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Studies on Fungal Communities Associated with Litter of Plant Cover at Al-Taif Province, Saudi Arabia

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Abstract. Twenty one fungal species belonging to ten genera were isolated from the litter of eight dominant plants cover of Al-Taif province, Saudi Arabia. The most frequently isolated fungal species were *Fusarium oxysporum* (100%), *Aspergillus alutaceus*, *A. niger*, and *Macrophomina phaseolina* (87.5%), *F. solani*, *Mucor racemosus*, *Penicillium glaburum* (75%), and *P. jaczewskii* (62.5%). The influence of temperature and pH on selected eleven isolates, representing the dominant fungal species, was estimated. The representative moulds failed to grow at 55ºC temperature. *A flavipes*, *A. niger* and *Emericella nidulans* showed thermotolerant activity (optimum growth at 35ºC). The selected fungal species responded differently to the tested pH values of Czapek-Dox medium; some appeared to be acidophilic (*P. jaczewskii*, *T. harzianum*, *F. niveus*, *A. flavipes* and *A. melleus*, pH 3.5-4.5), some alkaliphilic (*Emericella nidulans* and *Gliocladium roseum*, pH 8.1) and a third group attained their best growth values around neutral pH (5.9-6.8). The selected moulds were capable of producing cellulytic, pectinolytic and amylolytic enzymes, which indicate their major role in litter decomposition.

Keywords: Al-Taif, microfungi, plant litter, enzyme activity, growth conditions.

Introduction

The plant litter and its physical and chemical properties contribute to a considerable extent to the carbon cycling of the site, humus formation, soil structure and fertility, as well as, the nutrients and organic matter in soil (Lianne and Merriam, 1981; Nilsson, e*t al.*, 1999; and Berg and Meetemeyer, 2001). Sapro-

phytic 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 of ecologists to the relationship between fungal species diversity and ecosystem functioning, little is known about how decomposer fungi and their relative frequencies 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, and amylases (Celestino, *et al*., 2005; Dhouib, *et al*., 2005; and Jorgensen and Olsson, 2006).

The present work aimed to isolate fungal communities from the litter of dominant species of Al-Taif province 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

Al-Taif province is located on the Sarawat Mountains, south west of Saudi Arabia, at about 1800 m above sea level, with a mean annual precipitation of 500 mm and a mean of annual temperature of 24ºC and relatively moderate relative humidity (Meteorology and Environmental Protection Administration, 2000).

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

The dominant plant cover included, contains *Acacia asak*, *Asphodilus fistulosus*, *Eucalyptis rostata*, *Francoeuria crispa*, *Hypoestes forskalei*, *Juniperus excelsa*, *Opuntia ficus indica*, and *Shinus molls.* These plants were identified by the staff members of the Herbarium of Biological Sciences Department, Faculty of Science, King Abdulaziz University.

Isolation of Fungi

To obtain cultures for use in function tests with an estimate of frequency of occurrence for every taxon or isolate obtained, dilution series and litter plating techniques were used (Warcup, 1950). Litter was crushed and mixed thoroughly then 10 g were mixed with 90 ml sterile distilled water in 250 ml conical flasks,

shaked at 250 rpm for 20 min, thereafter serial dilutions were made. Czapek-Dox agar medium was inoculated with one ml (Warcup, 1955). Litter fragments were cut into 2 mm^2 pieces and about 0.001g (equivalent to 10^{-3} litter dilution) was placed on Czapek-Dox agar medium. Five replicate plates of each sample and dilution technique were prepared. The plates were incubated at 25ºC for 7 days. Fungi growing out of each dilution method or litter fragments were transformed to potato dextrose agar (PDA) medium slants. The isolated fungi were purified and identified based on their cultural and microscopic characteristics (Gilman, 1971; Barnett and Hunter, 1972, Stevens, 1984; Ellis and Ellis, 1985; and Moubasher, 1993).

Environmental Studies

The inoculum was in the form of disks, prepared using a sterile cork poorer (5 mm in diameter). The disks were obtained from homogenous growth of 4 days old cultures grown on PDA medium at 25ºC. Each treatment was carried out in five replica and the estimated results are the arithmetic mean.

The effects of incubation temperature and pH value on the growth of 11 isolates representing the dominant species of the isolated fungal genera were investigated.

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

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

Enzymes Activities

Cellulose, pectin and starch are among the main constituents of plant tissues. Therefore, cellulytic, pectinolytic, and amylolytic activities of the selected 11 isolates were estimated.

Aliquots (100ml) of cellulase promoting medium (Talboys and Busch, 1970), at pH 5, were dispensed in 250 ml Erlenmeyer flasks, inoculated with two disks of 7 days old culture for 14 days at 28ºC. 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 10 ml of 1.2% carboxy methyl cellulose (CMC) in phosphate buffer (pH 5.5), as enzyme

substrate, to which 5 ml of crude enzyme were added and the reaction time was 30 min at 30ºC. The Viscometer of Cannon Fenske type No 511 was used:

$$\% \text{ of loss in viscosity} = \frac{T_1A - T_2B}{T_1A - T_3B} \times 100$$

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

T_2 B = Time (in seconds) of flow of boiled crude enzyme mixture (15 ml, control).

T_3 W = Time (in seconds) of flow of 15 ml distilled water.

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).

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).

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

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

Highest fungal counts (72.7×10^3, 28.5×10^3, and 18.5×10^3 cfu/g litter were found in *A. asak*, *E. rostrata* and *A. fistulosus*, respectively. Fungal counts found in litter of other plant species were 1.5×10^3 and up to 6×10^3 cfu/g.

Twenty one fungal species belonging to ten different genera were isolated from the studied plant litter materials (Table 1). Fungal community was dominated by *Fusarium oxysporum* (100% frequency) and to a lesser extent *A. alutaceus*, *A. niger*, *Macrophomina phaseolina* (87.5% frequency), *F. solani*, *M. racimosus*, *P. glaburum* (75 % frequency), and *P. janczewskii* (62.5% frequency). While the rest of the isolates showed frequencies less than 38%. Soil mycoflora of different regions in 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 in Saudi Arabia (Abdel-Aziz and Mohammed, 1972; Fathi, *et al*., 1975; and Hashem and Al-Farraj, 1995).

Table 1. Frequency of fungal species isolation from litter of the dominant plants (15 samples each) at Al-Taif province.

Fungus	Plant species								Frequency (%) sample
	A. asak	*E. rostrata*	*J. excelsa*	*H. forsskalei*	*A. fistulosus*	*F. crispa*	*O. ficus indica*	*S. molls*	
Acremonium strictum	–	–	–	–	–	1	–	–	12.5
Aspergillus alutaceus	–	1	3	5	1	2	2	1	87.5
A. flavipes	–	–	–	–	–	–	1	–	12.5
A. melleus	–	–	1	–	–	–	1	–	25.0
A. niger	–	5	3	1	2	7	8	9	87.5
A. niveus	–	–	–	–	4	–	1	–	25
A. ustus	–	–	–	–	–	4	–	–	–
A. versicolor	1	–	–	2	–	–	–	1	37.5
Circinella muscae	–	2	2	–	2	–	1	–	50
Emericella nidulans	–	–	–	4	–	2	–	–	25
Fusarium oxysporum	5	5	6	1	7	6	5	1	100
F. solani	2	1	1	–	–	2	3	1	75
Gliocladium roseum	1	–	2	–	–	–	2	–	37.5
Macrophomina phaseolina	6	3	1	–	1	3	3	6	87.5
Mucor circinelloides	–	–	–	–	–	–	1	3	25
M. racemosus	–	2	2	5	–	2	2	3	75
Penicillium glabrum	1	2	2	2	–	1	–	1	75.0
P. freguentani	–	2	–	1	–	–	–	–	25.0
P. janczewskii	–	1	3	4	3	–	–	3	62.5
P. verrucosum	–	–	–	–	–	1	–	–	12.5
Trichoderma harzianum	–	–	–	–	–	6	1	1	37.5

Effect of Different Growth Media

The results indicated that Czapek-Dox, Malt, and Sabouraud media provided nutrients quality and/or quantity that were optimum for maximal linear growth of *Acremonium strictum* and *Mucor racemosus* at the sixth day of incubation. *Acremonium strictum* attained its maximal linear growth, on the tested five different media, at the sixth day of incubation. This indicated that it contains an active enzyme system capable of assimilating and using different ingredients in the route of its 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). 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 findings reflect the varied affinity of the tested fungi to utilize monomeric, oligomeric and polymeric sugars, as well as nitrogenous materials and ingredients of media (Griffin, 1981; and Al- Garni, 2006).

The results of Table 2 indicate that *Aspergillus flavipes*, *A. niger* and *Emericella nidulans* are thermotolerant which grow at temperature up to 40-45ºC, with the optimum growth at 35ºC. The thermotolerant activity of these fungi was reported by Abdel-Hafez (1982), Moubasher (1993) and Al-Fassi *et al.* (1994). While the rest of the tested fungi were mesophilic, where they attain their best growth values at 25ºC. This finding is in accordance with that reported by Yusef and Allam (1965) and Moubasher (1993). On the other hand, all the tested fungi failed to grow at 55ºC.

Effect of pH Value

The growth of the tested fungi (Table 3) responded differently to the hydrogen ion concentration of Czapek-Dox medium. They can be satisfactorily divided into three groups: acidophilic (attaining their best growth values at pH 3.5-4.5), as *Penicillium janczewskii*, *Trichoderma harzianum*, *Aspergillus flavipes*, *A. melleus* and *A. niveus*, alkaliphilic (pH 8.1), as *Emericella nidulans* and *Gliocladium roseum*, while the third group of the tested fungi attains its 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; while *A. flavipes* and *A. melleus* are acidophilic, *A. niger* is alkaliphilic. The influence of pH values on the fungal growth was reported by many workers (Yusef and Allam, 1965; Ramadani and Aggab, 1993; and Azmi and Seppelt, 1997).

Table 2. Effect of different incubation temperatures on the growth (mg/100ml) of the tested fungi for 12 days.

Fungus	D. wt (mg /100 ml medium)			
	15ºC	25ºC	35ºC	45ºC
Acremonium strictum	122	215	135	96
Aspergillus flavipes	78	122	197	188.0
A. melleus	230	420	384	0.0
A. niger	398	508	576	522
A. niveus	54	380	260	100
Circinella muscae	80	126	36	0.0
Emericella nidulans	98	308	492	456
Gliocladium roseum	168	532	316	98
Mucor racemosus	112	148	86	0.0
Penicillium janczewskii	206	414	309	170

Table 3. The pectinolytic, cellulytic and amylolytic activities of the tested fungi.

Fungus	Pectinolytic activity (U)	Cellulase activity (% of relative activity)	Amylase activity)
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
A. niveus	34.2	35.9	6.6
Circinella muscae	45.8	62.0	3.7
Emericella nidulans	50.0	66.4	4.9
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

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

Enzymatic Activity

In order to characterize the role that may be 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 in Table 3 revealed that the tested fungi have noticeable efficiencies to produce the tested hydrolytic enzymes, which indicate their major role in litter decomposition. *A. niger* and *G. roseum* 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 of the fungi showed lower activities (less than 40 En. U.) 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 associated with *A. flavipes*, *T. harzianum*, *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; and Jorgensen and Olsson, 2006).

References

Abdel-Aziz, M. and **Mohammed, Y.K.** (1972) Studies in the rhizospheric fungi of *Rhazya stricta*, *Bull. Fac. Sci., Riyad Univ*., **4:** 170-180.

Abdel-Hafez, S.I. (1981) Halophilic fungi of desert soils in Saudi Arabia, *Mycopathologia*, **75:** 75-80.

Abdel-Sater, M.A. (1994) Cellulase activity and succession of fungi in soil amended with sodium chloride, organic matter and Ca-superphosphate, *J. Basic Microbiology*, **34** (5): 283-302.

Abou-Heliah, A.N. (1985) Soil mycoflora of Saudi Arabia, II. Some microfungi in the forest soils of Asir region, *J. Biological Sciences Research*, **16**(2): 1-16.

Al-Fassi, F.A., Malibari, A.A. and **Moustafa, M.A.** (1994) Physiological studies on ten thermophilic and thermotolerant fungi isolated from different locations in the Western Region of Saudi Arabia, *Arab Gulf J. Scient. Res*., **12**(2): 321-340.

Al-Garni, S.M. (2006) Mycoflora associated with some textiles in Jeddah city, *Journal of King Abdulaziz University, Science* (In press).

Ali, M.I. and **Abou-Heliah, A.N.** (1984) On the fungal flora of Saudi Arabia, III. Some fungi in soils from eastern and southern regions, *J. Coll. Sci. King Saud Univ.*, **15** (2): 309-320.

Azmi, O.R. and **Seppelt, R.D.** (1997) Fungi of the Windmill islands, Continental Antarctica. Effect of temperature, pH and culture media on the growth of selected microfungi, *Polar Biology*, **18** (2): 128-134.

Barnett, H.L. and **Hunter, B.B.** (1972) *Illustrated Genera of Imperfect Fungi*, Burgess Publishing Company, New York.

Berg, B. and **Meetemeyer, V.** (2001) Litter fall in some European coniferous forests as dependent on climate: a synthesis, *Canadian Journal of Forest Research*, **31:** 292-301.

Cavalitto, S.F., Arcas, J.A. and **Hours, R.A.** (1996) Pectinase production profile of *Aspergillus foetidus* in solid state cultures at different acidities, *Biotech. Letters*, **18** (3): 251-256.

Celestino, S., Maria de Freitas, S., Medrano, F., Valle de Sousa and **Filho E.** (2005) Purification and characterization of a novel pectinase from *Acrophialophora nainiana* with emphasis on its physicochemical properties, *Journal of Biotechnology*. Available online at *www.sciencedirect.com.*

Deacon, L.J., Pryce-Miller, E.J., Frankland, J.C., Bainbridge, B.W., Moore, P.D. and **Robinson, C.H.** (2006) Diversity and function of decomposer fungi from a grassland soil, *Soil Biology and Biochemistry*, **38:** 7-20.

Dhingra, O.D. and **Sinclair, K.B.** (1985) *Basic Plant Pathology Method*, Florida, CRC Press. Inc., Boca Raton.

Dhouib, A., Hamza, M., Zouari, H., Mechichi, T., Hmidi, R., Labat, M., Martinez, M. and **Sayadi. S.** (2005) Autochthonous fungal strains with high ligninolytic activities from Tunisian biotopes, *African J. of Biotechnology*, **4** (5): 451-436.

Ellis, M.B. and **Ellis, J.P.** (1985) *Microfungi on Land Plants, An Identification Handbook*, London and Sydney, Croom Helm.

Fathi, S.M., El-Husseini, T.M. and **Abu-Zinada, A.H.** (1975) Seasonal variations of soil microflora and their activities in Riyadh region, Saudi Arabia, *Bull. Fac. Sci., Riyadh Univ.*, **7:** 17-30.

Frankland, J.C., Magan, N. and **Gadd, G.M.** (1996) *Fungi and Environmental Change*, Cambridge University Press, Cambridge.

Gilman, J. (1971) *A Manual of Soil Fungi*, The Iowa State University Press, Ames, Iowa, USA.

Griffin, D.H. (1981) *Fungal Physiology*, John Wiley and Sons, New York, Toronto.

Hashem, A.R. (1993) Soil analysis and mycoflora of the industrial Yanbu city, Saudi Arabia, *Arab Gulf J. Scient. Res*., **11** (1): 91-103.

Hashem, A.R. and **Parvez, S.** (1994) Mycoflora of aluminum rich soil of Hail region, Saudi Arabia, *Arab Gulf J. Scient. Res*., **12** (2): 341-350.

Hashem, A.R. and **Al-Farraj, M.M.** (1995) Mineral analysis of soil, Euphorbia hirta L. and mycoflora from the industrial Yanbu city, Saudi Arabia, *Qatar Univ. Sci. J*., **15** (1): 83-89.

Jorgensen, H. and **Olsson, L.** (2006) Production of cellulases by *Penicillium brasilianum* IBT 20888- Effect of substrate on hydrolytic performance, **38:** 381-390.

Joshi, S.R., Sharma, G.D. and **Mishra, R.R.** (1993) Microbial enzyme activities related to litter decomposition near a highway in a sub-tropical forest of north east India, *Soil Biology and Biochemistry*, **25** (12): 1763-1770.

Kartesz, Z.I. (1951) *The Pectic Substance*, New York. Inter. Science Publisher, p. 628.

Kvesitadze, E., Adeishvili, E., Gomarteli, M., Kvachadze, L. and **Kvesitadze, G.** (1999) Cellulase and xylanase activity of fungi in a collection isolated from the southern Caucasus, *International Biodeterioration and Biodegradation*, **43:** 189-196.

Lianne, M. and **Merriam, G.** (1981) Influence of topographic heterogeneity on deciduous litter decomposition, *Oikos*, **37:** 228-237.

Matta, A. and **Dimond, A.** (1963) Symptoms of *Fusarium* wilt in relation to quantity of fungus and enzyme activity in tomato stems, *Phytopathology*, **53:** 475-478.

Meteorology and Environmental Protection Administration (2000) *Scientific Information and Documentation Center*, Ministry of Defense and Aviation, Saudi Arabia.

Moubasher, A.H. (1993) *Soil Fungi in Qatar and Other Arab Countries*, University of Qatar, Qatar.

Nilsson, M.C., Wardle. D.A. and **Dahlberg, A.** (1999) Effects of plant litter species composition and diversity on the boreal forest plant-soil system, *Oikos,* **86** (1): 16-26.

Plummer D.T. (1987) *An Introduction to Practical Biochemistry*, London and New York, McGraw-Hill Bood Company.

Ramadani, A.S. and **Aggab, A.M.** (1993) Aldalophily among some filamentous fungi isolated from Saudi Arabia soils, *Arab Gulf J. Scient. Res*., **11** (3): 403-414.

Stevens, R.B. (1984) *Mycology Guidebook*, Seattle and London University of Washington Press.

Talboys, P.W. and **Busch, L.V.** (1970) Pectic enzyme produced by *Verticillium* sp., *Trans. Br. Mycol. Soc*., **55:** 367-381.

Ugwuanyi, J.O. and **Obeta, J.A.** (1977) Some pectinolytic and cellulytic enzyme activities of fungi causing rots of coccyams, *J. Science Food and Agric*., **73** (4): 432-436.

Warcup, J.H. (1950) The soil-plate method for isolation of fungi from soil, *Nature*, **166:** 117-118.

Warcup, J.H. (1955) Isolation of fungi from hyphae present in soil, *Nature*, **175:** 953-954.

Yusef, H. and **Allam, M.** (1965) Studies on the physiology of certain fungi, I. Temperature relations, II. pH relations, *Bull. Fac. Sci., University of Alexandria*, **7** (1): 137-162.

المجتمعات الفطرية المصاحبة للركام النباتي في محافظة الطائف بالمملكة العربية السعودية

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المستخلص. تم عزل ٢١ نوعا فطريا تابعة لعشرة أجناس من الركام النباتي، لثمانية نباتات برية، تشكل الغطاء النباتي في محافظة الطائف بالمملكة العربية السعودية، باستخدام بيئة أجارتشابك دوكس. وكان أكثر الأنواع الفطرية تواجداً *Fusarium oxysporum* بنسبة ١٠٠٪، حيث تم عزله من جميع عينات ركام النباتات المدروسة، يليه فطريات *Asper-gillus alutaceus* و *A. niger* و *Macrophomina phaseolina* بنسبة ٨٧,٥٪ ، ثم فطريات *Fusarium solani*، *Mucor racemosus* و *Penicillium glaburum* بنسبة ٧٥٪، وفقط *P. jaczewskii* بنسبة ٦٢,٥٪. كذلك تمت دراسة تأثير بعض الظروف المزرعية (الوسط الغذائي، درجة الحرارة، درجة الحموضة ودرجة الملوحة) على إحدى عشرة عزلة مختارة، تمثل الأنواع الفطرية السائدة.

وأوضحت الدراسة أن أجار تشابك دوكس، ومستخلص الشعير، وبيئة سابوراد كانت أفضل الأوساط المستخدمة في تقدير النمو الخطي لحوالي ٦٥٪ من الفطريات المختبرة. وقد أخفقت جميع الفطريات المدروسة في النمو عند درجة حرارة التحضين ٥٥°م ، وإن أظهرت فطريات *A. flavipes* و *A. niger* و *Emericella nidulans* قدرة على تحمل الحرارة العالية، حيث أعطت أعلى نمو عند ٣٥°م . كذلك تباينت بعض الأنواع الفطرية المختارة في درجة الحموضة في بيئة تشابك دوكس ، حيث أظهرت بعضها تفضيل الظروف الحمضية مثل *P. jaczewskii* ، *T. harzianum* ، *A. niveus* ، *A. flavipes* ، *A. melleus* (درجة حموضة ٣,٥-٤,٥)، في حين فضل البعض الآخر الظروف القاعدية مثل

Emericella nidulans و *Gliocladium roseum* (درجة حموضة ١ ,٨)، وكان أفضل نمو المجموعة الفطرية الثالثة عند درجة الحموضة بين ٩ ,٥ – ٦ ,٨. وأظهرت النتائج قدرة الفطريات المختبرة على إنتاج الأنزيمات المحللة للسليلوز والبكتين والنشا مما يدل على دورها الرئيس في تحليل الركام النباتي.