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## Isolation and Identification of Soil Keratinophilic Fungi from Different Area in South of Libya

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### Abstract

Keratinophilic fungi are environment worldwide fungi that degrade keratinous substrates to components of low molecular weight; they belong to Hyphomycetes and several other taxonomic groups. The objective of this study is to assessing the frequency of Keratinophilic fungi, potentially pathogenic fungi from different area at south Libya.

Thirty nine soil samples were sterile inoculated in sterile petri dishes were half filled with approximately 40-50 g of soil for each sample. Hair baiting technique was used for isolating the fungi. Plates were then incubated at 28±20 °C for a period of 3-4 weeks.

A total of 136 strains of different Keratinophilic fungi were isolated; Keratinophilic fungi were classified into 10 species belonging to 6 genera, *Aspergillus* species represent the highest occurrence of isolated. In conclusion, this study is the first of its kind in the south Libya, high incidence of *Aspergillus* species in the soil of the south Libya finding of public health significance.

**Key Words:** Keratinophilic fungi, Hair baiting technique, South Libya

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### Introduction

Keratinophilic fungi are found worldwide in environment that degrades keratinous substrates to components of low molecular weights; they belong to Hyphomycetes and several other taxonomic groups. Hyphomycetes include dermatophytes and a variety of non-dermatophytic Keratinophilic fungi.

Dermatophytes group are anthropophilic or zoophilic in their natural habitat, they cause superficial cutaneous infections of keratinized tissues (skin, hair and nails) of humans and animals [1, 2].

Keratins are divided into two types.  $\alpha$ -Keratin in the form of folder chain, present in wool, hair, and horn and  $\beta$ -Keratin in the form of polypeptide chain, present in feather [3].

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Soil provides a heterogeneous and complex environment for all soil inhabitants; it is main reservoir of different types of fungi. Soil is good source of Keratinophilic fungi and the probability of the incidence of such fungi increases manifold if it is rich in keratinous materials [4].

The 'hair baiting' technique initially developed by R Vanbreuseghem, a Belgian mycologist in 1952. A number of modifications have been developed later, but the basic principle remains the same i.e. use of natural keratin substrate as baits to recover these fungi from soil [5].

Surveys conducted in several countries in different parts of the world have demonstrated that the occurrence of a variety of keratinophilic fungi e.g. (Egypt [6], Australia [7], Palestine [8], Spain [9], India [10], Korea [11], Iran [12], Kuwait [13] and Malaysia [14])

So far, no data are available on the on the prevalence of keratinophilic fungi in south of Libya to date is not available.

The aim of this study was to assess the frequency of Keratinophilic fungi, in various soil samples collected from different area at south of Libya.

## **Material and Methods**

### *Collection of samples*

A Total 39 soil samples were collected randomly from different area in south of Libya during the period January-Jun 2013. Samples were collected from the superficial layer with depth ranged less than 10 cm in sterile labeled polyethylene bags by sterile disposable spoon and transferred immediately to the laboratory for processing. Sterile horse hair bait technique was applied to isolate Keratinophilic fungi from soil.

### *Isolation and Identification of Keratinophilic Fungi*

Different sterile petri dishes were half filled with approximately 40-50g of soil for each sample. Hair baiting technique design by Larone [15] was used for isolating the fungi. Sterilized distilled water was added to provide moisture to the soil with varied amount depending on the sample moisture content. Then small pieces of sterilized horse hair were distributed on the surface of soil. The plates were then incubated at  $28\pm 20$  °C for a period of 3-4 weeks.

The plates were examined for any fungal growth on the hair bait regularly. A small part of growth was picked up and examined by normal slain or Lactophenol cotton blue wet mount preparation microscopically. Sabouraud dextrose agar media containing chloramphenicol were used for isolation, purification and identification of the fungal growth.

Identification of fungal culture was done on the bases of their macroscopically and microscopically characteristics according to the microbiology manuals and atlases [15-18].

## **Results**

One hundred and thirty six fungal colonies were isolated and identified from all collected soil samples during the study period. The numbers of colonies were ranged between 4-12 (5.7) colonies per each plate. 136 different fungal strains were isolated with rang between 1-6 (3.8) strains per plate (Table. 1).

A total of 136 strains of different Keratinophilic fungi were isolated from 39 soil samples, the isolated Keratinophilic fungi were classified into 6 species.

*Aspergillus* species represent the highest occurrence of isolated Keratinophilic fungi with 58.9%, other isolates were; *Acremonium spp* 14.8%, *Chrysosporium spp* 8.9%, *Trichoderma spp* 5.8%, unknown mold 5.8%

and minimum in *Microsporum spp* 2.9%, *Mucor spp* 2.9% (Table. 2).

*Aspergillus* species distributed (Table 3) as (*A.niger* 37.5%, *A.nidululans* 35%, *A.fumigatus* 15%, *A.flavus* 8.8%, *A.terreus*3.7%).

Table 1: Number of isolated fungal colonies and strains.

	Number of colonies/ single plate	Number of strains/ single plate
<b>Rang</b>	4-12	1-6
<b>Average</b>	5.7	3.8

Table 2: Number and percentages of isolated fungal species

Fungal species	Number	Percentage
<i>Aspergillus spp.</i>	80	58.9%
<i>Acremonium spp.</i>	20	14.8%
<i>Chrysosporium spp.</i>	12	8.9%
<i>Trichoderma spp.</i>	8	5.8%
Unknown mold	8	5.8%
<i>Microsporum spp.</i>	4	2.9%
<i>Mucor spp.</i>	4	2.9%

Table 3: Number and percentages of isolated *Aspergillus spp*

<i>Aspergillus</i> species	Number	Percentage
<i>A.niger</i>	30	37.5%
<i>A.nidululans</i>	28	35%
<i>A.fumigatus</i>	12	15%
<i>A.flavus</i>	7	8.8%
<i>A.terreus</i>	3	3.7%

## Discussion

Soils enriched with keratin substrate like hair, feathers, horn, hoofs and skin which is extreme resistance to biological attack and represent good reservoir of Keratinophilic fungi as well as dermatophytes. These fungi having a good ability to cause human infections and attract interest of the researchers in the soil mycoflora that can degrade keratinized residues [19, 20].

The present study investigated large number of soil samples for the presence of Keratinophilic fungi and represents the first study of its kind in south of Libya.

Our study showed that, *Aspergillus* species were the highest isolated 58.9%, other genera were included; *Acremonium spp* 14.8%, *Chrysosporium spp* 8.9%, *Trichoderma spp* 5.8%, unknown mold 5.8% and minimum in *Microsporum spp* 2.9% and *Mucor spp* 2.9%.

Keratinophilic fungi can be different species in different countries dependent on nature of soil and environment [21]. Several reports in agree with our founding indicated that, *Aspergillus* species are among the most prevalent Keratinophilic fungi in the soils [21-23].

Our results are agree with *Kumari et al* [24] who isolated 46 fungi from 40 different soil samples included; *Cunninghamella*, *Fusarium*, *Aspergillus*, *Rhizopus*, *Penicillium* and *Paecilomyces*.

*Acremonium*, *Pseudoallescheria boydis* and other pathogenic fungi were isolated from Indian soil [25, 26].

The high occurrence of Keratinophilic fungi especially *Chrysosporium spp* in the different countries support the fact that these species are able to tolerate the high temperature of this region which is about  $45 \pm 2$  C° in summer. In our isolates only 8.9% was *Chrysosporium spp*.

*Shadzi et al* and his colleague isolated 214 Keratinophilic fungi from the soil of schools and parks in Iran, of which *Chrysosporium keratinophilum* was the most frequent [27].

*El-Said et al* studied dermatophytic and keratinophilic fungi associated with the hair of goat and sheep in Libya and they found that, *Chrysosporium* was the most frequent genus and emerged in 92% and 96% of the total isolates, respectively [28].

*Ali Zarei et al* and *Kachuei et al* tested soil for dermatophytes and related Keratinophilic fungi in Iran and they reported that, *Chrysosporium spp* was the most common Keratinophilic fungus which partial agree with our results [12, 29].

Other study in agree with our finding done by *Ali-Shtayeh*, who reported that *Chrysosporium spp* was the highest isolated Keratinophilic fungi in school playgrounds in the Nablus area [30].

*Itisha Singh* screened Keratinolytic fungi in soil of Himachal Pradesh and isolated different type of Keratinolytic fungi including, *Chyrosoprium*, *Acremonium*, *Microsporum*, *Malbranchea* and *Trichophyton* were represented by 15, 8, 7, 6 and 4 species respectively [31].

*Ritesh Kumar* and his colleague investigated Keratinophilic fungi from garbage waste soils in India and they isolated various type of fungi which include; *Penicillium chrysogenum* (15.62%), *Aspergillus niger* and *Rhizopus stolonifer* (14.06%), *Trichoderma harzianum* (12.50%), *Fusarium oxysporum* (10.94%), *Aspergillus spp* (7.81%), *Aspergillus flavus* and *Curvularia lunata* (5.97%), *Penicillium spp* (5.81%) and *Chrysosporium spp* (4.69%) [32].

Their finding similar to our result with slight differ in the species and there percentage, and that can explain by different in the nature of soil from almost desert in south of Libya and other area of the world. *Mucor spp* were the lowest in our isolates, few other studies were reported *Mucor spp* in there founding [33, 34].

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