

Full Length Research Paper

Isolation and screening of some medically important fungi from indoor environment: Studying the effect of some environmental and chemical factors on their growth and spore adhesion

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Isolation of some pathogenic fungi from indoor environment that may cause diseases to athletes was the goal of this work. The effect of different cloth materials and some environmental factors on the growth and adhesion of the isolated fungi as *Aspergillus sydowii*, *Cochliobolus hawaiiensis*, *Cochliobolus lunatus*, *Epicoccum nigrum*, *Nigrospora oryzae*, *Penicillium aurantiogriseum*, *Cladosporium sphaerospermum*, *Aspergillus niger*, *Cochliobolus australiensis*, *Stemphylium botryosum*, *Alternaria alternata*, *Fusarium chlamydosporum*, *Aspergillus flavus* and *Aspergillus versicolor* was investigated. By studying the effect of different cloth materials, at temperatures (18, 25 and 35°C) and at pH values (4, 5.6, 8), it was concluded that cloth material, 74% cotton - 25% polyester-1% elasthan (C.P.E) was the lowest in susceptibility to fungal attack. The fungal pathogens growth was favored at 35°C and pH 8 after two days of incubation while, after five days the growth was favored at 25 and 35°C at pH 5.6 and pH 8. *Alter. alternata* and *A. flavus* were selected for studying their spore adhesion on different cloth material samples. Also, their sensitivity for detergents and drugs on different cloth material samples was carried out.

Key words: Pathogenic fungi, athletes, fungal adhesion, antimicrobial activity.

INTRODUCTION

Concern of the quality health effect of indoor environment has grown in recent years, since many people now spend most of their time indoors, in locations such as indoor sports halls and gyms, where pollutants found in indoor environment, which can be inorganic or organic.

Skin and skin structures (hair, nail, and subcutaneous

tissues) in human are subjected to infection by several factors including organisms (mainly fungi), hormonal, and/or chronic inflammatory skin disorder. The disease is widely distributed all over the world with various degrees and is more common in men than in women (Mutwally et al., 2002).

For the purposes of this paper, clothes in sport is regarded as not ending with the stuff, but covers accompaniments or accessories such as shoes, socks, gloves, suspenders, a mask, helmet, shin guards, belts, etc. (Akintunde and Neil, 2010; Brandi et al., 2007;

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Viegas et al., 2009). The effects of heat on fungi depend on many factors, including the genus, species, and strain of the fungus, the amount of available water, kinds of nutrients, and many other environmental factors (Burge, 2006).

Cotton, wool, jute, and flax are reported to be most susceptible to fungal attack. They found that there were differences in the strength of cotton as the time, temperature, pH, and medium conditions changed. Growth of fungi is slower on synthetic fibers as compared to their natural counterparts because their polymer backbone does not retain much water (Wasif and Laga, 2009).

Further, clothes provide a hygienic barrier, keeping toxins away from the body and limiting the transmission of germs (Luo and Sun, 2008; Yao and Li, 2010).

The biggest chemical barrier to infectious organisms is the acid layer on the skin. Healthy skin has a pH of about five, making it slightly acidic. Our sweat (containing uric and lactic acids) and body oils promote this acidic environment. For this reason, sweat and oil do us good (Chandrasekar and Manavathu, 2008).

For soap or detergent to function effectively, it must be soluble in water. Thus, when the dirty materials are immersed in the soapy water, several reactions take place. The dirt is lifted and held in suspension until it is being rinsed off (Edoga, 2009). Soap which contains antifungal agent, is considered as preventative for fungal infections (Jun'ya and Yayoi, 2006). Antifungal drugs provide an effective means of preventing mold germination.

The aim of the present work was to study the fungi which are present in indoor environment as gyms and cause some skin diseases to athletes and the effect of different cloth materials, some environmental factors and antimicrobial substances on the fungal isolates growth and their adhesion on different cloth materials.

MATERIALS AND METHODS

Isolation of fungal strains

Fungal strains were isolated from the faculty of Physical Education for Men Alexandria University from clothes, solid surfaces, skin and air. They were identified in the Mycological Center, Assiut University, Egypt.

The modified sabouraud dextrose agar medium (SDA) (g/L): glucose, 20; peptone, 10; agar, 15 and chloramphenicol 500 mg in 10 ml of 95% ethanol was used for routine isolation, growth, and cultivation of dermatophytes (Martino and Luzi, 2008).

Cloth material samples preparation and sterilization

The cloth material samples were cut into 4 × 4 cm test specimens, sterilized by impregnation in 15 ml of normal saline solution (0.9% sodium chloride) and then autoclaved (Bishop, 2005).

Effect of some environmental factors

The growth of fungi was studied under different environmental conditions, the effect of different temperature degrees (18, 25 and 35°C), and different pH values (4, 5.6, 8) was determined.

The fungal adhesion on different cloth materials

Alter. alternata and *Aspergillus flavus* were selected to know their adhesion on different cloth material samples depending on their high growth visual observation (%) in different environmental conditions and on different cloth materials.

The number of adhesive spores on a cloth material = spore count per ml of control – spore count per ml of the residual of fungal suspension (Aberkane et al., 2002).

Antimicrobial activity

Alter. Alternata and *A. flavus* were selected to know the sensitivity against detergents and drugs on tested cloth material samples depend on their high growth visual observation (%) in different environmental conditions and on different cloth materials. Antimicrobial tests were carried out by disk diffusion method. Using 1 ml of fungal suspension spread on modified Sabouraud dextrose agar (SDA) medium, ten cloth material samples cut into 4 × 4 cm were impregnated in 30 ml of antimicrobial substance and were placed on the inoculated agar (Semwal et al., 2009).

Detergents and drugs used

Three different antifungal detergents were tested, Fungisalt, Red Lifebuoy™ and Betadine® (skin cleaner), Eight different antifungal liquid drugs were tested, Terbin®, Locasten®, Trozyd*, Mykotral®, Batrafen®, Locatret®, Dermofix® and Betnovate™. 0.05 and 0.01 g/ml of these detergents and drugs were prepared (Butron and Gaikwad, 2009; Henein, 2011); Registered (®) and Trademark (™,*).

RESULTS AND DISCUSSION

Fungal strains isolation, identification, counting and their pathogenicity

In this study, fungal strains were isolated from sport environment using modified SDA medium; were identified in the Mycological Center, Assiut University. Colonies of each fungal isolate were counted in all plates after 30 days of incubation and their pathogenicity are shown in Table 1. The results reveal the presence of some isolates which 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.

The sport environment work as fungi reservoirs and, their own users and professionals, which could carry in the body (commensal flora) or clothing, a great fungal species diversity (Viegas et al., 2009). Fungi ubiquity

Table 1. Fungal identification, counting and their pathogenicity.

Fungal identification	Number of colony	Pathogenicity
<i>Cochliobolus australiensis</i>	4	phaeohyphomycosis
<i>Epicoccum nigrum</i>	2	No documented infection in humans.
<i>Aspergillus flavus</i>	9	Etiologic agent
<i>Alternaria. alternata</i>	6	Phaeohyphomycosis. Cases of onychomycosis, sinusitis, ulcerated cutaneous infections, and keratitis
<i>Penicillium aurantiogriseum</i>	3	Penicilliosis, endophthalmitis, otomycosis, necrotizing esophagitis, pneumonia, endocarditis, peritonitis, and urinary tract infections
<i>Cladosporium sphaerospermum</i>	3	skin lesions, keratitis, onychomycosis, sinusitis and pulmonary infections
<i>Aspergillus sydowii</i>	1	nail infections
<i>Aspergillus niger</i>	3	otitis, pulmonary disease
<i>Aspergillus versicolor</i>	9	cutaneous disease, onychomycosis, otomycosis, osteomyelitis, pulmonary disease
<i>Fusarium chlamydosporum</i>	6	Etiologic agent
<i>Cochliobolus hawaiiensis</i>	1	Phaeohyphomycosis
<i>Stemphylium botryosum</i>	5	Phaeohyphomycosis
<i>Nigrospora oryzae</i>	2	Cutaneous lesions
<i>Cochliobolus lunatus</i>	1	Human pathogen, considered as keratinophilic fungi
Total	55	

in sport environment is also favoured by the organic matter accumulation, construction complexity, material selection, high temperatures and inadequate maintenance (Brandi et al., 2007). Thus, athletes are more likely to acquire skin lesions including those caused by fungi. The count and identification data showed variations in both fungal genera and species, this finding was reported by several workers (Neely and Orloff, 2001).

The effect of different cloth materials at different temperature degrees on the fungal isolates growth

Different cloth materials were collected from

common market and their effect was tested at three different temperatures (18, 25 and 35°C), to show the effect of temperature on the mean of visual observation of the fungal isolates growth on different cloth materials at pH 5.6 and after 2 and 5 days of incubation.

The data after 2 days of incubation are illustrated in Figure 1 and indicate that the fungal isolates growth was favored at 35°C and gave the highest mean of the visual observation of the fungal growth on all of the tested cloth material samples followed by the fungal growth at 25°C.

After 5 days of incubation, as shown in Figure 2, the fungal isolates growth was favored at 25 and 35°C and gave nearly, the same highest mean of the visual observation of the fungal growth on all

of the tested cloth materials.

From Figures 1 and 2, it was concluded that 74% cotton - 25% polyester- 1% elasthan (C.P.E) was the lowest in susceptibility to fungal attack at the three tested temperatures and it can be selected for sport wear.

Degradative processes, such as denaturation of proteins, including enzymes, are very slightly at low temperatures and do not become marked until moderate temperatures are attained, but then they increase rapidly (Belitz et al., 2009). Also, the cloth specification of the cloth material sample showed that they were already treated with antimicrobial substances, which reduced the fungal isolates growth on it (Dart and Obendorf, 2000).

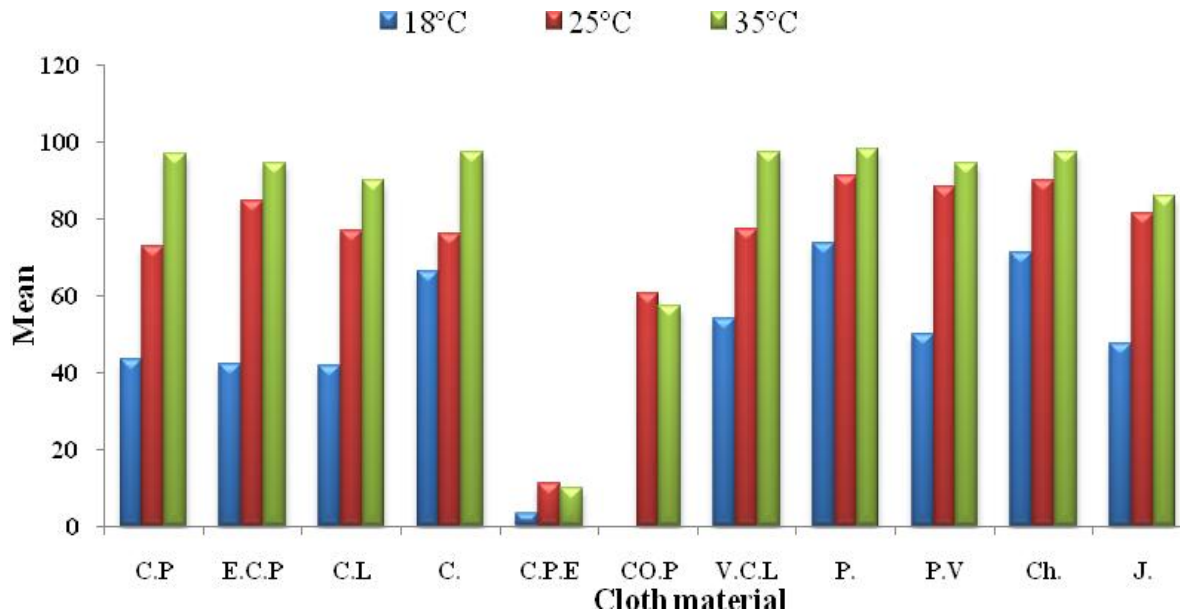


Figure 1. Effect of different cloth materials at different temperature degrees on the mean of visual observation (%) of the fungal isolates growth at pH 5.6 and after 2 days of incubation. C.P, 65% cotton - 35% polyester; E.C.P, 10% elasthan- 30% cotton 60% polyester; C.L, 98% cotton 2% lycra; C., 100% cotton; C.P.E, 74% cotton - 25% polyester- 1% elasthan; CO.P, 80% cotton - 20% polyester; V.C.L, 60% viscose - 20% cotton - 20% linen; P., 100% polyester; P.V, 65% polyester- 35% viscose; Ch., 100% chiffon; J., 100% denim.

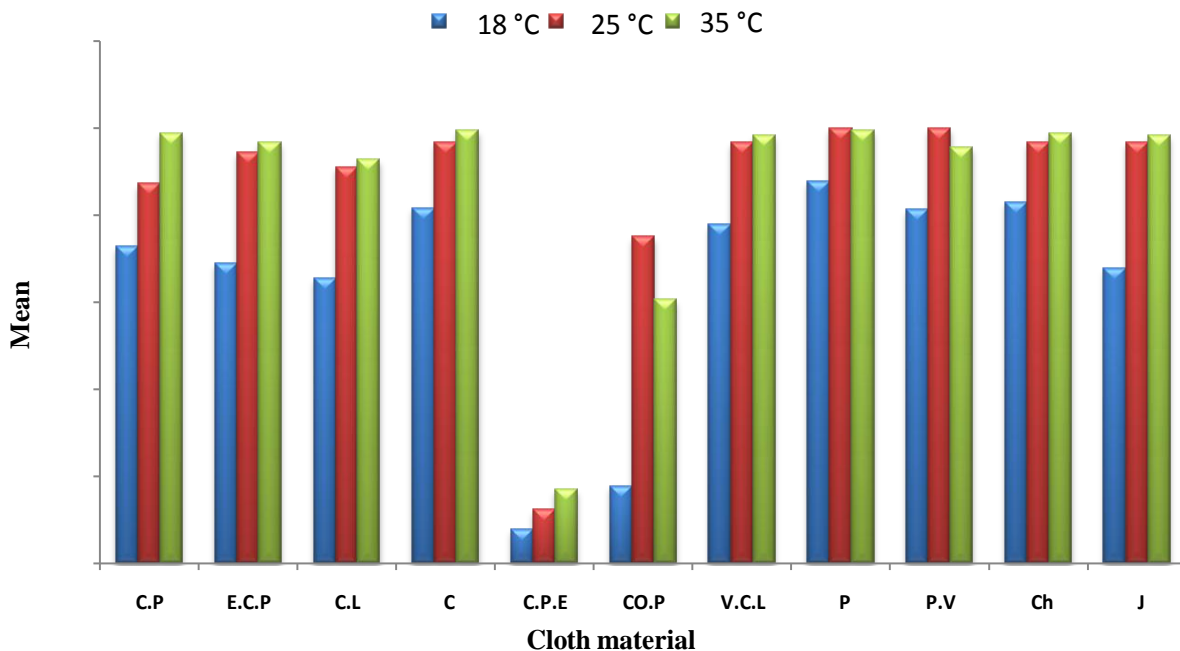


Figure 2. Effect of different cloth materials at different temperature degrees on the mean of visual observation (%) of the fungal isolates growth at pH 5.6 and after 5 days of incubation. C.P, 65% cotton - 35% polyester; E.C.P, 10% elasthan- 30% cotton 60% polyester; C.L, 98% cotton 2% lycra; C., 100% cotton; C.P.E, 74% cotton - 25% polyester- 1% elasthan; CO.P, 80% cotton - 20% polyester; V.C.L, 60% viscose - 20% cotton - 20% linen; P., 100% polyester; P.V, 65% polyester- 35% viscose; Ch., 100% chiffon; J., 100% denim.

The effect of different cloth materials at different pH values on fungal isolates growth

After 2 days of incubation, there were significant differences between the three pH values in mean of visual observation of the fungal isolates growth on some of the tested cloth materials while, there were no significant differences on the other cloth materials at temperature 25°C. The highest means of fungal isolates growth were recorded at pH 8 while, at pH 4, the lowest means of fungal growth were obtained (Figure 3).

After 5 days of incubation and at temperature 25°C as shown in Figure 4, there were significant differences between pH 5.6 and 8 which gave optimal values of fungal growth levels at most of the tested cloth materials from side and pH 4 from the other side which gave smaller values in mean of visual observation of the fungal isolates growth.

From Figure 3 and Figure 4, it was concluded that (C.P.E), 74% cotton - 25% polyester- 1% elasthan, was the lowest in susceptibility to fungal attack at the three tested pH values and it can be selected for sport wear.

The growth of the tested fungi responded differently to the hydrogen ion concentration of SDA. They grew satisfactory at pH values (4 to 9), and higher pH resulted in lower growth values. *A. flavus*, and *A. niger* could grow up to pH 12, while the rest fungi failed to grow under these conditions. SDA of pH 6 was optimal for the highest linear growth values of the tested fungi, except *Aspergillus* that attain the highest growth at pH 7. The influence of pH values on the fungal growth was reported by many workers (Al-Garni et al., 2007).

The effect of different cloth materials on fungal isolates spore adhesion

A. flavus and *Alter. alternata* were tested to know the effect of cloth material on their levels of spore adhesion and calculate the percentage of adhesive spores which was obtained from the number of adhesive spores on a cloth material = spore count per ml of control – spore count per ml of the residual fungal suspension, and then the percentage was calculated. Counting with a haemocytometer in three replicates was performed. The data are illustrated graphically in Figure 5 and indicates that the cloth materials were arranged according to their % of adhesive spores from maximum to minimum, for *A. flavus* [(C.), (C.P.E), (CO.P), (P.V), (V.C.L) and (J.), (C.L), (C.P), (E.C.P), (P.) and finally, (Ch.)]. While, for *Alter. alternata* the cloth materials were arranged as follows [(P.V), (CO.P), (C.), (C.P.E), (V.C.L) and (J.), (C.L), (C.P), (E.C.P), (P.) and finally, (Ch.)].

For *A. flavus*, the 100% cotton (C.) was the maximum cloth material sample with fungal spore adhesion because textile fibers have various spaces, sizes, and surface characters that can physically entrap spores and

because viscose fibers had higher moisture content than cotton. While, in the case of *Alter. alternata*, P.V was the largest cloth material sample in their fungal spore adhesion due to under normal conditions; polyester fibers have a low moisture content, which can lead to static problems; the polyester static problems results in spores adhering to the static charged polyester fiber. Thus, it was thought that spore retention and release are influenced by reduced surface contact area (Kan, 2007).

The effect of different detergents and drugs on the fungal isolates growth on different cloth materials

A. flavus and *Alter. alternata* were tested against many detergents and drugs on different cloth material samples depending on their high growth visual observation (%). Antimicrobial tests were carried out by disk diffusion method and antimicrobial activity was evaluated by measuring the zone of inhibition against the tested fungi around each cloth material sample. Data shown in Figure 6 for the effect of detergents on *A. flavus* and *Alter. alternata* at temperature 25°C and after 72 h indicate that, Betadine is more effective than red Lifebuoy in preventing fungal growth on different cloth material samples. The Fungi salt has an intermediate effect which is more than red Lifebuoy effect and less than Betadine effect in preventing *A. flavus* growth on different cloth material samples, while in the case of *Alter. alternata*, Betadine and red Lifebuoy were more effective than Fungi salt.

Data shown in Figure 7 for the effect of different drugs on *A. flavus* and *A. alternata* at temperature 25°C and after 72 h reveal that, Terbin, Locasten and Dermofix had the highest inhibitory effect on *A. flavus* and *A. alternata* while, Locatret and Betnovate had a limited effect on the two tested fungi.

Betadine is more effective than red Lifebuoy in preventing fungal growth on different cloth material samples. The carbolic acid, a phenol red Lifebuoy acts as a wide spectrum of activity against bacteria, viruses, and fungi but they have minimal sporicidal activity. Phenol classified as low-level to intermediate-level germicides, while povidone-iodine (Betadine) was effective against fungi for up to 72 h (Bodrumlu and Alaçam, 2006). 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 (Al-Garni et al., 2007).

Ergosterol, the predominant component of fungal cell membranes, is therefore an obvious and specific target for fungal inhibition (Clausen and Yang, 2008). Finally, Batrafen which belongs to the antimycotic drugs is used for the treatment of superficial mycoses. Batrafen might

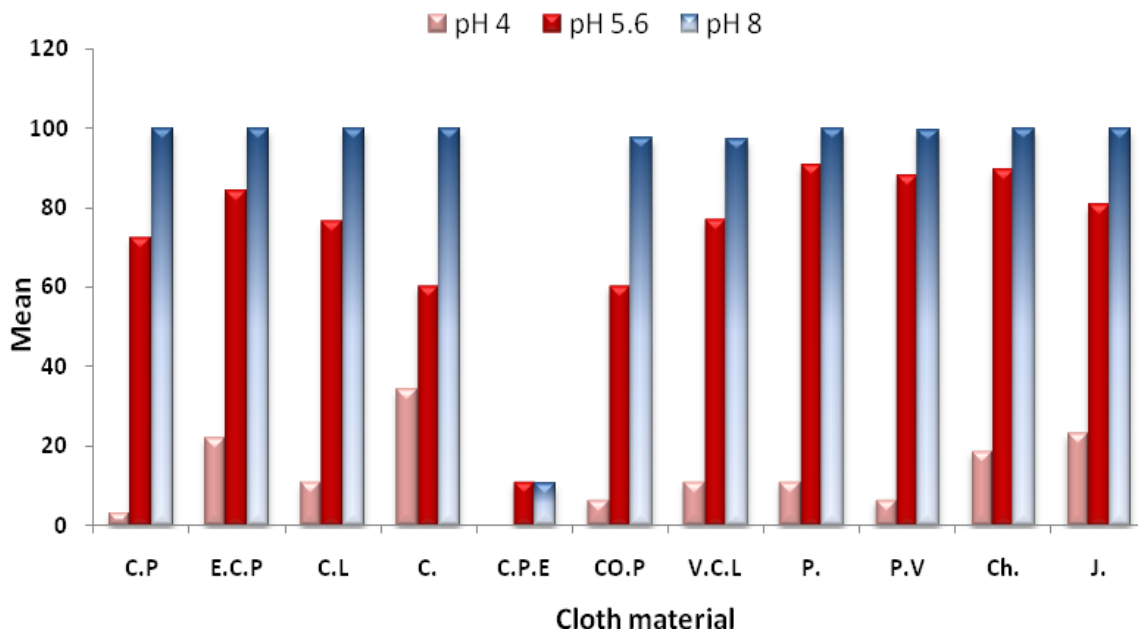


Figure 3. Effect of different cloth materials at three pH values on the mean of visual observation (%) of the fungal isolates growth after 2 days of incubation and at 25°C. C.P, 65% cotton - 35% polyester; E.C.P, 10% elasthan-30% cotton 60% polyester; C.L, 98% cotton 2% lycra; C., 100% cotton; C.P.E, 74% cotton - 25% polyester- 1% elasthan; CO.P, 80% cotton - 20% polyester; V.C.L, 60% viscose - 20% cotton - 20% linen; P., 100% polyester; P.V, 65% polyester- 35% viscose; Ch., 100% chiffon; J., 100% denim.

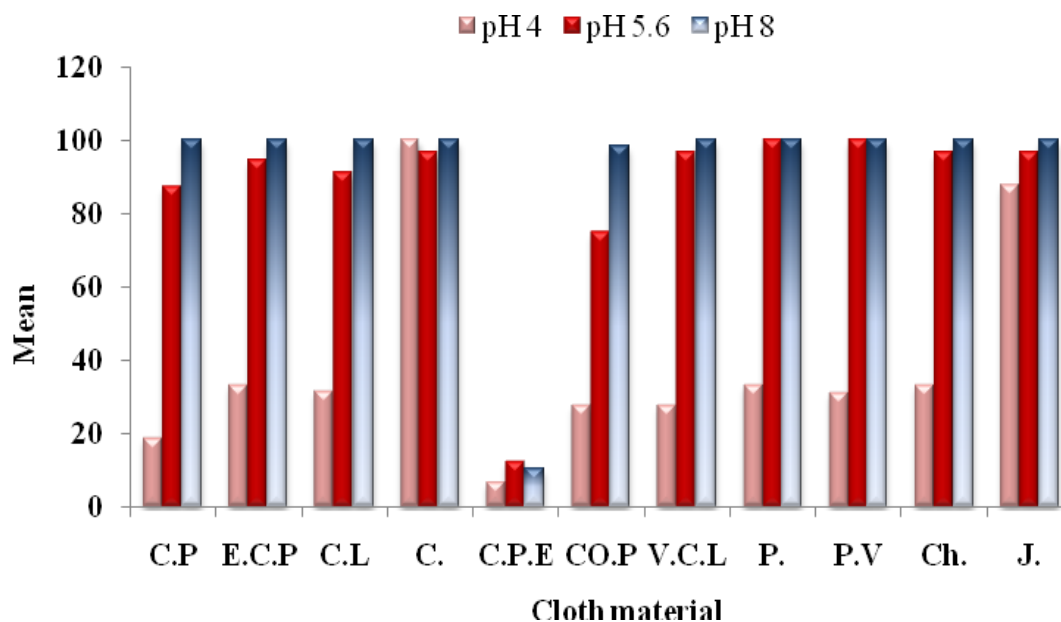


Figure 4. Effect of different cloth materials at three pH values on the mean of visual observation (%) of the fungal isolates growth after 5 days of incubation and at 25°C. { (C.P), 65% cotton - 35% polyester; (E.C.P), 10% elasthan- 30% cotton 60% polyester; (C.L), 98% cotton 2% lycra; (C.), 100% cotton; (C.P.E), 74% cotton - 25% polyester- 1% elasthan; (CO.P), 80% cotton - 20% polyester; (V.C.L), 60% viscose - 20% cotton - 20% linen; (P.), 100% polyester; (P.V), 65% polyester- 35% viscose; (Ch.), 100% chiffon and finally, (J.), 100% denim}.

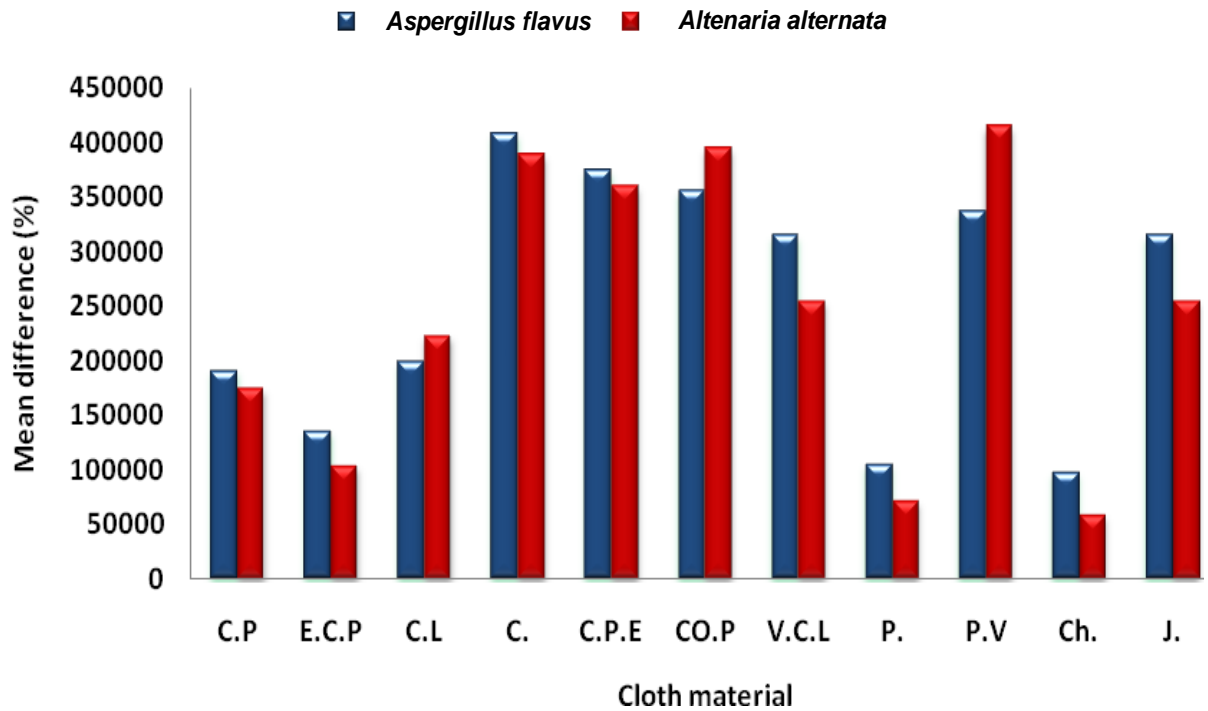


Figure 5. The effect of different cloth materials on the mean difference %, (percentage of adhesive spores) of *Aspergillus flavus* and *Alternaria alternata*. C.P, 65% cotton - 35% polyester; E.C.P, 10% elasthan- 30% cotton 60% polyester; C.L, 98% cotton 2% lycra; C., 100% cotton; C.P.E, 74% cotton - 25% polyester- 1% elasthan; CO.P, 80% cotton - 20% polyester; V.C.L, 60% viscose - 20% cotton - 20% linen; P., 100% polyester; P.V, 65% polyester- 35% viscose; Ch., 100% chiffon; J., 100% denim.

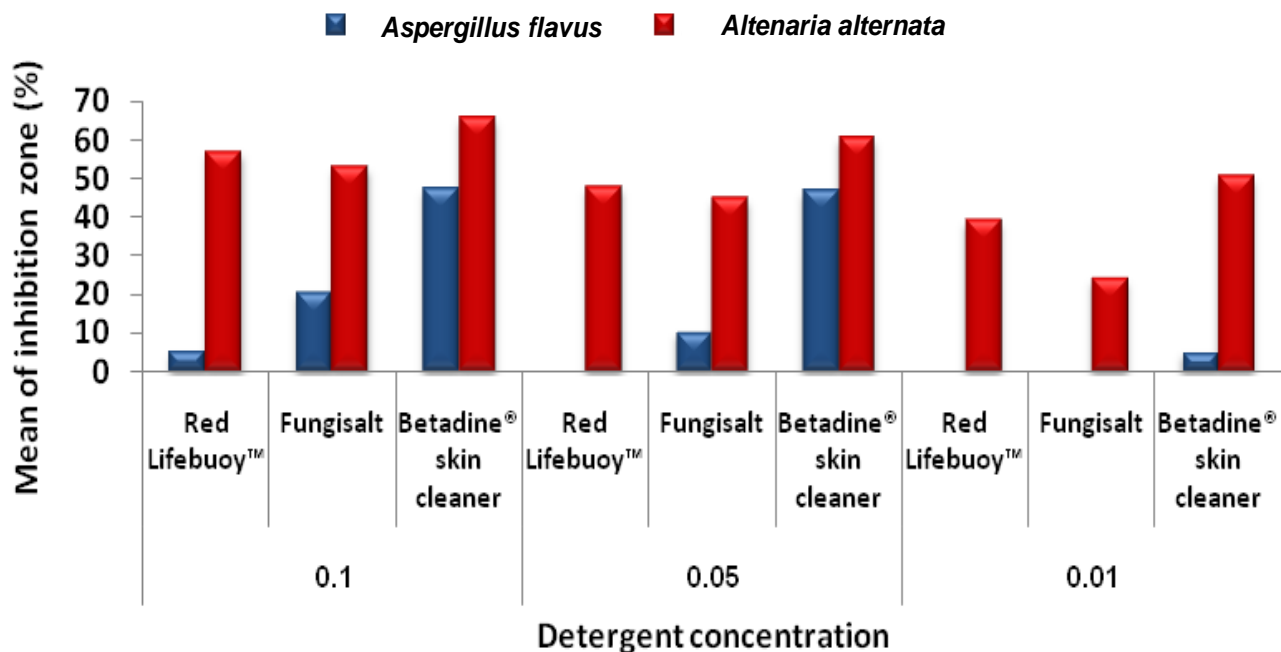


Figure 6. Antifungal activity of different detergents (0.1, 0.05, 0.01 g/ml) against *Aspergillus flavus* and *Alternaria alternata* tested based on disk diffusion method; the fungal response was evaluated by the mean of inhibition zone (%).

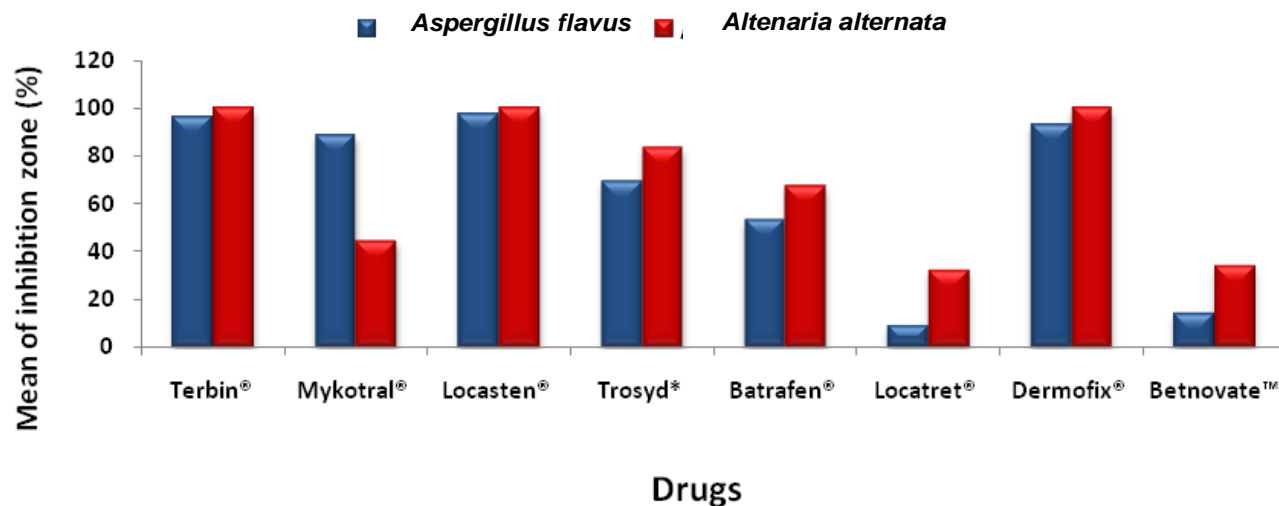


Figure 7. Antifungal activity of different drugs against *Aspergillus flavus* and *Alternaria alternata* tested based on disk diffusion method; the fungal response was evaluated by the mean of inhibition zone (%).

act as a chelator of iron ions. In addition, their degree of sensitivity is variable; they are used in the treatment of fungal skin diseases (Sterry et al., 2006), while resistance to Locatret and Betnovate has been seen, for Locatret. However, the tested fungi (*A. flavus* and *Alter. alternata*) are not causative agent of acne, they cause aspergillosis and alternariosis (Hedayati et al., 2007). Betnovate is used for treatment of the seborrhoeic dermatitis *Pityrosporum ovale* which is probably the causative agent (Milani et al., 2003) (Figure 6, 7).

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