

RESULTS

A- Survey of barley foliar diseases:

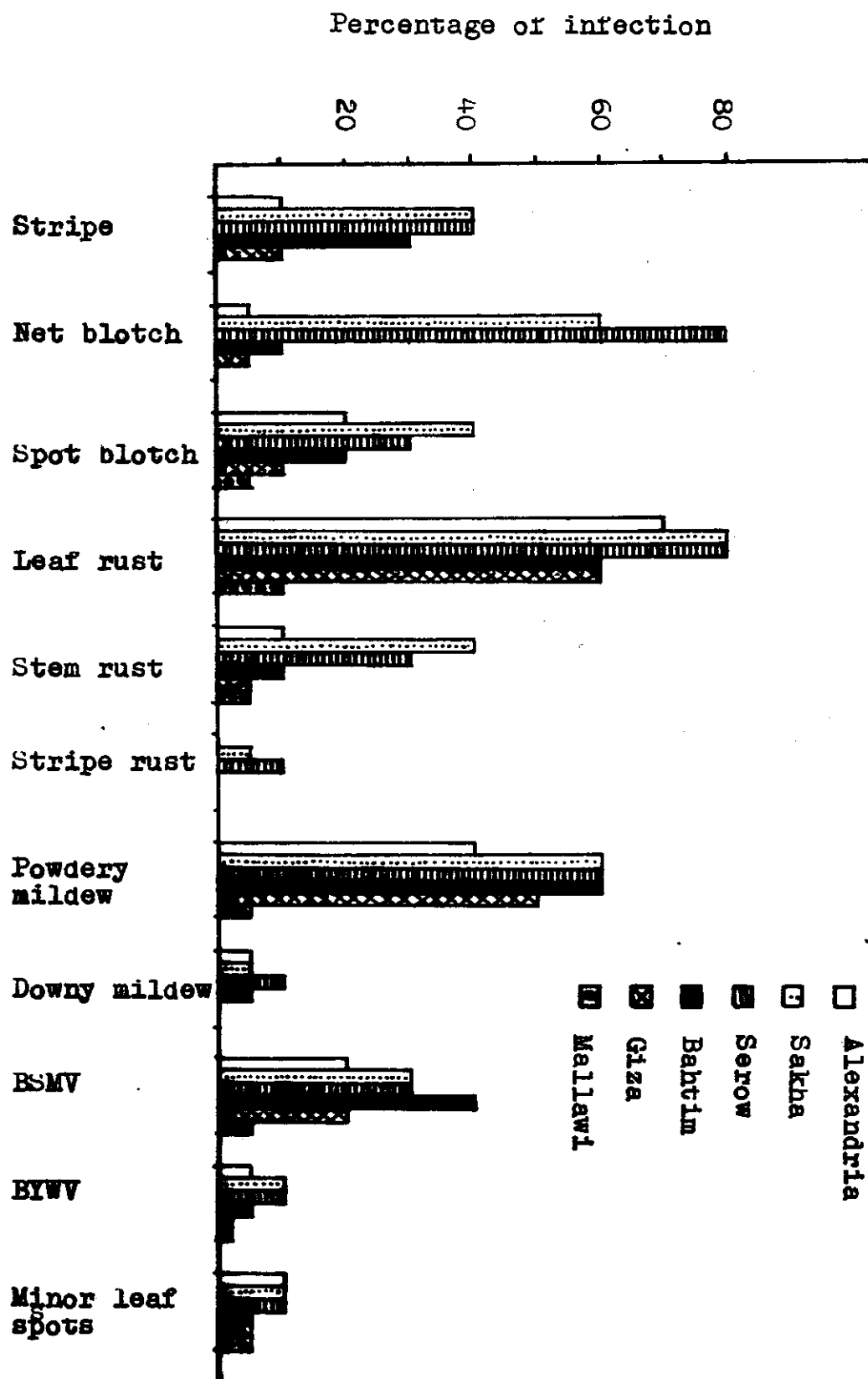
The general survey conducted during the growing seasons 1978-1979 at six different locations in Egypt showed that barley foliage is vulnerable to nine fungal and two viral diseases.

The fungal diseases were identified at Cereal Diseases Division, Plant Pathology Inst. Agric. Res. Center, Giza. Viral diseases were identified by R.G. Timian, USDA, SEA, AR, Dept. Plant Pathology, North Dakota State Univ., Fargo, E.A. Hockett and R.F. Eslic, Dept. Plant and Soil Sciences, Montana State Univ., Bozeman, Montana, USA. Prevalence of those diseases varied at the different locations (Table 3 and Fig. 2) and could be arranged in a descending order as follows: leaf rust (Puccinia hordei Otth.); powdery mildew (Erysiphe graminis hordei Em. Marchal); net blotch (Helminthosporium teres Sacc.); barley stripe mosaic virus (BSMV); leaf stripe (Helminthosporium gramineum Rabh.); spot blotch (Helminthosporium sativum Pem. King & Bakke); stem rust (Puccinia graminis Pers.); minor leaf spots (Alternaria spp., Stemphylium spp. and Helminthosporium spp.); barley yellow dwarf virus (BYDV); downy mildew (Sclerospora macrospora Sacc.) and stripe rust (Puccinia striiformis Westend.).

Table (3): Foliar disease occurred on barley at six locations during season 1978-1979

Diseases	% of infection at					
	Alexandria	Sakha	Serow	Bahthm	Giza	Mallawi
a- Helminthosporiosis						
Leaf stripe	10	40	40	30	10	0
Net blotch	5	60	80	10	5	0
Spot blotch	20	40	30	20	10	5
b- Rusts						
Leaf rust	70	80	80	60	60	10
Stem rust	10	40	30	10	5	5
Stripe rust	0	5	10	0	0	0
c- Mildews						
Powdrey mildew	40	60	60	60	50	5
Downy mildew	5	5	10	5	1	0
d- Viral diseases						
Barley stripe Mosaic Virus	20	30	30	40	20	5
Barley Yellow Dwarf Virus	5	10	10	5	2	2
e- Other diseases						
Minor leaf spots	10	10	10	5	5	5

Fig.2. Barley roller diseases at six location , 1978 - 1979 .



Successful attempts to transmit foliar diseases from diseased samples to ten days- old barley seedlings, variety Giza 119, were conducted. Leaf spots pathogens were isolated on PDA medium. Mycelial and spore suspensions were applied for inoculation of net blotch, spot blotch and minor leaf spots, whereas barley seeds were firstly, sprouted on mycelial growth of leaf stripe pathogen H. gramineum, and sown in pots. Mildews and rusts were, directly, inoculated on seedlings. Saps of infected leaves and insects were used for transmitting viral diseases. The first and fully developed symptoms on inoculated seedlings have been described and their occurrence times ^{were} recorded as shown in Table 4. Leaf spots and powdery mildew occurred, generally earlier than downy mildew, rusts and viral diseases. Barley stripe mosaic virus was found to transmit mechanically whereas yellow dwarf virus was transmitted by the apple grain aphids, Rhopalosiphum padi (L.).

The symptoms and specification of the surveyed diseases can be summarized as follows:

1) Leaf rust:

Leaf rust caused by Puccinia hordei Otth. appears on leaves only and seldom on stem. Uredia are small and round irregularly scattered pustules of light yellowish brown in colour. The telia are round to oblong, brown and covered by the epidermis.

2) Stem rust:

Stem rust (Puccinia graminis tritici Eriks, and Henn.) occurs on leaves and stem. Uredia are reddish-brown, while telia are black in color.

3) Net Blotch:

Net blotch (Helminthosporium teres Sacc.), the characteristic " netting " of the discolored areas on

Table (4): Developed symptoms of barley foliar diseases on seedlings.

Diseases	The first symptoms		Fully developed symptoms	
	Time (days)	Description	Time (days)	
Leaf stripe	15	(a) Yellow narrow stripes	35	Greenish black stripes.
Net blotch	3	(b) Light brown pen-point	12	Grown net-like pattern lesions, 1 x 15 mm.
Spot blotch	3	(b) Dark brown pen-point	12	Dark brown spots, 2 x 8 mm.
Leaf rust	7	(b) Flecking	14	Yellowish brown, round uredia.
Stem rust	7	(b) Flecking	15	Reddish brown, oblong uredia.
Stripe rust	8	(b) Flecking	18	Linear, citron-yellow uredia.
Powdery mildew	3	(b) Light gray pen-point	10	Gray, powdery -surfaced lesions.
Downy mildew	20	(c) Dwarf plants	45	Brown, dead tillers.
Barley stripe mosaic virus	6	(b) Chlorotic mottled stripe	45	Brown mottled stripe.
Barley Yellow Dwarf Virus	10	(b) Bright yellow blotches	40	Stunting plants with excessive tillering
Minor leaf spots	4	(b) Dark brown pen-point	12	Dark brown spots, 1 mm.

- (a) days after transplanting infected sprouted seeds.
 (b) days after inoculating seedlings (10 days-old).
 (c) days after sowing seeds in treated soil.

leaves, sheaths, glumes, spikelets and grain distinguishes this disease from the spot blotch. On seedling, the blotches are evident even on the first blade. As new blades form, these brown blotches are scattered over the surface of one or all blades of plants. The blotches are yellowish at the ends and at some time in their development show the brown "netting" in the central portions.

The mycelium is white to olivaceous and makes a very sparse tufted growth on media. Conidia usually cylindric and tapering slightly the ends. Conidia slightly constricted at the septa, subhyaline to greenish-fuliginous or yellow, 30-175 X 15-22 U, one to eleven-septate (Luttrell, 1951).

4) Spot blotch: (*Helminthosporium sativum* P.K. et B.):

Brown blotches appear on the seedling, as well as on older leaf blades, on roots, culms and glumes. Often entire blades are effected to such an extent that they curl up and cause the death of the plant.

Colonies at first white becoming brown with spore production. Conidiophores fasciculate 150-180 U long, 6-10 U in diameter, dark reddish-brown. Conidia straight or curved, tapering toward the ends, ends

rounded, divaricate 90-130 X 15-20 μ with 6-13-septate.
(Luttrell, 1951).

5) Powdery mildew (Erysiphe graminis hordei En-Marchal)

The disease develops on the epidermis of blades, leaf sheaths and floral bracts. The superficial mycelium and conidia are first light gray in color, the mycelium darkens with age and later numerous round dark perithecia develop on these areas.

The characteristic symptoms are the gray powdery surfaced lesions scattered or completely covering the leaf blade, with yellowing, browning and gradual drying out of the leaf tissue.

The conidia are light gray, ovoid, measure 25-30 X 8-10 μ and are borne in chains.

6) Downy mildew: (Sclerospora macrospora Sacc.)

The infected plants are erect, yellowish green, somewhat dwarfed. The leaves are thickened and develop in a close whorl around the culm. Many of the infected tillers turn brown and die.

The oospores are light yellow, large, about 60 μ in diameter, globose and smooth-walled (Dickson, 1956).

7) Barley Stripe Mosaic Virus (BSMV):

Seedlings showed chlorosis and chlorotic to brown stripes in the leaf blade, especially near the base, stunting and excessive tillering.

8) Barley Yellow Dwarf Virus (BYDV) :

Seedlings showed bright - yellow blotches, golden yellow developing from the leaf tip through the entire leaf blade and dark - green stripes extending into the yellow in the color - transition portion of the blade.

B) Studies on barley leaf stripe:

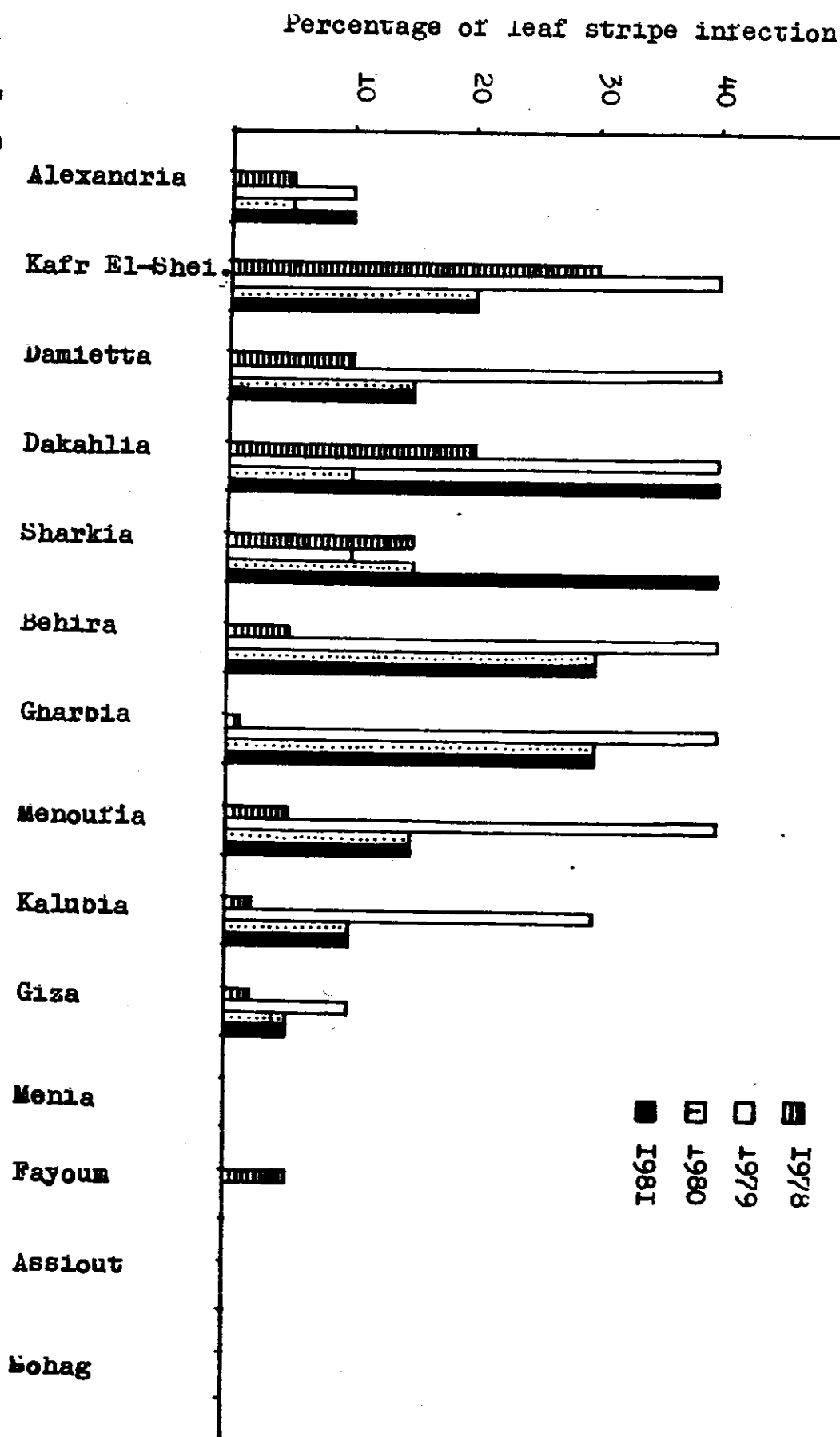
I- Distribution of barley leaf stripe in Egypt:

Percentage of stripe infection on commercial barley varieties as well as 47 varieties of trap nurseries at 14 Governorates of Egypt are shown in Table (5), and illustrated in Fig. (3). It could be concluded that barley leaf stripe disease appears heavily in the humid

Table (5): Percentage of barley stripe infection, Helminthosporium gramineum Rabh., at 14 governorates of Egypt during four years, 1978-81.

Governorates	Percentage of infection in			
	1978	1979	1980	1981
a- <u>Delta Region:</u>				
1- Alexandria	5	10	5	10
2- Kafr El-Sheikh	30	40	20	20
3- Damietta	10	40	15	15
4- Dakahlia	20	40	10	40
5- Sharkia	15	10	15	40
6- Behira	5	40	30	30
7- Gharbia	1	40	30	30
8- Menoufia	5	40	15	15
9- Kalubia	2	30	10	10
b- <u>Middle Region:</u>				
10- Giza	2	10	5	5
11- Menia	0	0	0	0
12- Fayoum	5	0	0	0
c- <u>Southern Region:</u>				
13- Assiout	0	0	-	-
14- Sohag	0	0	-	-

Fig. 3. Percentage of barley leaf stripe infection at 14 Governorates of Egypt, 1978-1981.



districts of Delta Region. Prevalence of stripe was higher at Kafr El-Sheikh, Dakahlia, Behira, Gharbia, Sharkia, Damietta, Menoufia, Kalubia and Alexandria, than at Giza and Fayoum, successively. Stripe occurred rarely in Middle Region, (Minia) and Southern Region (Assiout and Sohag).

Percentage of infection, however, varied in the different seasons, stripe disease was more distributed during 1979 than 1981, 1980 and 1978, respectively.

II- Isolation and Identification of the causal organism:

Out of 40 samples showed typical barley stripe symptoms collected from different locations, 12 isolates of Helminthosporium gramineum were obtained from Alexandria (isolate No. 1), Gharbia (No. 2), Sharkia (No. 3), Menoufia (No. 4), Behira (No. 5), Kafr El-Sheikh (No. 6 and 11), Kalubia (No. 7 and 12), Damietta (No. 8 and 9), Giza (No. 10).

Identification of isolates was conducted according to the key of Helminthosporium species reported by Luttrell (1951) depending on characters of conidiophores and conidia. Conidiophore's colour was dark brown and shiny, ranged from 100 to 300 μ and straight. Conidia were subhyaline to yellowish brown without

constrictions at the septa, straight, subcylindrical, slightly tapering towards the distal end and four septa were most common (Fig. 4). They ranged from 12 x 64 to 20 x 100 U. Germ tubes developed from terminal cells (polar) and less commonly the central cells (Fig.5).

The isolates were, also, compared with other isolates presented at Cereal Diseases Division, Inst. Plant Pathology, Giza, which have been, already, identified by the Commonwealth Mycological Inst., Kew, Surrey, England. In addition, symptoms produced on barley plants artificially inoculated with isolates, gave typical stripe symptoms.

III- Pathogenicity studies and Inoculation techniques:

The aim of this study was to determine the pathological behaviour of H. gramineum on the plants of susceptible barley variety Giza 119, using several techniques of artificial inoculation. When plants were sprayed with mycelial and spore suspension at successive plant ages, tiny brown flecks appeared on leaves, but never expanded further. Barley seeds, soaked in mycelial suspension or sprouted on cultures of H. gramineum, as well as that planted in artificially infested soil, developed seedlings with typical leaf stripe (Fig. 6).

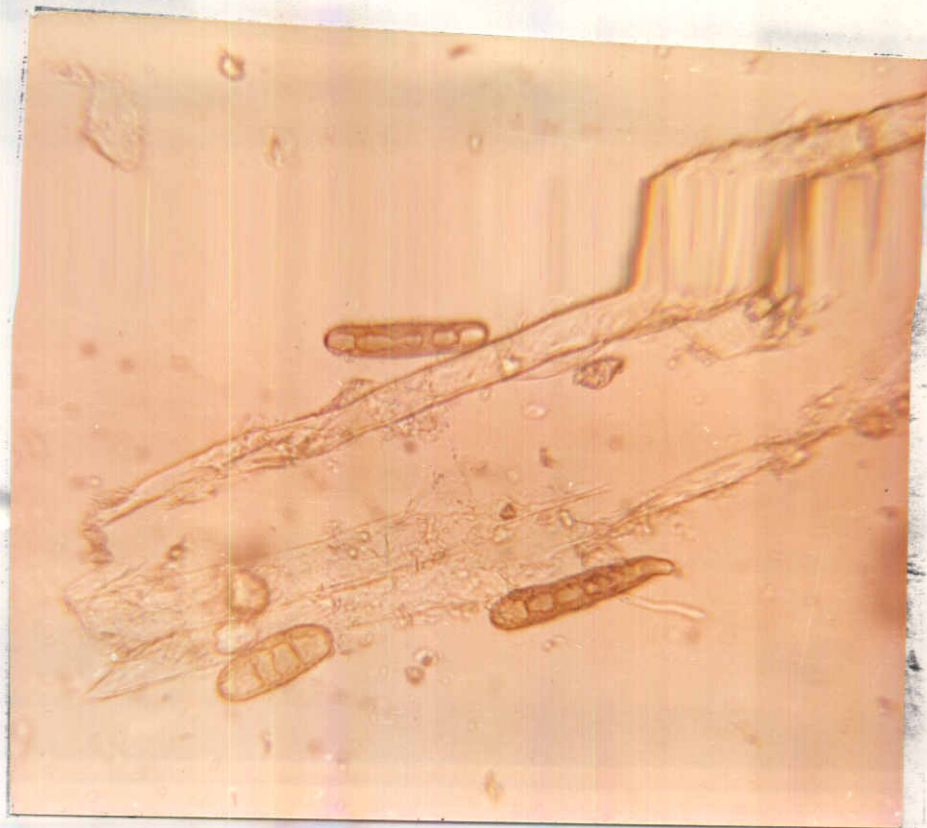


Fig. (4): Microscopic photograph showing structure of Helminthosporium gramineum conidia. X 250.



Fig. (5): Microscopic photograph illustrating development of germ tube (G) of terminal cell in H. gramineum conidia. X 250.



C

I

2

3

4

Fig. (6): Development of stripe typical symptoms on barley leaves.

C. Healthy leaf.

1. Yellow narrow stripes.

2. Clearly visible yellow stripes.

3. Brownish yellow stripes.

4. Greenish-dark brown stripes with a powdery appearance of developed conidia.

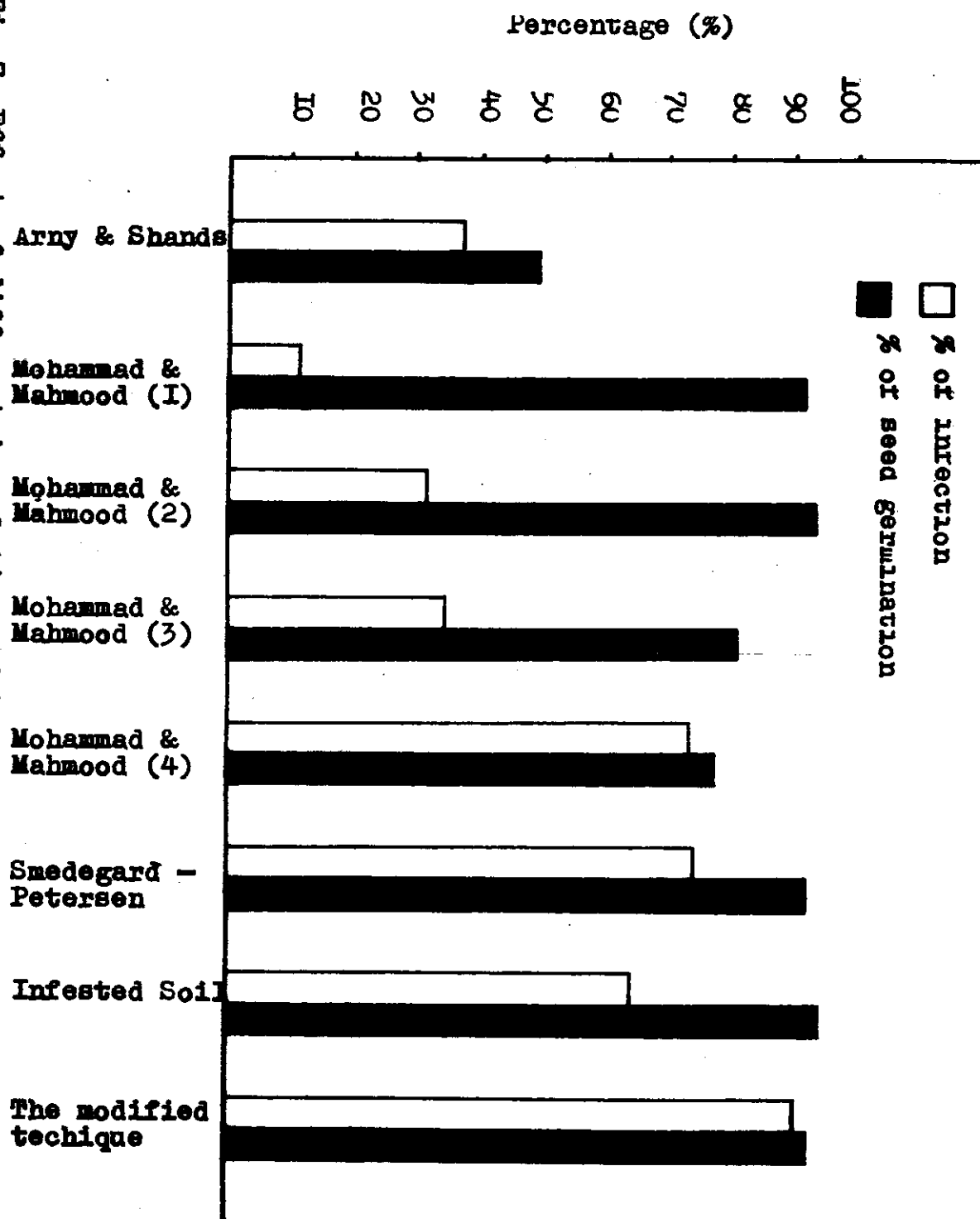
Symptoms started as yellow narrow stripes at the base of leaves, then longitudinally extended towards their tips and were clearly visible after 3-4 weeks. Those stripes turned brownish and became shredded with age. Finally they turned greenish-dark brown and showed a powdery appearance of developed conidia. Striped plants dried up gradually as they became older.

The various techniques of inoculation, however, resulted in different percentages of infection as shown in Table (6) and illustrated in Fig. 7. It is obvious that using the modified technique, gave the highest percentage of stripe infection, 90%, followed by 74.3% with Smedegard-Petersen, and 72.8% with Mohammad & Mahmood-4 technique. In the modified technique, healthy seeds were sprouted on an actively 8 days-old mycelial culture for ten days then transferred with culture and sown, whereas Smedegard-Petersen sprouted seeds for eight days and transferred without culture. In Mohammad & Mahmood-4 technique, dehulled seeds were sprouted in - between two layers of mycelial culture for 60 hrs and sown. The other percentages of infection may be arranged in a descending order as follows: sowing seeds in infested soil with, 64%; Army & Shands technique with 37.0% where seeds, which were mixed with fungus grows on autoclaved wheat grains and sown, the percentage

Table (6): Effect of different inoculation techniques on seed germination and barley leaf stripe infection.

No.	Inoculation techniques	Seed germination %	Infection %
1	Army and Shands	49.3	37.0
2	Mohammad and Mahmoud -1	92.5	11.8
3	Mohammad and Mahmoud -2	93.5	30.5
4	Mohammad and Mahmoud -3	80.5	34.0
5	Mohammad and Mahmoud -4	77.0	72.8
6	Smedegard-Petersen	92.3	74.3
7	Infested soil	94.3	64.0
8	The modified technique	92.3	90.0
<hr/>			
L.S.D. at 5%		5.3	17.8
1%		7.2	24.2

Fig. 7. Effect of different inoculation and barley leaf stripe infection.



of infection decreased to 34.0% in Mohammad & Mahmood-3 technique where hulled seeds were sprouted in between two layers of mycelial culture for 60 hrs. whereas gave 30.5% with Mohammad & Mahmood-2 technique, where dehulled seeds were immersed in conidial and mycelial suspension for 60 hrs and finally were 11.8% in Mohammad & Mahmood-1 technique, where hulled seeds were immersed in suspension for 60 hrs. Data in the same table showed that Army and Shands technique gave the lowest percentage of seed germination, whereas techniques No. 7, 3, 2, 6 and 8 could induce high percentage of seed germination, respectively.

IV- Variability in *Helminthosporium gramineum*:

Greenhouse and laboratory experiments were designed to study the pathogenicity of twelve isolates of *H. gramineum* on 11 barley varieties, as well as cultural and physiological characters of those isolates.

1- Variation of pathogenicity:

The tested eleven varieties were selected because of their different reactions to *H. gramineum* in various countries as several investigators have mentioned. Varietal reactions are shown in Table (7). It is clear that pathogenicity of tested isolates is completely different. Isolate No. 4 is considered the most

Table (7): Reactions of eleven barley varieties to 12 Egyptian isolates of Helminthosporiumgramineum.

No. Varieties	C.I. No.	Isolates No. of <u>H. Gramineum</u>											
		1	2	3	4	5	6	7	8	9	10	11	12
1 Nepal*	595	0	0	0	5	0	0	5	0	0	0	0	0
2 Kanchen	531	0	0	0	3	5	0	5	5	0	0	5	5
3 Abyssinian	1243	0	0	0	5	0	0	0	0	0	5	5	0
4 Tion	923	0	5	0	5	5	0	5	0	5	5	5	5
5 Excelsior	1248	0	5	0	5	5	0	5	0	5	5	5	5
6 Quinn	1024	5	5	0	5	0	0	5	0	5	5	0	5
7 Cutnml	1413	0	0	0	5	0	0	5	5	5	0	5	5
8 Trebl	936	-	5	0	5	-	0	5	5	5	0	5	5
9 Odessa	934	5	3	0	5	5	0	5	5	5	5	5	5
10 Black Tailless	1277	5	3	5	3	5	3	5	5	5	5	5	5
11 Oderbrucker	940	5	3	5	5	5	0	5	5	5	5	5	5

* Isolate (1): Alexandria (2): Charbia (3): Sherkia (4): Menoufia
 (5): Reitra (6 and 11): Kafr El-Sheikh (7 and 12): Kalubia
 (8 and 9): Damietta (10): Giza.

virulent isolate, it infected all inoculated varieties showing susceptible reactions. Most of those varieties were infected by isolate No. 7 with exception of the variety Abyssinian which showed resistant reaction. On the other hand, isolate No. 6 infected, only, one variety, i.e., Black Hulless. Two different varieties showed resistance against both isolates No. 11 and 12, whereas isolates No. 2, 5 and 10 failed to infect four varieties. Total 3, 5, 6 and 9 varieties were, respectively, resistant to isolates No. 9, 8, 1 and 3. Black Hulless was susceptible to all tested isolates, followed by Oderbrucker which showed resistance to isolate No. 6.

2- Variation of cultural characteristics:

The tested isolates were allowed to grow on PDA medium for ten days. Four cultural characteristics were comparatively studied and data are shown in Table 8. The isolates of H. gramineum gave different cultural characters, thus they could be classified into specialized cultural groups. Four isolates showed high rates of mycelial growth, whereas seven ones were moderate and only one isolate slowly grew.

With regard to the nature of mycelium, nine isolates were fluffy, and three were velvety. Colony's colour was mostly gray with the exception of four olivaceous isolates (Fig. 8).

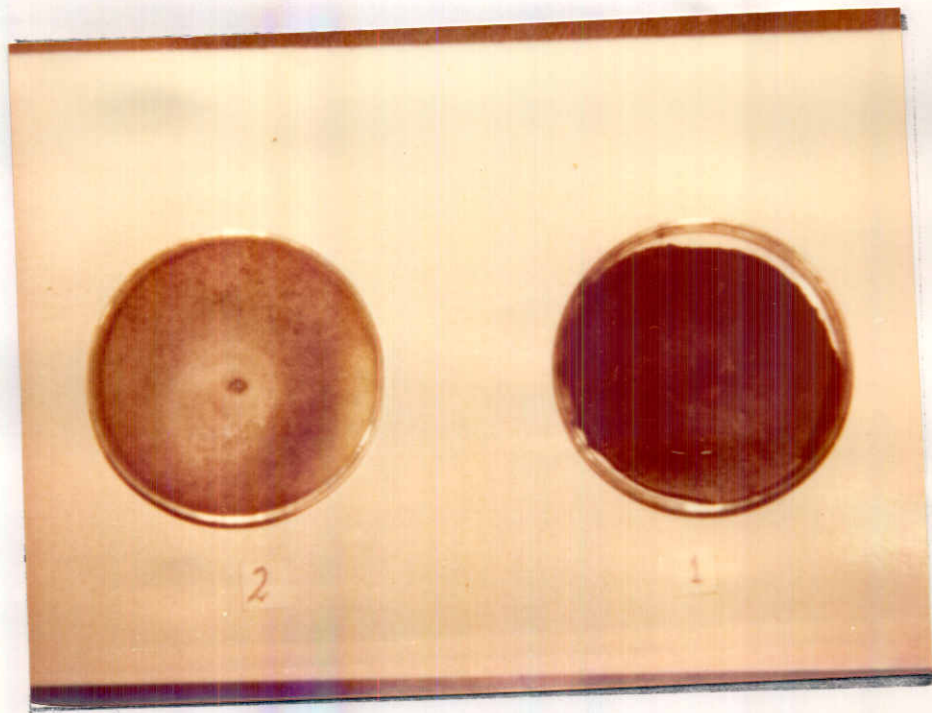


Fig. (8): Nature of mycelium and colour of Helminthosporium gramineum isolates on PDA medium.

1. Velvety olivaceous mycelium.
2. Fluffy gray mycelium.

Pigmentation were only noticed in three and four isolates, respectively.

3- Variation of physiologic characters:

Three different physiologic characters of H. gramineum isolates were studied to detect the variation among these isolates:

a) The activities of oxidative enzymes:

Changes of activities of polyphenol oxidase, peroxidase, catalase and ascorbic acid oxidase in supernatants of trituated matrix of 12 isolates were measured as absorbance changes per one minute ($\Delta A/\text{min.}$). Changes of polyphenol oxidase activity were entirely absent in all isolates.

Data obtained in Table (9) show the changes of activities of peroxidase, catalase and ascorbic acid oxidase. All tested isolates of H. gramineum showed changes in both catalase and ascorbic acid oxidase activities, whereas no changes were observed, in peroxidase activity of four isolates (Fig. 9). Change of ascorbic acid oxidase activity, was higher than that of peroxidase and catalase. Generally, isolates of H. gramineum may be classified into, at least, three categories; high, moderate and low or no changes of activities of peroxidase, catalase and ascorbic acid oxidase.

Table (9): Changes of peroxidase, catalase and ascorbic acid oxidase activities per one minute, measured in mycelial extracts of Helminthosporium gramineum isolates.

Isolates No.	Absorbance changes (ΔA / min.)		
	Peroxidase	Catalase	Ascorbic acid oxidase
1	0.014	0.004	0.045
2	0.021	0.007	0.045
3	0.007	0.002	0.045
4	0.008	0.007	0.030
5	0.010	0.004	0.055
6	0.000	0.004	0.080
7	0.000	0.008	0.025
8	0.006	0.007	0.035
9	0.000	0.007	0.040
10	0.002	0.007	0.070
11	0.000	0.006	0.040
12	0.010	0.007	0.040

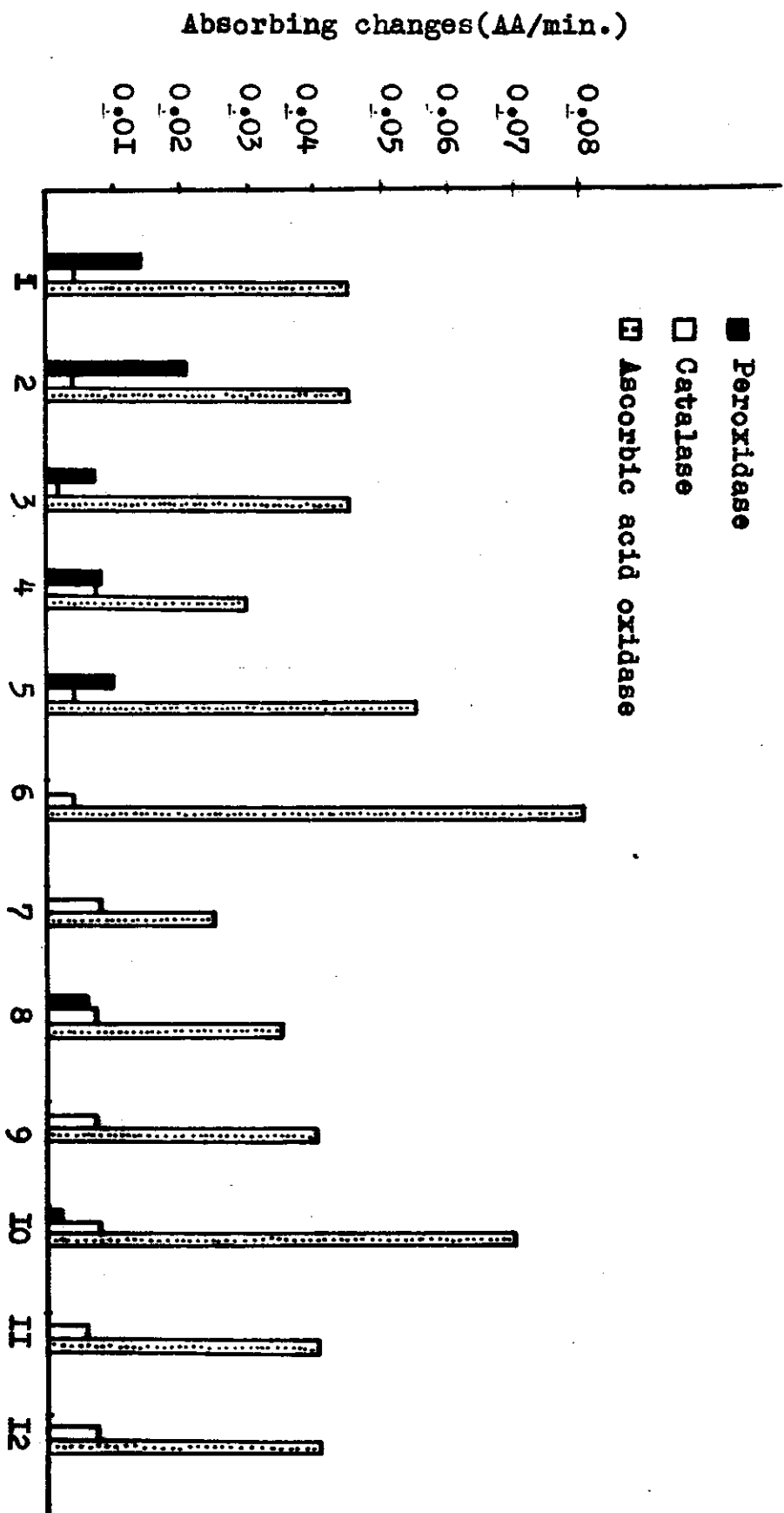


Fig.9. Changes of peroxidase , catalase , and ascorbic acid oxidase activities per one minute , measured in mycelial extracts of *H.Gramineum* isolates .

b) Production of pectolytic and cellulolytic enzymes:

Ability of the tested isolates of H. gramineum to produce cellulase and polygalacturonase were measured by estimating percentage of loss in viscosity of 1.2% pectin or CMC solution per five minutes during an incubation period with cultures filtrates at pH = 5.6 for 20 minutes. Pectin methyl estrase production was expressed as the mean values of 0.01N. sodium hydroxide required to neutralize the carboxylic groups produced of 1.2% pectin solution after 24 hrs. of mixing solution with equal amounts of the cultural filtrates.

Data obtained are tabulated in Table (10) and illustrated in Fig. (10). It is obvious that the isolates have different activities of cellulolytic and pectolytic enzymes. Data, however, indicate that it is not difficult to arrange those isolates in three groups. It is suggested to be one group contains the isolates which were highly active, another one of moderate, and the third group, is that isolates which produce low or not detectable amounts of pectolytic and cellulolytic enzymes, i.e., PG, PME and CX.

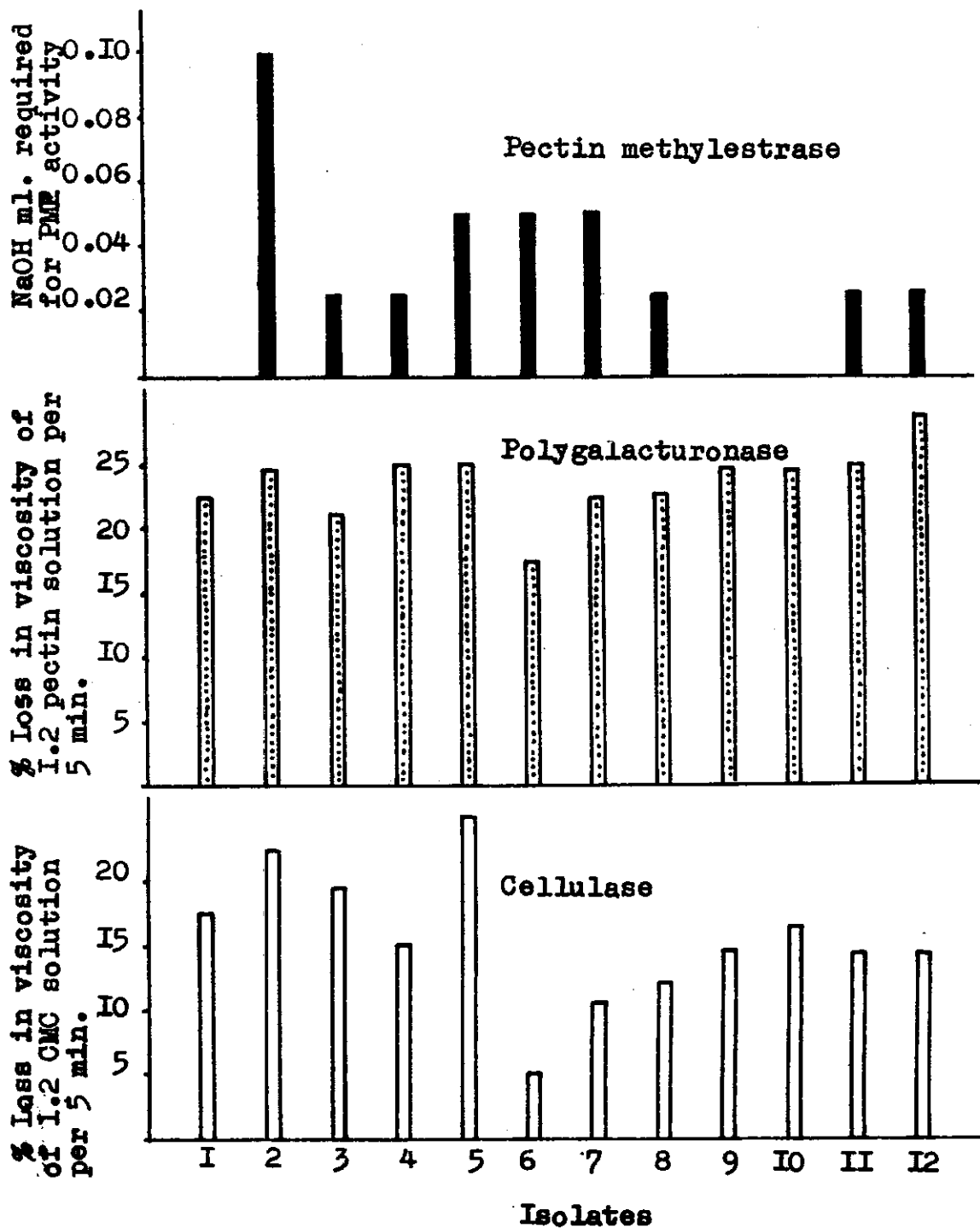


Fig. 10. Activity of cellulolytic and pectolytic enzymes in culture filterates of 12 isolates of *H. gramineum*.

c) Effects of cultural filtrates on barley seed germination and seedlings morphology:

The aim of this experiment is to find if there are any drastic effects of fungal filtrates of the tested isolates of H. gramineum on the percentage of germination of barley seed and seedling morphology.

Data, in Table (11) and Fig. (11), show that percentage of seed germination was 83% in case of untreated seeds. A drastic decrease was recorded as a result of application of isolates filtrates where two isolates gave less than 25%, one isolate resulted more than 50% and the rest isolates gave 25 to 50% seed germination. Similar results were obtained concerning both radicles and plumules length, where they decreased to be less than 10 cm and 5 cm respectively. Length of radicle and plumules were 61.3 and 65.5 cm in case of untreated barley seeds.

V- Host range of Helminthosporium gramineum:

Studies on host range of H. gramineum showed that Hordeum vulgare and H. distichon were susceptible showing characteristic disease symptoms (Table 12), while Triticum durum and Sorghum vulgare var gudanesse were slightly affected. However, the other species were free from symptoms. These results indicated that this fungus has a narrow host range under the Egyptian conditions.

Table (11): Effect of culture filtrates of 12 isolates of H. gramineum on seed germination and length of both radicle and plumule of barley seedlings.

Isolates	Seed germination (%)	Radicle length (cm.)	Plumule length (cm.)
1	12.0	1.8	0.4
2	45.8	9.8	3.7
3	52.5	5.0	4.4
4	46.3	4.8	3.3
5	36.5	5.2	3.1
6	35.0	5.0	2.8
7	32.0	3.4	3.5
8	35.0	6.4	2.5
9	30.0	4.1	3.0
10	23.0	3.5	2.2
11	39.0	3.5	3.0
12	32.0	1.8	3.5
Control	83.0	61.3	65.5

L.S.D.			
at 5%	20.6	6.7	1.7
1%	27.7	8.9	2.3

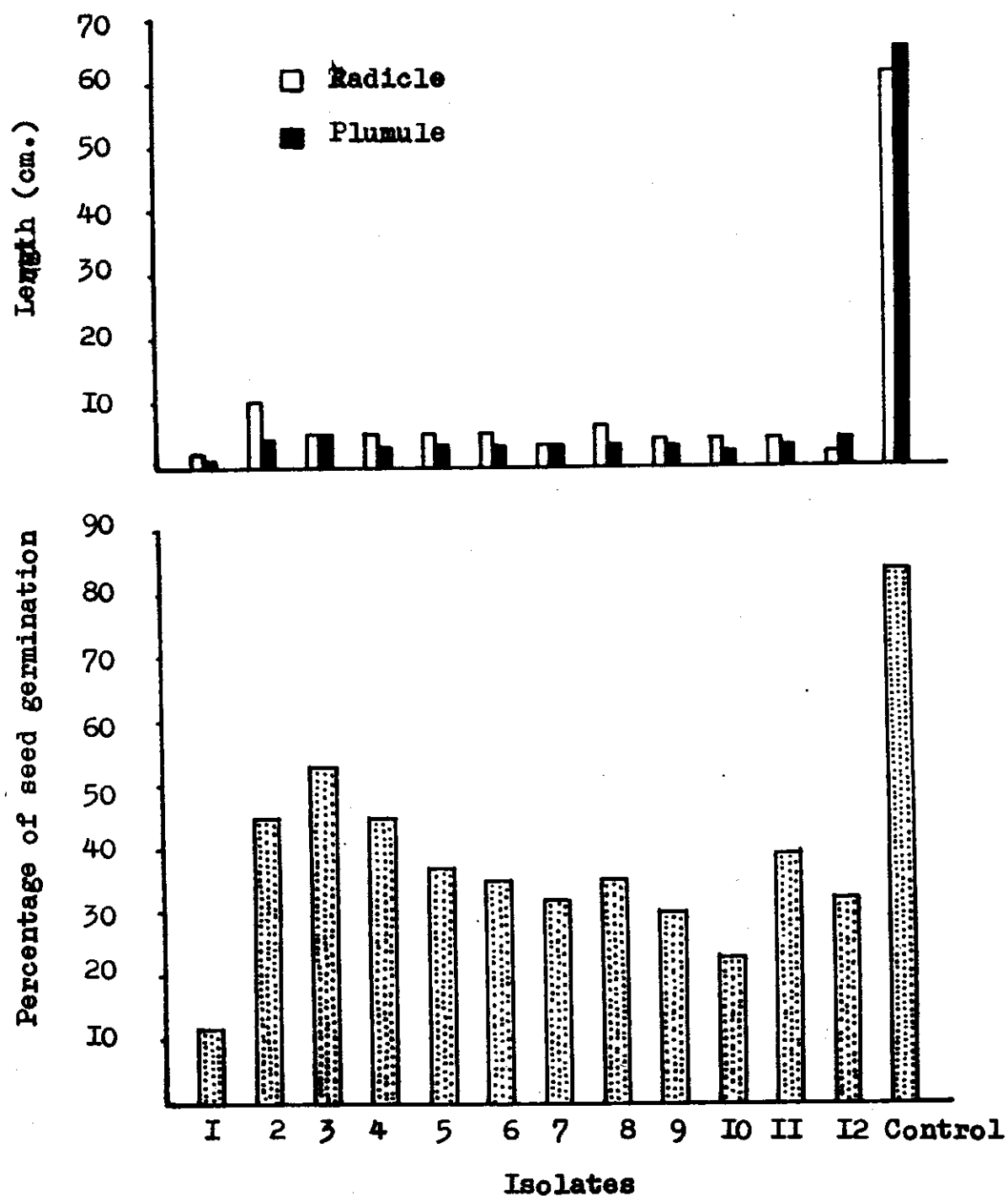


Fig.II. Effect of culture filtrates of 12 isolates of *H.graminum* on seed germination and length of both radicle and plumule of barley seedlings.

Table (12): Host range of Helminthosporium
gramineum.

No.	Genera of Gramineaceae	Infected plants
1	<u>Hordeum vulgare</u> L.	+ ⁺
2	<u>Hordeum distichon</u> L.	+
3	<u>Triticum durum</u> Vill.	+
4	<u>Triticum vulgare</u> Desf.	- ⁺⁺
5	<u>Oryza sativa</u> L.	-
6	<u>Zea mays</u> L.	-
7	<u>Sorghum vulgare</u> Pers.	-
8	<u>S. vulgare</u> var. <u>sudanese</u> Hit.	+
9	<u>Avena sativa</u> L.	-
10	<u>Secale cereale</u> L.	-
11	<u>Arrhenatherum elatius</u> L.	-
12	<u>Phalaris paradoxa</u> L.	-
13	<u>Phalaris tuberosa</u> L.	-
14	<u>Cynodon dactylon</u> L.	-

+⁺ Typical disease symptoms were produced

-⁺⁺ No symptoms observed.

VI- Effect of leaf stripe infection on chemical composition and the relationship between disease development and the chemical components:

Different compounds and changes of oxidative enzymes activities were determined in the first leaf of barley seedlings of the susceptible variety Giza 119, after 2, 4, 7 and 11 days of inoculation, representing four different stages of inoculation, i.e., beginning of infection, flecking, appearance of tiny brown spots and maximum size of spots, respectively.

Sugars, amino acids and phenols, as well as changes of activities of polyphenol oxidase, peroxidase, catalase and ascorbic acid oxidase were determined.

1. Determination of sugars:

Total, reducing and non-reducing sugars were quantitatively determined as mg equivalent of glucose per 100 g fresh weight, using a constructed standard curve. Data in Table (13) and Fig. (12) indicated that total, reducing and non reducing sugars were reduced as a result of fungal infection. The development of total sugar contents was, generally, fluctuated, then returned to resemble the first situation seven days after inoculation, whereas at late stage (11 days after inoculation), sugar contents were more higher in healthy

Table (13): Total, reducing and non-reducing sugars mg/100 g fresh weight of inoculated and uninoculated barley seedlings vr. Giza 119 after 2, 4, 7 and 11 days of inoculation with Helminthosporium gramineum.

Days After Inoculation	Sugar contents (mg/100 g fresh weight)					
	Total		Reducing		Non-reducing	
	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated
2	168.5	331.2	112.5	262.5	46.0	68.7
4	168.5	168.5	108.8	75.0	59.7	93.5
7	225.0	225.0	150.0	140.0	75.0	75.0
11	185.0	293.8	112.0	184.0	73.0	109.8

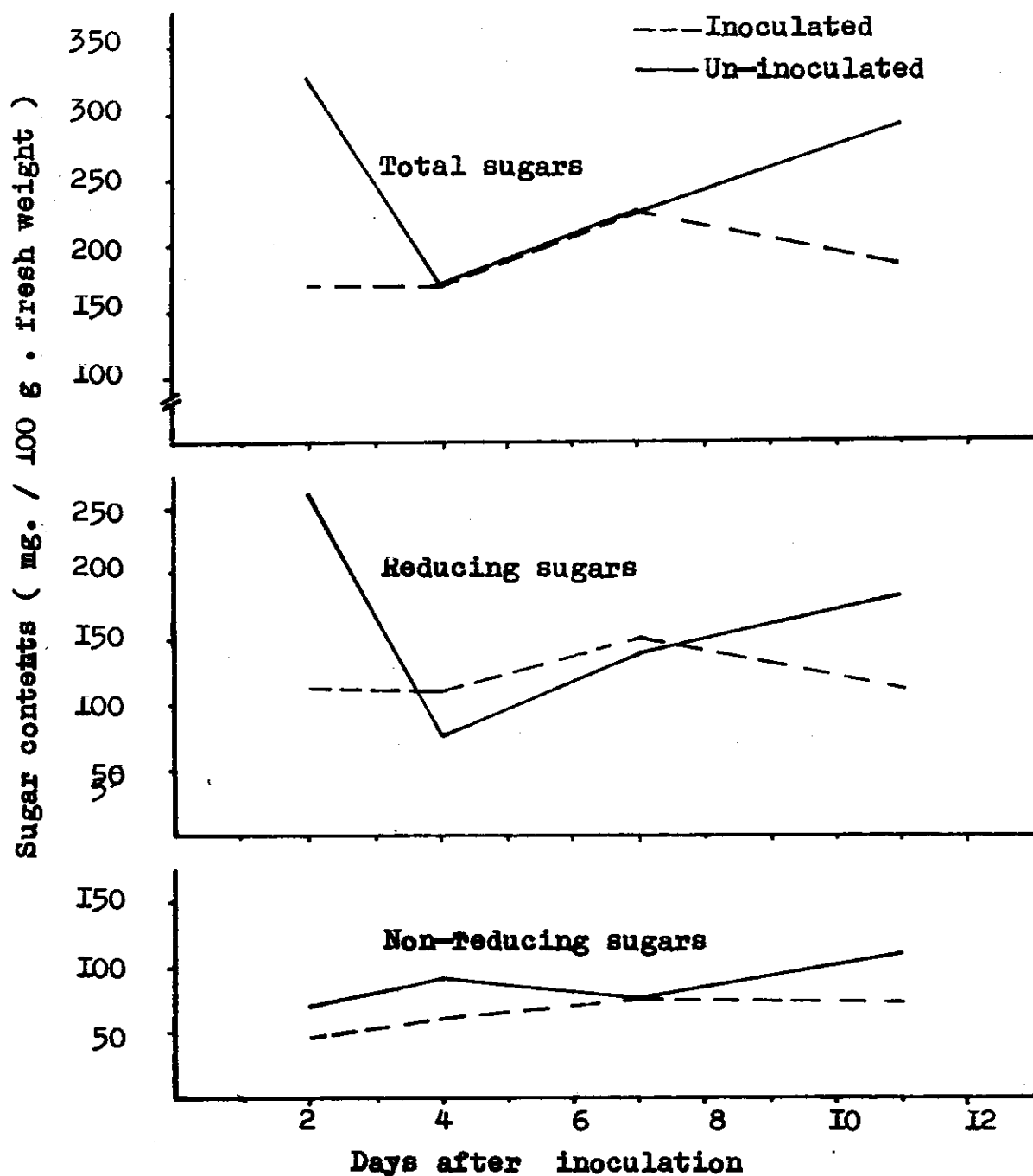


Fig.12. Total ,reducing and non-reducing sugar content mg/ 100g fresh weight of inoculated and un-inoculated barley seedlings with H.gramineum .

samples than in infected ones. During the flecking stage, reducing sugars increased in infected plants compared to that of healthy ones, whereas non-reducing sugars decreased, simultaneously.

2. Determination of free amino acids:

Total free amino acids were determined as mg per 100 g fresh weight, using the modified colorimetric ninhydrin method. Data presented in Table (14) and Fig. (13) show that the first leaf of inoculated barley seedlings contains total free amino acids less than those of uninoculated ones, two days after inoculation. During the next stage (four days after inoculation) amino acids started to increase, then gradually decreased (seven days after inoculation). Finally, concentrations of amino acids were found to be more in infected leaves than in healthy ones (11 days after inoculation).

Individual seventeen amino acids, i.e., cystine, lysine, histidine, arginine, aspartic acid, serine, glycine, glutamic acid, threonine, alanine, proline, tyrosine, methionine, valine, phenyl alanine, leucine and isoleucine, were quantitatively determined, using paper chromatography, seven and eleven days after inoculation, when development of infection seemed to be stopped. A modification of standard curves was adopted, using

Table (14): Total free amino acids mg/100 g
fresh weight of inoculated and
uninoculated barley seedlings vr.
Giza 119 after 2, 4, 7 and 11 days
of inoculation with Helminthosporium
gramineum.

Days after inoculation	Total free amino acids (mg/100 g) fresh weight)	
	Inoculated	Uninoculated
2	925	1075
4	1050	1025
7	750	850
11	1050	750

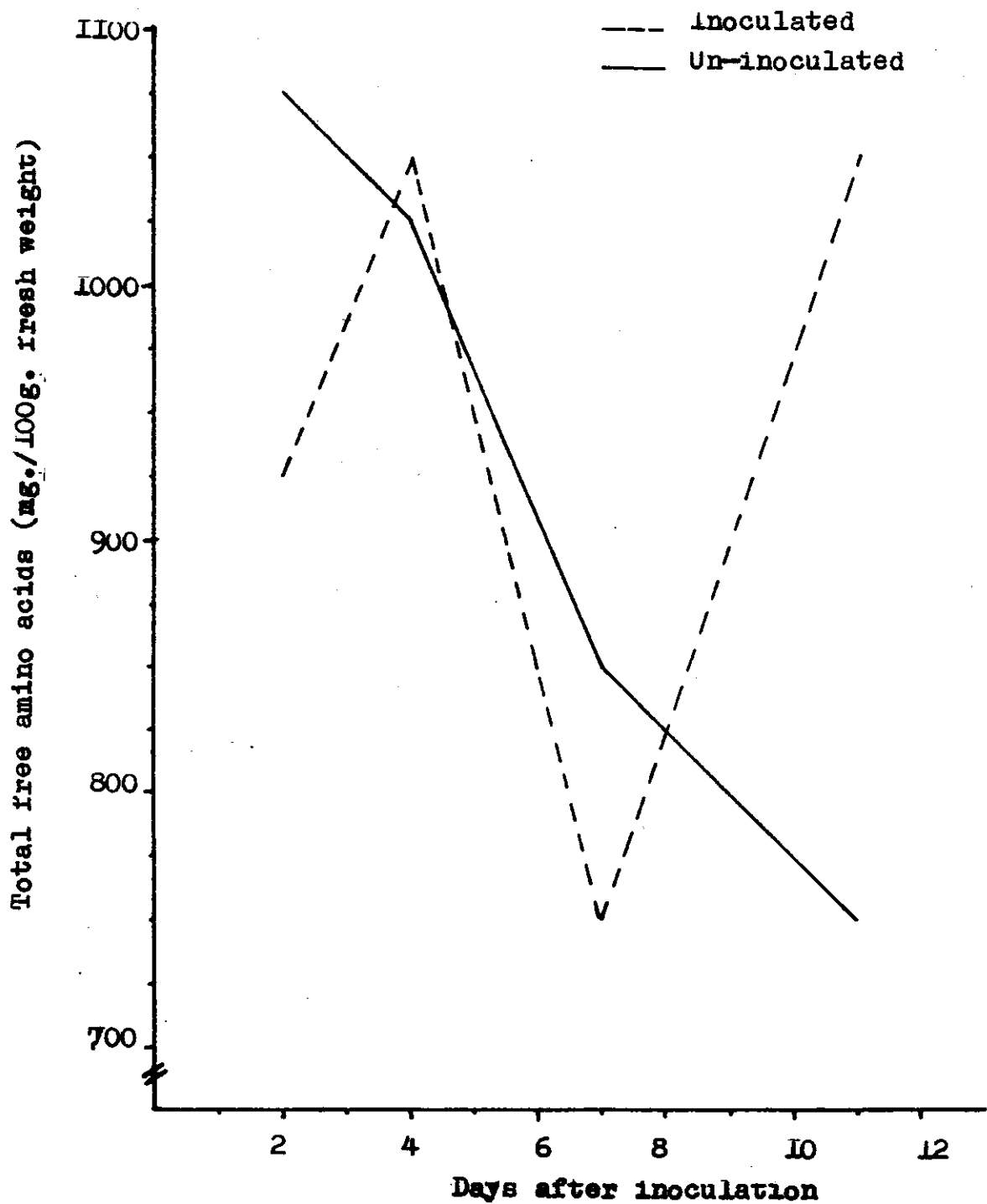


Fig.13. Total free amino acids mg./100g. fresh weight of inoculated and un-inoculated barley seedlings with *H.gramineum*.

special standard curve for each detected amino acid instead of one for all (leucine).

Amounts of amino acids as mg/100 g fresh weight are shown in Table (15) and Fig. (14). Concentrations of amino acids varied according to their kinds and stages of infection. Arginine showed the lowest concentration (seven and ten mg), respectively after seven and 11 days of inoculation, in the infected samples; whereas alanine was the highest one (100 and 192 mg). In the healthy samples, alanine showed the same situation (170 and 125 mg), whereas cystine and arginine, showed the lowest concentration (22.5 and 15 mg). Seven amino acids, i.e., cystine, tyrosine, methionine, valine, phenyl alanine, leucine and isoleucine, were higher in infected samples than in healthy ones (seven days after inoculation). Alanine, proline, methionine and valine as well as total amounts of aspartic acid, serine and glycine, and that of both glutamic acid and threonine, were also more concentrated in infected samples than in healthy ones (11 days after inoculation).

Concerning the development of amino acids concentrations, seven to 11 days after inoculation, two amino acids i.e., cystine and lysine were found to gradually decrease in both infected and healthy samples.

Table (15): Individual free amino acids mg/100 g fresh weight of inoculated and uninoculated barley seedlings vr. Giza 119 after 7 and 11 days of inoculation with H. graminum.

Individual amino acids	Amino acids contents (mg/100 g fresh weight)			
	7 days after inoculation		11 days after inoculation	
	Inoculated	Uninoculated	Inoculated	Uninoculated
1- Cysteine	40.0	10.5	10.5	15.0
2- Lysine	30.0	47.5	12.5	22.5
3- Histidine	45.0	77.5	70.0	85.0
4- Arginine	7.0	45.0	10.0	27.0
5- Aspartic acid				
+				
6- Serine	150.0	200.0	150.0	125.0
+				
7- Glycine				
8- Glutamic acid				
+	90.0	100.0	165.0	95.0
9- Threonine				
10- Alanine	100.0	170.0	192.0	125.0
11- Proline	60.0	80.0	117.5	82.0
12- Tyrosine	42.0	25.0	15.0	30.0
13- Methionine	72.5	70.0	117.0	97.5
14- Valine	95.0	80.0	95.0	92.5
15- Phenyl alanine	90.0	35.0	25.0	40.0
16- Leucine				
+	60.0	37.5	87.5	92.0
17- Iso-Leucine				

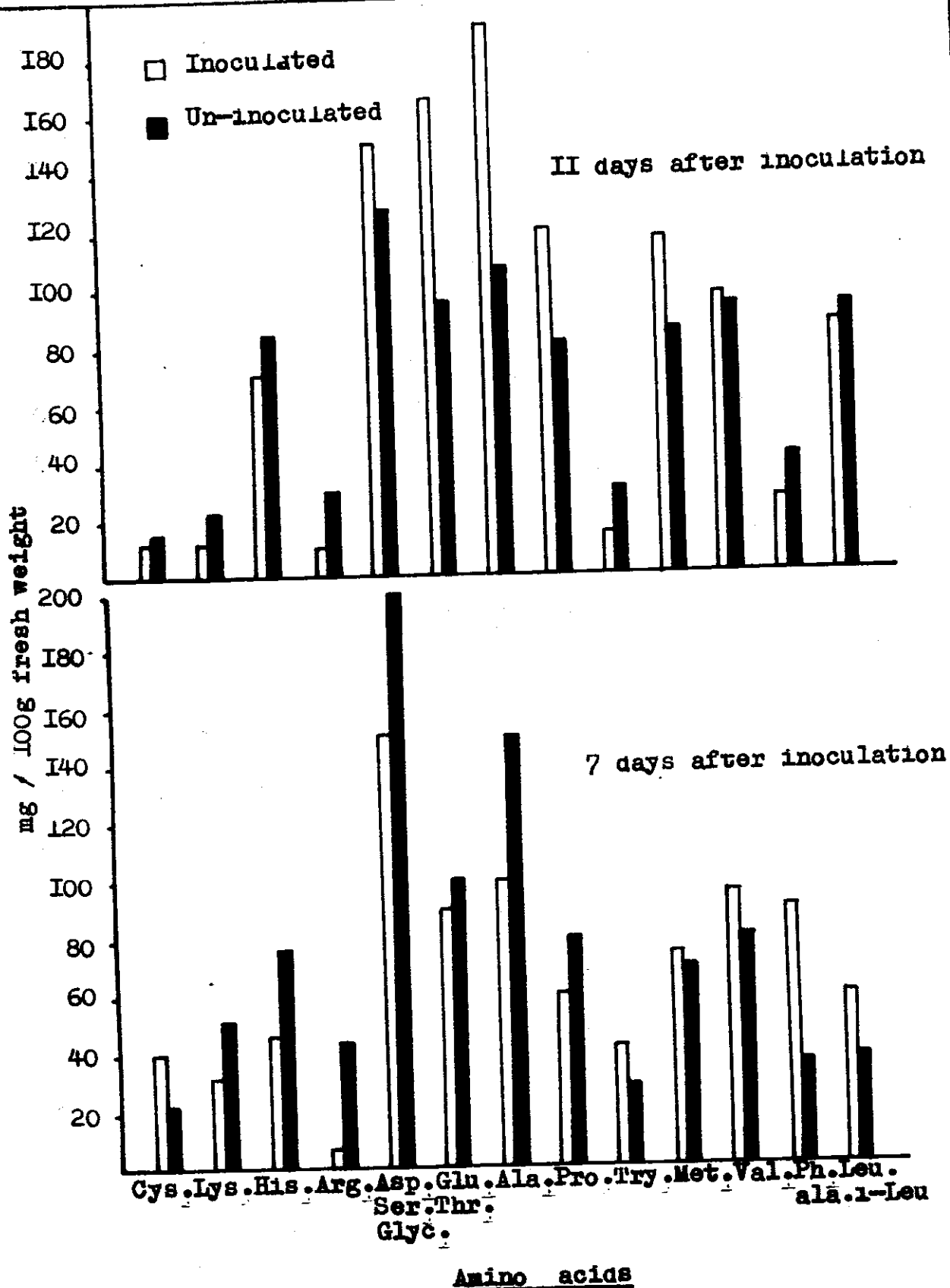


Fig. I4. Individual free amino acids mg/g fresh weight of inoculated and un-inoculated barley seedlings after 7 days and 11 days of inoculation with *H. gramineum*.

On the contrary, five amino acids, i.e., histidine, proline, methionine and total amounts of both leucine and isoleucine increased. Concentrations of valine and total amount of aspartic acid, serine and glycine, were equal in infected samples, but valine increased and the other amino acids decreased in healthy samples. The detectable aromatic amino acids, tyrosine and phenyl alanine, decreased in infected samples, and simultaneously increased in healthy ones, whereas arginine, alanine and total amount of glutamic acid and threonine increased and decreased in infected and healthy samples, respectively.

3. Determination of phenolic compounds:

Total, free and conjugated phenols, as well as ortho-dihydroxy phenols, were determined as mg equivalent of chlorogenic acid per 100 g fresh weight of leaves using standard curve. The obtained data are presented in Table (16) and illustrated by Fig. (15).

Total and free phenols were higher in inoculated leaves than that in uninoculated ones, whereas conjugated and ortho-dihydroxy phenols were lower, two days after inoculation. Maximum concentrations of phenolic compounds were generally recorded after four days in infected plants then reduced, comparing with that in

Table (16): Total, free, conjugated and ortho dihydroxy phenol contents mg/100 g fresh weight of inoculated and uninoculated barley seedlings vr. Giza 119 after 2, 4, 7 and 11 days of inoculation with H. Graminis.

Days after inocul- ation	Phenolic compounds (mg/100 g fresh weight)									
	Total		Free		conjugated		ortho dihydroxy phenol			
	In.*	Un.*	In.	Un.	In.	Un.	In.	Un.		
2	99	90	30	20	69	70	3.0	3.5		
4	180	66	70	25	110	41	2.5	1.9		
7	91	64	70	50	21	14	3.0	2.0		
11	88	60	50	55	38	5	4.8	2.0		

* In. = inoculated, Un. = uninoculated seedlings.

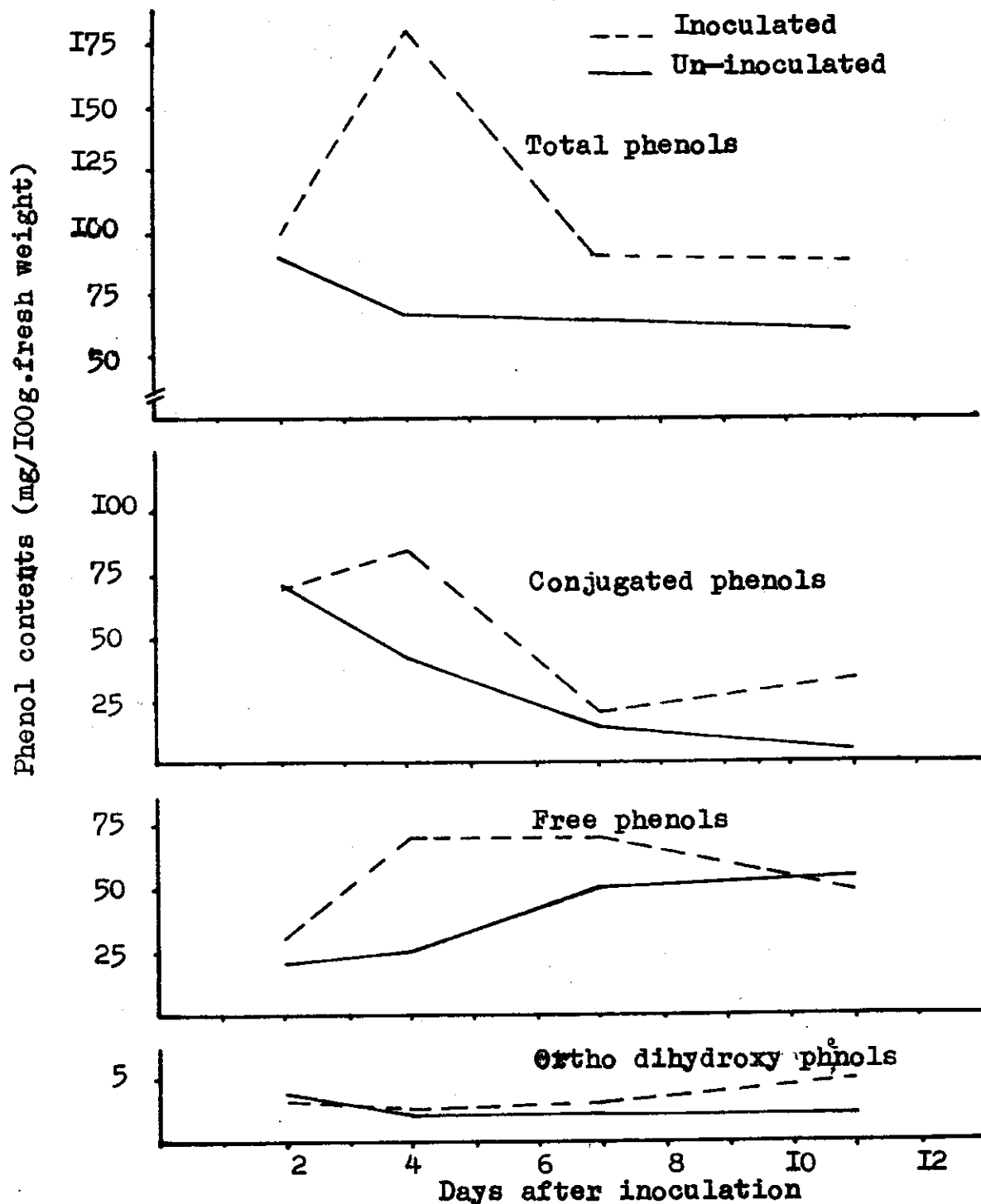


Fig.15. Total, free, conjugated, and orthodihydroxy phenols mg./100g. fresh weight of inoculated and un-inoculated barley seedlings with *H.gramineum*.

Table (17): Changes of activity of peroxidase, catalase and ascorbic acid oxidase per one minute, measured in inoculated and uninoculated barley seedlings var. Giza 119 after 2, 4, 7 and 11 days of inoculation with H. Graminum.

Days after inocula- tion	Absorbance changes ($\Delta A / \text{min.}$)					
	Peroxidase		Catalase		Ascorbic acid oxidase	
	Inoculated	Un- inoculated	Inoculated	Un- inoculated	Inoculated	Un- inoculated
2	0.080	0.020	0.005	0.005	0.023	0.019
4	0.020	0.090	0.008	0.005	0.000	0.023
7	0.011	0.030	0.010	0.009	0.000	0.000
11	0.007	0.000	0.000	0.000	0.000	0.007

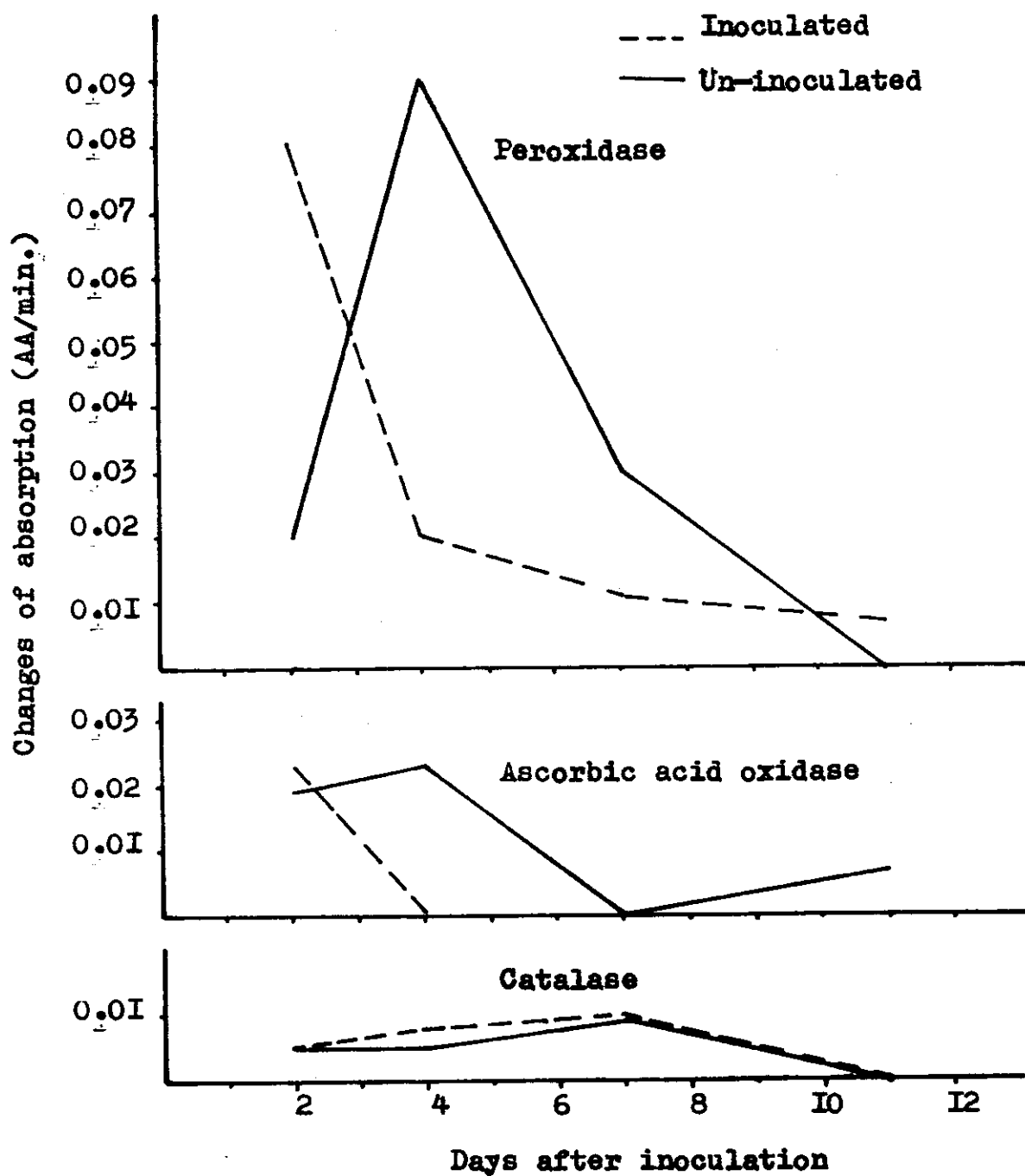


Fig.16. Changes of activity of peroxidase , catalase, and ascorbic acid oxidase per one minute ,measured in inoculated and un-inoculated barley seedlings with *H.gramineum*.

c) Catalase:

Slight increase was recorded in infected tissues compared to those of healthy ones, only after four and seven days of inoculation.

Development of activity changes, however, was, almost, similar in both inoculated and uninoculated samples.

d) Ascorbic acid oxidase:

Although the inoculated samples showed a high change of ascorbic acid oxidase activity, two days after inoculation, activity was absent afterwards. Change of activity, simultaneously increased in uninoculated samples and was found to be maximum, four days after inoculation, and reduced afterwards.

VII- Disease Control:

1- Varietal resistance:

This study was undertaken chiefly to assist plant breeders in choosing suitable material and in developing resistant varieties. Screening of the tested 100 barley varieties and crosses selected from various varietal collections under both artificial inoculation and natural infection (Table 18) indicated that 43 varieties and crosses showed immunity (score = 0) and five exhibited the score (1). Simultaneously, two and eleven varieties and crosses showed scores 2 and 3, respectively. In addition, 17 gave the score (4), while 22 varieties and crosses showed the score (5).

It can be concluded that the 48 varieties and crosses which gave resistant reactions can be used as resistant parents in breeding program for developing new resistant varieties.

Table (18): Reactions of 100 barley varieties and crosses to leaf stripe, H. gramineum.

No.	Varieties or cross and pedigree	C. I. No.	Source	Reaction
1	Beecher	6566	USDA	0
2	Bonus	11308	Sweden	0
3	Dayton	9517	PON	0
4	Deir Alla 103		Jordan	0
5	Giza 121		Egypt	0
6	Tequila		Mexico	0
7	Gorgan		Iran	0
8	Masurka		IBON (Portugal)	0
9	Proctor		RDISN	0
10	Ratana		RCB	0
11	Tokak	8655	Turkey	0
12	Trikedritt		RDISN	0
13	Union	1180	England	0
14	Vijay		RDISN	0
15	Yercil		RDISN	0
16	Coho-Zephyr		IBON	0
17	Vanguard-julia x Zephyr		IBON	0
18	Apam-Athenais x Godiva		IBON	0
19	CM 67 - Jet		IBON	0
20	Bussell		RDISN	0
21	Api-Benton		IBON	0
22	Ungi-Api ³ x 5107		RDISN	0
23	api-CM 67 x D 171		RDISN	0
24	SI-70/22385		RDISN	0
25	11012-2-Impala x Birence		IBON	0
26	Apam - 5106		IBON	0
27	Mezquite. P 71386		IBON	0
28	Apam -IB 65 x 11012.2		IBON	0
29	Api-CM 67 (For/Aprs-SV 02109 x Mari)		Mexico	0
30	H. 272 - 11012.2		IBON	0
31	M 67. 18. M 14 x 5106		IBON	0
32	As 46 - Kristina/apam - Dwarf		Mexico	0
33	NS 36		Mexico	0
34	Nopal 'S'		Mexico	0
35		4466	Mexico	0
36	Cheveron	1111	Switzerland	0
37	11012.2 - CM 67 x Por - n. SaSK 1800		Mexico	0
38	Sahuaro - Shikoku Hadaka x 5106		IBON	0
39	(Cq-Co mun x Apam x 12410) x Giza 134 - 2 L.		Turkey	0
40	Gr. 289/53/2 (Giza 117 x FAO. 86)		Egypt	0
41	Gr. 362/94 (Giza 117 x FAO. 78) x (Giza 118 x RR 801)		Egypt	0
42	Gr. 42517 (Giza 121 x C.I. 6246)		Egypt	0
43	Gr. 425/19		Egypt	0
44	Gem	7243	USA ID	1
45	Orge Paye 25		RDISN	1
46	CM 67 - apam x Godiva		RDISN	1
47	Peru	653	Peru	1
48		3909.2	RDISN	1
49	Arrivat		USA	2
50	Bonanza - Godiva		IBON	2

(Cont. Table 18):

51	Albacete		RDISH	3
52	Research		RDISH	3
53	Ds ² - Apro x (M 67/Apro x Sv. 02109 Mari		RDISH	3
54	Baladi 16	9206	Egypt	3
55	Gr. 115 - Pro x Kristina		IBON	3
56	Apam. P - 1046		IBON	3
57	DL 69 - Sultan		IBON	3
58	11012.2 Por x WPG 708.21		IBON	3
59	Gr. 345/6 (Giza 119 x Shiga Hakkobeus		Egypt	3
60	Gr. 366/13/1 (Giza 117 x Bahtim 52)(Giza 118 x Pao 86)		Egypt	3
61	Gr. 474/1 (Giza 117 x Giza 120)		Egypt	3
62	Athenais		Cyprus	4
63	Ceres	12036	France	4
64	Gumhuriyet 50		RDISH	4
65	Delisa		Holland	4
66	Martim		Tunisia	4
67	Solda		Algeria	4
68	Giza 117		Egypt	4
69	Gr. 115 - For x Beecher/apl - CM 67		RDISH	4
70	Mezquite x DL. 71		RDISH	4
71	Api - 5106		IBON	4
72	Odessa	2228	USSR	4
73	12201 - Attiki		RDISH	4
74	CQ - CM x Apam x V-2410		Mexico	4
75	M 69. 69 - Hja		Mexico	4
76	Himalaya	620	USA WI	4
77	Gr. - 366/1		Egypt	4
78	Gr. 366/131 ²		Egypt	4
79	apizaco		RCB	5
80	Arabic Akhda		Syria	5
81	Arabic Local white		RDISH	5
82	Attiki		Greece	5
83	Emir	11790	Netherland	5
84	Giza 119		Egypt	5
85	Giza 120		Egypt	5
86	Jyoti		RDISH	5
87	Numar		RDISH	5
88	Ogalitsu	7152	USA	5
89	Provenir		Mexico	5
90	RS - 6		RDISH	5
91	Strain 205		RDISH	5
92	CM 67 - Usask 1800 x Pro - CM 67		RDISH	5
93	Choya x DS - Apro 6B		IBON	5
94	Bco. Mr Dzoa - 391		IBON	5
95	Lion	2238	USSR	5
96	Trebi	936	Turkey	5
97	WI - 2274 - Benton		IBON	5
98	CM 67 - 70/22385 x B 4 -54		IBON	5
99	Cremleck	1215	RDISH	5
100	Oderlrueker	1272	USA WI	5

RDISH = Regional Disease and Insect Screening Nursery.
 IBON = International Barley Observation Nursery.
 RCB = Regional Crossing Block.
 PON = Preliminary Observation Nursery.

2- Chemical control:

Effect of six different fungicides was studied on both mycelial growth of H. gramineum and incidence of barley leaf stripe disease, using three different concentrations.

a) Effect of fungicides on mycelial growth:

Influence of three concentrations, i.e., 100, 200 and 400 ppm of the tested fungicides had been studied, according to Lab Bioassay for Eradicant Action of Fungicidal Chemicals. Colonies diameters were measured, eight days after inoculation, (Table 19 and Fig. 17).

It is obvious that the tested fungicides affected the mycelial growth of H. gramineum, where they reduced growth of mycelium on Czapek-Dox medium, comparing with the untreated control. High concentrations of fungicides caused decrease of mycelial growth more than low concentrations. Both high and low concentrations of Vitavax-thiram definitely prevented mycelial growth, as well as high concentrations, 400 ppm, of both Vitavax and Orthocide 83.

b) Effect of fungicidal seed dressers on disease incidence:

Healthy seeds of the susceptible barley variety

Table (19): Effect of three different concentrations of six fungicides on the mycelial growth of Helminthosporium gramineum.

Fungicides	Mycelial growth (cm.) in diameter		
	100 ppm	200 ppm	400 ppm
Allisan	60.8	53.2	37.6
Dexon	62.8	56.0	39.6
Orthocide 83	11.6	10.0	0.0
Dithane M-45	42.0	26.4	22.8
Vitavax	20.0	5.6	0.0
Vitavax thiram	0.0	0.0	0.0
<hr/>			
Control	90.0		

L.S.D. at 5% = 2.4

1% = 3.3

Giza 119 were treated with three rates of the tested fungicides, i.e., one, two and four grams/1 kilogram seeds, then inoculated and grown. Percentage of infection was recorded and data are presented in Table (20) and Fig. (17).

Results indicate that application of the tested fungicides as seed dressers may reduce the percentage of barley stripe infection, at least about one half, comparing with control. Using four grams of Vitavax and Vitavax - thiram per one Kg seeds have entirely prevented stripe infection.

No infection was observed when untreated seeds were grown without inoculation, indicating that seeds were healthy. Also, no harmful effect on percentage of germination was found when healthy seeds were treated with fungicides and grown.

It may be concluded that Vitavax-thiram is the best fungicide for controlling barley stripe, followed by Vitavax. Also, Dithane M-45 gave good control when four grams per one Kg seeds were applied.

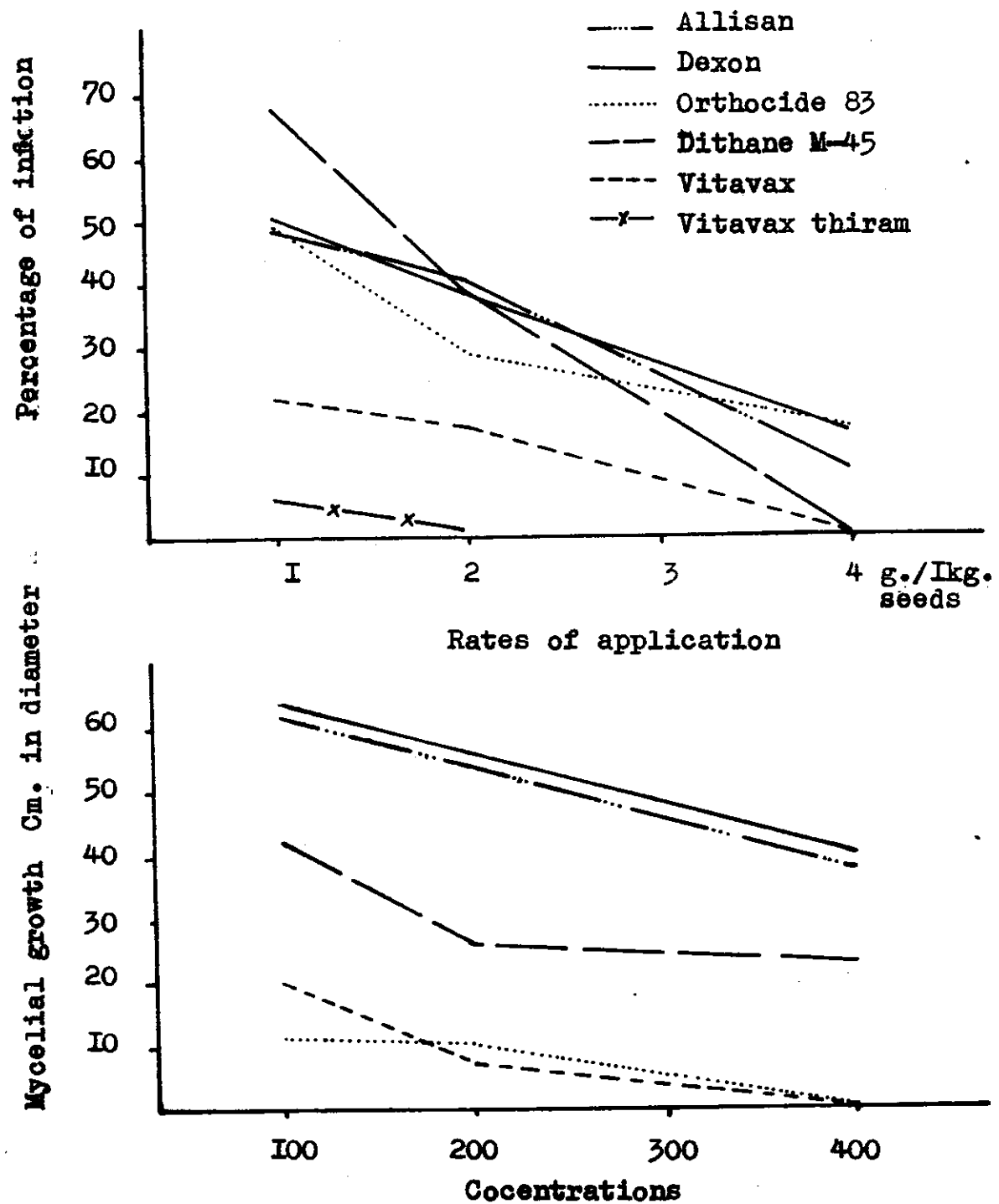


Fig. I7. Effect of six fungicides on mycelial growth of *H. gramineum* and percentage of barley leaf stripe infection .

Table (20): Effect of three different rates (1, 2 and 4 g per 1 kg seeds) of 6 fungicidal seed dressers on the percentage of barley leaf stripe infection.

Fungicides	Percentage of infection		
	1 g/l kg	2 g/l Kg	4 g/l Kg
Allisan	48.3	40.5	9.6
Dexon	49.6	38.5	15.9
Orthocide 83	48.7	29.4	16.8
Dithane M-45	68.1	39.3	1.4
Vitavax	21.7	16.9	0.0
Vitavax thiram	5.9	1.2	0.0
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Control	91.8		

L.S.D. at 5% = 3.16

1% = 1.25

DISCUSSION

Barley foliage is attacked by a relatively large number of diseases (Dickson, 1956), of these diseases, eleven diseases were found to attack barley in Egypt (Abdel-Hak and Ghobrial, 1977). Drechsler (1923) reported that Helminthosporium gramineum, H. teres and H. sativum attacked barley causing leaf stripe, net blotch, and spot blotch, respectively. Similar results were obtained in the present study. A general survey in Egypt exhibited that barley foliage is vulnerable to other six fungal diseases, i.e., downy mildew, Sclerospora macrospora; powdery mildew, Erysiphe graminis hordei; leaf rust, Puccinia hordei; stem rust, P. graminis; stripe rust, P. striiformis and minor leaf spots, Alternaria spp., Stemphylium spp. and Helminthosporium spp. This is in agreement with the findings of Oswald & Houston (1951), Reed (1909), Buchwald (1943), Shands (1939), Bever (1938), and Mehlar et al. (1976). Two viral diseases, Barley Stripe Mosaic Virus (Harborg, 1954), and Barley Yellow Dwarf Virus (Oswald & Houston, 1953), were also found to cause considerable damage in Egypt.

Some other diseases, i.e., stripe, net blotch, spot blotch, minorleaf spots, powdery mildew and leaf rust, were previously recorded in Egypt (Briton-Jhones,

1922; El-Helaly et al., 1956; El-Shafey, 1970; Sabet, 1972; Mehlar et al., 1976 and Ghobrial et al., 1977).

On the other hand, downy mildew, viral diseases, stem rust and stripe rust, were identified in Egypt for the first time. Although leaf rust, powdery mildew and net blotch, are common, in Egypt, more than stripe yet the latter is much more serious, where it attacks each leaf in turn from the seedling stage, causes damage of leaf, and sometimes prevents the emergence of heads from the leaf sheath. Dickson (1956) mentioned similar results. Barley leaf stripe appears heavily in the humid districts. It was found to occur widely in Kafr El-Sheikh, Dakahlia, Behira, Gharbia, Sharkia, Damietta, Menoufia, Kalubia and Alexandria, respectively. Its prevalence was low or entirely absent in southern Region of Egypt. These results are in agreement with that of El-Helaly et al. (1956), Ghobrial et al. (1976), and El-Sherif (1978). Moreover, percentage of stripe infection was found to vary during different years. This variation from season to season might be ^{due to} environmental ^{conditions} ^{be} favourable for disease development but not in the other. Concerning the isolation of H. gramineum from striped leaves, Sabet (1972) placed leaves on filter papers and incubated them under outdoor conditions, whereas El-Sherif (1978) placed the striped

leaves on P.D.A. plates then incubated at 20°C. In the present investigation, pieces of striped leaves were placed also on P.D.A. plates then incubated for 5 days at 20°C.

Conidiophores of H. gramineum were found to be dark brown, straight and ranged from 100 to 300 U. Conidia were subhyaline to yellowish brown, straight, sub-cylindrical, slightly tapering towards the distal end and four septa were most common without constrictions at the septa. ^a Measures of conidia ranged from 12 x 64 to 20 x 100 U. Germ tubes developed from terminal cells (polar) and less commonly the central cells. This is in harmony with the findings of Luttrell (1951), El-Helaly et al. (1956) and Sabet (1972).

Mycelial and spore suspension of H. gramineum were applied to barley leaves for inducing artificial inoculation. This procedure caused tiny brown flecks but never expanded further. Similar result was also obtained by Smedegard-Petersen (1972).

Several methods of artificial inoculation were adopted by many investigators in order to obtain typical symptom of barley leaf stripe. Hulled or dehulled barley grains were germinated in contact with mycelium

of H. gramineum (Ravn, 1900; Johnson, 1925; Isenbeck, 1930; Army and Shands, 1942; Smedegard-Peterson, 1972 and Mohammad and Mahmood, 1974). In the present work, a modified technique was used, as well as seven others adopted before. Application of this modified technique gave the highest percentage of stripe infection, comparing with the others. Thus the new adopted procedure will be of more advantage in artificial inoculation for research purposes.

The typical symptoms started as yellow stripes, then enlarged and were clearly visible after 3-4 weeks. They turned brownish and became shredded with age, then turned greenish-dark brown and showed a powdery appearance of developed conidia. This finding agrees with those of Drechsler (1923), El-Helaly et al. (1956) and Sabet (1972). H. gramineum may not be as highly specialized as rusts and smuts, but this specialization should not be overlooked in testing for varietal reactions (Shands and Army, 1944). Several attempts were conducted ^{to} study the specialization of the fungus according to different pathogenic cultural and physiologic characters. Pathogenic ability of different Egyptian isolates of H. gramineum was comparatively studied on several barley varieties during the course of this investigation. Distinct differences were recorded among

these isolates indicating that they may be related to different races. Similar results were obtained by Christensen and Graham (1932 and 1934), Singh (1971) and Mohammad and Mahmood (1973-b and 1976). On the contrary, Arny (1945-a) reported that specialization of this fungus is not clear-cut.

The tested isolates of H. gramineum were found to exhibit different cultural characters such as; rate of mycelial growth, nature of mycelium, colour of colony, and pigmentation. This is in accordance with the findings obtained by Pon (1949), Cappellini (1952), Singh (1958 and 1962), Singh (1971), Mohammad and Mahmood (1973-b) and Hammouda (1980).

Strains of some fungi were also distinguished using different physiologic characters, i.e., production of pectolytic and cellulolytic enzymes in special synthetic media and changes of oxidative enzymes activities in mycelium (Singh and Hussin, 1964 and Hammouda, 1980). The same characters were used during this course, where the tested isolates showed different abilities of producing pectolytic and cellulolytic enzymes, as well as changes of oxidative enzymes activities. Moreover, effects of cultural filtrates on barley seed germination and seedling morphology were used for the same purpose.

A drastic decrease was recorded in percentage of seed germination and length of both radicle and plumules. These results resemble those found by Luke and Gracen (1972), El-Bigawi (1976), Smedegard-Petersen (1976) and El-Sherif (1978). Cultural filtrates, of the tested isolates of H. gramineum showed distinctly different effects on barley seed germination and length of radicles and plumules. This finding indicated that H. gramineum isolates may be categorized according to effects of their cultural filtrates on seed germination as well as radicles and plumules length. Four species (three genera) of Gramineae i.e. Hordeum vulgare, H. distichon, Triticum durum and Sorghum vulgare var. sudanese were found to be susceptible to infection with H. gramineum. Typical symptoms, however, developed only on both Hordeum vulgare and H. distichon indicating that this fungus has a narrow host range under Egyptian conditions. Braverman (1960) and Sabet (1972), previously reported that only barley (Hordeum spp.), was infected with this pathogen.

Further studies on the effect of stripe infection on chemical composition (sugars, free amino acids, phenolic compounds and oxidative enzymes in plant tissues) and the relationship between disease development and these components, however, are required, to declare the

dynamic nature of disease development. In the present study, concentrations of total, reducing and non-reducing sugars were determined in barley seedlings, where artificial inoculation with mycelial and spore suspension of H. gramineum gave tiny brown flecks, which never expanded to produce the characteristic symptoms of stripe disease, in spite of using susceptible variety, Giza 119. Stripe infection was found to reduce the concentration of sugars in plant tissues and this coincides with that of Caldwell et al. (1934), Norman et al. (1961), Garg and Mandahar (1976) and Hammouda (1980). On the contrary, Nazim (1975), found initial increase within six days after inoculation. It seems that no clear-cut relationship exists between sugar contents and the abnormal development of infection.

Free amino acids were evaluated as a total, in addition to separated individuals. Many investigators found that free amino acids may increase in susceptible varieties when inoculated with fungi, whereas their concentrations may decrease in inoculated resistant ones (Shaw & Colotelo, 1961; Garg and Mandahar, 1976 and Hammouda, 1980). During the present course, amounts of free amino acids were found to decrease in the first leaf of barley seedlings inoculated with H. gramineum, in comparison with uninoculated ones. Concentrations

of amino acids, however, increased only from four to seven days after inoculation indicating that barley seedlings inoculated with mycelial and spore suspension of H. gramineum behave as resistant variety in early and late stages of infections, whereas showing reverse behavior, when tiny brown flecks started to appear on inoculated leaves. This conclusion may demonstrate the abnormal development of stripe symptoms. Concerning individual amino acids, seven show similar behaviour, i.e., total amounts of aspartic acid, serine, glycine, both glutamic acid and threonine, alanine and proline. Concentration of aromatic amino acids, tyrosine and phenyl alanine clearly increase in infected tissues seven days after inoculation. The previous conclusions mentioned that this phenomenon may characterize resistant varieties (Kim and Rohringer, 1959; Garg and Mandahar, 1976 and Hammouda, 1980).

Total, free, conjugated and ortho-dihydroxy phenols, were determined in inoculated and uninoculated tissues, since they were reported to be involved in pathogenicity and have marked fungistatic property (Ramsay et al., 1946; Scott et al., 1957; Beckman and Müller, 1970 and El-Hayatmy, 1973). Total and free phenols were found to increase in barley tissues invaded by H. gramineum. Similar results were reported by Frakas and Kiraly (1962) and Nazim (1975). The

maximum concentrations of phenolic compounds were generally recorded in infected barley seedlings, four days after inoculation. Abo-Shosha (1977) found also an increase of phenols in corn varieties infected with Helminthosporium maydis, especially at early stages of infection.

It can be concluded that phenolic compounds may play an important role concerning the inhibition of development of stripe infection as using mycelial and spore suspension for artificial inoculation.

Changes of polyphenol oxidase activity were, entirely , absent in the first leaf of barley seedlings inoculated with H. gramineum. Similar results were reported by Toyoda and Suzuki (1960) and Parkas and Kiraly (1962).

An appreciable increase of peroxidase activity was recorded, two days after inoculation in inoculated seedlings compared ^{with} that of uninoculated ones. This result agrees with that achieved by Macko et al. (1968) and Price and Fuchs (1970). The situation changed after four and seven days, when changes of peroxidase activity were higher in uninoculated tissues than that in inoculated ones. This is in disagreement with

the finding of Hammouda (1980) who found that changes of peroxidase activity increase in the infected tissues with advancement of infection. It may be another reason for the inhibition of barley stripe development.

Changes of catalase activity were generally, more in inoculated tissues than in the uninoculated ones. Hammouda (1980) mentioned similar results.

Concerning changes of ascorbic acid oxidase activity, a sharp decrease was recorded in inoculated tissues, compared to that in uninoculated ones, especially four and eleven days after inoculation.

Control of barley leaf stripe may be done along several lines. Resistant varieties offer the best means of controlling barley stripe (Dickson, 1956). In the present work, 100 cultivars and crosses were artificially inoculated with H. gramineum then their reactions were recorded. A number of these varieties were tested before, by several investigators, whereas others tested for the first time. Forty-eight varieties showed resistance. Cheveron, Bonus and Union were resistant, exactly as before-mentioned by Shands (1939) and Ghobrial et al. (1976). On the contrary, Oderbrucker showed susceptibility. Similar result was found by Shands et

al. (1933) and Shands and Army (1944). Although Trebi, Lion and Odessa were mentioned as resistant varieties, yet they ^{were} found to be susceptible during the present work. Out of five Egyptian barley varieties, Giza 121 was resistant, whereas Baladi 16, Giza 117, Giza 119 and Giza 120 were susceptible. Resistant varieties may be included in breeding program.

Various workers conducted numerous experiments to find a satisfactory seed treatment for the control of stripe using different chemicals such as, copper sulphate, Formaldehyde, Uspulun, Germisan, Corbin, chlorophenol mercury, Semesan, Corona, Ceresan, Betoxin 61, Panogen DGSV, Aagrano, Agrosan, Mercuran, Granosan, Thiram, Semex, Vitavax, Maneb, Dithane M-45, Fl-129, Quinolate Y-4-X and Panacotine (Johnson, 1916; Kiessling 1918; Müller et al. 1920; Remy and Vasters, 1923; Adams, 1924; Johnson et al. 1924; Reddy & Burnett, 1930; Andren, 1951; Lee, 1955; El-Helaly et al. 1956; ^{and Rapparini,} Uzunov, 1964; Olmo, 1968; Kingsland, 1971; Hansen, 1975; Eframova, 1976; Navushchanov, 1978 and Liska, 1978). In the present investigation, Dithane M-45, Vitavax and Vitavax-thiram, were also used as well as three other fungicides, i.e., Allisan, Dexon and Orthocide 83. Seeds had been artificially inoculated before they were treated during this course, whereas most of

the other investigators depended on natural infection. In lab. experiments, the tested fungicides inhibited growth of H. gramineum onto Czapek-Dox medium. Similar results were obtained by Grewal and Vir (1958) by using Fusariol, Agrosan GN and Agrosan 5W. Moreover, Vitavax-thiram, definitely prevented mycelial growth at 100 ppm, as well as Vitavax and Orthocide 83 at 400 ppm. Vitavax-thiram ranked, as seed dresser, on top of the tested fungicides for controlling leaf stripe disease. It may be concluded that ^a mixture ^{of} fungicides such as Vitavax-thiram proved more efficient to control barley stripe than either systemic fungicides, as vitavax, or contact ones as Dithane M-45, Orthocide 83, Dexon and Allisan. This result agrees with that of Baicu and Nagler (1974). Vitavax and Dithane M-45, however, gave an excellent control at 4 g/ Kg seeds. Hansen (1975) and Navushchanov (1978) found similar effects.