# STUDIES ON FUNGI ASSOCIATED WITH LABORATORY ANIMAL 'GOLDEN HAMSTER' AND ANTIBIOTIC EFFECTS OF ALOE SAP, GARLIC EXTRACT AND ONION OIL

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*SUMMARY: Healthy hair samples from golden hamsters were examined for the presence of dermatophyte and non-dermatophyte using baiting technique and direct inoculation. Thirty four species and 2 varieties attributed to 17 genera were recovered. Paecilomyces variotii (isolated from 84.4% of the examined hair) and A. niger (81.3%) were more frequently on Sabouraud's dextrose agar (SDA) without cycloheximide. Our results have clearly demonstrated that the hair of hamster was free from true dermatophyte. Using the dilution plate method many different fungal species were isolated from cage material (7 genera and 10 species + 1 variety); from faeces (10 genera and 17 species); from standard chow (3 genera and 6 species) of hamster on SDA without cycloheximide. P. variotii which was the most frequent fungus in the preceding 3 substrates; was completely absent in the presence of cycloheximide in SDA. The present study has demonstrated for the first time the isolation of Trichophyton rubrum from hamster faeces. Also several saprophytic and cycloheximide resistant fungi were isolated. In the air of hamster cage Cladosporium cladosporioides, Penicillium chrysogenum, Alternaria alternata and Scopulariopsis brevicaulis were the most dominant species on SDA with or without cycloheximide.*

*Using the agar diffusion method; Aloe sap, onion oil, garlic bulb extract and aqueous leaf extracts of Andropogon citratus, Euphorbia sp. and Ruta graveolens were tested for their antifungal activity on 10 Fungal species. It was observed that onion oil exhibited a high inhibitory effect against most of the tested fungi.*

*Key Words: Saprophytic fungi, cycloheximide, hamster, hair, faeces, antifungal activity.*

## INTRODUCTION

The skin of animals is contaminated by numerous fungi, some of which are opportunistic pathogens or allergens. Several investigations have reported the occurrence of dermatophytes on the apparently healthy skin of domestic and wild animals (1-3). Also animal pens and animal faeces represent a good habitat for keratinophilic and saprophytic fungi (4,5).

The objective of this work, was to determine:

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1.The existence of dermatophytes and non-dermatophytes, in the skin of hamster employed for biomedical research.

2. Occurrence of these fungi in cage material, faeces and standard chow of hamster.

3. Incidence of these fungi in air of hamster's cage.

4. Antifungal activity of the extracts of six plants against some fungal species.

## MATERIALS AND METHODS

**Animals:** Adult male golden hamsters (Mesocricetus auratus) weighing approximately 110 g were obtained from Schistosoma Biological supply programme, Theodor Bilhars Research Institute, Imbaba, Egypt. The animals were maintained for at least 4 weeks before experiments in temperature controlled room (24°C + 3). Light was provided by Philips 40 w fluorescent tubes between 06.00 and 20.00 h (L:D. 14:10). The hamsters received standard laboratory chow and tap water *ad libitum*.

**Isolation Medium:** Sabouraud's dextrose agar (6) supplemented with antibiotics (Chloramphenicol-0.05 mg/ml and cycloheximide-0.05 mg/ml) and without cycloheximide were used in all experiments carried out during the present investigation.

**Isolation of fungi from hamster's hair:** Thirty two samples of hamster hair were collected. These samples were placed separately in clean plastic bags and then transferred directly to the laboratory and kept in a cool place (3-5°C) till fungal assay. All samples were tested with 10% KOH for direct examination. Two different techniques were used: hair baiting as recommended by Vanbreuseghem (7). Pieces of hair were sprinkled on the surface of double sterilized soil. The soil was moistened with sterilized distilled water and remoistened whenever necessary and incubated at room temperature for up to 4 weeks. The moulds which appeared on the baits were transferred onto agar medium without cycloheximide. The other technique was direct plating of the hair onto the agar media. Plates were incubated at 28°C for 10-21 days and the cultures were examined periodically for fungal growth.

**Isolation of fungi from cage, faeces and chow of hamster:** Three samples each of cage material (wood shavings was the sole constituent of hamster cage), faeces and chow were also collected. The dilution plate method (8) was employed. Ten plates (five plates for each medium) were used for each sample and were incubated for 7-15 days. The developing fungi were identified and counted per 9 gram dry sample.

**Determination of air borne fungi in hamster's cage:** Ten plates (five for each medium) of 9 cm diameter were used for each exposure. The plates were exposed to the air in hamster's metal cage weekly at 11 a.m. for 10 min. The plates were incubated at 28°C for 7-15 days during which the developing fungi were identified and counted. The count of fungi were calculated per 20 plates in 4 exposures. Several books and mycological papers were used for identification (9-12).

**Antifungal preparations:** For the *in vitro* studies Aloe leaf sap, onion (*Allium cepa L.*) oil (from El-Nasr Company, Egypt), garlic (*Allium sativum L.*) bulb extract and aqueous leaf extracts, prepared as mentioned by Hasan and Abdel-Mallek (13) of each of *Andropogon citratus* Hort., *Euphorbia sp.* and *Ruta graveolens L.* were screened for their activities against 10 fungal species (*Trichophyton rubrum*, *Chrysosporium keratinophilum*, *Scopulariopsis brevicaulis*, *Acremonium strictum*, *Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Paecilomyces variotii* and *Penicillium chrysogenum*). The agar diffusion technique (14) was employed with some modification. One ml portions of spore suspension of each tested fungus were pipetted in sterilized plates followed by the addition of 20 ml of Sabouraud's dextrose agar. After solidification similar holes (5mm) were made in the agar plates with cork borer. 0.2 ml extract of each tested plants was placed inside the holes. The antifungal agent trosyd (1%, manufactured by Pfizer, Egypt) was used as standard. Three plates were used for each plant for trosyd per fungus species. Cultures were incubated at 28°C for 10-15 days, after which the inhibition zones around the holes were measured. The relative inhibitory power of extracts was calculated as % inhibition in comparable with trosyd drug.

## RESULTS

**Fungi isolated from hamster's hair:** Thirty four fungal species and 2 varieties which belong to 17 genera were isolated using hair baiting and direct plating techniques (Table 1).

**Hair baiting technique:** A total of 9 genera, 15 species and 1 variety were collected. *Aspergillus niger* (59.4% of the examined hair), *Paecilomyces variotii* (56.3%) and *A. flavus* (50.0%) were highly frequent. Three species showed low incidence viz: *A. fumigatus*

Table 1: Incidence of fungi in hamster's hair on Sabouraud's dextrose agar at 28°C.

| Species | Hair baiting tecnique | | | Direct plating technique | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | S | | | S | | | S+Cy | | |
| | NCl | % | OR | NCl | % | OR | NCl | % | OR |
| Acremoniumstrictum W. Gamus | - | - | - | - | - | - | 1 | 3.1 | R |
| Alternaria alternata (Fr.) Keissler | - | - | - | 4 | 12.5 | L | 1 | 3.1 | R |
| A. tenuissima (Kunze ex Pers.) Wiltshire | - | - | - | 1 | 3.1 | R | 3 | 9.3 | R |
| Aspergillus candidus Link | - | - | - | - | - | - | 1 | 3.1 | R |
| A. flavus Link | 16 | 50.0 | H | 5 | 15.6 | L | 14 | 43.8 | M |
| A. flavus var. Columnaris Raper and Fennel | 1 | 3.1 | R | - | - | - | - | - | - |
| A. fumigatus Fresenius | 6 | 18.8 | L | 8 | 25.0 | M | - | - | - |
| A. niger van Tieghem | 19 | 59.4 | H | 26 | 81.3 | H | 16 | 50.0 | H |
| A. ochraceus Wilhelm | 1 | 3.1 | R | - | - | - | - | - | - |
| A. sydowii (Bain. and Sart.) Thom and Church | 1 | 3.1 | R | 3 | 9.3 | R | 7 | 21.8 | L |
| A. ustus (Bain.) Thom and Church | - | - | - | - | - | - | 2 | 6.2 | R |
| A. versicolor (Vuill.) Tirab | - | - | - | - | - | - | 2 | 6.2 | R |
| Candida spp. | - | - | - | 1 | 3.1 | R | 3 | 9.3 | R |
| Chaetomium globosum Kunze ex Fries | - | - | - | - | - | - | 1 | 3.1 | R |
| Chrysosporium inops Carmichael | - | - | - | - | - | - | 1 | 3.1 | R |
| C. keratinophilum (Frey) Carmichael | 1 | 3.1 | R | 1 | 3.1 | R | 1 | 3.1 | R |
| Cladosporiumcladosporioides (Fres.) De Vries | 1 | 3.1 | R | 6 | 18.8 | L | 10 | 31.3 | M |
| C. herbarum (Pers.) Link ex Gray | - | - | - | 1 | 3.1 | R | - | - | - |
| C. sphaerospermum Penz. | - | - | - | 1 | 3.1 | R | - | - | - |
| Drechslera spicifera (Bain.) von Arx | - | - | - | - | - | - | 1 | 3.1 | R |
| Fusarium moniliforme Scheldon | 1 | 3.1 | R | - | - | - | - | - | - |
| Geotrichum candidum Link | - | - | - | 1 | 3.1 | R | - | - | - |
| Humicola grisea Traaen | 1 | 3.1 | R | - | - | - | - | - | - |
| Paecilomyces variotii Bain. | 18 | 56.3 | H | 27 | 84.4 | H | 1 | 3.1 | R |
| Penicillium brevicompactum Dierckx | - | - | - | - | - | - | 1 | 3.1 | R |
| P. chrysogenum Thom | 3 | 9.3 | R | 1 | 3.1 | R | 11 | 34.4 | M |
| P. citrinum Thom | 4 | 12.5 | L | 3 | 9.3 | R | 6 | 18.8 | L |
| P. corylophilum Dierckx | 3 | 9.3 | R | 4 | 12.5 | L | 1 | 3.1 | R |
| P. decumbens Thom | - | - | - | - | - | - | 1 | 3.1 | R |
| P. duclauxii Delacrolx | - | - | - | 2 | 6.2 | R | - | - | - |
| P. frequentans Westling | - | - | - | 2 | 6.2 | R | - | - | - |
| P. funiculosum. Thom | - | - | - | 1 | 3.1 | R | - | - | - |
| P. goldewskii Zaleski | - | - | - | 1 | 3.1 | R | - | - | - |
| Penicillium spp. | 2 | 6.2 | R | 2 | 6.2 | R | 3 | 9.3 | R |
| Rhizopus stolonifer (Ehrenb. ex Fries) Lind | 4 | 12.5 | L | 2 | 6.2 | R | 1 | 3.1 | R |
| Scopulariopsis brevicaulis (Sacc.) Bain. | 1 | 3.1 | R | 2 | 6.2 | R | - | - | - |
| Sterile mycelium | - | - | - | 1 | 3.1 | R | 1 | 3.1 | R |
| Talaromyces flavus var flavus (Klöcker) Stolk and Samson | - | - | - | 2 | 6.2 | R | - | - | - |
| Trichotheciumroseum (Pers.) Link | - | - | - | 1 | 3.1 | R | 1 | 3.1 | R |
| Number of species | 15 + 1 variety | | | 22 + 1 variety | | | 23 | | |
| Total number of species | | | | 34 + 2 variety | | | | | |

S= Sabouraud's dextrose agar medium, S+Cy= Sabouraud's dextorse agar medium + cycloheximide (Actidione), NCI= Number of cases of isolation (out of thirty two hair samples, % = Percentage frequency of occurrence (calculated/32 samples), OR= Occurrence remarks, H= High occurence (more than 16 samples), M= Moderate occurence (between 8-15 samples), L= Low occurrence (between 4-7 samples), R= Rare occurence (less than 4 samples)

(18.8%), *Penicillium citrinum* and *Rhizopus stolonifer* (12.5%, each). The remaining species (9 species + 1 variety) were rare frequent as listed in Table 1.

**Direct plating tecnique:** On Sabouraud's dextrose agar (SDA) without cycloheximide 21 species and 1 variety were isolated (Table 1). *Paecilomyces variotii* and *A. niger* were isolated in high frequency. They emerged from 27 and 26 out of 32 hair samples, respectively. *A. fumigatus* was moderately isolated being present in 8 samples. Four fungal species showed low incidence viz: *Cladosporium cladosporioides* (6 samples, 18.8% of the samples), *A. flavus* (5 samples, 5.6%), *Alternaria alternata* and *Penicillium corylophilum* (4 samples, 12.5%, each). The remaining fungal species (15 species + 1 variety) were rare in hamster's hair.

Twenty three cycloheximide resistant species were recovered. *A. niger* was the most common fungus species, occuring in 50% of the hair samples. *A. flavus, P. chrysogenum* and *C. cladosporioides* were recovered in moderate frequency, they encountered in 43.8%, 34.4% and 31.3% of the hair samples, respectively. *A. sydowii* (21.8%) and *P. citrinum* (18.8%) were low frequent. Sixteen species were isolated with rare frequency including *Chrysosporium keratinophilum, C. inops, Paecilomyces variotii, Acremonium strictum* and *Alternaria alternata* (3.1% of the examined hair).

**Fungi isolated from hamster's cage:** Wood shavings was the sole constituent of the hamster's cage. The results of Table 2 show that the total fungal count isolated on SDA without cycloheximide was 1191.9 colonies (calculated per g dry sample) whereas on SDA with cycloheximide was 244.7 colonies. Seven genera and 10 species in addition to 1 variety were recovered on SDA without cycloheximide. *Paecilomyces variotii* was the most common fungus giving rise to 71.5% of total fungal count. *A. niger* was the second frequent species accounting for 17.2%.

The following five fungal species could not be isolated on SDA without cycloheximide but appeared on SDA supplemented with cycloheximide: *Chrysosporium keratinophilum* (77.8 colonies, 31.8% of total fungal count), *Geotrichum candidum, P. citrinum, P. jensenii* (11.1 colonies, 4.5%, each) and *F. moniliforme* (3.6 colonies, 2.3%).

**Fungi isolated from hamster's faeces:** A total of 10 genera, 17 species of saprophytic fungi were isolated from hamster's faeces on SDA (Table 2). These numbers were higher than those isolated on SDA with cycloheximide (7 genera and 8 species). *Paecilomyces variotii* was also the most frequent fungus, accounting for 67% of total fungi on SDA without cycloheximide, whereas it was completely absent on SDA with cycloheximide. *Candida* was isolated in 19.7% and 63.2% of total fungal count on the above two mentioned media, respectively.

Six cycloheximide resistant species were isolated from hamster's faeces namely: *Chrysosporium keratinophilum* (6.7%), *Trichophyton rubrum, Fonsecae compactum, Gymnoascus reessii, Aspergillus ustus* and *Penicillium citrinum* (3.4%, each).

**Fungi isolated from hamster's chow:** From chow of hamster, six fungal species were recovered on Sabouraud's agar viz: *Paecilomyces variotii* (44.4 colonies/g dry sample, 30.7% of total fungal count), *A. niger* (33.3 colonies, 23%), *A. flavus, A. fumigatus, A. ustus* and *Cladosporium cladosporioides* (16.7 colonies, 11.6%; each). Whereas *P. chrysogenum, A. flavus, P. citrinum* and *Rhizopus stolonifer* were isolated on SDA with cycloheximide. They occurred in 56.6%, 23.3%, 10.0%, and 10.0% of total fungal count, respectively.

**Air borne fungi in hamster's cage:** Air borne fungi were estimated using two agar media (Sabouraud's dextrose agar with or without cycloheximide). The results of Table 3 reveal that the number of genera and species obtained on SDA with cycloheximide (7 genera, 10 species and 1 variety) was markedly lower than that obtained on SDA free from cycloheximide (16 genera, 25 species and 1 variety). Similarly the count of total fungi on SDA with cycloheximide (61 colonies/20 plates for 4 exposures) was lower than that on SDA without cycloheximide (218 colonies). However, the most dominant species on both media are alike e.g. *Cladosporium cladosporioides, Penicillium chrysogenum, Alternaria alternata, Aspergillus flavus* and *Scopulariopsis brevicaulis.* In addition to the above mentioned species 19 fungal species were isolated only on SDA without cycloheximide including *A. niger* (22%), *Paecilomyces variotii* (8.7%) and *Botryotrichum piluliferum* (28%).

Table 2: Count (per g dry sample) and percentage count (calculated to the total count) of fungal species isolated from cage material, faeces and chow of hamster.

| Species | Cage | | | | Faeces | | | | Chow | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | | S+ Cy | | S | | S + Cy | | S | | S + Cy | |
| | C | % | C | % | C | % | C | % | C | % | C | % |
| Alternaria alternata | - | - | - | - | 5.6 | 0.5 | - | - | - | - | - | - |
| Aspergillus flavipes (Bain. and Sart.) Thom and Church | - | - | - | - | 11.1 | 1.0 | - | - | - | - | - | - |
| A. flavus | 27.8 | 2.3 | 27.8 | 11.4 | 11.1 | 1.0 | 16.7 | 10.0 | 16.7 | 11.6 | 38.9 | 23.3 |
| A. flavus var. columnaris | 5.6 | 0.5 | 16.7 | 6.8 | - | - | - | - | - | - | - | - |
| A. fumigatus | - | - | - | - | 5.6 | 0.5 | 5.6 | 3.4 | 16.7 | 11.6 | - | - |
| A. niger | 205.6 | 17.2 | - | - | 10.6 | 0.9 | - | - | 33.3 | 23.0 | - | - |
| A. terreus Thom | 5.6 | 0.5 | - | - | 5.6 | 0.5 | - | - | - | - | - | - |
| A. ustus | - | - | - | - | - | - | 5.6 | 3.4 | 16.7 | 11.6 | - | - |
| A. versicolor | - | - | - | - | 5.6 | 0.5 | - | - | - | - | - | - |
| Candida spp. | - | - | - | - | 222.3 | 19.7 | 105.5 | 63.2 | - | - | - | - |
| Chrysosporium keratinophilum | - | - | 77.8 | 31.8 | - | - | 11.1 | 6.7 | - | - | - | - |
| Cladosporium cladosporioides | 5.6 | 0.5 | 16.7 | 6.8 | 5.6 | 0.5 | - | - | 16.7 | 11.6 | - | - |
| Drechslera spicifera | 5.6 | 0.5 | - | - | - | - | - | - | - | - | - | - |
| Fonsecae compactum Carriõn | - | - | - | - | - | - | 5.6 | 3.4 | - | - | - | - |
| Fusarium moniliforme | - | - | 5.6 | 2.3 | 5.6 | 0.5 | - | - | - | - | - | - |
| Geotrichum candidum | - | - | 11.1 | 4.5 | 5.6 | 0.5 | - | - | - | - | - | - |
| Gymnoasous reessii Baran | - | - | - | - | - | - | 5.6 | 3.4 | - | - | - | - |
| Mucor hiemalis Wehmer | 5.6 | 0.5 | - | - | - | - | - | - | - | - | - | - |
| M. racemosus Fresenius | - | - | - | - | 5.6 | 0.5 | - | - | - | - | - | - |
| Paecilomyces variotii | 852.8 | 71.5 | - | - | 755.6 | 67.0 | - | - | 44.4 | 30.7 | - | - |
| Penicillum chrysogenum | 11.1 | 0.9 | 66.7 | 27.3 | 5.6 | 0.5 | - | - | - | - | 94.4 | 56.6 |
| P. citrinum | - | - | 11.1 | 4.5 | - | - | 5.6 | 3.4 | - | - | 16.7 | 10.0 |
| P. corylophilum | 38.8 | 3.3 | - | - | 11.1 | 1.0 | - | - | - | - | - | - |
| P. frequentans | - | - | - | - | 38.9 | 3.4 | - | - | - | - | - | - |
| P. funiculosum | - | - | - | - | - | 5.6 | - | - | - | - | - | - |
| P. jensenii Zaleski | - | - | 5.6 | 4.5 | 5.6 | 0.5 | - | - | - | - | - | - |
| Penicillium spp. | 16.7 | 1.4 | - | - | - | - | - | - | - | - | - | - |
| Rhizopus Stolonifer | - | - | - | - | 5.6 | 0.5 | - | - | - | - | 16.7 | 10.0 |
| Syncephalostrum racemous (Cohn) | | | | | | | | | | | | |
| Schroeter | 11.1 | 0.9 | - | - | - | - | - | - | - | - | - | - |
| Sterile mycelium | - | - | 5.6 | 4.5 | 5.6 | 0.5 | - | - | - | - | - | - |
| Trichophyton rubrum (Castellani) Sabouraud | - | - | - | - | - | - | 5.6 | 3.4 | - | - | - | - |
| Gross total count | 1191.9 | | 244.7 | | 1127.9 | | 166.9 | | 144.5 | | 166.7 | |
| Number of species | 10+1 variety | | 8+1 variety | | 17 | | 8 | | 6 | | 4 | |
| Total number of species | 27 +1 variety | | | | | | | | | | | |

C= Count of fungi

%= Percentage count of fungi

Table 3: Count (per 20 plates in 4 exposures) and percentage count of air borne fungi in hamster's cage.

| Species | S | | S+ Cy | |
|---|---|---|---|---|
| | C | % | C | % |
| Acrophilophora fusispora (Saksena Samson | 1 | 0.5 | - | - |
| Alternaria alternata | 8 | 3.7 | 16 | 26.2 |
| Aspergillus flavus | 7 | 4.8 | 6 | 9.8 |
| A. flavus var. columnaris | 1 | 0.5 | 1 | 1.6 |
| A. niger | 50 | 22.9 | - | - |
| A. ochraceus | 1 | 0.5 | - | - |
| A. sydowii | 3 | 1.4 | - | - |
| A. ustus | 1 | 0.5 | 1 | 1.6 |
| A. verzicolor | 5 | 2.3 | - | - |
| Botryotrichum piluliferum Sacc. and March. | 6 | 2.8 | - | - |
| Candida spp. | 3 | 1.4 | - | - |
| Cladosporium cladosporioides | 66 | 30.3 | 8 | 13.1 |
| C. herbarum | 1 | 0.5 | - | - |
| Drechslera spicifera | 1 | 0.5 | - | - |
| Fusarium moniliforme | 2 | 0.9 | 1 | 1.6 |
| F. solani (Mart.) Sacc. | 1 | 0.5 | - | - |
| Gliocladium roseum Bain | 1 | 0.5 | - | - |
| Mucor racemosus | 1 | 0.5 | - | - |
| Paecilomyces variotii | 19 | 8.7 | - | - |
| Penicillium brevicompactam | - | - | 4 | 6.6 |
| P. chrysogemun | 14 | 6.4 | 11 | 28.8 |
| P. citrinum | - | - | 7 | 11.5 |
| P. corylophilum | 2 | 0.9 | - | - |
| P. duclauxii | 1 | 0.5 | - | - |
| P. funiculosum | 2 | 0.9 | - | - |
| Penicillium spp. | 4 | 1.8 | 1 | 1.6 |
| Rhizopus stolonifer | 6 | 2.8 | - | - |
| Scopulariopsis brevicaulis | 1 | 0.5 | 4 | 6.6 |
| Sterile mycelium (dark colour) | 7 | 4.8 | - | - |
| Torula herbarum Pers. ex Gray | 2 | 0.9 | - | - |
| Trichothecium roseum | 1 | 0.5 | - | - |
| Ulocladium alternariae (Cke.) Simmons | - | - | 1 | 1.6 |
| Gross total count | 218 | | 61 | |
| Number of species | 275 + 1 variety | | 10 + 1 variety | |

Table 4: Inhibitory effect (% inhibition comparable with trosyd) of Aloe leaf sap, aqueous garlic bulb extract and onion oil on ten fungal species[a].

| Species tested | % Inhibition | | |
|---|---|---|---|
| | Aloe sap | Garlic extract | Onion oil |
| Trichophyton rubrum | 69.8 | 69.8 | 73.0 |
| Chrysosporium keratinophilum | 33.3 | 81.6 | 89.8 |
| Scopulariopsis brevicaulis | 135.0 | 135.0 | 141.5 |
| Acremonium strictum | 104.5 | 106.0 | 90.9 |
| Alternaria alternata | 33.3 | 46.7 | 133.3 |
| A. niger | 101.3 | 106.0 | 111.4 |
| A. flavus | - | 39.0 | 121.9 |
| A. fumigatus | - | - | 139.5 |
| Paecilomyces variotii | - | 144.3 | 144.3 |
| Penicillium chrysogenum | - | - | 50.0 |

a: None of the aqueous extract of *Andropogon citratus, Euphorbia sp.* and *Ruta graveolens* inhibited growth of the test fungi.

**Antifungal activity:** The results of Table 4 show the *in vitro* antifungal activity of some natural compounds against 10 fungal species. It was noticed that onion oil was the most active one against *Paecilomyces variotii* (144.3% inhibition compared with trosyd). *Scopulariopsis brevicaulis* (141.5%), *Aspergillus fumigatus* (139.5%), *Alternaria alternata* (133.3%), *Aspergillus flavus* (121.9%) and *A. niger* (111.4%). However, *Acremonium strictum* and *P. chrysogenum* were inhibited with onion oil less than trosyd. Also, it was observed that *Aloe sap* and garlic extract showed better antifungal activity against *Acremonium strictum, A. niger* and *Scopulariopsis brevicaulis*. On the other hand, *Paecilomyces variotii* was affected with garlic extract more than trosyd, whereas it was not affected with *Aloe sap*. The above 3 mentioned plants showed less antifungal activity against *Trichopyton rubrum* and *Chrysosporium keratinophilum*.

The remaining aqueous leaf extracts were ineffective against all tested fungi.

## DISCUSSION

In the present study, three different techniques (hair baiting, direct inoculation and dilution plate) and Sabouraud's dextrose agar with or without cycloheximide (Actidione) were employed. This indicate that a careful examination of the hair, cage material, faeces and chow of hamster by the use of different types of tecniques and media gives a better idea about the real fungal content of these samples and allows the isolation of a wide spectrum of fungi. The mycological analysis of hamster hair revealed the isolation of 34 species and 2 varieties which belong to 17 genera of saprophytic fungi. Bagy and Abdel-Mallek (15) isolated 23 genera and 53 species from the hair of small mammals (rabbits, guinea pigs, mice, cats and rats). In this respect, Aho (16) isolated the saprophytic fungi with suspected dermatophytes from the hair of domestic and laboratory animals (dog, cat, horse, cow, guinea pig, parakeet, goat, rat, lesser panda and mink). The commonest isolated genera in order of frequency were *Penicillium, Cladosporium, Aspergillus, Mucor, Aureobasidium, Alternaria, Scopulariopsis and Trichothecium*. He also suggested that the presence of saprophytic fungi on hair and skin creats an opportunity for them under special circumstances to become invasive to the skin or hair and thus cause primary or secondary infection. In the present study, *Paecilomyces variotii* and *Aspergillus niger* were recovered with high frequent

either by using the hair baiting or direct inoculation techniques. They occurred in 84.4% and 81.3% of the examined hair, respectively. Twenty three cycloheximide resistant fungal species were obtained from hamster's hair of which *A. niger* (50% of the samples) was the most common one. *A. flavus, P. chrysogenum* and *C. cladosporioides* were moderately isolated. Whereas *Chrysosporium inops, C. keratinophilum, Paecilomyces variotii, Acremonium strictum* and *Alternaria alternata* were recovered with rare frequent. Moubasher *et al.* (17) isolated 47 species and twenty five genera of non-dermatophyte cycloheximide resistant fungi from patients of skin diseases. Of which *Penicillium* and *Aspergillus* were the most abundant followed by *Scopulariopsis, Alternaria, Thermoascus, Chrysosporium and Cladosporium*. Our results have clearly demonstrated that hamster's hair was free from true dermatophyte. However, Stenwig (18) isolated *Microsporum canis* from hamster but the reported that *M. canis* infection in the hamster should not be unexpected. In a review by Dvorak and Otcenasek (19) *Trichophyton mentagrophytes* was listed as the only dermatophyte isolated from this species. In the same review, more than 30 animals hosts, among them rodents, were considered to be susceptible to *M. canis* infection. In Egypt, most of the preceding genera and species were isolated previously from healthy hair of dog, donkey and cow (20), from camel and goat (21), from small mammals (15), from human axillary (22) and from diseased camels (23).

From metal cage material (wood shavings) of hamster 7 genera, 10 species and 1 variety were recovered on SDA without cycloheximide. *Paecilomyces variotii* (71.5% of total fungi) was the most common fungus followed by *A. niger* (17.2%). Whereas Moharram *et al.* (24) isolated *Paecilomyces variotii* in rare frequency from animal and bird pens. They also reported that *A. niger, A. fumigatus, A. flavus, A. terreus, A. sydowii, S. brevicaulis, P. chrysogenum and P. funi-culosum* were the most common species in animal and bird pens. Also, Hubalek *et al.* (25) isolated *A. flavus, A. fumigatus, A niger, A. sydowii, A versicolor and Penicillium spp.* from birds' nests in the nest boxes.

Five fungal species were recovered only on SDA plus cycloheximide viz: *Chrysosporium keratinophilum* (31.8% of total fungal count), *Geotrichum candidum, P. citrinum, P. jensenii* (4.5%, each) and *F. moniliforme* (2.3%). Hubalek *et al.* (25) isolated, *C. keratinophilum* (22.8%) from birds' nest in boxes. Pugh and Evans (26) reported that *Chrysosporium spp.* collectively represented 32% of the isolates from 59% of the nests. They also reported that *Chrysosporium spp.* were much more common in the nests than in the soils. These fungi were also isolated from animal and bird pens after baiting the samples with sterile camel wool (27) and from chicken and wild sparrow nests (28).

A total of 10 genera, 17 species of saprophytic fungi were isolated from hamster's faeces on SDA without cycloheximide. Paecilomyces variotii was also the most frequent species (67% of total fungal count) followed by Candida (19.7%). Bagy *et al.* (29) isolated 14 genera and 38 species from camel dung on glucose agar, among them *Paecilomyces variotii* was recovered with rare frequent. Seven cycloheximide resistant fungi were isolated viz. *Candida* (63.2%), *Chrysosporium keratinophilum* (6.7%), *Trichophyton rubrum, Fonsecae compactum, Gymnoascus reessii, A. ustus and P. citrinum* (3.4%, each).

It is worth mentioning that the demonstration of *T. rubrum* from hamster's faeces does not seem to have been reported earlier. Currah (30) reported that Arthroderma and Nannizzia contain both saprophytic species found on dung and in soil enriched with keratin, and species that cause ringworm.

The following fungal species were recovered from hamster's chow on SDA: *Paecilomyces variotii,* the most common one (30.7% of total fungal count), A. niger (23%), *A. flavus, A. fumigatus, A. ustus and Cladosporium cladosporioides* (11.6%, each). Ogundero (31) isolated *A. candidus* (16%) of the samples and *A. fumigatus* (50%) from poultry feeds. On the other hand, most of these fungi were isolated from poultry feed stuff ingredients (32).

Five cycloheximide resistant species were the most dominant species in air of hamster's cage and these were *Cladosporium clodosporioides, P. chryso-genum, Alternaria alternata, Aspergillus flavus and Scopulariopsis brevicaulis*. Della Fraco and Caretta (33), isolated *A. flavus, Alternaria alternata, Cladosporium* (6 species), *Penicillium* (3 species) and many others which are resistant to antibiotic from the air at *Pavia*, Italy. In addition to the previous species 19 fungal species were isolated only on SDA without cycloheximide among them *A. niger*, *Paecilomyces variotii* and *Botryotrichum piluliferum*. Bagy (34) in Egypt also recovered these fungi from the air in chicken's pens.

From the preceding results and discussion it can be concluded that *Paecilomyces variotii* was the most dominant fungus species in the air and in the hair, cage, faeces and chow of hamster on SDA without cycloheximide. However, it was completely absent in the presence of cycloheximide. This clearly indicates that this species was more sensitive to the antibiotic cycloheximide.

When the antifungal activity of *Aloe sap*, onion oil, garlic bulb extract and the aqueous leaf extracts of 3 plants was tested; it was noticed that onion oil exhibited a high inhibitory effect (compared with the effect of trosyd) on the in vitro growth of *Paecilomyces variotii* (144.3%), *Scopulariopsis brevicaulis* (141.5%), *Aspergillus fumigatus* (139.5%), *Alternaria alternata* (133.3%), *Aspergillus flavus* (121.9%) and *A. niger* (111.4%). El-Shanawany (35) reported that the mycellial growth of Scopulariopsis brevicaulis was greatly suppressed by 100, 200, 400 ppm of onion oil. However, A. niger was not significantly affected by any level of onion oil.

*Aloe sap* and garlic extract show better antifungal activity against *Acremonium strictum*, *Aspergillus niger* and *Scopulariopsis brevicaulis*. Yashida *et al.* (36) observed that the growth of both *A. niger* and *Candida* albicans was inhibited by ajaene (derived from garlic) at <20 mg/ml. On the other hand El-Shanawany (35) noticed that *Scopulariopsis brevicaulis* was not significantly affected by any concentration (1000, 2000, 4000 ppm) of garlic extract. In the present study, *Paecilomyces variotii* was affected with garlic extract more than trosyd, whereas it was not affected with *Aloe sap*.

The above 3 mentioned plants showed less antifungal activity against *Trichophyton rubrum* and *Chrysosporium keratinophilum*.

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