Microfungal communities in litter of dominant plants cover at Al- Baha region, Saudi Arabia

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Abstract

Twenty five mould species belonging to eleven genera were isolated from the litters of dominant plants cover (Twelve species) at Al-Baha region, Saudi Arabia on Czapek-Dox agar medium. Fungal community was dominated by *Aspergillus niger* and *Fusarium oxysporum* (100% frequency) and to a lesser extent *Trichoderma harzianum*, *Mucor racemosus* and *Fusarium acuminatum* (83.3 to 66.7 % frequency). While, *A. flavus*, *A. terreus*, *Botryotrichum piluliferum*, *Emericella nidulans* and *Macrophomina phaseolina* showed only 8.3 % frequency. The effect of growth medium, incubation temperature, pH value and salinity, on the growth of most dominant fungal species(13 species) were estimated. Czapek-Dox agar medium was optimum for maximal linear growth of *Acremonium strictum*, *M. racemosus*, and *T. koningii*, after six days of incubation. However, *A flavipes*, *Circinella muscae* and *Penicillium janczewskii* have the least activities to assimilate the ingredients of tested media in favor their growth. Tested moulds failed to grow at 55°C and *A. flavipes*, *A. niger* and *E. nidulans* showed thermotolerant activity. Selected species responded differently to the tested pH values of Czapek-Dox broth. *Penicillium jaczewskii*, *T. harzianum*, *T .koningii*, *F. niveus*, *A. flavipes* and *A. melleus* were acidophilic(pH 3.5 to 4.5). While, *E .nidulans* and *G. roseum* were alkaliphilic(pH 8.1) and the rest moulds gave their best growth values around neutrality(pH 5.9 to 6.8). The growth of tested fungi responded differently to salinity(0- 14% NaCl). *Circinella muscae* appeared to be halophilic(attained best growth at 14% NaCl). Cellulytic, pectinolytic and amylolytic activities of tested fungi indicated that they have noticeable efficiencies to produce them. This finding indicated their major role in litter decomposition.

Key words: *Al-Baha, Microfungi, Plant litter, Enzyme activity.*

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Introduction

The ecological impact of litter derived from the tree stand and under storey vegetation is considerable in the ecosystem. Litter has an effect on nutrient budgets of plants and nutrient cycling, and provides a physical substrate and nutrient source for soil microbes. The litter and its physical and chemical properties regulate a considerable extent of the carbon cycling of the site, humus formation, soil structure and fertility, as well as, it is a source of nutrients and organic matter in soil (Lianne and Merriam, 1981; Nilsson et al, 1999; Berg and Meetemeyer, 2001) . Saprophytic fungi (decomposers) play a major role in the carbon and nutrient cycling in ecosystems and impacts of environmental change on fungal diversity could influence ecosystem function via decomposition (Frankland et al, 1996). Despite the substantial interest to ecologists of the relationship between fungal species diversity and ecosystem functioning, little is known about how the high species richness of decomposer (saprophytic) fungi and their relative frequencies of occurrence influence the decomposition of organic matter (Deacon et al, 2006). Fungi are recognized for their superior aptitudes to produce a large variety of extracellular enzymes as cellulases, pectinases, ligninases, amylases (Celestino et al, 2005; Dhouib et al, 2005; Jorgensen and Olsson, 2006).

The present work aimed to isolate fungal communities from the litter of dominant species of Al-Baha region plant cover and to study the effect of some growth parameters on the dominant fungal species, as well as, their ability to produce some extracellular enzymes, as cellulases, pectinases and amylases.

Materials and Methods

Study area

Al- Baha province is located on the Sarawate Mountains, south west of Saudi Arabia, of about 900-1800 meters above sea level, with a mean annual precipitation of 111.5mm and a mean of annual temperature of 22.7° C and relatively moderate relative humidity (Meteorology and Environmental Protection Administration, 2000).

Plant litter

Litter of the dominant species of plant cover at Al-Baha province was aseptically collected in sterile bags from the site under each plant (about 300g litter for each, 5 samples from different plants of the same species were collected). It contains deciduous leaves, twigs, flowers, seeds, fruits, and plant bark, beside other dead materials. The litter of the same plant species was mixed and crushed thoroughly inside the bags, then kept in a refrigerator until use.

The dominant plant cover contains *Acacia abyssinica, A. farnesiana Casuarina equistifola, Conchrus ciliaris, Cupressus sempervirens, Erica arborea, Ficus carica, Juniperus phoenicea, Mentha longifolia, Olea chrysophylaa, Pinus pinea and Tamarix aphylla.* These plants were identified by the staff members of the Herbarium of Biological Sciences Department, Faculty of Science, King Abdulaziz University.

Moisture content of litter

Ten grams of each litter sample were used to determine moisture content at 105° C until constant weight.

Isolation of fungal cultures

To obtain cultures for use in function tests with an estimate of frequency of occurrence for every taxon or isolate obtained, dilution and litter plating techniques were used (Warcup, 1950). Where 10g of thoroughly mixed crushed litter were mixed thoroughly with 90ml sterile distilled water in 250ml conical flasks, shaked at 250 rpm for 20 min, thereafter serial dilutions were made. One ml was used to be inoculated with Czapek-Dox agar medium (Warcup, 1955). Litter fragments were cut into 2 mm^2 pieces and about 0.001g (equivalent to 10^{-3} litter dilution) was pushed into Czapek-Dox agar medium. Five replicate plates of each sample and dilution technique were prepared. The plates were incubated at 25 °C for 7 days. Taxa growing out of each dilution method or litter fragments were isolated onto potato dextrose agar (PDA) medium slopes (Deacon et al, 2006). The developing fungi were purified and identified based on their macro and microscopic characteristics (Gilman, 1971; Barnett and Hunter, 1972, Stevens, 1984; Ellis and Ellis, 1985; Moubasher, 1993). If a fungus occurred on the litter of one of the twelve common plant cover, at Al-Baha region, its isolation frequency was 8.33%.

Environmental studies

The inoculum was in the form of disks, prepared using a sterile cork poorer (5mm in diameter). The disks were obtained from homogenous growth of 4 days old cultures grown on PDA medium at 25 °C. Each treatment is carried out in 5 replica and the estimated results are the arithmetic mean. The effect of some cultural conditions as nutrient media, incubation temperature, pH value and salinity on the growth of the selected 13 isolates representing the dominant species of the isolated genera, was carried out.

Effect of different nutrient media

Tested fungi were cultivated on five different agar media of Difco (PDA, malt extract, Czapek Dox, Rose-Bengal and Sabouraud) and linear growth was estimated regularly for 24 days of incubation.

Effect of growth temperature

The selected fungi allowed to grow in 250ml Erlenmeyer flasks containing 100ml of Czapek Dox medium inoculated with two disks of fungal growth and incubated stagnantly at different temperatures ranging from 15-55° C for 12 days. Thereafter, the growth was separated by centrifugation at 3000 rpm for 20 min and the dry weight was estimated.

Effect of initial pH values

The influence of different pH values (3.5-9.5) on the biomass output (dry weight) of the test fungi on Czapek-Dox medium, after 12 days of incubation at 25° C, was tested.

Influence of salinity

The effect of different salinity levels (0.0-14%) using NaCl on growth yields (dry weight) of tested fungi on Czapek-Dox medium after 12 days of incubation at 25° C, was estimated.

Enzyme activity

Cellulose, pectin and starch are of the main constituents of plant tissues. So cellulytic, pectinolytic, and amylolytic activities of the selected 13 isolates were estimated.

Cellulytic activity

Aliquots (100ml) of cellulase promoting medium (Talboys and Busch, 1970), at pH5, were dispensed in 250ml Erlenmeyer flasks, inoculated with two disks of 7 days old culture for 14 days at 28° C. The crude enzyme (filtrate) was isolated by centrifugation at 10,000 rpm for 20 min using refrigerated centrifuge (Denly BR401). The enzyme activity was determined as loss in viscosity of 10ml of 1.2% carboxy methyl cellulose (CMC) in phosphate buffer (pH 5.5), as enzyme substrate, to which 5ml of crude enzyme was added and the reaction time was 30 min at 30° C . Viscometer of Cannon Fenske type No 511 was used:

$$\% \text{ of loss in viscosity} = \frac{T_1A - T_2B}{T_1A - T_3W} \times 100$$

T_1A = Time (in seconds) of flow of active crude enzyme mixture (15 ml)

T_2B = Time (in seconds) of flow of boiled crude enzyme mixture(15 ml, control)

T_3W = Time (in seconds) of flow of 15 ml distilled water.

Amylolytic activity

Amylases of the tested fungi were estimated using enzyme promoting medium of the following composition (g/l): soluble starch, 20; ammonium sulphate, 4; KH_2PO_4, 1.5; $MgSO_4 .7H_2O$, 0.5; $MnSO_4$, 0.05; Fe $SO_4.5H_2O$, 0.005. The enzyme activity was determined in the filtrate as µmol maltose / min / ml crude enzyme. The produced maltose was estimated using dinitrosalicylic acid (Plummer, 1987).

Pectinolytic activity

The ability of the selected fungi to produce pectin methyl esterase was tested using enzyme promoting mineral medium containing apple pectin (Dhingra and Sinclair, 1985). The activity of the crude enzyme (filtrate) was determined titrametrically using 0.1N NaOH to neutralize the carboxyl

groups of the liberated galacturonic acid (Kartesz, 1951; Matta and Dimond, 1963).

$$\text{Enzyme units} = \frac{\mu g \text{ of galacturonic acid x dilution}}{\text{Time of enzyme incubation(min)}}$$

Three replica at least of each treatment were carried out and the recorded results are the arithmetic mean.

Results and Discussion

Microfungal community of plant litters

Total fungal count (colony forming unit, CFU) per one gram dry litter of different plants (Table 1) revealed that no consistent correlation between litter moisture content and fungal count, either the plant is a herb (as *Conchrus ciliaris* and *Mentha longifolia*) or a shrub (as *Tamarix aphylla* and *Erica arborea*) or a tree (as *Acacia abyssinica*, *Acacia farnesiana*, *Casuarina equistifola*, *Cupressus sempervirens*, *Ficus carica*, *Juniperus phoenicea*, *Olea chrysophylaa*, *Pinus pinea*).

Thus, *Tamarix aphylla* litter, with lowest moisture content (4.9%) and the highest CFU (95×10^3) and *Acacia farnesiana, Olea chrysophylaa* and *Cupressus sempervirens* litters with the highest moisture contents and lower fungal counts. However, the different species of the same genus *(Acacia abyssinica and A. farnesiana*) showed almost parallel fungal counts in spite of the differentiation of moisture content. These results may indicated that the fungal count is correlated with the structure and ingredients of the litter.

Twenty five different fungal species belonging to eleven different genera were isolated from the studied litter materials (Table 2). Fungal community was dominated by *Aspergillus niger* and *Fusarium oxysporum* (100% frequency) and to a lesser extent *Trichoderma harzianum, Mucor racemosus and Fusarium acuminatum* (83.3, 75 and 66.7% frequencies, respectively). While, *A. flavus, A. terreus, Botryotrichum piluliferum, Emericella nidulans, and Macrophomina phaseolina,* representing the least dominant mycoflora in the tested litters (8.3 % frequency). Soil mycoflora

of different regions at Saudi Arabia was isolated and identified by many workers (Ali and Abou-Heliah, 1984; Abou-Heliah, 1985; Hashem, 1993; Hashem and Parvez, 1994). While other researchers isolated fungi from the rhizosphere of many plants from different localities at Saudi Arabia (Abdel-Aziz and Mohammed, 1972; Fathi et al, 1975; Hashem and Al-Farraj, 1995).

Effect of different growth media

The results (Table 3) indicated that Czapek-Dox medium provided nutrients quality and/or quantity that were optimum for maximal linear growth of *Acremonium strictum*, *Mucor racemosus* and *Trichoderma koningii* at the six day of incubation. Both *Acremonium strictum* and *Trichoderma koningii* almost attained their maximal linear growth, on the tested five different media, at the six day of incubation, i.e.: they may have active enzyme systems capable of assimilating and using different ingredients in the route of their growth. However, *Aspergillus flavipes*, *Circinella muscae* and *Penicillium janczewskii* appeared to have the least activities to assimilate the different ingredients of the growth media, under the tested conditions, where their linear growth, either needed more than 24 days of incubation or ceased at earlier ages (less than 24 days). The results indicated that under the tested conditions the nutritional requirements of the tested fungi were not dependent on the genus of fungus, but in its species. Whereas, ingredients of malt extract and Sabouraud media were stimulatory for higher growth values of *Aspergillus niger* and to a lesser extent *A. melleus*, they were unfavorable for *A. flavipes* growth. The above mentioned finding reflect the varied affinity of the tested fungi to utilize monomer, oligomeric and polymeric sugars, as well as nitrogenous materials and ingredients of media (Griffin, 1981; Al- Garni, 2006).

Effect of growth temperature

The results (Table 4) indicated that *Aspergillus flavipes*, *A. niger* and *Emericella nidulans* are thermotolerant which grow at temperature up to 40- 45 °C, with the best growth at 35 °C. The thermotolerant activity of these fungi was reported (Abdel-Hafez, 1982; Moubasher, 1993; Al-Fassi et al, 1994). While the rest of the tested fungi were mesophilic, where they attain their best growth values at 25 °C, this finding was in accordance with that reported (Yusef and Allam, 1965; Moubasher, 1993). On the other hand, all the tested fungi failed to grow at 55°C.

Effect of pH value

The growth of the tested fungi (Table 5) responded differently to the hydrogen ion concentration of Czapek-Dox medium. They can be satisfactory divided into three groups: acidophilic (attain their best growth values at pH 3.5-4.5), as *Penicillium janczewskii*, *Trichoderma koningii*, *T. harzianum*, *Fusarium niveus*, *Aspergillus flavipes* and *A. melleus*, alkaliphilic (pH 8.1), as *Emericella nidulans* and *Gliocladium roseum*, while the third group of the tested fungi attain their best growth yields around the neutral pHs (5.9-6.8). The results revealed that the optimal pH for fungal growth depends on the fungal species and not fungal genus. Where *A. flavipes* and *A. melleus* are acidophilic, while *A. niger* is alkaliphilic. On the other hand as pH 5.9 was optimal for *F. acuminatum*, pH 4.5 was so for *T. harzianum*. The influence of pH values on the fungal growth was reported by many workers (Yusef and Allam, 1965; Ramadani and Aggab, 1993; Azmi and Seppelt, 1997).

Effect of Salinity

The effect of different concentrations of NaCl (salinity) on the growth of the tested fungi indicated that they can tolerate up to 14% NaCl, except *Acremonium strictum* and *T. harzianum* can tolerate up to 6% NaCl (Table 6) However, *F. niveus*, *G. roseum* and *T. koningii* can grow up to 10% NaCl. On the other hand, *C. muscae* appeared to be halophilic where the best growth values were estimated at 14% NaCl and to a lesser extent, *A flavipes*, *A. niger*, *E. nidulans*, *M. racemosus* and *T. koningii* appeared to be highly halotolerant. In accordance with these findings, it was reported that the last five fungal species are halotolerant and isolated from salt marches (Moubasher, 1993). It was also indicated by many workers that the tested fungi were isolated using media containing 5% NaCl (Abdel-Hafez, 1981; Al-Fassi et al, 1994; Omar et al, 1994).

Enzymatic activity

In order to characterize the role that may played by the isolated fungi in litter decomposition and humus formation, as well as, mineralization of complex organic compounds, that increase soil fertility and hence plant growth, the effect of hydrolytic enzymes on cellulose, pectin and starch (as of the main constituents of plant residues) were estimated. The results (Table 7) revealed that the tested fungi have noticeable efficiencies to produce the tested hydrolytic enzymes, which indicate their major role in litter decomposition. *A. niger*, *G. roseum* and *F. acuminatum* synthesize pectin methyl esterase with the highest activity (60-70 En. U.), while *E. nidulans*, *C. muscae*, *Acremonium strictum* and *M. racemosus* were with moderate activities (40 -50 En. U.). However, the rest fungi showed lower activities (less than 40 En.μ.) As for cellulase yielding the most active

enzyme system was produced by *G. roseum*, *A. niger*, *E. nidulans*, and *C. muscae* (in descending order). The highest amylase activity was retained with *A. flavipes, T. harzianum T. koningii, F. niveus* and *A. niger*. The production of amylases, pectinases and cellulases by fungi was reported by many workers (Joshi et al, 1993; Abdel-Sater, 1994; Cavalitto et al, 1996; Ugwaunyi and obeta, 1997; Kvesitadze et al, 1999; Celestino et al, 2005; Jorgensen and Olsson 2006).

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Table(1). Total count (CFU/g dry litter) of fungi isolated from the litter of the dominant plant cover of Al-Baha region.

Plant cover	CFUX10^3	Moisture content (%)
Acacia abyssinica	6.5	6.5
Acacia farnesiana	6.4	9.8
Casuarina equistifola	62.5	7.2
Conchrus ciliaris	53.2	6.0
Cupressus sempervirens	3.1	8.9
Erica arborea L.	2.2	5.5
Ficus carica L.	8.2	5.3
Juniperus phoenicea L.	12.3	5.1
Mentha longifolia L.	35.0	6.1
Olea chrysophylaa	6.4	9.5
Pinus pinea	3.5	6.8
Tamarix aphylla L.	95.0	4.9

Table(2). Frequency of fungal species isolation from litter of the dominant plants (15 samples each) at Al-Baha region.

Fungus	Tested plant litter												Frequency (%)
	Acacia abyssinica	*Acacia farnesiana*	*Casuarina equistifola*	*Conchrus ciliaris*	*Cupressus sempervirens*	*Erica arborea*	*Ficus carica*	*Juniperus phoenicea*	*Mentha longifolia*	*Olea chrysophylaa*	*Pinus pinea*	*Tamarix aphylla*	
Acremonium murorum	-	-	2	-	-	-	-	-	-	-	-	2	16.7
A. strictum	-	1	-	-	-	-	-	-	-	1	-	1	25.0
Aspergillus	1	-	-	-	-	-	-	-	2	-	-	-	16.7
A. flavus	-	-	-	-	-	3	-	-	-	-	-	-	8.3
A. flavipes	-	-	-	5	2	-	-	-	6	-	1	2	41.7
A. melleus	-	-	1	-	1	-	1	1	2	-	-	-	41.7
A. niger	8	3	2	8	2	3	1	1	5	1	2	4	100.0
A. sulphureus	6	-	2	-	-	-	-	-	-	3	-	1	33.3
A. terreus	-	-	2	-	-	-	-	-	-	-	-	-	8.3
A. versicolor	-	-	2	2	2	-	-	-	-	-	-	2	41.7
Botryotrichum piluliferum	-	-	-	-	-	-	2	-	-	-	-	-	8.3
Circinella muscae	-	2	6	-	-	1	-	-	3	-	-	-	33.3
Emericella nidulans	-	-	1	-	-	-	-	-	-	-	-	-	8.3
Fusarium acuminatum	3	1	1	1	-	1	-	1	-	-	-	2	66.7
F. niveus	-	-	-	2	-	1	-	-	1	-	-	-	25.0
F. oxysporum	5	4	3	4	4	4	6	7	5	5	3	2	100.0
F. solani	-	1	3	-	-	-	-	-	1	-	-	-	25.0
Gliocladium roseum	-	1	-	-	1	-	-	-	-	-	-	-	16.7
Macrophomina phaseolina	1	-	-	-	-	-	-	-	-	-	-	-	8.3
Mucor circinelloides	-	4	2	-	-	3	1	-	-	3	-	-	41.7
M. racemosus	4	1	1	3	1	-	1	-	4	-	2	3	75.0
Penicillium glabrum	1	-	-	2	-	-	-	3	5	-	-	4	41.7
P. janczewskii	-	-	-	-	-		1	-	-	2	-	-	16.7
Trichoderma harzianum	4	2	1	6	2	2	-	4	7	-	3	1	83.3
T. Koningii	-	2	1	-	-	-	-	-	2	1	-	-	33.3
No. of isolates	33	22	30	33	15	18	13	17	43	18	11	24	-
No of species	9	11	15	9	8	8	7	6	12	9	5	11	-

Table (3): Effect of different growth media on the linear growth (cm) of the tested fungi for 24 days of incubation.

Fungus	Growth medium	Average of linear growth (cm) every 2 days											
		2	4	6	8	10	12	14	16	18	20	22	24
Acremonium strictum	Czapek,s	4.9	8.6	*									
	Malt	5.8	8.6	*									
	PDA	5.8	8.6	*									
	Rose- Bengal	3.5	8.6	*									
	Sabouraud	4.8	8.6	*									
Aspergillus flavipes	Czapek,s	1.6	2.2	3.5	4.4	6.0	7.0	7.6	8.0	8.6	*		
	Malt	1.5	2.1	2.5	2.9	3.6	4.5	5.5	6.7	7.1	7.6	**	
	PDA	1.3	2.0	2.4	2.7	3.4	3.8	4.4	5.3	6.2	7.1	**	
	Rose- Bengal	1.4	2.0	2.6	3.1	4.6	5.2	6.3	6.8	7.0	**		
	Sabouraud	1.5	2.2	2.6	2.9	3.3	3.7	4.0	4.6	4.8	5.0	**	
A .melleus	Czapek,s	2.3	4.3	6.4	7.4	8.6	*						
	Malt	2.7	4.9	6.6	8.0	8.6	*						
	PDA	2.1	3.0	3.5	3.7	4.1	4.5	4.8	5.6	6.8	**		
	Rose- Bengal	1.8	3.3	4.4	5.4	6.4	7.3	7.7	**				
	Sabouraud	3.3	6.6	8.4	8.6	*							
A .niger	Czapek,s	3.1	7.4	8.0	8.6	*							
	Malt	3.6	7.5	8.0	8.6	*							
	PDA	2.8	5.6	6.3	6.9	8.1	**						
	Rose- Bengal	1.9	3.8	5.8	6.9	7.6	**						
	Sabouraud	3.2	7.2	8.6	*								
Circinella muscae	Czapek,s	1.3	1.6	1.8	2.0	3.1	3.8	4.4	5.1	5.5	5.9	6.6	7.3
	Malt	1.1	1.5	1.7	2.0	2.5	3.0	3.7	4.4	4.8	5.2	5.6	**
	PDA	1.2	1.5	1.7	1.9	2.3	2.7	2.9	3.3	3.8	4.3	5.5	**
	Rose- Bengal	1.1	1.5	1.6	1.8	2.5	3.0	3.4	4.3	4.9	5.5	6.3	**
	Sabouraud	1.4	1.6	1.9	2.1	2.4	2.5	2.9	3.2	3.2	**		
Emericella nidulans	Czapek,s	2.0	6.0	7.9	8.6	*							
	Malt	2.5	6.3	7.8	8.6	*							
	PDA	1.9	3.5	5.1	5.7	5.8	6.0	**					
	Rose- Bengal	2.5	5.7	8.0	8.6	*							
	Sabouraud	2.7	5.2	7.7	8.6	*							

Table 3 continued

Fungus	Growth medium	Average of linear growth (cm) every 2 days											
		2	4	6	8	10	12	14	16	18	20	22	24
Fusarium niveus	Czapek,s	1.5	2.5	3.2	3.9	4.7	5.3	5.7	6.3	6.7	6.9	7.3	**
	Malt	1.9	3.5	5.0	6.0	7.4	8.1	8.6	*				
	PDA	2.0	3.6	5.3	6.3	7.3	7.7	7.8	**				
	Rose- Bengal	1.2	2.0	2.5	2.8	3.5	4.0	4.3	4.7	5.0	5.4	7.0	**
	Sabouraud	2.0	4.0	5.9	7.1	8.1	8.2	8.6	*				
Fusarium acuminatum	Czapek,s	3.1	4.8	5.7	6.4	7.5	8.1	8.6					
	Malt	3.3	5.6	6.4	7.8	8.6	*						
	PDA	2.4	3.8	4.0	4.3	5.3	6.1	7.3	8.6	*			
	Rose- Bengal	2.5	4.5	5.5	6.5	7.6	8.0	8.6	*				
	Sabouraud	2.9	4.8	5.6	6.3	7.6	8.0	2.6	*				
Gliocladium roseum	Czapek,s	3.2	7.6	8.6	*								
	Malt	3.0	7.0	8.6	*								
	PDA	2.8	4.0	4.9	5.6	6.7	8.6	*					
	Rose- Bengal	1.9	3.9	5.9	7.5	8.6	*						
	Sabouraud	2.6	6.3	8.0	8.3	8.6	*						
Mucor racemosus	Czapek,s	7.0	8.6	*									
	Malt	6.0	8.6	*									
	PDA	4.6	7.7	8.6	*								
	Rose- Bengal	3.5	6.5	7.5	7.7	8.6	*						
	Sabouraud	6.1	8.6	*									
Penicillium janczewskii	Czapek,s	1.2	1.6	1.9	2.1	2.5	3.0	3.4	3.8	4.1	4.5	5.2	**
	Malt	1.3	1.6	1.7	1.8	2.4	2.9	3.3	3.9	4.0	4.3	**	
	PDA	1.2	1.3	1.7	2.0	2.3	2.7	3.0	3.3	3.9	4.6	5.3	**
	Rose- Bengal	1.2	1.4	1.7	1.8	2.2	2.7	3.2	3.5	3.8	4.2	5.4	**
	Sabouraud	1.2	1.6	1.8	2.1	2.6	3.2	3.4	4.5	5.1	5.5	**	
Trichoderma harzianum	Czapek,s	2.2	5.0	7.0	8.2	8.6	*						
	Malt	1.7	3.7	5.1	7.5	8.6	*						
	PDA	2.8	5.6	7.6	8.6	*							
	Rose- Bengal	2.3	4.4	5.9	7.2	8.3	8.6	*					
	Sabouraud	2.3	5.3	7.0	7.8	8.6	*						
T .koningii	Czapek,s	4.3	8.6	*									
	Malt	8.6	*										
	PDA	8.4	8.6	*									
	Rose- Bengal	4.6	8.6	*									
	Sabouraud	6.2	8.6	*									

* The growth completed in the Petri-dish. ** The growth ceased in the Petri-dish.

Table(4). Effect of different incubation temperatures on the growth (mg/100ml) of the tested fungi for 12 days.

Fungus	D. Wt (mg /100 ml medium)				
	15 C	25 C	35 C	45 C	55 C
Acremonium strictum	122	215	135	96	0.0
Aspergillus flavipes	78	122	197	188.0	0.0
A. melleus	230	420	384	0.0	-
A. niger	398	508	576	522	0.0
Circinella muscae	80	126	36	0.0	--
Emericella nidulans	98	308	492	456	0.0
Fusarium acuminatum	68	290	174	0.0	-
Fusarium niveus	54	380	260	100	0.0
Gliocladium roseum	168	532	316	98	0.0
Mucor racemosus	112	148	86	0.0	-
Penicillium janczewskii	206	414	309	170	0.0
Trichoderma harzianum	288	298	80	0.0	-
Trichoderma koningii	196	312	321	108	0.0

Table(5). Effect of different pH values on the growth (mg/100ml) of the tested fungi for 12 days.

Fungus	D. wt. (mg/100ml) medium					
	3.5	4.5	5.9	6.8	8.1	9.5
Acremonium strictum	186	220	251	366	278	156
A. flavipes	182	355	288	182	92	72
A. melleus	298	448	362	289	184	90
A. niger	296	324	554	648	278	158
Circinella muscae	74	106	143	86	60	37
Emericella nidulans	186	193	204	317	426	280
Fusarium acuminatum	208	356	482	380	260	180
F. niveus	254	380	310	260	120	80
Gliocladium roseum	232	432	486	540	660	425
Mucor racemosus	120	156	170	143	104	85
Penicillium janczewskii	462	298	184	92	60	40
Trichoderma harzianum	346	444	380	230	140	70
T. koningii	570	340	170	158	118	88

Table(6). Effect of Salinity on the growth (mg/100ml medium) of the tested fungi for 12 days.

Fungus	NaCl %				
	0.0	2	6	10	14
Acremonium strictum	166	188	310	-	-
A. flavipes	144	250	510	617	340
A. melleus	196	408	666	490	148
A. niger	420	564	677	770	226
Circinella muscae	140	211	250	276	320
Emericella nidulans	150	376	442	560	280
Fusarium acuminatum	180	506	396	346	228
F. niveus	160	324	460	136	-
Gliocladium roseum	370	736	536	346	-
Mucor racemosus	80	110	236	320	338
Penicillium janczewskii	86	206	542	380	-
Trichoderma harzianum	54	300	388	-	-
T. koningii	72	158	372	488	-

Table (7). The pectinolytic, cellulytic and amylolytic activities of the tested fungi.

Fungus	Pectinolytic activity (U)	Cellulase activity (% of relative activity)	Amylase activity (U)
Acremonium strictum	43.0	30.5	2.1
A. flavipes	37.5	49.9	10.1
A. melleus	34.5	47.9	4.0
A. niger	70.8	69.7	6.4
Circinella muscae	45.8	62.0	3.7
Emericella nidulans	50.0	66.4	4.9
Fusarium acuminatum	59.7	36.7	3.6
F. niveus	34.2	35.9	6.6
Gliocladium roseum	60.4	84.0	4.8
Mucor racemosus	41.7	16.4	3.5
Penicillium janczewskii	31.3	24.0	4.3
Trichoderma harzianum	31.7	46.6	9.6
T. koningii	38.8	38.7	7.4

* Pectin methyl esterase activity unit (U) = µg galacturonic acid/ min /ml crude enzyme.

* * amylase activity unit (U) = µmol maltose / min /ml crude enzyme.