Mycoflora Associated with Some Textiles in Jeddah City

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Abstract. The Mycoflora of eight different fabrics (natural, synthetic, and blended), collected from warehouses in Jeddah, Saudi Arabia, were isolated by different methods, contact plate, dilution, Stomacher, and moisturization that followed by contact plate method. Twenty three different species were isolated. Aspergilli were the most dominant (35%), followed by penicilli and fusaria (13% each). The highest fungal count (1932 CFU/cm²) was concomitant with cotton fabrics, even when blended with polyester. Wool textiles came in the second order of fungal counts (1421 CFU/cm²). While natural silk fibers, was less susceptible for fungal attack, polyamide textiles had moderate affinity toward fungal colonization.

The influence of some environmental conditions (temperature, growth medium, pH and relative humidity) on the linear growth of 10 representative isolates, that may have health hazards to man (Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Fusarium oxysporum, Geotrichum sp., Gliocladium artum, Penicillium chrysogenum, Rhizopus nigricans and Trichoderma viride), were tested. The cellulytic and proteolytic activities of these fungi indicated that T. viride, Geotrichum sp. A. niger, A. alternata and P. chrysogenum were with the highest activities. Antimycotic activity of two detergents was studied and indicated that the tested fungi responded differently to the detergent type and its concentration.

Introduction

The importance of textile fibers has changed over the years and moreover differs according to culture [1]. In Europe and North America, by 1900, cotton provided about 80% and the rest was wool, flax and silk. Since that time, manmade fibers have been developed and increased in importance [2]. So, the

degradation of cellulosic textiles and the prevention of biogenous decay have changed. The use of man-made fiber textiles in the place of natural fibers does not automatically mean that they are "biologically resistant" [3]. Many synthetic fabrics may be easily attacked and decomposed by microorganisms [4].

Fabrics are suitable environments for the growth and multiplication of microorganisms. Many investigations were made on mycoflora found on textiles exposed to indoors and outdoors. It has been shown that microbial population in air "aeromycoflora" is similar to that communities of the organisms found in soil [5-10].

Aeromycoflora are biopollutants and have important impacts on medicine. They cause various health hazards in man and other organisms; they cause a variety of mycoses and allergies [11-13].

Mycoflora have the priority within the microorganisms that attack fabrics and hydrolyze their components by their hydrolyzing enzymes as cellulases and proteases, especially under favorable conditions of temperature, humidity and pH^[14].

In Saudi Arabia there are poor records on the mycoflora of different types of fabrics ^[15]. So the present work aimed to characterize the mycoflora associated with some natural, synthetic and blended fibers and to study the effect of some environmental conditions on the isolated fungi, as well as their cellulytic and proteolytic activities.

Materials and Methods

Two hundred samples of natural fibers (cotton, lenin, silk and wool textiles), synthetic fibers (nylon of polyester and polyester of polyamide textiles), and blended textiles (75% cotton with 25% polyester and 65% cotton with 35% polyester), were collected from warehouses at Jeddah, Saudi Arabia (twenty five samples of each textile). The samples were collected in sterile plastic bags and stored at 4°C till analysis.

Moisture Content

Twelve grams of each sample were used to determine the moisture content at 100°C, until constant weight. The percentages of moisture contents were 4.3, 3.5, 0.25, 5.25, 0.5, 0.75, 2.7 and 2.5 for cotton, lenin, silk, wool, nylon, polyester, cotton with polyester (75 with 25%) and cotton with polyester (65 with 35%), respectively.

Estimation of Textile Associated Fungi

Five different methods were used to isolate the textiles mycoflora using potato dextrose agar (PDA) medium, to which penicillin and streptomycin were added to prevent bacterial growth [16-20]. These methods were:

1 - Contact Plate

Petri-dishes with PDA medium were used. They were pressed onto the fabric test samples (1 cm²) and then incubated at 28°C for 2 weeks. There after, the number of colonies was counted.

2-Dilution

Pieces of tested fabrics (1 cm² each) were mixed thoroughly with 10 ml sterile distilled water in 50 ml conical flasks, shaked at 250 rpm for 20 min. One ml was used to inoculate Petri-dish containing PDA medium, then incubated for 2 weeks at 28°C. Colony counts were then carried out.

3-Stomacher

A fabric test sample (100 cm²) was placed in a sterile polyurethane bag with 100 ml 0.9% NaCl. The bag was scaled and run for 3 min in a stomacher 400®. One ml fluid was spread onto PDA plates which then incubated at 28°C for 2 weeks.

4-Moisturization

Ten pieces (1 cm², each) were moistening with sterile distilled water in empty Petri-dish, incubated at 28°C for six months. Moistening was carried out weekly.

5 - Contact Plate Method for Moisturized Fabrics

Petri-dishes containing PDA were pressed onto the fabric sample of the last method (moisturization) and then incubated at 28°C for 2 weeks. The number of colonies was then counted. The isolated fungi by the above five methods are maintained on PDA slants at 5°C with monthly intervals subculture.

Identification of the Isolated Fungi

The developing fungi, by the five used isolation methods, were purified and identified. Identification was based on macro and microscopic characters [21-27]. The fungal counts were the sum of the used five isolation methods.

Environmental Studies

The effect of some cultural conditions as, incubation temperature, nutrient media, pH value and relative humidity on the linear growth of selected representative 10 isolates that may have health hazards in man (Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Fusarium oxysporum, Geotrichum sp., Gliocladium atrum, Penicillium chrysogemum, Rhizopus nigricans and Trichoderma viride), was carried out. 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 28° ± 2°. Each treatment was carried out in 5 replica and the estimated results were the arithmetic mean.

Effect of Growth Temperature

The linear growth (cm) of the tested moulds on PDA medium was estimated at incubation temperatures ranging from 10-55°C for 14 days.

Effect of Different Nutrient Media

The tested moulds were cultivated on five different media of Difco (PDA, Malt Extract, Czapek's, Rose Bengal agar and Sabouraud) and the linear growth was estimated regularly for 24 days of incubation or until the completion of growth in Petri-dishes.

Effect of Initial pH Value

The influence of different pH values (4-12) on the linear growth of the test fungi on PDA medium, after 14 days of incubation at 28° ± 2°C, was estimated.

Influence of Relative Humidity (R.H)

Levels of R.H (14.5 – 100%) were maintained using different amounts of KOH and NaOH [28]. The test mould was inoculated to PDA, then the Petri-dish upside down, where the tested R.H (5ml) was placed in the lid. Incubation for 14 days at 28° ± 2°C, there after the linear growth was recorded.

Antimicrobial Activity of Detergents

Two different detergents (Tide and Ariel of Procter and Gamble) were tested for their antifungal activity. Three different levels were used (4,8 [recommended level] and 16 g/l) The tested detergent level was mixed aseptically with PDA (at 50°C) poured in sterile Petri-dishes and after solidification inoculated with the tested fungal growth (disks), incubation for 14 days at 28° ± 2°C, where the linear growth was estimated.

Enzymatic Activity

The cellulytic and proteolytic activities of the selected 10 isolates were estimated.

Cellulytic Activity

Aliquots (100ml) of cellulases promoting medium [29], at pH4, were dispensed in 250ml Erlnmeyer flasks, inoculated with 1 ml spore suspension (10⁶ spore/ml) of 5 days old culture and incubated for 12 days at 2°C. The crude enzyme (filtrate) was isolated by centrifugation at 3000 rpm for 20 min and the enzyme activity was determined, using CMC substrate [30,31], as μmol glucose /h/ml crude enzyme. The produced glucose was estimated colorimetrically using dinitrosalicylic acid [32-34].

Proteolytic Activity

The proteases of the tested fungi were estimated using modified Czapek's medium of the following composition (g/l): maltose, 20; casien, 4; K₂HPO₄, 1; KCl, 0.5; ferric chloride 0.005. The enzyme activity was determined in the filtrate and the enzyme units were calculated using tyrosine standard [32-34], as follows:

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

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

Results and Discussion

Mycoflora of Different Textiles

Twenty three different fungal species were isolated from the tested fabrics (Table 1). Aspergilli represent about 35%, penicilli and fusaria were about 13% of each and the rest species were represented by only one species (4.4%). It was reported that aspergilli, penicilli and fusaria were the most dominant moulds in textiles [15]. The cellulosic textiles of cotton represent the highest fungal counts 1932 CFU/cm², i.e., cotton fibers represent a prime substance for microbial attack and/or cotton fibers were more contaminated during the different stages of cotton processing, since in fields up to the form of textile. Even cotton in its blended form with polyester resulted in about 3.8, 2.4 fold increase of fungal count when mixed with 75, 65%, respectively, as compared with synthetic textiles of polyester only. Wool, as a natural fiber, was in the second order from the stand point of fungal count, while silk textiles were the less susceptible natural fibers for fungal attack. It is noteworthy that the polyamide textiles have

Table 1. Total count (CFU/cm²) of fungi isolated from 8 different fabrics (25 sample of each), with 5 different laboratory methods after 14

Fungus	ů	Cotton	Le	Lenin	SIIK	K	We	Wool	ž	Nylon	Polyester		Blended or Polyeste	Blended cotton (75%) Polyester (25%)	Blended cotton (65%) Polyester (35%)	ended cotton (65%) Polyester (35%)
	Total	Freq.*	Total	Freq.*	Total	Freq.*	Total	Freq.*	Total	Total Freq.*	Total	Freq.*	Total	Freq.*	Total	Freq.*
Alternaria alternate	10	1	1	1	6	i	T.	i	£3	ì	1	ï	26	3	16	-
Aspergillus	1204	25	574	25	307	19	631	23	247	12	100	90	622	91	409	14
A. farms	86	2	33	2	28	150	Ξ	9	.1	Ţ	ij	1	19	4	991	9
A. fumigatus	250	4	187	3	40	9	274	99	J	1	Ť	1	144	4	100	3
A. Isumicola	.1	1	40	7	1	1	1	1	1	1	1	1	1	1	,	1
A. niger	410	6	201	90	62	3	148	7	71	4	30	*	183		143	3
A. terrues	20	4	78	1	130	63	E	E	14	-	T.	ľ	į.	ı	Ŀ	1
A. asstus	190	2	20	-	100	-	31	г	83	2	53	53	74	5	t	1
A versicolor	210	1	15	-	14	67	19	1	8	8	17	3	160	3	1	1
Fusarium	82	7	t	-	10	1	71	2	280	33	1	ì	1	ï	30	-
F. dimerum	X	m	1.	1	1	71	c)	1	1	1	1	ì	1	ı	Į.	
F. oxyaporum	1	ï	7	1	T	1	10	1	130	2	T	1	1	1	30	-
F. powa	30	4	1	1	£	1	E	1	110	-	1	1	ı	1	T:	1
Geotrichum sp.	E	-	t	-	Ţ	-	Ţ	1	13	1	Ţ	-	138	2	Ŀ	1
Gilocladium атым	1	1	1	1	10	1	I,	ı	22	8	ľ	1	1	1	41	3
Maror musedo	22	**	=	2	40	,	127	y	10	,			36	,	19	

Table 1. Contd

Fungus	Cot	Cotton	И	Lenin	Silk	*	Wool	To.	Ny	Nylon	Polyester	ster	Blended co Polyeste	Blended cotton (75%) Polyester (25%)	Blended cotton (65%) Polyester (35%)	ton (65%)
	Total	Freq.*	Total	Freq.*	Total	Freq.*	Cotal	Freq.*	Total	Freq.*	Total	Total Freq.*	Total	Freq.*	Total	Freq.*
Penicillium	372	6	190	2	140	3	255	15	203	4	178	9	380	9	166	7
P.chrysogenum	340	9	180	4	140	3	215	6	203	4	148	4	380	9	991	7
P. janczewskii	20	1	ı	1	1	1	33	5	1	ì	1	1	1	ī	1	1
Penicillium sp.	12	61	10	1	1	1	7	1	T	ì	30	2	î	1	1	1
Rhizoctonia	120	4	20	*	11	-	84	6	119	5	162	4	1	T	1	1
Rhizoctonia solani	120	4	8	3	Ξ	-	13	61	119	2	1	1	ï	ı	1	1
Rhizoctonia sp.	1	1	1	i	ા	1	71	1	1	1	162	4	1	1	31)
Rhizopus nigricans	70	4	J	1	1	-	312	5	1	1	1	.1	110	3	109	80
Spondylockidm xhsogenim	- 1	1	-	1	T	- 1	1	1	- 1	- 1	- 1	- 1:	130	2	14	-
Sporotrichum laxum	1	ा	1	1	1	1	1	1	40	2	1	1	70	3	1	1
Trichodernas viride	99	5	12	1	1	1	1	1	1	1	1	1	-	1	130	2
Total	1932	57	837	36	498	25	1421	54	935	31	400	18	1514	37	626	37

*Frequency = $\frac{\text{Times of furgus isolation}}{\text{Number of fabric samples (25)}} \times 100$

moderate affinity toward mycoflora attack as compared to natural, synthetic and blended textiles. It was reported that the high fungal counts in textiles usually due to polluted storage places, transportation, and handling of textiles [36,37]. The last factors beside the hot humid atmosphere of Jeddah city, almost allover the year, encourage fungal attack, growth and multiplication.

The results revealed the presence of some isolates as Aspergillus fumigatus, A. niger, A. flavus, A. terreus, Fusarium sp., Geotrichum sp. and Mucor sp. that cause various health hazards to human being such as allergies, respiratory diseases and cutaneous diseases when in contact with human body during cloth wearing, as well as formation of mycotoxins [38-40].

The count and identification data showed variations in both fungal genera and species, this finding was reported by several workers [8,40,41].

Environmental Studies

Effect of Growth Temperature

The results (Fig. 1) indicated that each fungus has an optimum growth temperature and needs a certain range of temperature to attain detectable growth values. Seven moulds namely, Alternaria alternata, Aspergillus flavus, A. niger, Fusarium oxysporum, Rhizopus nigricans, Geotrichum sp. and Trichoderma viride attain their optimum linear growth at 25°C, while A. fumigatus, Gliocladium atrum and Penicillium chrysogenum accept their optimum growth at 35°C. However, the tested fungi failed to grow at 10 and 55°C. These results indicated that the tested fungi are considered to be mesophilic [42-44].

Effect of Different Growth Media

The results (Table 2) indicated that under the tested conditions the nutritional requirements of the tested fungi were not dependent on the genus of the fungus, but on the mould species. Whereas, potato dextrose agar (PDA) medium provided nutrient quality and/or quantity that were optimum for the linear growth of Alternaria alternata, Aspergillus fumigatus, Geotrichum sp., Rhizopus nigricans and Trichoderma viride, rose bengal medium provided ingredients in harmony with the least growth of A. alternata, A. flavus, Fusarium oxysporum and Penicillium chrysogenum. While, A. flavus and A. niger gave the highest growth on sabouraud and malt extract media. The ingredients of PDA and rose bengal media were not in harmony to their growth. On the other hand, PDA and sabouraud media stimulated the highest growth of P. chrysogenum. However, malt medium was the most adequate for G. atrum growth. These findings reflect the varied affinity of the tested fungi to utilize monomer, oligomeric and polymeric sugars, as well as nitrogenous materials and other ingredients of media [45]. According to

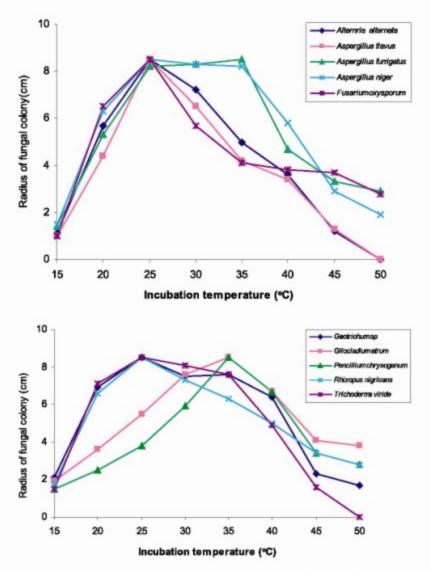


Fig. 1. Effect of incubation temperature on the linear growth (cm) of the tested fungi.

Table 2. Effect of different growth media on the linear growth (cm) of the tested fungi for 24 days of incubation.

Fungus	Growth medium					Incub	ation	perio	d (day)				
rungus	Growth medium	2	4	6	8	10	12	14	16	18	20	22	24
Alternaria alternata	Czapek's Malt PDA Rose-Bengal Sabouraud	1.5 2 2.1 1.5 1.9	2.5 3.5 4.5 2.4 2.3	3.8 4.9 8.5* 3.3 4.5	4.5 5.7 4.1 6.8	6.3 6.7 4.9 7.5	7.5 8.5* 5.5 8.5*	8.5°	6.9**				
Aspergillus Janus	Czapek's Malt PDA Rose-bengal Sabouraud	23 25 21 1.5 4.7	4.4 5.1 4.7 2.3 6.3	5.8 8.5° 6.3 3.8 8.5°	6.8 7.5 4.7	7.5 8.5* 5.6	8.5° 6.7	7.1	8.5*				
Aspergillus famigatus	Czapek's Malt PDA Rose-Bengal Sabourand	3.7 3.5 4.5 3.5 4.7	4.8 6.1 8.5* 4.5 6.9	6.7 7.5 5.9 8.5°	7.5 8.5* 7.8	8.5° 8.5°							
Aspergillus niger	Czapek's Malt PDA Rose-bengal Sabouraud	2.5 2.5 2.6 2 3.2	5.3 6.6 4.5 3.7 5.8	7.3 8.5° 5.7 4.3 8.5°	8.5* 6.3 5.6	7.1 7.3	8.5* 8.5*						
Fusaritan охупрогит	Czapek's Malt PDA Rose-Bengal Sabouraud	25 25 23 1.5 29	4.3 4.5 4.4 2.6 5.5	5.3 6.3 5.7 3.1 8.5°	6.4 7.2 7 4.4	7.8 8.5* 8.5* 5.2	8.5*	6.7	7.2	7.8**			
Geotricum sp.	Czapek's Malt PDA Rose-bengal Sabouraud	6.5 5.5 7.3 5.7 6.5	7.5 7.5 8.5* 7.5 7.8	8.5° 8.5° 8.5°							9 2		
Gliocladium atrum	Czapek's Malt PDA Rose-Bengal Sabouraud	2.5 2.5 3.5 2.1 2.3	4.7 6.1 5.9 4.3 4.8	5.3 8.5* 7.1 6.8 6.3	6.2 6.2 8.5* 7.1 7.2	7.7 7.7 8.5* 8.5*	8.5* 8.5*						
Panicillium chrysogenum	Czapek's Malt PDA Rose-Bengal Sabouraud	2.1 2.5 1.2 2.3	3.2 3.8 5.3 2 4.6	4.5 4.7 6.5 2.5 5.7	5.7 5.3 7.5 2.9 7.1	6.8 6.4 8.5* 3.1 8.5*	7.1 7.5 3.8	8.5° 8.5° 4.3	4.9	5	5.8	6.1	6.7*

Table 2. Contd.

Fungus	Growth medium					Incub	ation	period	(day)				
rungus	Crown medium	2	4	6	8	10	12	14	16	18	20	22	24
Rhizopus nigricans	Czapek's Malt PDA Rose-bengal Sabouraud	6.1 5.5 6.5 5.2 4.7	7.9 7.5 8.5* 7.8 7.6	8.5° 8.5° 8.5° 8.5°									
Trichoderma viride	Czapek's Malt PDA Rose-Bengal Sabouraud	5.5 5.8 6.2 4.5 4.9	7.4 7.4 8.5* 7 7.1	8.5° 8.5° 8.5° 8.5°									

[&]quot;The growth completed in the Petri-dish.

the time required for each fungus to attain its highest linear growth on the tested medium, two groups can be generally recognized. First: fast growing moulds that attain their growth within 2-6 days of incubation (Geotrichum sp., R. nigricans and T. viride). Second: moderate growing fungi, which reached their highest growth within 8-16 days, these included the rest tested fungi. However, the maximum growth of both F. oxysporum and P. chrysogenum on rose bengal medium was attained after 18 and 24 days of incubation, respectively.

Effect of pH Value

The growth of the tested fungi (Fig. 2) responded differently to the hydrogen ion concentration of PDA. They can grow satisfactory at pH values: 4-9, and higher pH resulted in lower growth values. A. flavus, A. niger and G. atrum can grow up to pH 12, while the rest fungi failed to grow under these conditions. However, F. oxysporum, Geotrichum sp. and P. chrysogemum aborted at pH11. PDA of pH6 was optimal for the highest linear growth values of the tested fungi, except aspergilli that attain, the highest growth at pH7. The influence of pH values on the fungal growth was reported by many workers [42,46].

Influence of Relative Humidity (R.H)

The linear growth of test fungi was influenced differently under the tested R.H (Fig. 3). The growth of A. fumigatus, A. niger, Geotrichum sp., R. nigricans and T. viride increased regulary with R.H increasing up to 100%. The same figure was reached for F. oxysporum, G. atrum, and P. chrysogenum but at 95% R.H. However, A. alternate and A. flavus can grow satisfactory at lower humidity (85%). It was reported that moulds that have the ability to hydrolyze

^{**}The growth ceased in the Petri-dish.

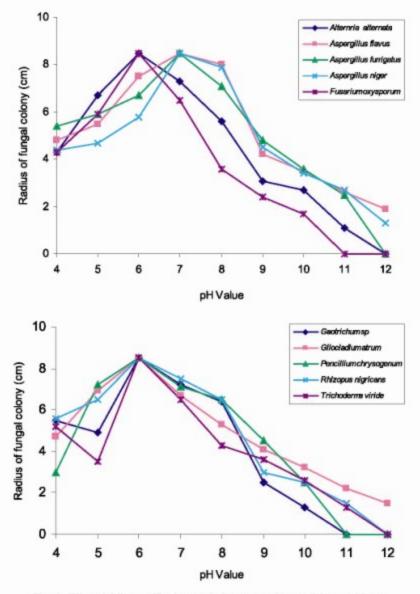


Fig. 2. Effect of different pH values on the linear growth (cm) of the tested fungi.

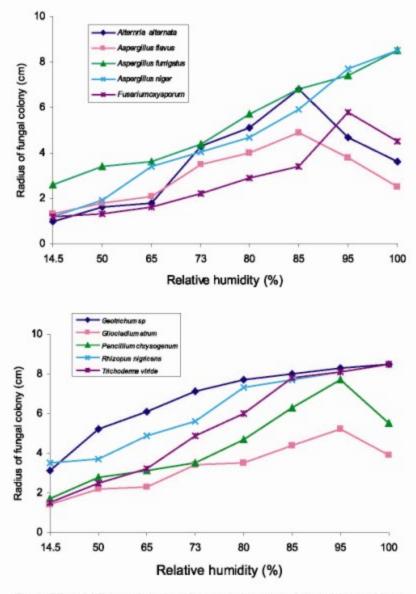


Fig. 3. Effect of different relative humidities on the linear growth (cm) of the tested fungi.

natural materials such as wood, textiles and papers stimulated at R.H more than 75% and their hydrolyzing activities increased with R.H increasing [47,48].

Enzymatic Activity

The previous study indicated that the tested environmental conditions (incubation temperature, pH and R.H) had great influence on the growth of the isolated fabrics mycoflora. In order to study how many of these fabrics can stand out the deterioration risk that can be attained by their mycoflora, the cellulytic and proteolytic activities were studied.

Cellulytic Activities

The data indicated that of the tested fungi (Fig. 4), T. viride has the priority to produce cellulases (0.387 µmol glucose/h/ml crude enzyme) followed by Geotrichum sp. and A. niger (0.354 and 0.321 µmol glucose/h/m, respectively). While, R. nigricans, A. alternata and P.chrysogemum were with lower activities (0.25 and 0.3 µmol glucose/h/ml). However, the rest fungi showed the least cellulytic activities (less than 0.25 µmol glucose/h/ml). The cellulytic activities of different fungi were reported by many workers [49,54].

Proteolytic Activities

The results (Fig. 5) revealed that *T. viride* has the highest proteolytic activities (58.6 En.U.). While *A. alternata*, *Geotrichum* sp., *A. niger* and *P. chrysogeman* were with moderate activities (37.6 – 43 En.U.). However, the rest of the tested fungi showed lower activities (less than 25 En.U.). The proteolytic activities of fungi were reported by [55-59].

The cellulytic and proteolytic activities showed that T. viride, Geotrichum sp., A. niger, A. alternata and P.chrysogenum (in descending order) have higher activities of both cellulases and proteases, so the risk of fabrics (natural, synthetic and blended) deterioration by them is highly expected when they present as textile mycoflora. The rest of the isolated fungi also showed both cellulytic and proteolytic activities but to a lesser extent and with different capabilities for the two systems of enzymes. Therefore, the risk of textiles deterioration by their mycoflora is expected when the environmental conditions of the fabrics permit their growth.

Antimycotic Activity of Some Detergents

In order to prevent the biodeterioration of textiles and at the same time to avoid human health hazards, the mycoflora of textiles must be leached, before and during fabrics usages, two different detergents were tested. The results (Fig.

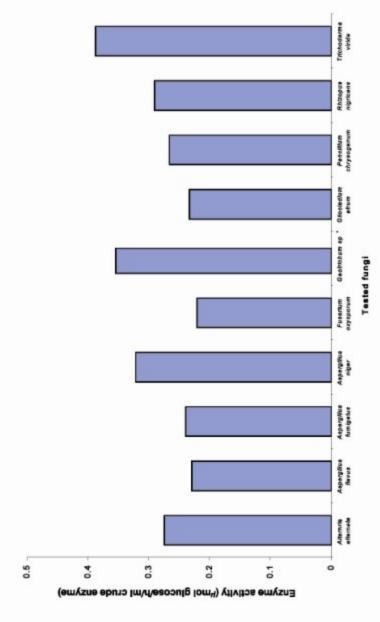


Fig. 4. Cellulytic activity of the tested fungi.

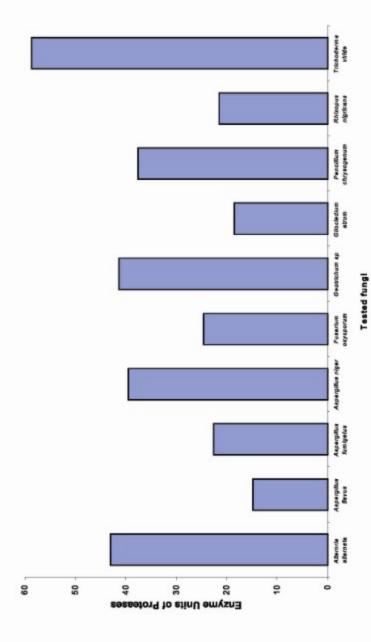


Fig. 5. Proteolytic activity of the tested fungi.

6) indicated that the tested fungi responded differently to the tested detergents, and their levels. A. flavus was the most sensitive fungus to Tide detergent and the same figure was recorded with P. chrysogenum, but under Ariel detergent treatment. On the other hand, the two detergents were conducive to inhibit the growth of T. viride especially at 16 g/l level. However, the increase of detergent level was concomitant with parallel inhibition to the fungal growth. R. nigricans was the least sensitive to the detergents treatments. The inhibitory effect of detergents may attribute to the toxic effect of some ingredients that elongate the fungal lag phase, inhibit normal cell elongation and spore germination. Detergents as surface active agents have detectable influences in permeability of the cell walls to different materials and metals [58,60-62].

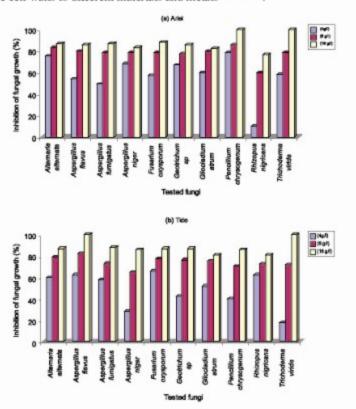


Fig. 6. Effect of two detergents (a - Ariel, b - Tide) at different concentrations on linear growth of the tested fungi.

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