SEASONAL FLUCTUATION OF NON-RHIZOSPHERE SOIL FUNGI IN WHEAT FIELDS IN EGYPT

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Key Words: Glycophilic (1% glucose), Mesophilic, Osmophilic (5% Sucrose) and Thermophilic soil fungi.

ABSTRACT

The study aims at intensive survey of fungal floras of non-rhizospheric soil in wheat fields in El-Minya Governorate over the periods December 1976 - May 1977 and December 1977 - May 1978.

At 28°C, using 1% glucose-Czapek's agar the soil samples were rich in fungal population during May 1977 and 1978. Also the periods of February 1978 and December and April 1977 showed fairly high fungal population. These high fungal counts were mainly contribution of Aspergillus. The most frequent species were A. niger. A. terreus, A. fumigatus, A. flavus, A. versicolor, Humicola grisea, Fusarium solani. F. oxysporum, Trichoderma viride and Stachybotrys chartarum.

On 50% sucrose-agar the highest fungal counts were recorded during May and April 1978. A. niger, A. terrus, A. fumigatus, A. flavus, A. chevalieri, A. sydowii, P. lilacinum, P. citrinum, P. chrysogenum, P. cyclopium, P. jensenii, P. nigricans, Epicoccum purpurascens, Humicola grisea, Fusarium solani, Stachybotrys chartarum and Scopulariopsis brevicaulis were the most common species.

At 45°C, on both media, the highest counts were recorded in March and January 1978. These high fungal populations were basically contributed by *Aspergillus*. The most frequent species on 1% glucose agar were *A. niger*, *A. fumigatus*, *A. terreus*, *A. flavus*, *E. purpurascens*, *S. brevicaulis* and *H. grisea* var. thermoidea.

INTRODUCTION

Many investigations have been carried out on the occurrence, of Egyptian soil fungi in agricultural and desert soils collected from different localities in Egypt, and during the different seasons of the year (Moubasher and his collaborators), but none of them studied the association between a particular crop in the field and the population of fungi during the different seasons of the year.

The present investigation was an intensive study of fungi in wheat fields in 2 consecutive wheat growing seasons in El-Minya Governorate, Egypt (December 1976 - May 1977 and December 1977 - May 1978).

MATERIALS AND METHODS

Samples of soil collected every month under wheat plants according to the method described by Johnson *et al* (1959) essentially.

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The organic matter, total soluble salts, Na, K, Ca and Mg contents and pH values of soil samples were estimated.

For the determination of soil fungi, the dilution plate method as described by Johnson *et al.* (1959) essentially was used. 1% glucose and 50% sucrose-Czapek's-agar were employed as isolation media to which was added rosebengal (1/30.000) combined with streptomycin ($30\mu g$ / 1ml.) as bacteriostatic agents (Martin, 1950). Five plates were used for each sample which were incubated at 28°C and the developig fungi were counted and identified. For isolating thermophilic fungi similar numbers of plates were incubated at 45°C, and excess volumes of agar medium were poured in the plates to avoid rapid dryness. Also, the humidity of the air bound the plates in the incubator was increased, by putting open container of water in the incubator (Cooney & Emerson, 1964).

RESULTS AND DISCUSSION

The soil samples were generally poor in organic matter content (1.25 - 2.4%) and with low contents of toal soluble salts (0.1. - 1.2%). pH values were slightly alkaline (7.5-8.2) Contents of Ca, Mg, K. and Na did not show appreciable differences (Table 1).

Years		О.М.	T.S.S.		-11			
	Months	0.111.		Ca	Mg	K	Na	рН
1976	Dec.	1.60	0.10	0.18	0.14	0.5	0.30	7.8
1977	Jan.	1.40	0.15	0.21	0.16	0.4	0.32	8.0
	Feb.	1.70	0.19	0.25	0.15	0.3	0.40	7.7
	Mar.	1.38	0.17	0.24	0.20	0.4	0.35	8.1
	Apr.	1.25	0.18	0.29	0.17	0.4	0.35	8.2
	May	2.40	0.24	0.31	0.07	0.5	0.50	8.0
	Dec.	1.46	0.15	0.20	0.18	0.3	0.35	7.0
1978	Jan.	1.80	1.20	0.20	0.01	0.4	0.40	8.0
	Feb.	1.40	0.15	0.30	0.14	0.5	0.35	7.7
	Mar.	1.24	0.17	0.32	0.17	0.5	0.04	8.0
	Apr.	2.40	0.20	0.37	0.18	0.4	0.55	7.9
	May	1.29	0.15	0.31	0.14	0.3	0.55	7.9

Table 1

Total soluble salts (T.S.S.), organic content (O.M.) and elements of calcium, Magnesium potassium and sodium (expressed as mg/g soil) and pH values of the soil samples tested.

The water contents of the soil samples tested were generally moderate (20 to 40% W.H.C.). The highest temperature of the experiment were recorded in May 1977 and 1978 and the lowest in December - February of the two seasons (Figure 1).

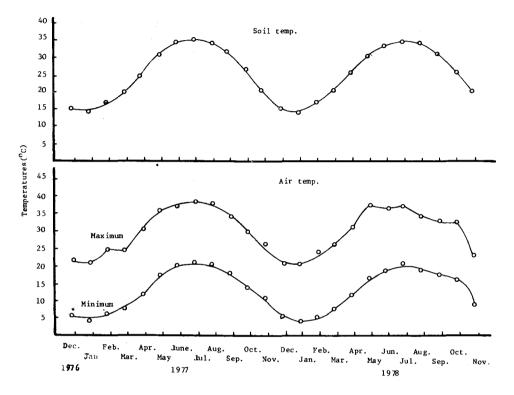


Figure 1. Monthly average maximum temperatures of the soil and the average minimum and maximum temperature of the air during the periods December 1976 - November 1978

A. Mesophilic fungi (recovered at 28° C)

Fifty species and 2 species varieties in addition to an unidentified Verticillium species which belong to 20 genera were isolated and identified (Table 2).

Generally, the soil samples were rich in their fungal population during May 1977 and 1978, and May and April 1978 on 1% glucose and 50% sucrose, respectively. Also December and April 1977 and February 1978 (1% glucose), and March 1977 (50% sucrose) showed fairly high fungal population and these high counts were mainly contribution of *Aspergillus* as shown in Figure (2).

Table 2

Percentage count, %C (calculated to total Fungal count out of 12 months) and the number of cases of islation (NCI) fungal genera and species recovered on 1% glucose and 50% sucrose)Czapek's agar media at 28% during the periods December 1976 - May 1977 and December 1977 - May 1978

	19	% Gluco	se	50% Sucrose		
Genera and species	%С	NCI	OR	%C	ÑCI	OR
Total count		36195		26380		
Aspergillus	52.8	12	H	70.2	12	Н
A. niger Van Tieghem	30.3	12.	Н	38.9	12	Н
A. terreus Thom	5.0	12	H	7.0	12	Н
A. fumigatus Fresenius	13.6	12	Н	13.4	11	Н
A. flavus Link	1.7	11	Н	7.2	10	Н
A. nidulans (Eidam) Winter	0.3	4	Μ	0.3	2	L
A. Sydowii (Bain. & Sart.) Thom & Church	0.2	4	М	0.9	7	Н
A. chevalieri (Mangin) Thom & Church	-	—		0.9	9	Н
A. versicolor (Vuillemin) Tiraboschi	1.6	6	Н	0.8	5	Μ
A. ochraceus Wilhelm	0.2	4	Н	0.3	- 4	Μ
A. terricola var. indicus (Mehrotra & Agnihotri) N. Comb.	_	—	_	0.1	2	L
A. amstelodami Thom & Church		—	· —	0.3	3	Μ
A. chevalieri var. intermedius (Thom & Raper)	-		_	0.3	2	L
A. ustus (Bain.) Thom & Church	0.1	1	R	_	_	
Humicola grisea Traaen	23.9	12	Н	3.2	7	Н
Fusarium	9.8	12	Н	3.8	9	Н
F. solani (Mart.) Appel & Wollen	4.3	7	Н	2.2	7	Н
F. oxysporum Schlecht.	1.4	6	Н	1.8	4	Н
F. moniliforme Scheldon	2.2	4	Μ	_	_	—
F. semitectum Berkeley & Ravenel	0.8	5	Μ	-		—
F. equiseti (Corda) Sacc.	0.9	2	L	-	_	_
F. poae (Peck) Wollenweber	0.2	2	L	0.7	4	М
F. nivale (Fr.) Cesati	0.1	1	R	_		_
Penicillium	6.9	9	Н	14.8	12	н
P. lilacinum Thom	0.7	3	M	2.1	11	н
P. chrysogenum Thom	1.7	5	Μ	3.4	8	Н
P. cyclopium Westling	1.2	4	Μ	1.8	8	Н
P. citrinum Thom	0.3	3	Μ	2.5	9	Н
P. jensenii Zaleski	1.6	-3	Μ	1.5	6	Н
P. funiculosum Thom	0.1	1	R	_		
P. corylophilum Dierckx	0.1	2	L	0.1	1	R
P. brevi-compactum Dierckx	1.8	2	L	1.0	3	Μ
P. nigricans (Bainier) Thom	0.3	2	R	1.3	6	Н
P. miczynski Zaleski	0.1	1	R	0.6	2	L
P. oxalicum Currie & Thom	-	—		0.5	3	Μ
P. camemberti Thom	-	_	_	0.1	1	R
Trichoderma viride Pers ex, S.F. Gray	0.9	9	Н	0.5	4	Μ
Stachybotrys chartarum (Ehrenb. ex Link) Hughes	0.6	6	Н	1.2	7	H

Contd. Table 2

Genera and species	1% Glucose			50% Sucrose		
Othera and species	%C	NCI	OR	%C	NCI	OR
Curvularia	0.7	6	Н	0.5	4	Μ
C. spicifera (Bainier) Boedijn	0.6	5	R	0.3	3	М
C. lunata (Wakker) Boedijn	0.1	1	R	0.1	1	R
Scopulariopsis brevicaulis (Sacc.) Bain.	0.1	5	Μ	0.8	7	Н
Epicoccum purpurascens Ehrenb ex Schlecht	0.3	2	L	2.4	10	Н
Rhizopus	0.3	5	Μ	0.3	2	L
R. stolonifer (Ehrenb. ex Fr.) Lindt	0.2	3	Μ	0.1	1	R
R. oryzae Went. & Prinsen Geerlings	0.1	2	L	0.2	1	R
Trichothecium roseum (Pers) Link ex Fr.	0.8	5	Μ	_	_	-
Drechslera	0.4	3	Μ	_	_	—
D. hawaiiensis (Bugn.) Subram & Jain	0.2	2	L	-		
D. sativus (Ito & Kurib) Drechsler	0.2	2	L	_	<u> </u>	
Ulocladium botrytis Preuss	0.3	3	М	0.1	1	R
Paecilomyces variotii Bainier	0.2	3	M	0.6	3	М
Cladosporium herbarum (Pers) Link ex F. Gray	1.4	1	R	—	_	
Botryotrichum piluliferum Sacc. & March.		_	_	0.1	1	R
Nigrospora oryzae (Berk. & Br.) Petch	0.1	2	L		_	_
Gliocladium roseum Bainier	_	_	_	0.6	1	R
Circinella musca (Sorok.) Berl. DeToni		_	—	0.3	1	R
Verticillium sp.	0.1	1	R	_	_	
Stemphylium botryosum Wallroth	0.1	1	R	_	_	
Sterile mycelium	0.5	5	М	_	_	

OR = Occurrence remarks:

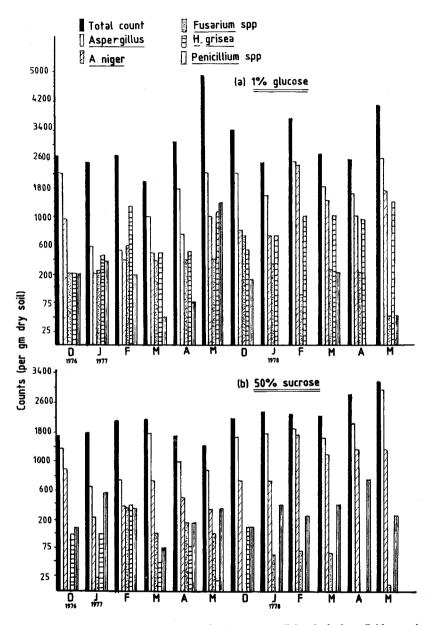
M = Moderate occurrence; between 3-5 cases.

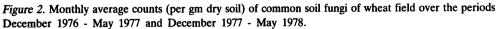
- L = Low occurrence; 2 cases.
- R = Rare occurrence; one case.

Aspergillus was the most frequent in the two media. It was collected in all months with total count representing 52.8 and 70.2% of the gross total counts of all fungi in 1% glucose and 50% sucrose-agar media respectively (Table 2). The highest counts of the genus were observed in February 1978 and December and May 1977 on 1% glucose and in March 1977 and May 1978 on 50% sucrose which represented 92.7 and 42.2% and 81.8 and 88.8% of the total fungi in those months respectively. On the other hand, the lowest counts of Aspergillus were recorded in February and January 1977, and January, and February 1977 on the two media respectively.

Eleven species and 2 species varieties of the genus were identified, of which A. niger, A. terreus, A. fumigatus and A. flavus were collected in high frequencies of occurrence on the two media whereas A. sydowii and A. chevalieri were only isolated in high occurrence on 50% sucrose. In other species of Aspergillus were isolated in less frequencies than the preceding ones (Table 2). Most of these species were also reported as common organisms in Egyptian soils

H = High occurrence; more than 5 cases out of 12.





a) Glycophilic fungi (on 1% glucose Czapek's agar medium) at 28°C (Mesophilic fungi).

b) Osmophilic fungi (on 50% sucrose Czapek's agar medium) at 28°C (Mesophilic fungi)

(Moubasher & Moustafa, 1970; Moubasher and Mazen, 1971 & 1972; Abdel-Hafez, 1974 and El-Maghraby, 1980), and from Jordanian soils (Moubasher *et al.*, 1977). On the other hand, Raper and Fennell (1965) in their treatise on the genus *Aspergillus*, reported that *A. glaucus* group (to which belong *A. chevalieri* and *A. amstelodami*) and most species of *A. restrictus* group are osmophilic. Moustafa & Al-Musallam (1975) stated that these two species were highly osmophilic. Abdel-Hafez *et al.* (1977), Moubasher *et al.* (1979), El-Maghraby (1980) and Moubasher *et al.* (1981) found that these species were of high or moderate frequency of occurrence in Egyptian and Jordanian soils and on grains on 40% sucrose-agar. Abdel-Hafez. *et al.*, (1977) found that *A. niger* and *A. terreus* were the most frequent species recovered from Egyptian saline soils on 40% sucrose.

Fusarium was the third on 1% glucose and fourth on 50% sucrose most common genus. It was recovered in 12 and 9 months, but with considerable low population representing 9.8 and 3.8% of the total count of all fungi respectively. The richest months in Fusarium population were December and February 1977 (on 1% glucose) and February 1977 (on 50% sucrose). Seven and three species of Fusarium were identified on the two media respectively, from which F. solani and F. oxysporum were the most prevalent species. Moubasher & Abdel-Hafez (1978 a & b), El-Maghraby (1980) and Mazen et al. (1984) found that F. oxysporum and F. solani were of high seasonal occurrence in Egyptian cultivated soils. Booth (1971) reported that F. oxysporum, F. moniliforme, F. graminearum, F. avenaceum and F. culmorum are serious pathogenus of many Gramineae. Also, the strains of F. solani are of world-wide occurrence as causal agents of root-rot. On the other hand, Moustafa (1975), isolated F. equiseti, F. concolor and F. acuminatum in moderate or low frequences from Kuwait salt marsh soils. Moubasher et al. (1977) and Moubasher et al. (1981) recorded that Fusarium spp. are common fungi in Jordanian soils on 1% glucose and 30% sucrose agar.

Humicola (H. grisea) was ranked second and fifth most frequent fungus as it recorded in 12 and 7 months contributing 23.9 and 3.2% of total fungi on the two media respectively. The highest counts of the species were estimated in February and May 1977, and May 1978 (on 1% glucose) and February 1977 (on 50% sucrose) as shown in Figure. (2).

H. grisea was also recorded in this laboratory either on 1% glucose or 40% sucrose agar but with moderate or low frequencies of occurence (Moubasher & Mazen, 1972; Abdel-Fattah, 1973; Abdel-Hafez, 1974; Moubasher et al., 1977; El-Maghraby, 1980; and Moubasher et al., 1981).

Pencillium was also of high occurrence on the two media (fourth and second respectively). The most prevalent species of the genus were *P. chrysogenum* (on 1% glucose), and *P. lilacinum*, *P. citrinum*, *P. chrysogenum*, *P. cyclopium*, *P. jensenii* and P. nigericans (on 50% sucrose). Other species of *Pencillium* were isolated as shown in Table (2).

The majority of these species were collected and identified in this laboratory on 1% glucose-Czapek's agar at 28°C from desert or cultivated Egyptian soils and from Jordanian soils by Moubasher and his collaborators. Also, Moustafa (1975), obtained 7 species of *Pencillium* in

various frequencies and these were *P. chrysogenum*, *P. notatum*, *P. crustosum*, *P. brevicompactum*, *P. frequentans*, *P. lanosum* and Pencillium sp. (from *P. reistrickii* series). Moustafa and Al-Musallam (1975) also reported *P. notatum*, *P. chrysogenum* and *P. crustosum* as strong osmotolerant species (best growth on 20 - 40% sucrose).

Trichoderma viride and Stachybotrys chartarum; and S. chartarum, Scopulariopsis brevicaulis and Epicoccum purpurascens were also isolated in high frequency of occurrence on 1% glucose and 50% sucrose agar respectively.

On 1% glucose, Curvularia spicifera, Rhizopus stolonifer, Scopulariopsis brevicaulus, Trichothecium roseum, Ulocladium botrytis and Paecilomyces variotii were identified in moderate frequencies. On 50% sucrose, Trichoderma viride, Curvularia spicifera and Paecilomyces variotii were encountered in moderate frequencies.

Other genera and species than the preceding ones were encountered in low or rare frequencies of occurrence on the two media as shown in Table (2).

Most of the genera and species, recovered in the present investigation, exhibited various osmophilic abilities (Mazen *et al.* 1981). Also most of these organisms were reported as osmophilic or osmotolerant organisms from Kuwait salt marsh soils (Moustafa, 1975).

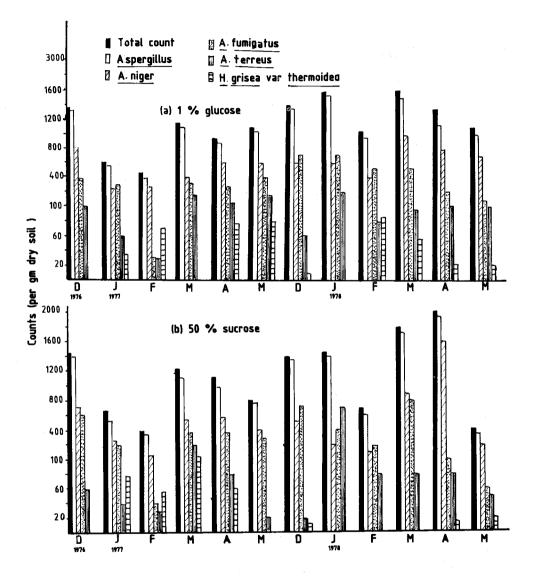
B. Thermophilic fungi (recovered at 45°C)

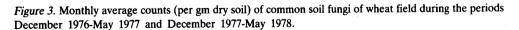
Generally, the fungal populations of non-rhizospheric soil at 45°C were markedly lower than respectives at 28°C either on 1% glucose or 50% sucrose-Czapek's agar (13830 and 13700 and 36195 and 26380 colonies per gm dry soil respectively).

The highest counts of thermophilic fungi were recorded in January and March 1978; and March and April 1978 on the two media respectively (Figure 3) these high populations were basically contributed by *Aspergillus* (96-98% of the total fungi recorded in these months). The least counts of fungi were also obtained during low or moderate temperature months, January and Feb. 1977 (1% glucose) and February 1977 and March 1978 (50% sucrose), which means no regular seasonal periodicity.

Aspergillus was the most dominant fungus at 45° C (Table 3) and contributed 93.7 and 92.9% of total fungi on the two media respectively. The most prevalent species of the genus were A. niger, A. fumigatus, A. terreus and A. flavus. A. ochraceus, A. nidulans and A. versicolor appeared on the media but with lower frequencies than the previous species. A. chievalieri appeared only on 50% sucrose in high frequency of occurrence and was completely absent on 1% glucose agar.

All these species were isolated previously from Egyptian soils (Abdel-Fattah, 1973; Mazen, 1973; Abdel-Fattah *et al.*, 1977; El-Maghraby, 1980; and Moubasher *et al.*, 1982 a & b), Jordanian soils (Moubasher *et al.*, 1981) peanut seeds (Moubasher *et al.*, 1977) and from wheat and broad bean straw composts (Moubasher *et al.*- 1982). Also El-Maghraby (1980) reported these species as thermotolerant or thermophilic on 40% sucrose agar medium from Wadi-Ber El-Ain soils. *A. fumigatus* has long been known as thermophilic fungus, but Crisan (1959) and





- a) Glycophilic fungi (on 1% glucose Czapek's agar medium) at 45°C (Thermophilic fungi).
- b) Osmophilic fungi (on 1% glucose Czapek's agar medium) at 45°C (Thermophilic fungi).

Table 3

Percentage count, % C (calculated to total fungi) and the number of cases of isolations (NCI) of fungal genera and species recovered on 1% glucose and 50% sucrose Czapek's agar at 45°C during the periods December 1976-May 1977 and December 1977 - May 1978.

	19	50% Sucrose				
Genera and species	%C	NCI	OR	%C	NCI	OR
Total Count		13830		13700		
Aspergilus	93.7	12	Н	92.9	12	Н
A. niger	44.8	12	Н	47.0	12	Н
A. fumigatus	29.6	12	Н	29.1	12	H
A. terreus	9.0	12	н	10.7	12	H
A. flavus	1.6	7	H	2.6	7	Н
A. nidulans	0.8	4	Μ	0.4	2	L
A. ochraceus	0.7	4	Μ	0.6	4	Μ
A. versicolor	0.9	4	Μ	1.0	4	Μ
A. ustus	0.1	1	R	—	_	
A. chevalieri		_		1.5	8	Н
Humicola grisea var. thermoidea Cooney & Emerson	3.2	8	Н	2.1	6	Н
Scopulariopsis brevicaulis	0.5	5	М	1.2	7	н
Epicoccum purpurascens	0.3	2	L	2.6	9	Н
Paecilomyces varitoii	2.0	4	M	1.0	4	М
Sterile mycelium	0.3	1	R	0.2	1	R

OR = Occurrence remarks:

H = High occurrence; more than 5 cases out of 12.

M = Moderate occurrence; between 3-5 cases.

L = Low occurrence; 2 cases.

R = Rare occurrence; one case.

Cooney and Emerson (1964) consider it as themotolerant as it has a maximum near to 50°C, but a minimum well below 10°C. A. niger and A. terreus show thermophilic tendencies as reported by Abdel-Fattah (1973), Moustafa et al. (1976) and Moubasher et al. (1976) and Moubasher et al (1981).

One species variety of the genus *Humicola*, *H. grisea* var. *thermoidea* was recorded in high frequency of occurrence (8 and 6 months) but with low population (5.2 and 2.1% of total fungi) on 1% glucose and 50% sucrose-agar respectively.

Scopulariopsis brevicaulis was ranked third most frequent fungus on both media (5 and 7 months respectively).

Epicoccum purpurascens appeared in high frequency of occurrence of 50% sucrose (9 months) and in low frequency on 1% glucose (2 months).

Paecilomyces varictii was also encountered in moderate frequency of occurrence on the media (4 months each).

All these latter organisms were isolated in this laboratory as thermophilic or thermotolerant organisms (Moubasher and his collaborators).

The results of our study generally reveals that all species of *Penicillium* and *Fusarium* and most species of the other genera could not appear at 45°C.

A. chevalieri, A. terricola var. indicus, A. chevalieri var. intermedius and A. amstelodami were only recorded on 50% sucrose. Some species were more frequently isolated on 50% sucrose than on 1% glucose (e.g. A. sydowii. P. lilacinum, P. citrinum, P. chrysogenum, P. cyclopium, P. jensenii, P. nigricans and Epicoccum purpurascens) whereas other species were more frequently isolated on 1% glucose (e.g. Humicola grisea, Fusrium oxysporum, Trichoderma viride and Trichothecium roseum).

Humicola grisea var. thermoidea was only recovered at 45°C and was completely absent at 28°C on the two media.

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وكانت أكثر الفطريات انتشاراً على وسط شابكس المتصلب والمحتوي على ١٪ جلوكوز ، ٥٠٪ سكروز هى الاسبرجلس نيجر ، الاسبرجلس فيوميجاتس ، الاسبرجلس تيريس ، الاسبرجلس فلافس ، هيوميكولا جريزياً صنف ثيرمويدي . كما كانت كل من الاسبرجلس شيفيليرى ، الابيكوكم بيربيروا سنس ، الاسكوبيولاريوبسس بريفيكولاس أكثر شيوعاً فقط على الوسط المحتوي على ٥٠٪ سكروز .

التغيرات الموسمية للفطريات في تربة خالية من الجذور في حقول القمح في مصر

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في هذا البحث تمت دراسة الفلورا الفطرية في تربة خالية من الجذور في حقول القمح لمحافظة المنيا خلال الفترة من ديسمبر ١٩٧٦ _ مايو ١٩٧٧ ، ديسمبر ١٩٧٧ _ مايو ١٩٧٨م ... ويمكن تلخيص النتائج فيما يلي :

١ - عند درجة ٢٨م[°] ، باستخدام وسط شابكس المحتوى على ١٪ جلوكوز أو ٥٠٪ سكروز كانت التربة غنية بمحتواها الفطري في مايو ١٩٧٧ ، ١٩٧٧ ، كما أظهرت أيضاً أشهر فبراير ١٩٧٨ ، ديسمبر وأبريل ١٩٧٧ ارتفاعاً نسبياً في تعداد الفطريات . وعند استخدام ١٪ جلوكوز شابكس آجار كانت أكثر الفطريات انتشاراً هى الاسبرجلس (٥ أنواع) ، فيوزاريوم (نوعين) ، نوع واحد لكل من هيوميكولا (ه- . جريزيا) تريكود يرما (ت . فيريدى) ، ستاكيبوس (س . كارتارم) .

وعند استخدام وسط شابكس المحتوي على ٥٠٪ سكروز سجل أعلى تعداد فطري خلال شهري مايو وأبريل ١٩٧٨م . وكانت أكثر الفطريات انتشاراً هى البنسيليام (٦ أنواع) ، الاسبرجلس ، (٥ أنواع) ، نوع واحد لكل من الابيكوكم (أ . بيربيرو أسنس) ، هيوميكولا (هـ . جريزيا) ، فيوزاريوم (ف . سولناى) ستاكيبوترس (س . كارتارم) سكوبيولاريوبسس (س . بريفيكولاس) .

٢ - عند ٥٤٩ سجل أعلى تعداد كلي للفطريات خلال شهرى مارس ويناير ١٩٧٨م . عند استخدام
٢ الأوساط الغذائية المختلفة .