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CHARACTERIZATION OF KERATINOPHILIC FUNGAL SPECIES AND OTHER NON-DERMATOPHYTES IN HAIR AND NAIL SAMPLES IN RIYADH, SAUDI ARABIA

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ABSTRACT

The presence of fungal species on skin and hair is a known finding in many mammalian species and humans are no exception. Superficial fungal infections are sometimes a chronic and recurring condition that affects approximately 10-20% of the world's population. However, most species that are isolated from humans tend to occur as co-existing flora. This study was conducted to determine the diversity of fungal species from the hair and nails of 24 workers in the central region of Saudi Arabia. Male workers from Riyadh, Saudi Arabia were recruited for this study and samples were obtained from their nails and hair for mycological analysis using Sabouraud's agar and sterile wet soil. A total of 26 species belonging to 19 fungal genera were isolated from the 24 hair samples. *Chaetomium globosum* was the most commonly isolated fungal species followed by *Emericella nidulans*, *Cochliobolus neergaardii* and *Penicillium oxalicum*. Three fungal species were isolated only from nail samples, namely, *Alternaria alternata*, *Aureobasidium pullulans*, and *Penicillium chrysogenum*. This study demonstrates the presence of numerous fungal species that are not previously described from hair and nails in Saudi Arabia. The ability of these fungi to grow on and degrade keratinaceous materials often facilitates their role to cause skin, hair and nail infections in workers and other persons subjected to fungal spores and hyphae.

Keywords: ***keratinophilic fungi, non-dermatophytes, mycobiota, hair, nails.***

INTRODUCTION

Fungi with affinities to attack keratinized tissue are called "Keratinophilic fungi". These fungi are present in the environment of all over the world, specifically in keratin containing habitats where humans and animals are living. The biological function of keratinophilic fungi in the soil is the degradation of keratinized materials such as hides, furs, hair, feather, claws, nails, horns and skin debris of dead animals (Bisen and Tewari, 2015). The Keratinophilic fungi are basically saprophytes but occasionally becomes potentially pathogenic to man and animals. The pathogenic forms of fungi are known as "dermatophytes" and are known to cause superficial cutaneous infections (dermatophytoses) of keratinized tissues of humans and animals. Such fungi have better growth at temperatures of 25-28˚C with warm

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and humid conditions. Infections by fungi are relatively common in tropical countries due to environmental, social and economic conditions. Superficial fungal infections are often chronic and recurring affecting approximately 10-20% of the world's population is affected during their lifetime (Abanmi *et al.*, 2008). Fungal infections of the skin, hair and nails are acquired via direct contact of other people, infected animals or fomites (Alsheikh, 2009). Dermatophytosis can either be caused by true dermatophytes (Microsporum, Trichophyton and Epidermophyton), yeasts (Candida), or moulds (e.g., Aspergillus, Alternaria, and Fusarium) (Sahin *et al*., 2004).

In various geographical locations, several studies have been conducted on characterization of fungi isolated from human hair and nails. In Turkey, *T. rubrum*, *T. mentagrophytes*, *T. verrucosum* and *T. violaceum*. *Microsporum canis*, *M. gypseum*, Epidermophyton floccosum, were commonly isolated from the hair and nails of students (Metintas **and** Kiraz**, 2004). In northern Egypt, the most prevalent species included Aphanoascus, Aspergillus, Penicillium, Paecilomyces and Chrysosporium (Gherbawy *et al*., 2006). . In Northeast India, *T. rubrum*, *T. mentagrophytes* and *M. gypseum* were also the common isolates (Sarma, 2007). In Northern Greece, dermatophytes including *Trichophyton rubrum* (53.9%), *Trichophyton mentagrophyte*s (17.6%), and *Microsporum canis* (22.5%) were the most common isolates (Nasr *et al*., 2016). These studies demonstrate the wide variety of fungal species that exist as normal flora or possibly as colonizing non-pathological organisms.**

Although, dermatophytic infections are a commonly encountered problem in Saudi Arabia, very few studies are available about the specific species that cause these infections and even fewer exist describing the species (Al-Sogair *et al*., 1991 and Venugopal, 1992). In a study conducted among patients in Central Saudi Arabia, *T. mentagrophytes*, *Candida spp.* and *Aspergillus spp*. were found to be the most likely isolated species causing onychomycosis (Abanmi *et al*., 2008). Another study conducted in 2009 among patients clinically diagnosed with dermatophytic infections in an Eastern province of Saudi Arabia showed a variety of species including *Epidermophyton floccosum*, *Trichophyton rubrum*, *Trichophyton schoenlenii*, and the non-dermatophytes *Candida albicans* and Fusarium (Alsheikh, 2009) . Reports from Madina, Saudi Arabia (Hanafy, 2012) revealed that the most frequently isolated causal agents of cutaneous mycoses were *Microsporum canis* (15.4%), *Trichophyton metagrophyte*s (11.7%), and *Trichophyton violaceum* (11%). Screening for keratinolytic activity showed that *M. canis* and *T. verrucosum* recorded the highest value. In Riyadh City, Khaled *et al.* (2015) showed that *Tinea capitis* infection had the highest prevalence among the patients (22.3%) while *Tinea barbae* had the lowest. The identified dermatophyte isolates were *Trichophyton violaceum*, *Trichophyton verrucosum*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Trichophyton schoenleinii*, *Trichophyton concentricum*, *Microsporum canis*, *Microsporum audouinii* and *Epidermophyton floccosum*. Non dermatophyte fungi included 5 isolates from Aspergillus, 4 isolates from Acremonium and 15 isolates from Candida. *M. canis* was the most common species (25% of isolated dermatophytes). In Hail region of Saudi Arabia, Moursi (2016)

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conducted an epidemiological study of dermatophytic diseases and found that *Trichophyton spp.*, are the predominant (82.11 % of cases) followed by *Epidermophyton spp*. (16.55 %) but *Microsporum spp.*, were the lowest (1.34%). Among *Tricophyton spp.*, *T. mentegrophytes* was more prevalent than *T. rubrum* (68.55% and 31.45%, respectively). More recently, Moursi *et al.* (2018) reported that dermatophytes are the major pathogens causing onychomycosis in Hail region. The prevalent yeast was represented by *Candida albicans* whereas the common non-dermatophytic mold was *A. niger*.

The heterogeneity of the distribution pattern of dermatophytes in different parts of the world has been attributed to various factors, including climate, lifestyle, and the prevalence of immunodeficiency diseases in the community, as well as the reluctance of patients to seek treatment because of embarrassment or the minor nature of disease unless the condition became sufficiently serious to affect the quality of life (Al-Sogair *et al.*, 1991 and Enugopal, 1992). Even fewer studies have attempted to understand the heterogeneity of the commensal fungi of the region due to their relative non-pathogenicity compared to the bacterial flora of the human body (Efuntoye and Fashanu, 2002). Hence, this study was conducted to determine the diversity and distribution of the commensal mycobiota from people living in Saudi Arabia in an attempt to characterize, classify and document these species and further understand their biology. The ability of some species to utilize keratin as an energy source i.e. keratinophilic fungi may aid in furthering our understanding of the interactions that fungi have with humans.

MATERIALS AND METHODS

1) Collection of hair and nail samples:

Twenty-four workers in Riyadh, Saudi Arabia were recruited as participants between January and March 2016. Their jobs included construction, menial work and operation of gas and petrol stations. Most of the recruited workers had spent at least a year in Saudi Arabia. We informed the participants of the aim and objectives of the study and obtained written informed consent. The study protocol was reviewed and approved by the Princess Nourah bint Abdulrahman University Research Ethics Committee IRB No. H-01- R-059. Hair and nail samples were obtained from each worker using sterile instruments and collection bags.

2) Mycological analysis of human hair samples:

i- Plating on Sabouraud`s Dextrose Agar (SDA)

Hair samples were individually placed on the surface of Sabouraud`s Dextrose agar (SDA) which contained (g/l) glucose, 20, peptone, 10, agar, 20 and chloramphenicol, 0.5 according to the procedure described by Ellis *et al.* (2007). Chloramphenicol was incorporated into the medium to suppress bacterial growth. Cultures were incubated at 28°C for 1-3 weeks during which the growing fungi were examined and identified. Pure cultures of fungi were kept on slants containing the same medium for preservation and revision (Efuntoye and Fashanu, 2002).

ii- Plating on sterile wet soil

The hair baiting technique originally described by Vanbreuseghem (1952) was employed. A medium of About 1 kg of a clayey soil sample was autoclaved twice and

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distributed into sterile plastic Petri plates (30 grams/plate). Five ml of sterile distilled water was added to each. Fragments of hair samples were distributed on the soil surface, followed by incubation at 28°C and rewetting sterile water as required.. The plates were then examined for fungal growth and the fungi appearing on hair fragments were obtained and streaked on SDA medium for further identification. Slant cultures of fungal strains were also prepared for preservation.

3) Mycological analysis of human nails samples:

Nail samples were placed on the surface of SDA. Samples were incubated at 28°C for 1-3 weeks, during which the growing fungal cultures were examined and identified.

4) Imaging of fungal species

Wet slide preparations of fungal isolates were made using lactophenol cotton blue stain (LPCB). Fungi were examined under low and high magnification with an Axiostar binocular research microscope (Carl Zeiss Microscopy, GmbH, Germany). Images were taken with a Canon Power shot G6 digital camera (Canon, New York, USA).

5) Identification of fungal cultures

Fungi were identified based on their macroscopic and microscopic features using the following references: (Ellis, 1971; Moubasher, 1993, de Hoog *et al.*, 2000, Domsch *et al.*, 2007, and Ellis *et al.*, 2007).

RESULTS

A total of 24 male workers participated in the study. The mean age was 34.1 ± 5.8 years and their ages ranged between 23 to 50 years. Twenty out of 24 (83.3%) hair samples analysed produced fungal colonies when incubated and examined. The total number of isolates including those grown on SDA and in soil cultures was 49. Of all the isolates, 26 species attributed to 19 fungal genera were identified. Isolates that grew dark sterile mycelia and budding yeasts were also included as shown in Tables (1 & 2). The number of isolates per sample ranged from 1 to 9 with the majority of samples yielding 1 or 2 species. Three samples yielded 3 species while the remaining positive samples produced 4, 6 or 9 fungal species (one sample for each).

Considering the frequency of occurrence of individual fungi on hair samples the present data showed that *Chaetomium globosum* was the most commonly isolated fungal species (7 samples representing 29% of hair samples matching 14.29% of total isolated fungi), followed by *Emericella nidulans* (4 samples representing 16.6% of samples matching 8.16% of fungal isolates). Each of *Cochliobolus neergaardii* (anamorph= Bipolaris neergaardii) and *Penicillium oxalicum* appeared on 3 samples sharing with 6.12% of total isolated colonies. Unidentified yeasts were recovered from 5 hair samples cultured on SDA (Tables 1, 2 and Fig. 7).

When hair samples were cultured on sterile wet soil, 7 fungal strains appeared on the hair baits including *Chaetomium globosum, Chrysosporium keratinophilum* (Teleomorph= *Aphanoascus fulvescens*), *Cladosporium cladosporioides, Cochliobolus neergaardii*, C. spicifer, *Curvularia papendorfii, Stachybotrys chartarum* in addition to an isolate of dark sterile myclia. This indicates the capacity of these isolates to degrade keratin and can be considered pathogenic causing skin and hair infections.

Regarding the fungi isolated from nail samples, only three species were identified

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including *Alternaria alternata, Aureobasidium pullulans* and *Penicillium chrysogenum.*

Microscopic examination and imaging of 19 different fungal strains from hair samples cultured on SDA or on sterile wet soil can be observed in 6 figures. Figure (1) illustrates the characteristic dark flexuous conidiophores and ellipsoidal conidia of *Cochliobolus neergaardii,* the dark geniculate conidiophore and cylindrical conidia of *Cochliobolus spicifer* (anamorph= *Bipolaris spicifera*), and the chlamydospores and ellipsoidal conidia with transverse septa of *Embellisia chlamydospora*.

Figure (2) shows the pigmented conidiophore of *Aspergillus nidulans* (Teleomorph= *Emericella nidulans*), the hyphae, microconidia and polyphialides o*f Fusarium chlamydosporum* and the black, shining, smooth-walled conidia of *Nigrospora oryzae* (Teleomorph= *Khuskia oryzae*).

Figure (3) illustrates the conidiogenous cells and conidia of *Nodulisporium acervatum*, the long metulae, cylindrical phialides and elliptical conidia of *Penicillium oxalicum,* the rebranched conidiophores of *Penicillium chrysogenum* and the dark pycnidium of *Phoma herbarum.*

Figure (4) shows the dark rosette–shaped phialides, conidiophores and dark conidia of *Stachybotrys chartarum* as well as the dark coloured geniculate conidiogenous cells and the solitary muriform conidia of *Ulocladium botrytis*.

Figure (5) shows the following: a. *Alternaria alternata* producing branched chains of dark conidia with transverse and longitudinal septa; b. *Aspergillus sydowii* with hyaline vesiculate conidiophores, biseriate conidial heads, metulae and phialides producing chains of echinulate conidia; c. the pigmented conidiophores of *Aspergillus ustus* with biseriate conidial heads and rough-walled conidia; and d. growth of *Chaetomium globosum* on a human hair fragment showing dark perithecial ascoma and ascospores.

Figure (6) illustrates the following: a. *Chaetomium globosum* with dark subglobose perithecial ascomata with lateral and terminal hairs. Dark olive-brown lemon shaped ascospores; b. Fungal growth on human hair fragments plated on wet sterile soil; c. growth of *Chrysosporium keratinophilum* on human hair showing hyaline hyphae that produce intercalary and lateral ovoid spores each with truncate base; and d. growth of *Curvularia papendorfii* on a human hair fragments.

DISCUSSION

Human skin, which includes structures such as hair and nails, supports the growth of a varied fungal flora, not only dermatophytes and yeasts but also other species of moulds. The biggest group of organisms that can utilize keratin as the sole source of carbon and nitrogen are the keratinophilic fungi. In the present study the diversity of this interesting group of fungi was studied in hair and nail samples from 24 male workers in Riyadh. These fungi cab easily transmitted from the environment to human hair and nails as well as to other body sites. Pandey *et al.* (1989) conducted a survey of pathogenic fungi in 45 soil samples collected from forest, riverside and residential garbage soil in Jabalpur, India. They isolated 66 fungal species classified in 35 genera which included representative species of Chrysosporium (78%), Fusarium (69%), Aspergillus (47%), Penicillium (11%), Cladosporium (7%) and Chaetomium (4%).

Among the 26 fungal species isolated and identified during the present study

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Chaetomium globosum was the most common fungus (29% of samples). This species was previously described as a cause of onychomycosis in Spain (Aspiroz *et al.*, 2007), Czeck Republic (Hubka *et al.*, 2011), Korea (Kim et al., 2013) and China (Shi *et al.*, 2016). *C. globosum* produces mycotoxins, particularly chaetoglobosins A and C when cultured on building materials (Fogle *et al.*, 2007). Together with *Stachybotrys charatarum*, *C. globosum* was frequently found growing on wooden materials and was involved in fungal infections amongst construction workers causing a disease named as "sick building syndrome" (Straus, 2011).

Emericella nidulans which occurred in 16.6% of our samples constituting 8.16% of fungal isolates has been recently found to cause endophthalmitis after cataract surgery which showed no improvement with vigorous topical and intravitreal therapy (Mutlu *et al.*, 2016). *Cochliobolus neergaardii* is a fungus that is associated with rice seeds and is usually found in the Asian temperate zones such as Saudi Arabia and the Arabian peninsula, and has been known to cause devastating disease epidemics on food crops, such as rice, wheat and maize (Otaka *et al.*, 2016). *Penicillium oxalicum* was found in 12.5% of our samples. Exposure to spores of *Penicillium oxalicum* may provoke adverse health effects such as allergic rhinitis, bronchial asthma or extrinsic allergic alveolitis (Lugauskas *et al.*, 2004).

Onychomycosis is documented to result from *Aspergillus sydowii* and *Ulocladium botrytis* (Romano *et al.*, 2004; Nouripour-Sisakht *et al.*, 2015). Cases of fungal endophthalmitis from *Aspergillus terreus* and Emericella nidulans were reported by Mutlu *et al.* (2016) and Puah *et al.* (2016). Pulmonary infections from *Aspergillus ustus* and *Stachybotrys charatarum* (Hodgson *et al.*, 1998; Cabada *et al.*, 2010), infection of the lymphatic system from *Aureobasidium pullulans* (de Morais *et al.*, 2011), haemorrhagic pneumonia from *Cladosporum cladosporioides* (Grava *et al.*, 2016), perinephric abscesses from *Fusarium chlamydosporum* (Sidhu *et al.*, 2013) and intestinal disseminated disease from *Penicillium chrysogenum* (Barcus *et al.*, 2005) were frequently documented. The large percentage of the isolated species that could potentially cause a human infection should be seriously considered. Mycological infections usually receive less attention than bacterial and viral infections, but the potential for these fungi to infect humans with their added ubiquity should be taken seriously (Tsoumani *et al.*, 2011).

Additional studies on fungal isolation from hair and nails in different parts of the world have been conducted in Turkey (Metintas *et al.*, 2004), India (Sarma, 2007), Czech Republic (Lysková, 2007) and in Northern Greece (Nasr *et al.*, 2016), The non-dermatophytes characterized in our study have also shown more diversity and were more prevalent in our samples. This diversity in fungal isolates supports the hypothesis that heterogeneity of the distribution may be due to differences in climate and lifestyle (Al-Sogair *et al.*, 1991; Enugopal, 1992; Alsheikh., 2009). More recently, Kutwal and Sambali (2016) were able to isolate *Aspergillus sydowi*, *A. ustus*, *A. stellatus* (= *Emericella vriecolor*) and *Cladosporium cladosporioides* as active keratinolytic fungi growing on human hair. Reports from Libya (Altayyar *et al.*, 2016) showed that *Aspergillus species* were the highest isolated (58.9%) followed by

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Acremonium spp. (14.8%), *Chrysosporium spp*. (8.9%), *Trichoderma spp*. (5.8%), *Microsporum spp.* (2.9%) and *Mucor spp.* (2.9%).

As mentioned by Piraccini and Alessandrini (2015) onychomycosis is the most common nail infective disorder, and it is responsible for about 50% of all consultations for nail disorders. Onychomycosis has been reported as a gender- and age-related disease, being more prevalent in males and increasing with age in both genders. In the elderly, onychomycosis may have an incidence >40%. Predisposing factors are diabetes mellitus, peripheral arterial disease, immunosuppression due to HIV or immunosuppressive agents. In most cases, this infection is caused by anthropophilic dermatophytes, in particular by *Trichophyton rubrum*, followed by *Trichophyton mentagrophytes* var. interdigitale. Non-dermatophyte molds, like *Scopulariopsis brevicaulis* and *Aspergillus spp.*, can be involved in onychomycosis as primary pathogens or as contaminant agents and secondary pathogens (Gherbawy *et al.*, 2006). Other molds that have been isolated from affected nails include *Fusarium spp.*, *Acremonium spp.* and *Alternaria spp.* The estimated worldwide prevalence of non-dermatophyte molds onychomycosis is 10%–15%. Yeasts, like *Candida albicans* and *Candida parapsilosis*, represent the third cause of nail fungal infection, and they occur only when predisposing factors are present, mainly immunosuppression and diabetes.

CONCLUSIONS

A diverse population of potentially pathogenic and non-pathogenic non-dermatophyte fungal species was isolated from the hair and nails of Saudi Arabian workers. These fungi were characterized and identified microscopically. The presence of these fungal species, their distribution amongst human hosts, their contributions to the normal flora of the skin and its appendages and their possible pathogenicity warrant further large scale study. Identifying these species and describing them morphologically with high definition images makes this study the first of its kind in the region.

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Table 1. Fungal species isolated from human hair samples on SDA (A) and sterile wet soil (B) and their frequency out of 24 samples.

Fungal species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	F
Alternaria alternata (Fries) Keissler		-	-	-		-	-	-	-	-		-	A	-		-	-	-	-	-	-	-	-	-	1
Aspergillus niger van Tieghem		-	A	-		-	-	-	-	-		-	-	-		-	-	-	-	-	-	-	-	-	1
Aspergillus sydowii (Bainier & Sartory) Thom and Church		-	-	-		-	-	-	-	-		-	-	A		-	-	-	-	-	-	-	-	-	1
Aspergillus terreus Thom		-	-	-		-	-	-	-	-		-	-	-		-	-	-	-	-	-	A	-	-	1
Aspergillus ustus (Bainier) Thom and Church		-	-	-		-	-	-	-	-		-	A	-		-	-	-	-	-	-	-	-	-	1
Aureobasidium pullulans(de Bary) Arnaud		-	-	A		-	-	-	-	-		-	-	A		-	-	-	-	-	-	-	-	-	2
Chaetomium globosum Kunze		B	-	-		-	A	A	-	-		-	-	-		-	B	-	A	A	-	-	-	A	7
Chrysosporium keratinophilum (Frey) Carmich.		-	-	-		-	-	-	-	-		-	B	B		-	-	-	-	-	-	-	-	-	2
Cladosporium cladosporioides (Fresenius) de Vries		-	-	A		-	-	-	B	-		-	-	-		-	-	-	-	-	-	-	-	-	2
Cochliobolus spicifer Nelson		-	-	-		-	-	-	B	-		-	-	-		-	-	-	-	A	-	-	-	-	2
Cochliobolus neergaardii Danquah		-	-	-		-	-	-	-	-		A	-	B		-	-	-	A	--	-	-	-	-	3
Curvularia papendorfii van der Aa		-	-	-		-	-	-	-	-		-	-	B		-	-	-	-	-	-	-	-	-	1
Embellisia chlamydospora (Hoes, Bruehl & Shaw) Simmons		-	-	-		-	A	-	-	-		-	-	A		-	-	-	-	-	-	-	-	-	2
Emericella nidulans (Eidam) Vuillemin		-	-	-		-	-	A	A	-		-	A	-		-	-	-	-	-	-	-	A		4
Emericella variecolor Berkeley & Broome		-	-	-		-	-	-	A	-		-	-	-		-	-	-	-	-	-	-	-	-	1
Fusarium chlamydosporum Wollenweber & Reinking		-	-	-		-	-	-	-	-		-	-	A		-	-	-	-	-	-	-	-	-	1
Nigrospora oryzae (Berkeley & Broome) Petch		-	-	-		-	-	-	A	-		-	-	-		-	-	-	-	-	-	-	-	-	1
Nodulisporium acervatum (Massee) Deighton		-	-	-		-	A	-	-	-		-	-	-		-	-	-	-	-	-	-	-	-	1
Penicillium chrysogenium Thom		-	A	-		-	-	-	-	-		-	-	-		-	-	-	-	-	-	-	-	-	1
Penicillium glabrum (Wehmer) Westling		-	-	-		-	-	-	-	A		-	-	-		-	-	-	-	-	-	-	-	-	1
Penicillium oxalicum Currie & Thom		A	-	-		A	-	-		A		-	-	-		-	-	-	-	-	-	-	-	-	3
Phoma herbarum Westend.		-	-	-		-	-	-	A	-		-	-	-		-	-	-	-	-	-	-	-	-	1
Stachybotrys charatarum (Ehrenberg) Hughes		-	-	-		-	-	-	-	-		-	-	B		-	-	-	-	-	-	-	-	-	1
Ulocladium botrytis Preuss		-	-	-		-	-	-	-	-		-	-	-		A	-	-	-	-	-	-	-	-	1
Dark sterile mycelium		-	-	-		-	-	-	-	-		-	-	-		A	B	-	-	-	-	-	-	-	2
Budding yeasts		-	-	A		-	-	-	-	-		-	-	A		A	-	A	-	-	A	-	-	-	5
Number of species/sample		2	2	3		1	3	2	6	2		1	4	9		3	2	1	2	2	1	1	1	1	49

Hair samples showing negative results are highlighted (four samples)

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Table 2: Incidence (out of 24 samples from male workers) of fungal species recovered from hair and nail samples on SDA and sterile wet soil (SWS).

Fungal species	Hair			Nails
	SDA	SWS	Total	SDA
Alternaria alternate (Fries) Keissler	1	-	1	1
Aspergillus niger van Tieghem	1	-	1	-
Aspergillus sydowii (Bainier&Sartory) Thom and Church	1	-	1	-
Aspergillus terreus Thom	1	-	1	-
Aspergillus ustus(Bainier) Thom and Church	1	-	1	-
Aureobasidium pullulans (de Bary) Arnaud	2	-	2	1
Chaetomium globosum Kunze	5	2	7	-
Chrysosporium keratinophilum (Frey) Carmich.	-	2	2	-
Cladosporium cladosporioides (Fresenius) de Vries	1	1	2	-
Cochliobolus spicifer Nelson	1	1	2	-
Cochliobolus neergaardii Danquah	2	1	3	-
Curvularia papendorfii van der Aa	-	1	1	-
Embellisia chlamydospora(Hoes, Bruehl& Shaw) Simmons	2	-	2	-
Emericella nidulans (Eidam) Vuillemin	4	-	4	-
Emericella variecolor Berkeley & Broome	1	-	1	-
Fusarium chlamydosporum Wollenweber & Reinking	1	-	1	-
Nigrospora oryzae (Berkeley & Broome) Petch	1	-	1	-
Nodulisporium acervatum (Massee) Deighton	1	-	1	-
*Penicillium chrysogenum*Thom	1	-	1	1
Penicillium glabrum(Wehmer) Westling	1	-	1	-
Penicillium oxalicum Currie & Thom	3	-	3	-
Phoma herbarum Westend.	1	-	1	-
Stachybotrys charatarum (Ehrenberg) Hughes	-	1	1	-
Ulocladium botrytis Preuss	1	-	1	-
Dark sterile mycelium	1	1	2	-
Budding yeasts	5	-	5	-
Total number of fungal strains	39	10	49	3

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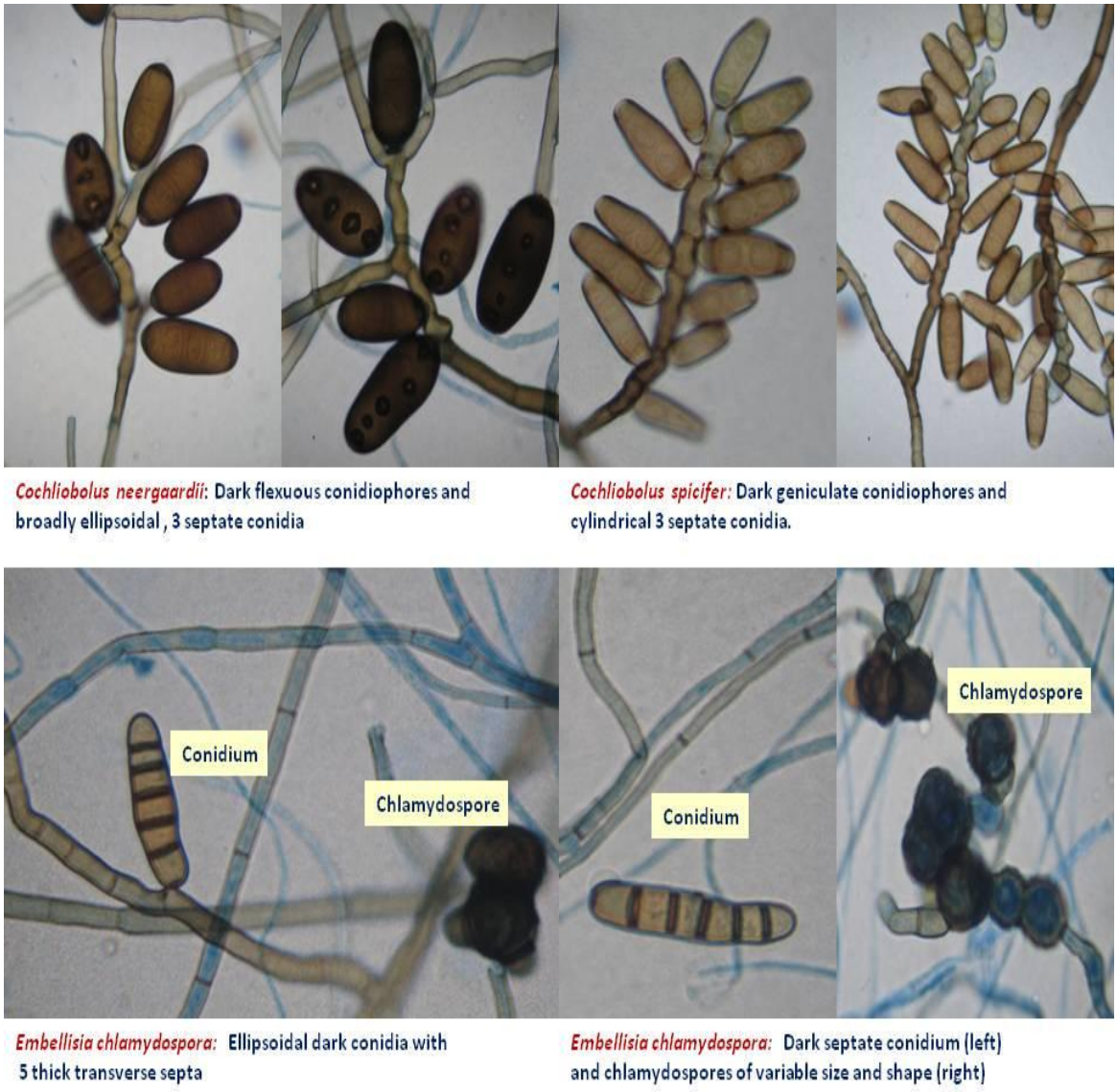

Cochliobolus neergaardii: Dark flexuous conidiophores and broadly ellipsoidal , 3 septate conidia

Cochliobolus spicifer: Dark geniculate conidiophores and cylindrical 3 septate conidia.

Embellisia chlamydospora: Ellipsoidal dark conidia with 5 thick transverse septa

Embellisia chlamydospora: Dark septate conidium (left) and chlamydospores of variable size and shape (right)

Figure 1. Microscopic images of some fungal species belonging to Cochliobolus, and Embellisia (X1000) isolated from the hair of workers

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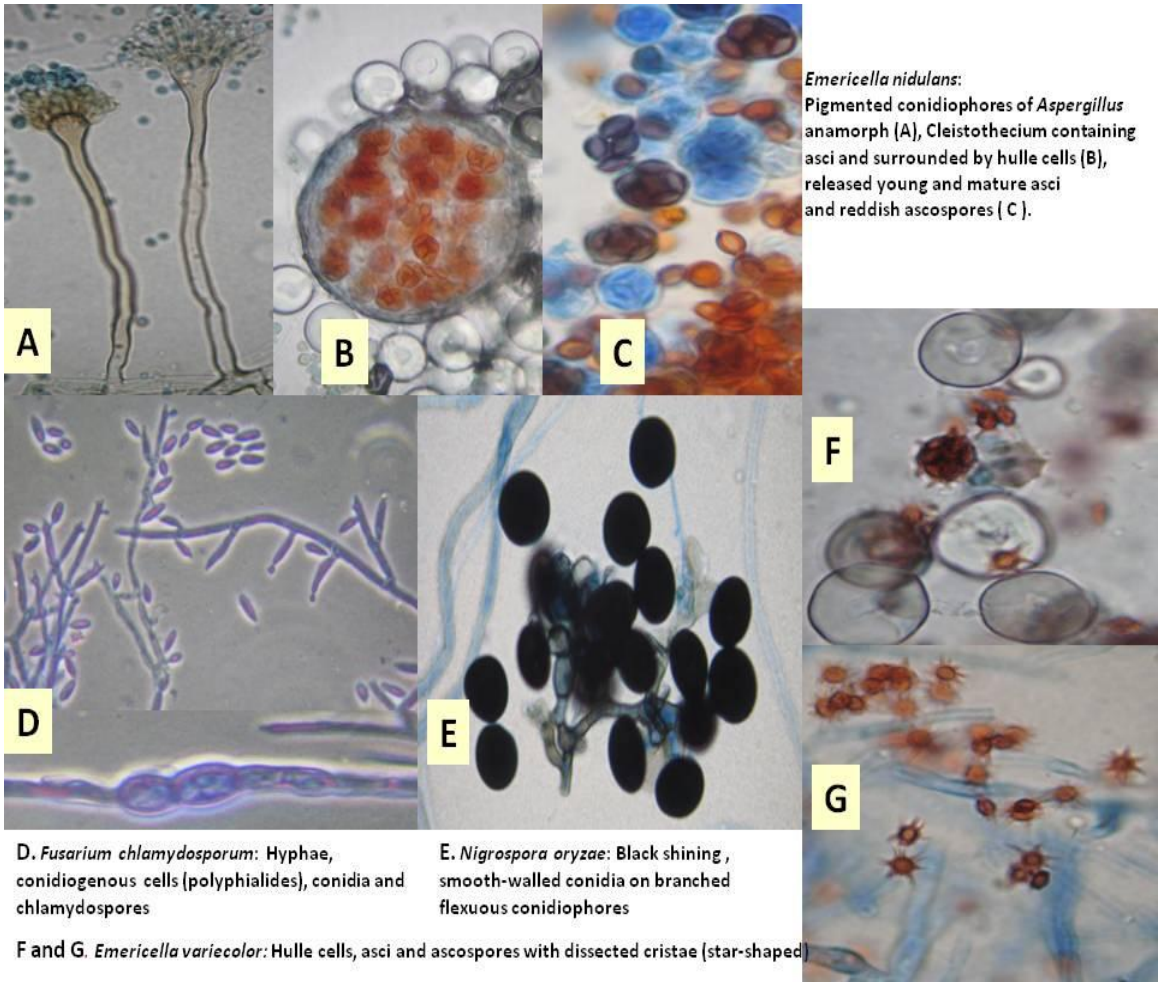

Figure 2. Microscopic images of some fungal species belonging to Emericella, Fusarium and Nigrospora (X1000) isolated from the hair of workers

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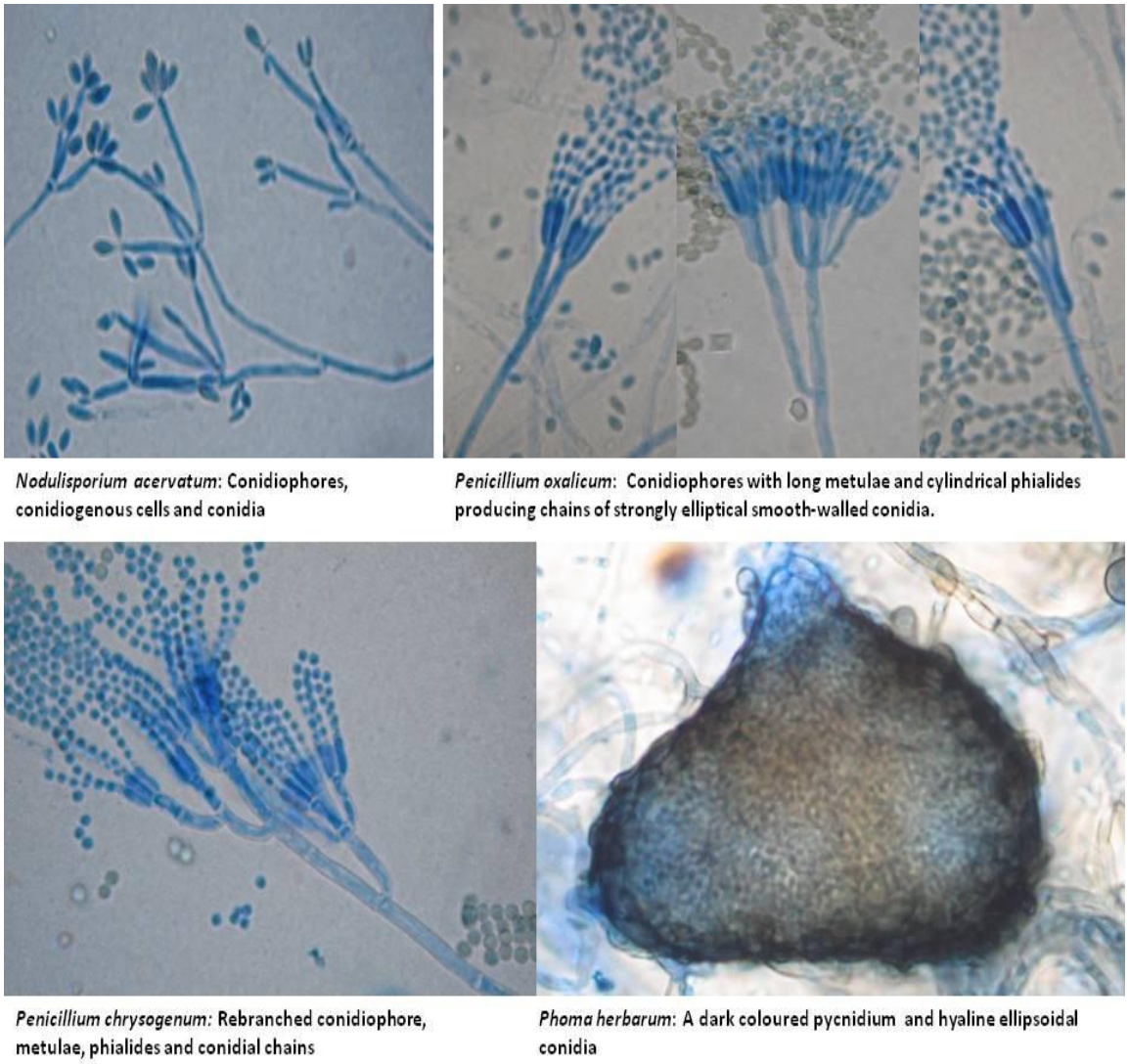

Figure 3. Microscopic images of some fungal species belonging to Nodulisporium, penicillium and Phoma (X1000) isolated from the hair of workers

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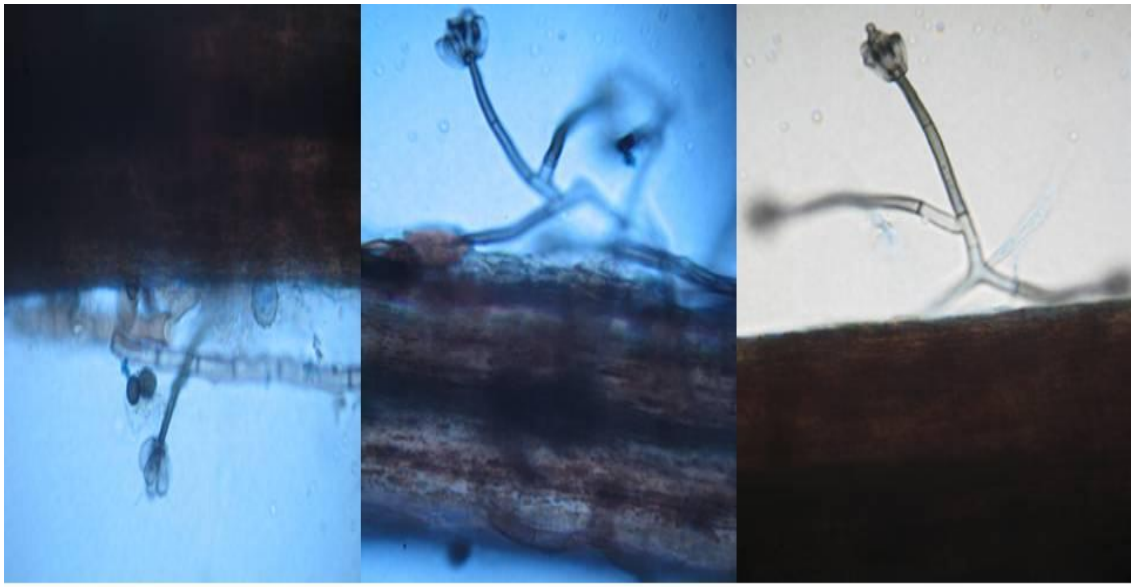
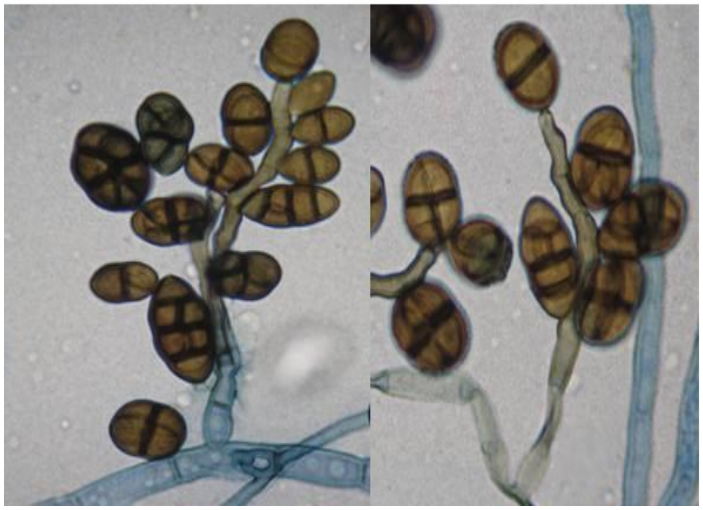


Growth of *Stachybotrys chartarum* on human hair fragment showing dark phialides and conidiophores.



Ulocladium botrytis:
Dark conidiophores (geniculate)
and solitary muriform conidia

Figure 4. Microscopic images of some fungal species belonging to Stachybotrys and Ulocladium (X1000) isolated from the hair of workers

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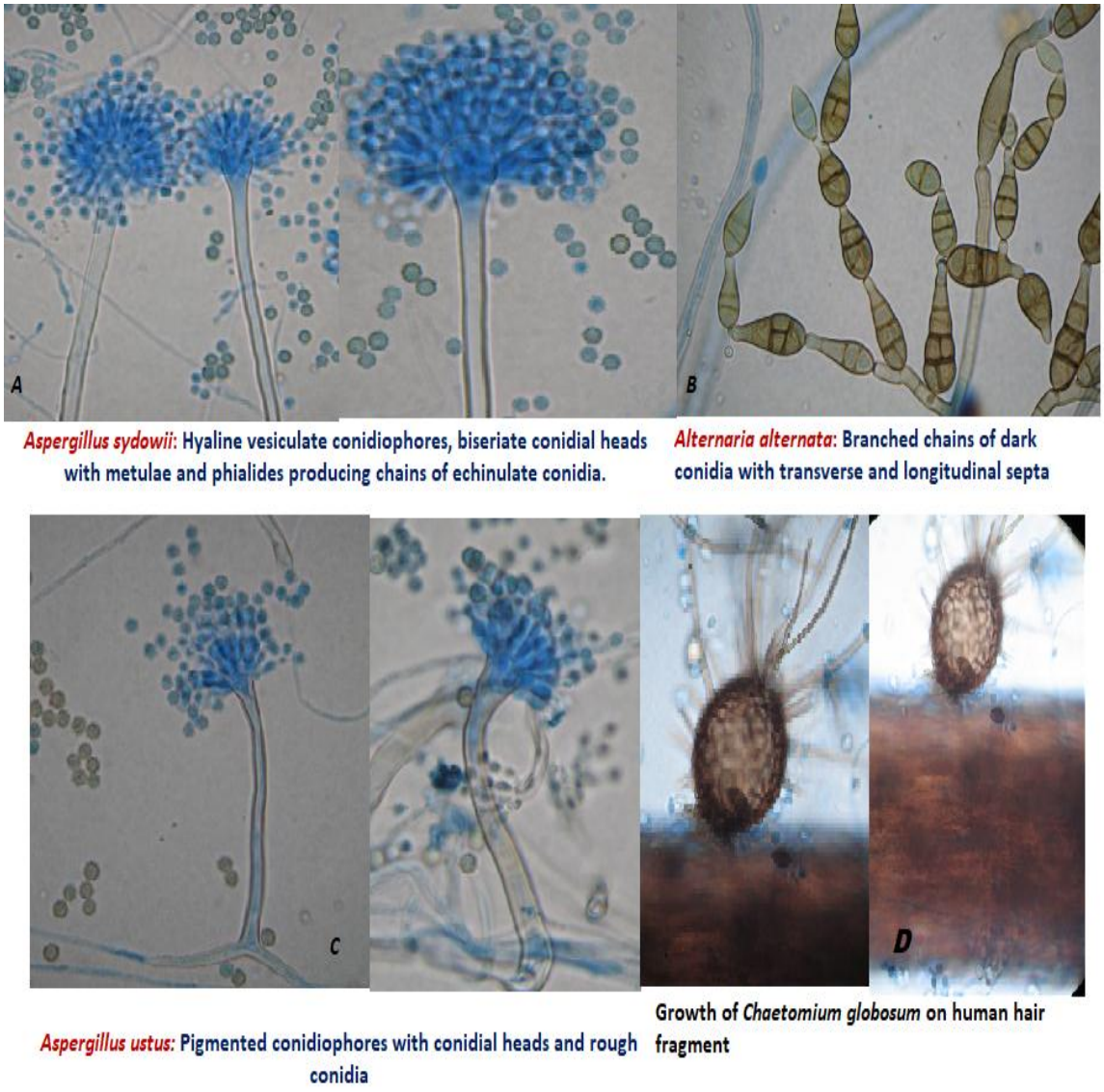

Figure 5. Microscopic images of some fungal species belonging to Aspergillus, Alternaria (X1000) and Chaetomium (X1000 left and X 400 right) isolated from the hair of workers

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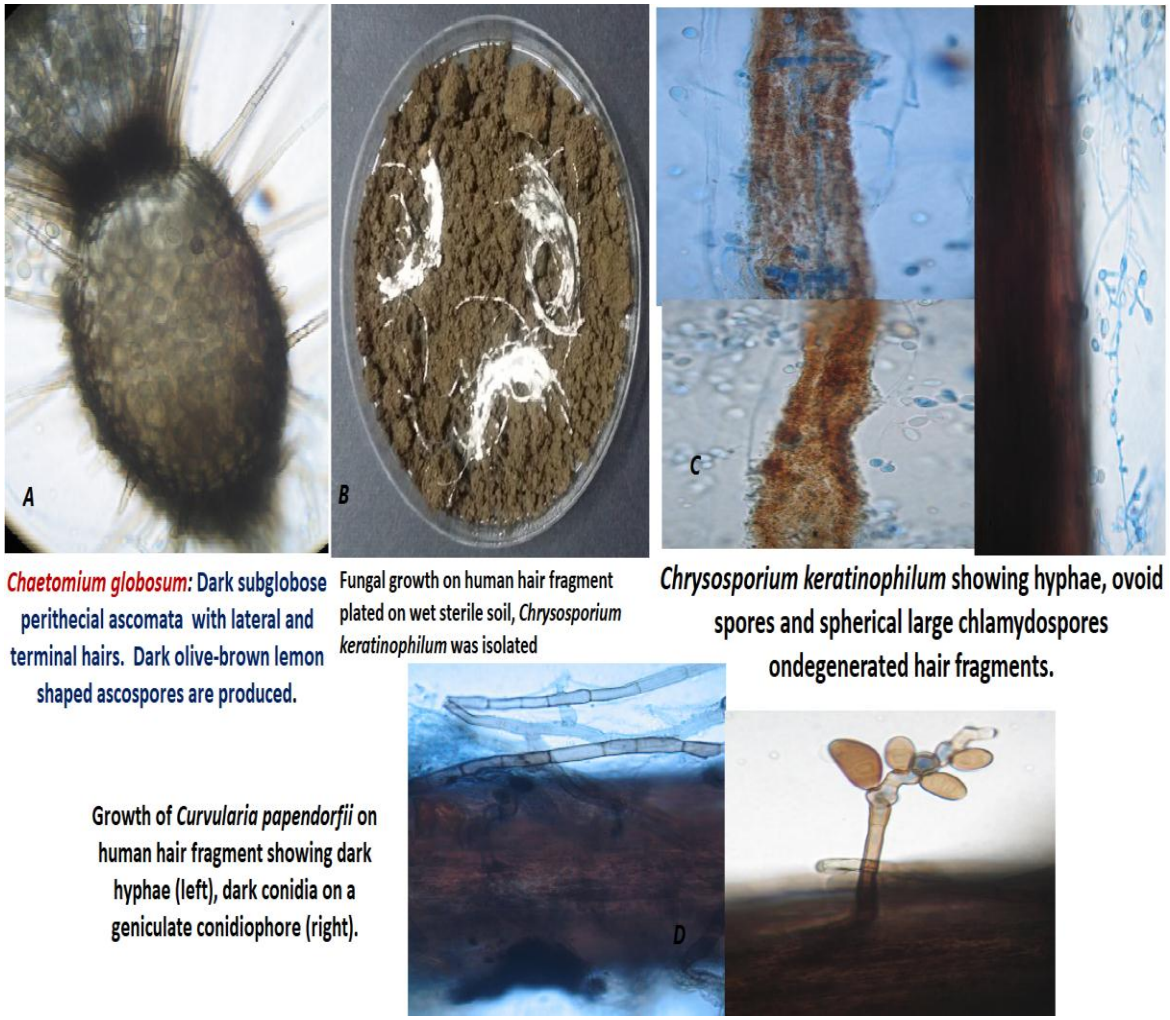

Figure 6. Microscopic images of some fungal species belonging to: A- Chaetomium (X1000), C- Chrysosporium (X400) and D- Curvularia (X1000) isolated from the hair of workers. Soil plate culture with human hair baits producing white growth of Chrysosporium is included (B).

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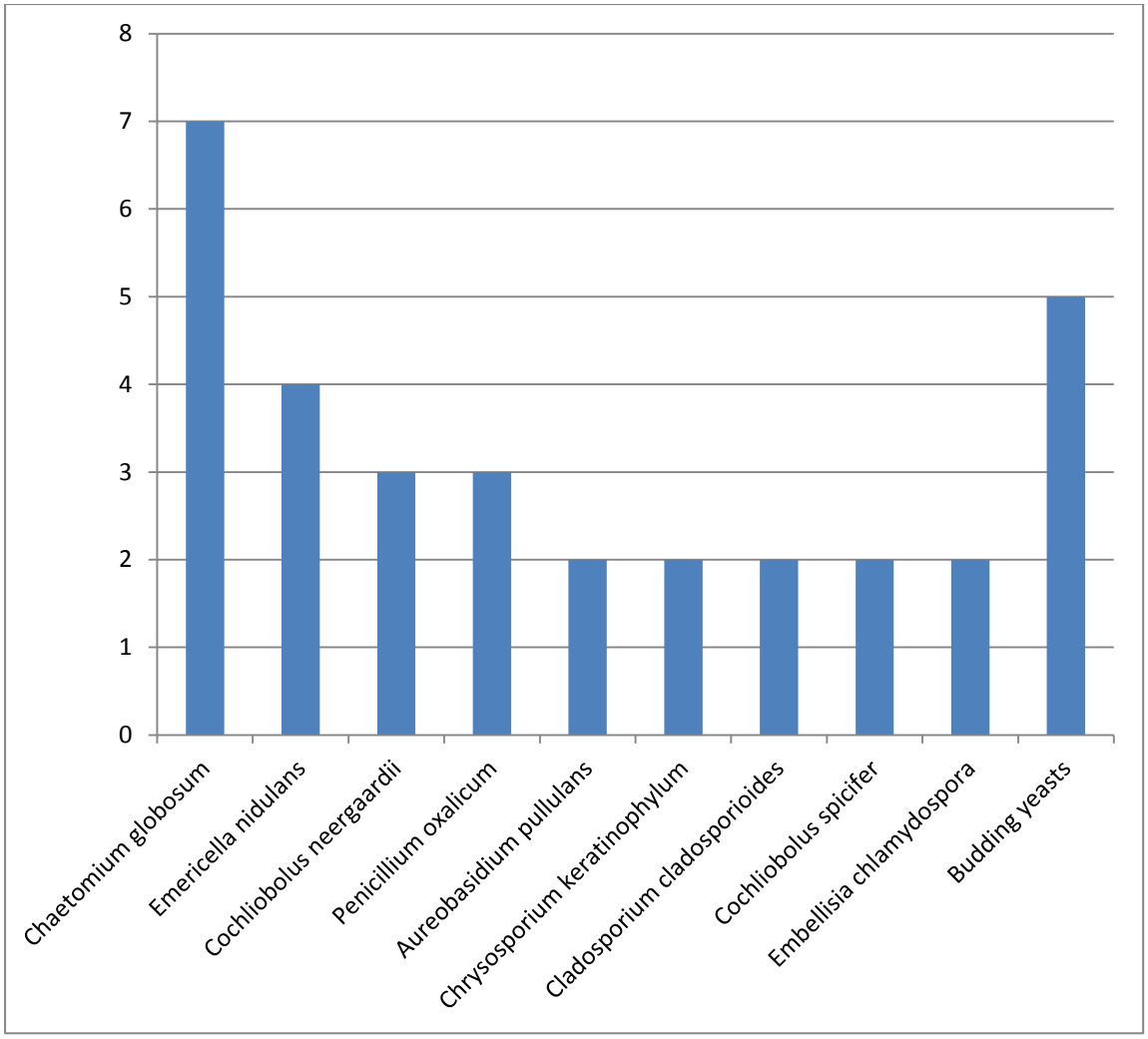

Figure 7: Frequency of keratinophilic fungi in hair samples (Fungi that appeared once were omitted from this chart)