American-Eurasian J. Agric. & Environ. Sci., 1 (3): 260-267, 2006
ISSN 1818-6769

Studies on Fungal Pollution on Houses Walls and Their Enzymes Activities in Jeddah (Kingdom of Saudi Arabia)

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Abstract: It is aims to study the fungal colonization of air conditioners and old houses walls during November 2002 to December 2003 from various sites in Jeddah, Saudi Arabia, in order to determine the pollution that causes Sick Building Syndrome (SBS) accompanying sickness symptoms, using the Settle plate and swap methods for the isolation of fungi from the collected samples, the total fungal counts and the frequency occurrence were determined. The exoenzymatic activities for the four fungi isolated were also studied. The statistical analysis of the deteriorative fungal colonization at different sites showed significant variations. Elaboration of cellulase and pectinase were detected using the four fungal isolates *Aspergillus niger*, *Aspergillus ustus*, *Penicillium duclauxii* and *Trichoderma harzianum* which have a bad effect on cellulose materials. It is found that the humidity and temperature affect the level of both cellulase and pectinase activity. *A. niger* was the most frequent isolate detected in all locations all over the year.

Key words: Fungal air spora · air-condition · biodeteriorative

INTRODUCTION

Fungal contamination has been known in the etiology of respiratory allergic diseases. This contamination often contributes to building-related diseases, including both infectious and hypersensitivity diseases, such as allergic, in addition, acute toxicosis and cancer have been attributed to respiratory exposure to mycotoxins [1].

In Saudi Arabia, a few investigations were carried out on the air-borne fungal mycoflora. This lack information is typical of most countries outside the north and south temperate regions of the world [2] and reflects the generally accepted view that probably about 95% of all species of fungi are still waiting to be discovered [3].

Some air-borne fungal in Saudi Arabia at different governorates were investigated [4-9]. Two studies were recorded in Jeddah buildings, Abu-Zinada [10] found fungi, on painted walls and Gashgari [11] of ambient polluting fungi in Binzger art Museum.

The aim of the present study was to study the fungal colonization at walls houses from various sites in Jeddah, Saudi Arabia, in order to determine the pollution that causes Sick Building Syndrome (SBS) for the accompanying sickness symptoms, The exoenzymatic activities for the four fungi isolated were also studied.

MATERIALS AND METHODS

Site description: Jeddah is 1200 square kilometer in area with approximately 2 million populations. in the western region of kingdom Saudi Arabia, which lies on the cost of the Red Sea, temperature in the summer hot range between 37-45°C, in winter, the temperature is moderate, there is high air relative humidty.

Sample collection and identification of fungal species: Air samples from fungus-contaminated wet walls and old painted walls houses, as well as clean control rooms (25 buildings) were collected during November 2002 until December 2003 from various sites in Jeddah city. These localities were central, east, north, south and west using two methods described by Bokhary and Parvez [6] as follows:

Settle plate method, Twenty plates were put in each room, or suspect surfaces, i.e. those that on visual inspection appeared to have fungal growth, were sampled with sterile cotton swabs (Fischer Scientific) were cultured under standard conditions on two different culture media were used for each sample, carboxymethyl Cellulose medium (CMC) and Potato Dextrose Agar (PDA), in each of culture media Penicillin (0.6 g l^{-1}) and streptomycin sulphate (1 g l^{-1}) were added in order to inhibit the

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growth of bacteria. The plates were incubated for 7 days at 28°C. Fungi were identified based on their morphology according to Gilman [12], Raper and Thom [13], Raper and Fennell [14], Ellis [15, 16], Simmons [17] and Sutton *et al*. [18].

Studying the effect of some environmental conditions such as:

Effect of temperature: Temperature of the medium could be adjusted at (25, 35, 45 and 55°C) on growth rate of four fungi tested *A. niger*, *A. ustus*, *P. duclauxii* and *T. harzianum.*

Effect of moisture: Moisture of the medium could be adjusted by adding different concentrations of sulphuric acid (35, 55, 75, 95 and 100%) according to Solomen [19].

Effect of water activity: Water activity (a_w) of both medium used adjusted at (0.990, 0.950 and 0.850) by adding glycerol according to Marin *et al.* [20] and Ni and Streett [21].

Enzymatic activity of fungal isolates: Four tested fungi in this study were screened for their abilities to produce extracellular enzymes in solid media.

For pectinase production: The method was carried out as described by Hankin *et al.* [22].

For cellulase production: The medium described by Eggines and Pugh [23].

Statistical analysis: All experiments replicate three times. T. test was done, significance of differences was defined at $p<0.05$.

RESULTS AND DISCUSSION

The genera and species identified from the different sites using two different culture media for the whole year are presented. Depending upon their frequency of appearance and the number in the sample, genera were grouped as "major components" and "minor components". Major components included most frequently encountered genera such as *Aspergillus.* The percentage of various components in the air are presented in (Table 1 and Fig. 1). The mean total (number) Colonies Forming Units (CFU) of *Aspergillus* colonies isolated was 193 that constituted 23.11% of the total fungi

Table 1: Total count, occurrence, percentage of fungi in PDA medium at 28°C in 8 day's

Fungi	No.	Occurrence	%
Aspergillus	193		23.11
A. flavus	39	++	4.67
A. flavus var. *columnaris*	7	++	0.84
A. flavipes	1	+	0.12
A. niger	108	++++	12.93
A. ustus	7	++	0.84
A. sydowii	7	++	0.84
A. versicolor	6	++	0.72
A. ochraceus	5	+	0.50
A.oryzae	9	++	1.08
A. tamarii	4	+	0.48
Alternaria	93		11.14
Al. alternate	93	++++	11.14
Aureobasidium	1	+	0.12
Au. pullulans	1		0.12
Acremonium	3	+	0.36
Ac. sp	3	+	0.36
Choetomium	7		0.84
Cho. globosum	7	++	0.84
Cochliobolus	2		0.24
Co. spicifer	2	+	0.24
Cladosporium	41		4.91
Cl. oxysporium	41	+++	4.91
Curvularia	12		1.44
Cu. ovoidea	12	++	1.44
Emericella	23		2.75
E. nidulans	10	++	1.20
E. quadrilineata	1	+	0.12
E. versicolor	12	++	1.44
Eurotium	1		0.12
E. amstelodami	1	+	0.12
Penicillium	128		12.33
P. duclauxii	101	++++	12.10
P. purpurogenum	2	+	0.24
P. pinophilum	8	++	0.96
P. funiculosum	8	++	0.96
P. echinulatum	9	++	1.08
Trichoderma	110		13.17
T. harizinum	110	++++	13.17
Rhizopus	110		13.17
R. arrhizus	110	++++	13.17
Nigrosporium	1		0.12
N. sp	1	+	0.12
Sterial myceium	110	++++	13.17
Total count	835		

+Low Occurrence, ++Moderate occurrence, +++Good occurrence, ++++High occurrence

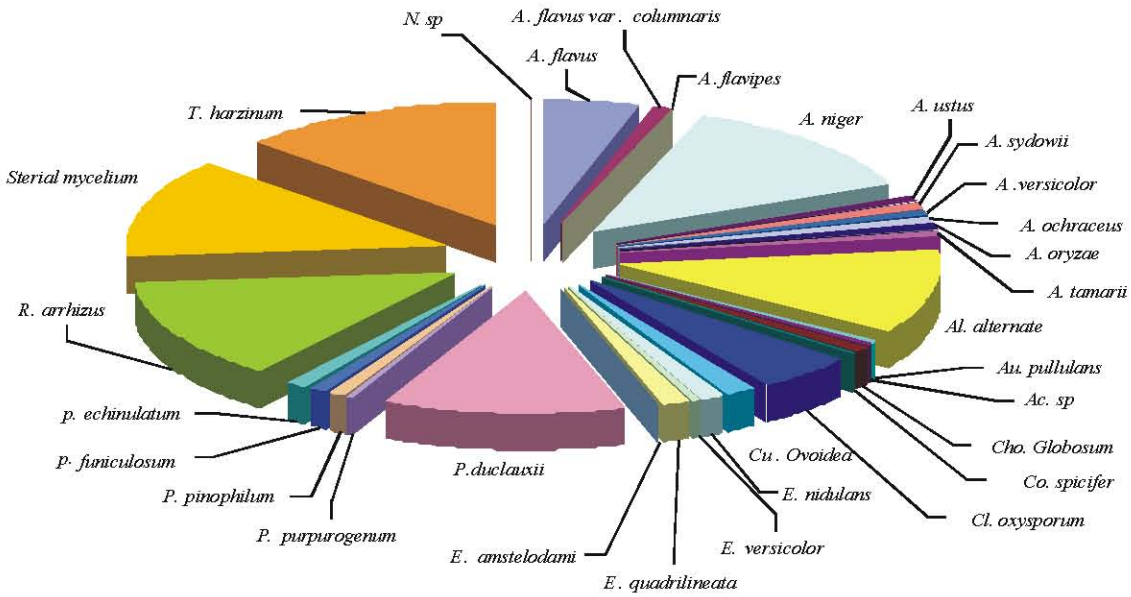

Fig. 1: Occureance of fungal species isolated from different buildings in PDA medium at 28°C in 8 day's

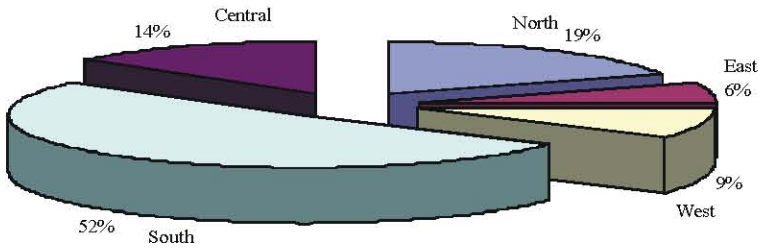

Fig. 2: Percentage of fungal colonies collected from buildings at different sites of Jeddah area

isolated, represented in 10 species. The total number of colonies of *Penicillium* (5 species) was 128 which constituted 12.33% of the total fungi isolated. The mean total number of *Trichoderma*, *Rhizopus* and Sterial mycelium (1 species) were 110 (13.17%), The total number of *Alternaria* (1 species) was 93 which (11.14%). While minor components included less frequent and sporadic types such as *Cladosporium*, *Emericella*, *Choetomium*, *Cochliobolus*, *Acremonium*, *Eurotium*, *Nigrosporium* and *Aureobasidium* (1 species). These results are comparable to those from previous study in Jeddah [10, 11] and other studies in Riyadh [7-9, 24], Cairo, Egypt [25, 26], Kuwait city, Kuwait [27] and Japan [28, 29] as well as studies in America [1].

Aspergillus niger exhibited the highest number of colonies per unit of areas followed by *A. flavus* and *A. oryzae*. *Aspergillus* species such as *A. flavus*, *A.niger* are common buildings fungi and are a high risk for causing deep-seated aspergillosis in immunocomromised persons [30].

The total number of fungal colonies per plate collected from buildings at different sites of south Jeddah area, give higher number of fungal colonies consist 52% of total fungi isolated (south is a developed area thickly populated, higher concentration of buildings, etc.). It mainly consists of old commercial buildings, roads and street are narrow, crowded and populated. There are a number of traditional homes in the area (Fig. 2) than buildings in other localities, a modern, new and less populated area. It has a very modernized shopping center and villas with open planned streets which are planted and irrigated regularly.

The highest number of colonies per settle plate was found in the bathrooms followed by kitchens walls and the lowest number was found in the bedrooms and living rooms (data not shown). The number of colonies per plate

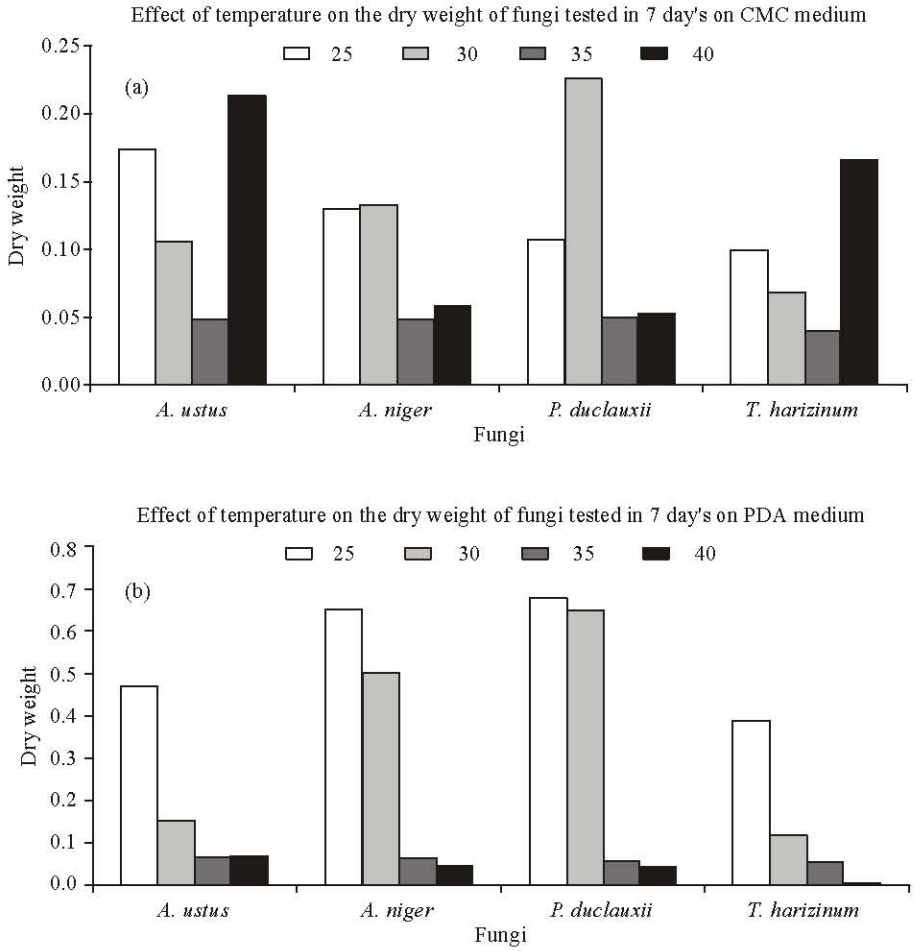

Fig. 3 a-b: Effect of different temperature of four tested fungi in PDA and CMC media

was in general higher in densely populated areas than in less populated areas. Fungi also adjust to growing on windows especially in bathrooms where water leakage was found to enhance its profound growth. In addition, the modern building system has added to pollution problems and the growth of fungi due to thermal insulation, double glazed windows to provide desired temperature in addition to central air-conditioning systems where there is no natural ventilation which creates a suitable environment for fungal growth. This was shown clearly by the number of fungi that have been isolated from ventilation filters in central air-conditioning systems [1, 31-34].

The variation of numbers of fungal isolates present in buildings show that *A. ustus*, *A. niger*, *P. duclauxii* and *T. harzinum* were detected in all seasons of the year. Physiological studies on that four fungi included effect of temperature; relative humidity and water activity selected based on the quantitative and qualitative information about the ability of fungi on growth in CMC and PDA were observed. Growth were compared at 25, 35, 45 and 55°C (Fig. 3a and b) data revealed that the maximum, optimum temperature in PDA medium of *A. niger*, *A. ustus*, *P. duclauxii* and *T. harzinum* were 25 and 35°C, suggests that the moderate temperature is optimum for growth in PDA, while in case of CMC medium were 55°C in *A. ustus* and *T. harzinum,* while 30°C in *A. niger* and *P. duclauxii.* This was similar with Ferreira and Filho [35] showed the mesophilic fungi *T. harzinum* strain T4 when grown on wheat bran as the carbon source and showed that the β-mannanase activity was most active at 55°C. This means the optimum temperatures differ greatly from one species to another; however, no difference appears clearly between strains belonging to the same species values were significantly affected by type of media. This influence of temperature was dependent on the species and affected by the composition of media [36].

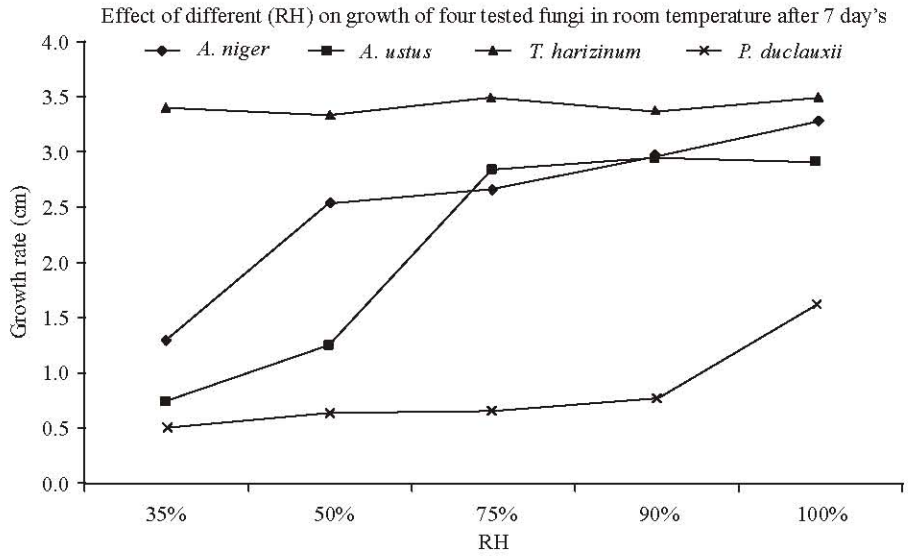

Fig. 4: Effect of different relative humidity of four tested fungi in PDA media in room temperature

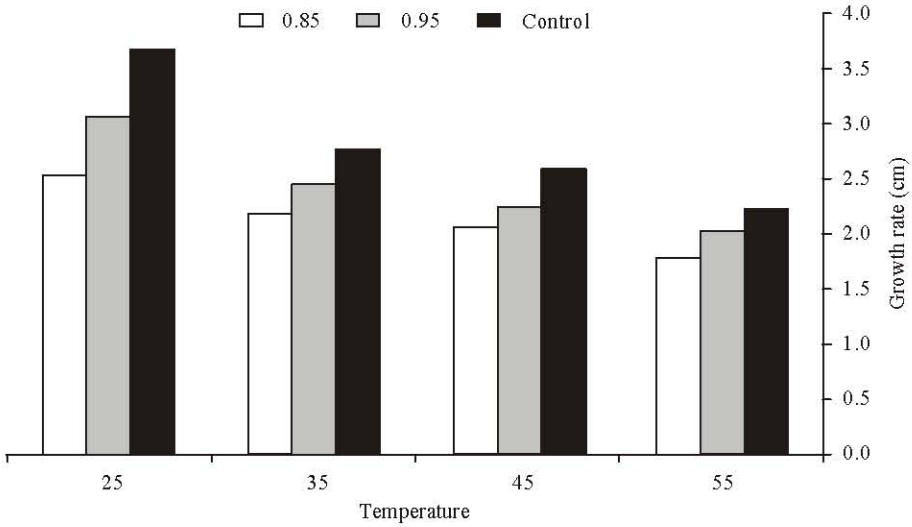

Fig. 5: Effect of water activity (a_w) on growth rate of *A. niger* in different temperature

Meanwhile the effect of Relative Humidity (RH) on the growth of the four tested fungi showed that all of them can grow on large range of RH from (35-100%) (Fig. 4). *T. harzinum* was more active than others high levels of (RH) favourd by these moulds. Maximum growth found at the earlier growth stages (5 day's for (*Trichoderma harzinum* and *Aspergillus niger*) and two had a 7-13 day's for aggregate (*Aspergillus ustus* and *Penicillium duclauxii*), these conclusions similar with that previously reported by Nicolajsen [37] and Canhoto *et al.* [38] that the maximum air relative humidity get growth of wood decaying fungi and results obtained were better at the higher humidity.

Water activity (a_w) effect on the growth of the four tested fungi showed that all of them can grow on large range of (a_w) from (0.850-1). *T. harzinum* and *A. niger* were more active than others (Fig. 5). Growth rate of all the fungi tested increased as water availability in the medium increased and had the fastest growth rate at highest a_w levels (0.995). Growth also varied with temperature and decreased as temperature increased from 25 to 55°C. *T. harzianum* grew faster at 25 and 35°C than *A. niger*, *A. ustus* and *P. duclauxii* at a_w levels of 0.950 and 0.850, these conclusions similar with that previously reported by Canhoto *et al.* [38] were carried out a cellulose based agar at two water activities (a_w, 0.975, 0.995) and on three

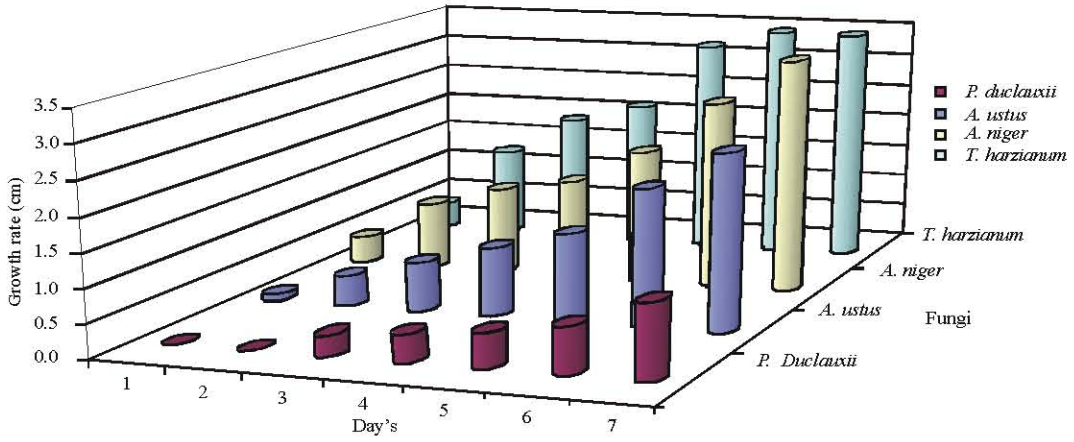

Fig. 6a: Cellulase production

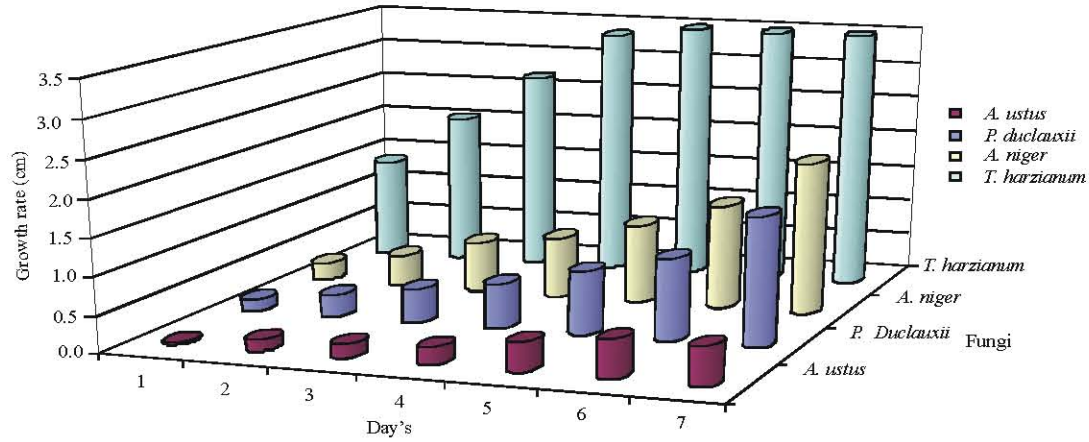

Fig. 6b: Pectinase production

types of paper at two relative humidities (75, 100% RH) for the potential for differentiation of contamination and colonization by *A. terreus*, *A. holandicus* and *Eurotium chevalieri* gave different responses to each of these species when grown on cellulose agar at both a_w levels. each of these fungi was different of each other and of the control.

Enzyme activity were studied of four tested fungi *T. harzinum* and *A. niger* revealed that they had the power to secrete analysing enzymes for cellulose and pectin in different degree, provided cellulose and pectin bait.

Enzyme concentration affects cellulase and pectinase activity in fungi, in case of *T. harzianum* enzyme activity increased than other species (Fig. 6a and b). that cellulose secretion of this isolate increased in PDA medium, this indicates that the enzyme secretion was dependent on the species. Various cellulosic materials as wallpapers, paintings and books in the liberary were very badly affected by cellulolytic fungi in damp weather as well as water seepage through the wall. These conclusions similar with that previously reported by Wang and Hung [39] and Karunasena *et al.* [40].

The variation in the levels of both cellulolytic and pectolytic activities in fungi revealed that *T. harzinum*, *A. niger*, *A. ustus* and *P. duclauxii* produce high activities of both enzymes in both medium, with high frequency occurrence %. High amount of enzymes was noticed in CMC then PDA medium and this might be the result of content of media for fungal growth. These conclusions similar with that previously reported by Cheng-Fang *et al.* [41] the optimal activity conditions with Carboxymethyl Cellulose (CMC) as the substrate were pH 6.0 and 50°C in fungus *Piromyces rhizinflata*.

ACKNOWLEDGEMENTS

The authors thank the General Directorate of Research Grants Programs KACST, for supported Kloud Saadi under post graduate project no. AT.13-19.

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