78	2.57	33.32	4.35	22.96	6.75
Giza-6	30.53	3.41	35.84	4.15	26.39	6.20
Giza-20	42.25	2.74	46.91	3.45	30.96	5.40
L.S.D.at level 5%	9.24	N.S.	N.S.	N.S.	N.S.	0.60

9. CHEMICAL ANALYSIS OF EVALUATED ONION CULTIVARS IN RELATION TO PINK ROOT ROT DISEASE :

1. Determination of individual free amino acids :

Free amino acids and the total of their groups were determined in the root and bulb tissues after 30; 60 and 90 days from transplanting using diseased and healthy three onion Cvs. under artificial inoculation with the most virulent isolate of *Pyrenochaeta terrestris* and greenhouse conditions at 27°C. ± 2.

After 30 days from transplanting it is clear from data presented in Tables (36 & 39) and illustrated in Figs. (30;33 and 34) that, total amino acids of the susceptible cultivar Giza-20 was decreased in the whole inoculated onion transplants than those in the non-inoculated ones, as it reached 18.959 mgs. in the diseased transplants and 22.148 mgs. in the healthy transplants. Resistant Cv. Shandaweel-1 showed the same decrease in the total free amino acids determined in the inoculated transplants than those of healthy ones, as it reached 16.980 mgs. in the diseased transplants and 18.963 mgs. in the healthy transplants however, the rate of decreasing of the latter cultivar was less than detected in the former one. On the other hand, in moderatly resistant cultivar Giza-6, it was clear that inoculation increased the total free amino acids of the whole infected transplants rather than the healthy ones, as it reached 20.632 mgs. in infected transplants and 18.443 mgs. in the healthy transplants. In the same Table it is also clear that, free amino acids of the infected susceptible roots of

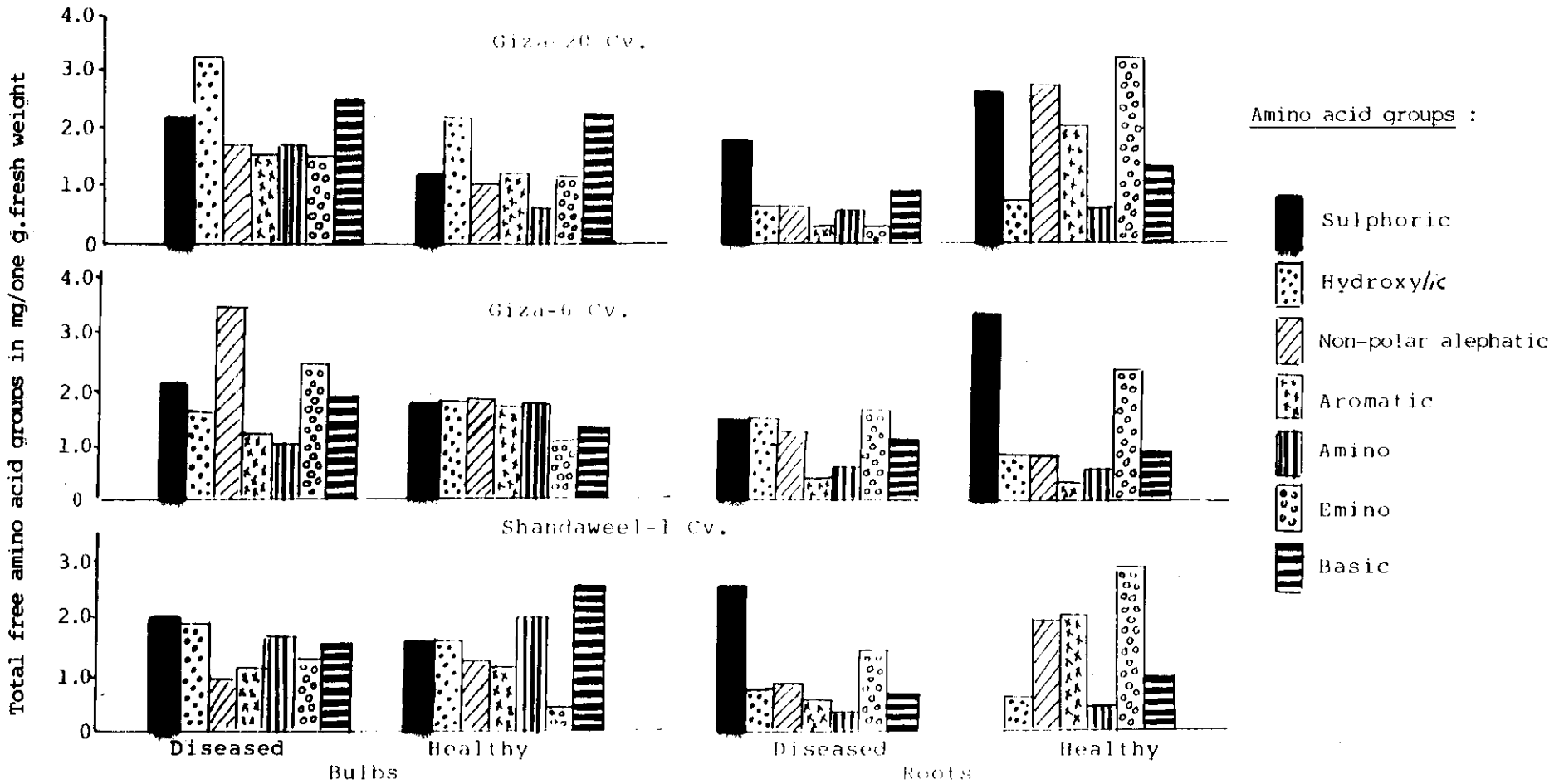

Fig. (30): Total free amino acid groups in mg./one g. fresh weight in roots and bulbs of three onion cultivars after 30 days from transplantation under artificial inoculation with the most virulent isolate of *Pyrenochaeta terrestris* and greenhouse conditions at 27^{o}C. $\pm$ 2.

Giza-20 sharply decrease from 12.817 mgs. to 4.534; whereas this decrease was limited in the inoculated roots of resistant and moderate resistant cultivars. Determinations of free amino acids on the bulbs of all evaluated Cvs. revealed that the quantities of these contents were constant in the resistant Cv. Shandaweel-1 whilst, the total free amino acids in the bulbs of infected transplants of both susceptible and moderate resistant Cvs. were increased particularly in the susceptible Cv. Giza-20 and this directly attributed to the decrease of such contents in the root tissues of both Cvs. respectively.

It is also very clear from data summarized in Table (36) that, all free amino acids groups decreased in the inoculated roots of the resistant Cv. Shandaweel-1 except for sulphoric group particularly L-Cystine which sharply increased in the inoculated transplants than those determined in the non-inoculated ones. The susceptible Cv. Giza-20 showed different reaction with regard to sulphoric group especially L- Cystine which was clearly decreased according to infection. Also, alephatic (in particular with DL- α -Alanine and Glycine); Aromatic (particularly DL-Treptophan); emino (especially proline) and basic (particularly L-lycine- HCl) groups were obviously decreased. Also, hydroxylic and amino groups showed limited decrease. In moderately resistant Cv. Giza-6, it was shown that some groups such as sulphoric group (especially L-Cystine); amino group (particularly DL-Aspartic acid) and aromatic (in particular with DL-Phenyl alanine) were clearly decreased, however other amino acids groups i.e. hydroxylic,

(especially 3, (3,4) Dihydroxy alanine); alephatic (particularly DL- ∝ -Alanine); emino and basic groups were clearly increased in the inoculated roots. It was also noticed that the different amino acid groups in the bulbs of the resistant Cv. Shandaweel-1 showed different reactions after 30 days from transplantation, sulphoric (especially L-Cystine); hydroxylic (particularly DL-Thrionine and 3, (3,4) Dihydroxy alanine) and emino groups (especially L-Proline) were clearly increased. On the other hand, the alephatic (in particular with DL- ∝ -Alanine); amino (especially L-Glutamic and Aspartic acid) and basic groups were obviously decreased. Only the aromatic group remained constant in both inoculated and non-inoculated bulbs. The pink root rot disease infection increased all amino acid groups in the bulbs of susceptible Cv. Giza-20 particularly alephatic; aromatic; amino and emino groups. With regard to moderately resistant Cv. Giza-6, amino acid groups were generally increased in the bulbs of inoculated plants with exception of aromatic and amino acid groups which clearly decreased as a result of the infection.

Data presented in Tables (37 & 39) and illustrated in Figs. (31, 33 and 34) show the individual free amino acids and totals of their groups after 60 days from transplantation. It was noticed that total amino acids of the whole transplants was increased in resistant diseased Cv. Shandaweel-1 than those in resistant healthy ones, as it reached 22.890 mgs and 17.200 mgs. respectively, whereas, such total determined in both susceptible Cv. Giza-20 and moderatly resistant Cv. Giza-6 showed reverse

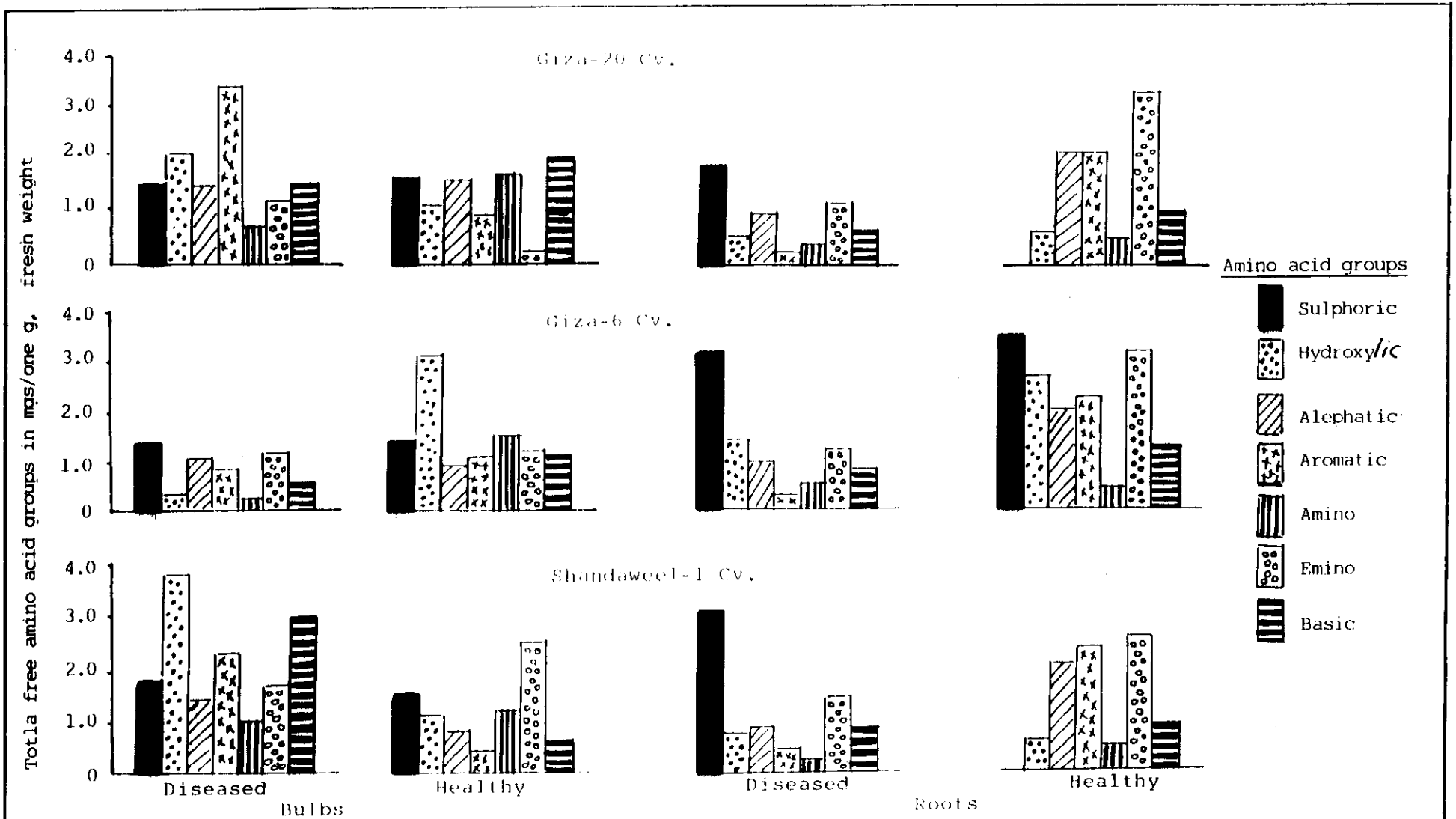

Fig. (31): Total free amino acid groups in mg./one g. fresh weight in roots and bulbs of three onion cultivars after 60 days from transplantation under artificial inoculation with the most virulent isolate of *Pyrenochaeta terrestris* and greenhouse conditions at 27°C. ± 2.

reaction as it decreased in diseased transplants rather than those of the healthy ones. Concerning amino acid groups, it was noticed that all groups in roots of resistant Cv. Shandaweel-1 were decreased except for sulphoric group which was increased very sharply, as a result of infection. The same trend was also observed in susceptible Cv. Giza-20. However, in the moderatly resistant Cv. Giza-6 sulphoric; hydroxalic; non-polar alephatic; aromatic; emino and basic groups were decreased, whereas amino group was slightly increased in the roots of diseased transplants than those detected in the healthy ones after 60 days and this was reverse to which determined after 30 days from transplantation. According to total of amino acid groups in bulbs of diseased susceptible Cv. Giza-20, it was noticed that sulphoric; alephatic; amino and basic groups were decreased in the diseased transplants than those of healthy ones, whereas hydroxylic; aromatic and emino groups were increased. In this respect it was noticed that sulphoric; alephatic; amino; emino and basic groups were decreased after 60 days from transplantation rather than those determined after 30 days from transplantation. However, the reverse was true concerning the hydroxylic and aromatic groups. The bulbs of diseased resistant Cv. Shandaweel-1 contained less concentrations of amino and emino groups rather than those of healthy transplants and in the same time sulphoric; hydroxylic; alephatic; aromatic and basic groups were sharply increased as a result of infection. In the moderate resistant Cv. Giza-6, it was noticed that hydroxylic and basic groups were sharply decreased and also aromatic and amino

groups. On the other hand, alephatic group was increased, however both sulphoric and emino groups remained constant. In this respect sulphoric; hydroxylic; alephatic and emino groups were clearly decreased after 60 days from transplantation rather than those detected after 30 days from transplantation.

Concerning the third period — after 90 days from transplanting — it is shown from data in Tables (38 and 39) and illustrated in Figs. (32; 33 and 34) that the total free amino acids in the whole diseased transplants of the susceptible Cv. Giza-20 was sharply increased than healthy transplants. This increase in the total free amino acids was also recorded in higher concentration if compared with those detected after 60 days from transplantation. On the other hand, it was observed that diseased transplants of resistant Cv. Shandaweel-1 revealed slight increase in the total free amino acids rather than both controls after 60 and 90 days from transplanting.

Reverse reaction was recorded in the moderate resistant Cv. Giza-6 as the total free amino acids were clearly decreased in the diseased transplants compared with the healthy ones. Also, this observation was previously recorded after 60 days from transplantation.

It worthy to mention that according to the resistant Cv. Shandaweel-1, only the sulphoric group (especially L-Cystine) and emino group (particularly L-Hydroxy proline) were increased in its root tissues, however sulphoric (L-Cystine); hydroxylic (DL-Thrionine and DL-Tyrosine); aromatic (DL-Treptophan) and

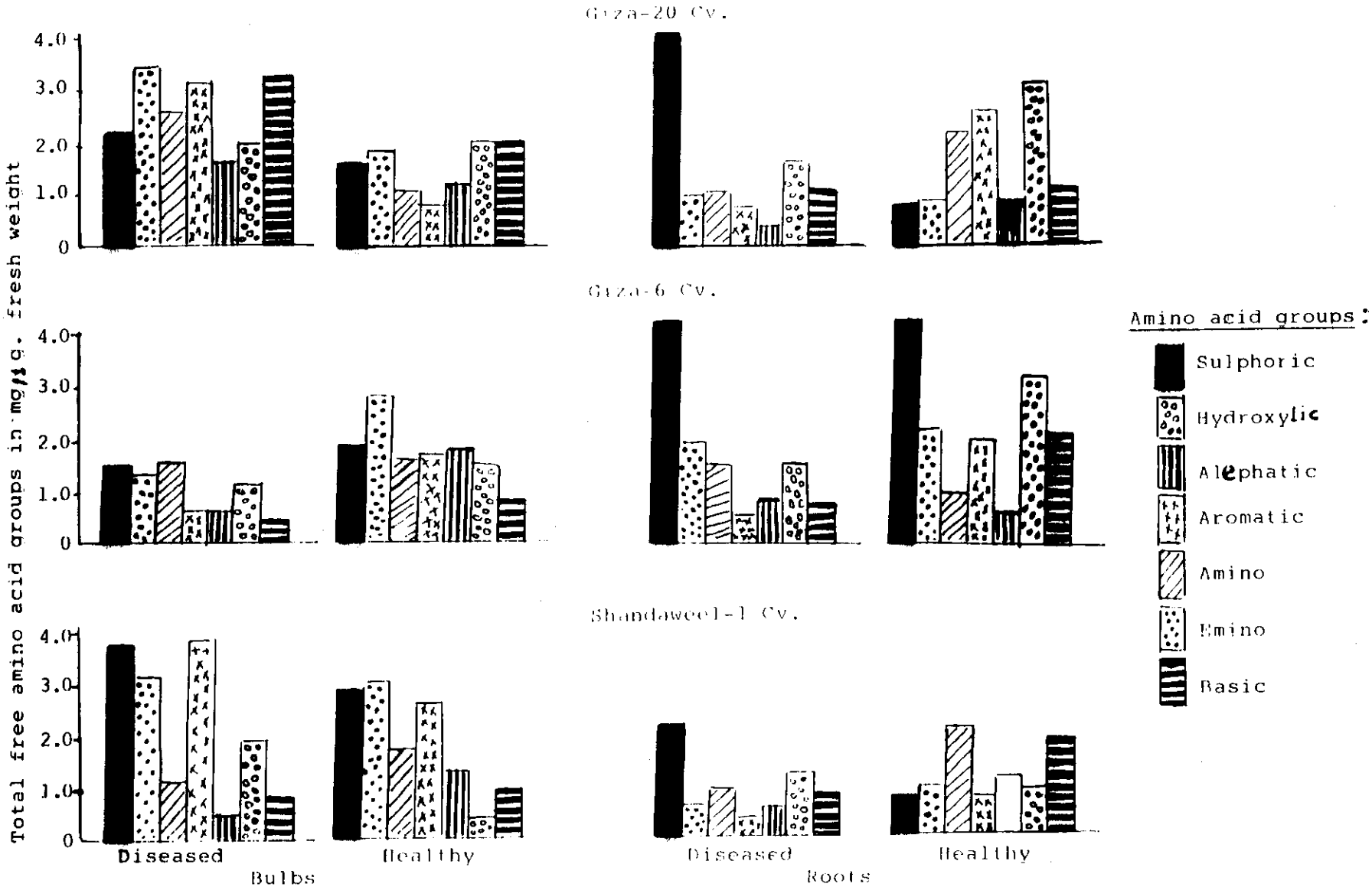

Fig. (32): Total free amino acid groups in mg./gm. fresh weight in roots and bulbs of three onion cultivars after 90 days from transplantation under artificial inoculation with the most virulent isolate of *Pyrenochaeta terrestris* and greenhouse conditions at 27° C. $\pm$ 2.

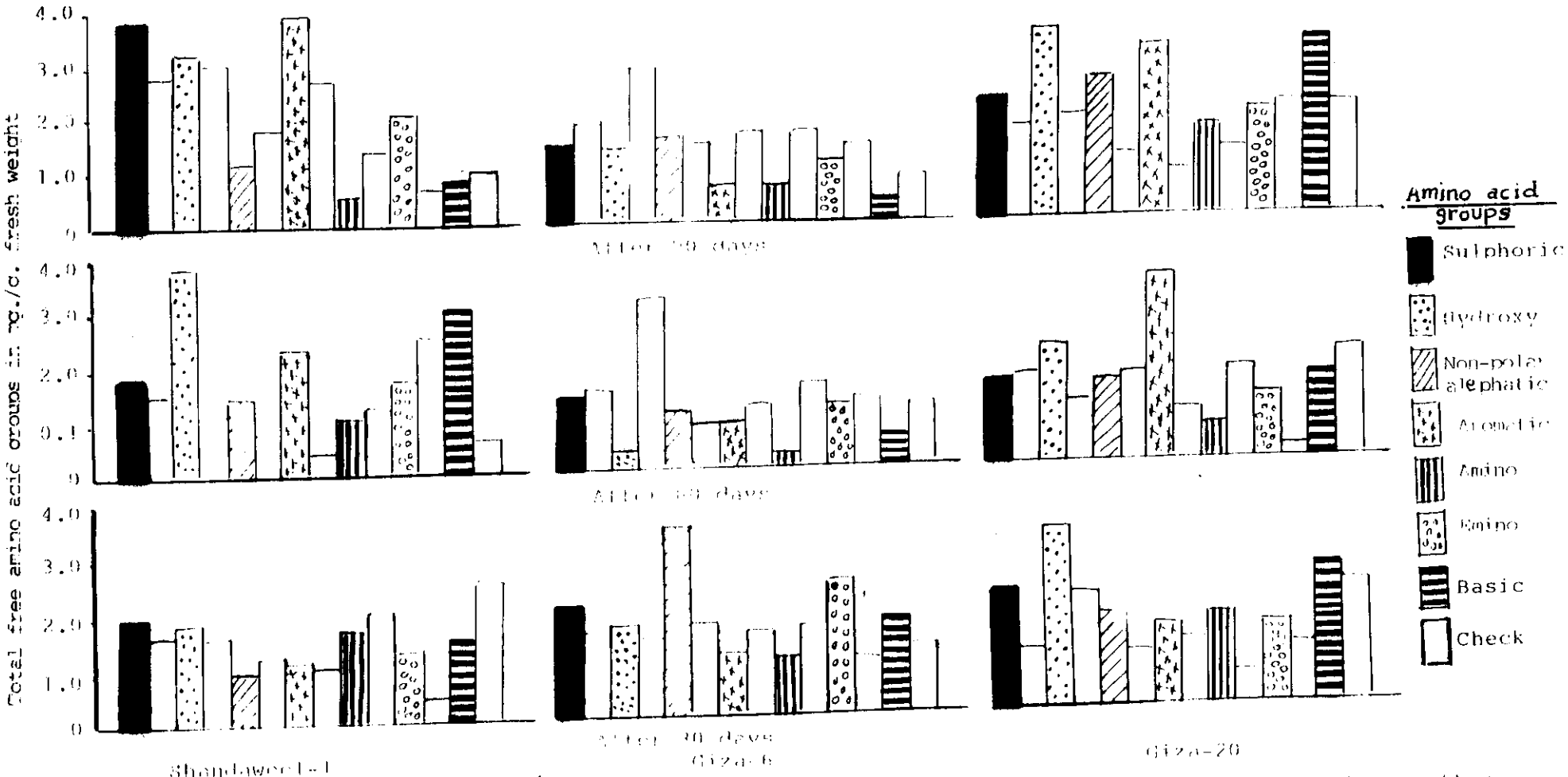

Fig. (33): Total free amino acid groups in mg./g. fresh weight in bulbs of three onion cultivars after 30; 60 and 90 days from transplantation under artificial inoculation with the most virulent isolate of *Pyrenochaeta terrestris* and greenhouse conditions at 27°C. ± 2.

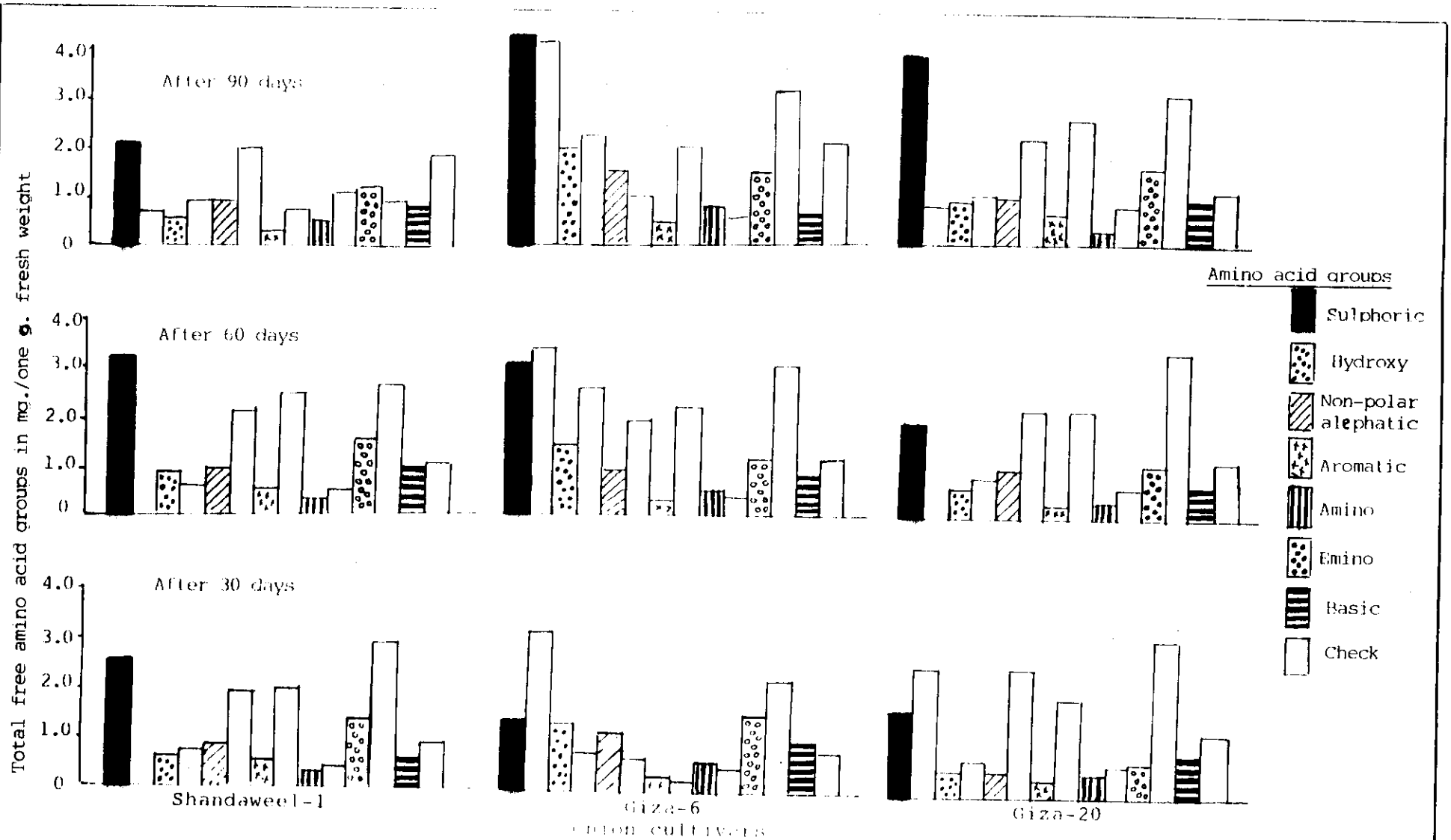

Fig. (34): Total free amino acid groups in mg./one g. fresh weight in roots of three onion cultivars after 30; 60 and 90 days from transplantation under artificial inoculation with the most virulent isolate of *Pyrenochaeta terrestris* and greenhouse conditions at 27°C. ± 2.

emino (L-Proline) groups were found in higher content in the diseased bulbs than those determined in healthy ones. Concerning to the susceptible Cv. Giza-20, only sulphoric group (especially L-Cystine) was increased in its roots as a result of pink root rot infection. However, all amino acid groups were increased in the tissues of its diseased bulbs except for emino group (L-Hydroxy Proline). In moderately resistant Cv. Giza-6, sulphoric group (especially L-Cystine); non-polar alephatic (particularly DL- ∝ Alanine) and amino group (in particular with L-Glutamic acid) were increased in its infected root tissues, whilst only non-polar alephatic group (especially Glycine) was increased in its bulbs as a result of infection.

2. Determination of phenolic compounds :

Data shown in Table (40) indicate the phenolic compounds which determined in 0.01 mg. catechol/one gram fresh weight of both diseased and healthy transplants matter of three onion Cvs. under artificial inoculation with the most virulent isolate of *Pyrenochaeta terrestris* and greenhouse conditions at $27^{o}C \pm 2$. It is very clear that total phenols in the healthy root tissues of the least infected Cv. Shandaweel-1 was relatively higher than those determined in the root tissues of the tolerant Giza-6 and the susceptible Giza-20 Cvs. after 30 days from transplantation. Inoculation with the fungus *Pyrenochaeta terrestris* increased the total phenols in both roots and bulbs of all tested Cvs., however the rate of total phenolic compounds increase was rather high in the tolerant and susceptible Cvs. than that happened in the resistant one.

Table (40): Free; conjugated and total phenols in 0.01 mg. catechol/one g. fresh weight of diseased and healthy three onion cultivars after 30; 60 and 90 days from transplantation under artificial inoculation with the most virulent isolate of *Pyrenochaeta terrestris* and greenhouse conditions at 27°C. ± 2.

Onion cultivars	Days after transplantation																Av. % of								
	30 days						60 days						90 days				Infection after:			Disease severity according to : Infected plants after:			Tested plants after		
	Bulbs			Root			Bulbs			Roots			Bulbs			Roots									
	F.	C.	Total	F.	C.	Total	F.	C.	Total	F.	C.	Total	F.	C.	Total	F.	C.	Total	30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
Shandaweel-1 Diseased (R)	11.50	4.00	15.50	28.75	12.00	40.75	14.25	2.05	16.30	31.25	8.00	39.25	18.20	2.80	21.00	32.80	13.20	46.00	15.96	29.12	36.68	6.70	19.80	22.60	1.30	4.80	8.00
Shandaweel-1 Healthy	14.65	0.35	15.00	29.75	9.75	39.50	13.00	1.80	14.80	31.25	9.25	40.50	13.50	4.60	18.10	40.10	10.60	50.70									
Giza - 6 Diseased (T.)	11.25	12.25	23.50	35.63	6.87	42.50	10.40	11.60	22.00	37.00	8.75	45.75	13.20	10.40	23.60	38.80	7.60	46.40	26.32	36.48	53.84	18.30	25.70	20.60	5.80	10.00	14.50
Giza - 6 Healthy	12.00	4.50	16.50	32.88	5.87	38.75	12.75	2.75	15.50	35.00	11.50	46.50	16.50	6.80	23.30	36.20	12.00	48.20									
Giza - 20 Diseased (S.)	16.90	0.10	17.00	35.63	5.12	40.75	11.25	5.75	17.00	35.75	5.00	40.75	10.80	18.80	29.60	40.40	6.60	47.00	34.16	46.16	79.36	31.20	40.00	56.80	11.30	24.00	50.50
Giza - 20 Healthy	11.00	4.50	15.50	34.13	3.37	37.50	14.65	3.35	18.00	31.00	5.25	36.25	18.40	5.60	24.00	30.20	9.00	39.20									
L.S.D.; 0.05 for infection and severity													for Cultivars (C.) or Periods (P.)						15.82			11.12			15.50		
													for Interaction (C.) X (P.)						N.S.			19.25			26.90		

F. : Free phenols
C.: Conjugared phenols

(R.): Resistant Cv.
(T.): Tolerant Cv.
(S.): Susceptible Cv.

Free phenols were increased in the root tissues of both tolerant and susceptible diseased transplants, whereas the reverse was true according to those of resistant ones. Moreover, the conjugated phenols were increased in the root tissues of all tested Cvs. after inoculation. In the bulb tissues of the resistant Cv. Shandaweel-1, it is very clear that free phenols were obviously decreased whilest conjugated ones were clearly increased, so, the total phenols were relatively the same in both diseased and healthy transplants.

Similar trend was observed in the bulb tissues of the tolerant Cv. Giza-6, as the free phenols were slightly decreased whereas conjugated ones were highly increased, so very clear increase was recorded in the total phenolic compounds of diseased than healthy bulb tissues of this Cv. On the other hand free phenols of its root tissues were clearly increased and so total ones. The susceptible Cv. Giza-20 showed reverse reaction concerning the phenolic compounds of its bulb tissues. It is enough clear that the free phenols were accumulated in its bulb tissues according to the infection whereas the conjugated ones were obviously decreased. However the total phenolic compounds were also increased according to infection. The same reaction which previously recorded in the bulb tissues of both the resistant and tolerant Cvs. was also observed in the bulb tissues of the susceptible Cv. as the free; conjugated and total phenols were found in higher concentrations in the diseased than those determined in the healthy transplants.

After 60 or 90 days from transplantation in infested soil with the causal agent, it is very clear that total phenols of root tissues of both resistant and tolerant Cvs. were decreased, whilest a clear increase of total phenolic compounds of the root tissues of the susceptible Cv. was recorded. However, the total phenols of the bulb tissues of all tested Cvs. were increased but to different rates particularly after 90 days from inoculation, as the bulb tissues of the diseased susceptible Cv. contained the higher concentration of total phenols, whereas the resistant Cv. showed moderate increasing of total phenols, however the tolerant Cv. showed very slight increase of total phenolic compounds in its bulb tissues.

Concerning free phenols, data indicate that such compounds were clearly decreased in the root tissues of the resistant Cv., moderately increased in the tolerant one, and clearly increased in the susceptible Cv. Reverse results were recorded according to free phenols found in the bulb tissues of the three Cvs. Such compounds were increased in the bulb tissues of the resistant Cv. and were decreased in both tolerant and susceptible ones.

These different reactions were observed either after 60 or 90 days from transplantation. According to conjugated phenols, it is clear that these compounds were increased in the root tissues of the resistant Cv. after 90 days only and were decreased in the root tissues of the tolerant and the susceptible ones, while the reverse was true according to those found in the bulb tissues of the mentioned Cvs.

3. Quantitative analysis of sugars :

Data written in Table (41) indicate that reducing sugars were slightly increased in the resistant Cv. Shandaweel-1; tolerant Cv. Giza-6, or the susceptible one Giza-20, either in the bulb or root tissues after 30 days from inoculation except for those of the susceptible bulb tissues. Non-reducing sugars were decreased in the bulb and root tissues of the resistant Cv. Shandaweel-1, decreased and slightly increased in the bulb and root tissues of the tolerant Cv. Giza-6, respectively. Meanwhile, such compounds were increased in both root and bulb tissues of the susceptible Cv. Giza-20. Total soluble sugars were also increased in the diseased root tissues of either susceptible or tolerant Cvs. and was being constant in the inoculated and healthy roots of the resistant Cv. Shandaweel-1 . Different reaction was found concerning the total soluble sugars of the bulb tissues of the three tested Cvs. as the total sugars of the bulb tissues of diseased transplants were shaeply increased, slightly decreased and clearly decreased in the susceptible; tolerant and resistant Cvs. respectively.

After 60 and 90 days from transplantation, data introduced an evidence that reducing; non-reducing and total sugars determined in both bulb and root tissues of the resistant Cv. Shandaweel-1 were clearly decreased. Similar trend was observed in the reaction of the tolerant Cv. Giza-6 with the exception for the sugars contents of the root tissues after 90 days from transplantation. Concerning the susceptible Cv. Giza-20 the

Table (41): Reducing; non-reducing and total soluble sugars in mg. glucose/one g. fresh weight of diseased and healthy three onion Cvs. after 30; 60 and 90 days from transplantation under artificial inoculation with the most virulent isolate of *Pyrenochaeta terrestris* and greenhouse conditions at 27°C. ± 2.

Onion cultivars	Days after transplantation																Av. % of Infection after			Disease severity according to:-					
	30 days						60 days						90 days							Infected plants after:			Tested plants after :		
	Bulbs			Roots			Bulbs			Roots			Bulbs			Roots									
	R.	N.	Total	R.	N.	Total	R.	N.	Total	R.	N.	Total	R.	N.	Total	R.	N.	Total	30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
Shandaweel-1 * Diseased (R)	5.56	17.64	23.20	2.30	1.60	3.90	1.56	12.04	13.60	2.30	1.60	3.90	4.60	12.00	16.60	2.10	1.40	3.50	15.96	29.12	36.68	6.70	19.80	22.60	1.30	4.80	8.00
Shandaweel-1 Healthy	4.24	24.80	29.04	2.00	1.90	3.90	3.72	23.48	27.20	2.10	2.90	5.00	5.70	20.20	25.90	3.00	3.40	6.40									
Giza-6 Diseased (T.)	7.36	7.68	15.04	3.20	4.30	7.50	1.96	5.08	7.04	2.60	2.60	5.20	3.70	4.90	8.60	3.50	3.00	6.50	26.32	36.48	51.84	18.30	25.70	20.60	5.80	10.00	14.50
Giza - 6 Healthy	4.60	11.48	16.08	3.00	1.00	4.00	4.24	20.56	24.80	2.90	2.40	5.30	4.50	22.50	27.00	4.20	2.70	6.90									
Giza - 20 Diseased (S.)	3.20	24.00	27.20	2.40	2.80	5.20	4.72	12.32	17.04	2.30	3.30	5.60	6.40	18.20	24.60	3.80	3.00	6.80	44.16	46.16	79.36	31.20	40.00	45.80	11.30	24.00	50.50
Giza - 20 Healthy	4.04	13.00	17.04	2.00	2.60	4.60	2.72	17.76	20.48	2.40	2.50	4.90	4.50	21.60	26.10	2.70	2.60	5.30									
L.S.D. for infection and severity at level 5% :-													for Cultivars (C.) or Periods (P.)						15.82			11.12			15.50	
													for Interaction (C.) X (P.)						N.S.			19.25			26.90	

R: Reducing sugars
N.: Non-reducing sugars

*(R.) Resistant Cv.
(T.) Tolerant Cv.
(S.): Susceptibible Cv.

sugar contents were generally increased in the root tissues according to pink root rot infection but its concentration were decreased in the bulb tissues except for the reducing sugars which found in the bulb tissues of this Cv.