INFLUENCE OF LICID ON FUNGI OF HUMAN HAIR AND KERATIN DEGRADATION

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SUMMARY: The effect of licid lotion (recommended by professionals for killing and protection against head lice and nits) on the presence of fungi on 96 human hair samples were studied by plating the untreated and licid-treated hair directly on Sabouraud's dextrose agar medium and by using soil plating technique. Licid caused an inhibition in frequency of occurrence of all fungal genera recovered on treated hair samples using soil plating technique, when compared with those of un-treated specimens. However, the frequency of occurrence of each of Aspergillus, Penicillium and Rhizopus was promoted by licid using direct plating technique. The capabilities of selected four fungal species to degrade keratin in licid-treated liquid medium were also tested. The quantities of amino-N and protein-N in culture media (containing 2.6% human hair as a keratinaceous substrate) inoculated with each of Alternaria alternata, Chrysosporium indicum, C. keratinophilum and C. tropicum and supplemented with different concentrations of licid (500, 1000 and 1500 µg ml^{-1}) were increased.

Key Words: Alternaria alternata, Aspergillus, Penicillium funiculosum.

INTRODUCTION

Licid lotion is an insecticide manufactured by Advanced Biochemical Industries-S.A.E., Egypt, and recommended by professionals for killing and protection against head lice and nits. It is used widely among students. For maximum efficiency, it is important to repeat the preparation twice weekly for a month. The possibility that this lotion may affect the occurrence of fungi in human head hair has been recognized. Therefore, the purpose of this investigation is to report the influence of licid on;

(a) the occurrence of fungi on human hair,

(b) the capabilities of four fungal species to degrade keratin.

MATERIALS AND METHODS

Ninety-six human hair samples were collected from barbers in Assiut City, Egypt. These samples were placed in clean plastic bags, transferred immediately to the laboratory and stored in a refrigerator (3-5°C) until examined.

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In order to estimate the effects of licid on the occurrence of fungi, each sample was divided into two parts, one was treated with licid, as mentioned below, and the other remains free from the licid lotion.

Licid Formula

Licid lotion contains: 0.6% N - (hydroxymethyl) - 1 - cycl - ohexene 1,2 - dicarboximide 2,2 - dimethyl - 3 - (2 - methylpropenyl) cyclopropane carboxylate, and 2.4% methylene dioxy - 6 - propyl benzyl - 2 - butyl diethylene glycol ether.

Treatment of hair with licid

Each hair sample was placed in sterilized Petri-dish. The mass of the hair was saturated with licid and left overnight (for about 12 hour).

Two techniques were used for isolation of fungi from human hair:

(a) Direct plating technique

Pieces of each hair sample (about 1 cm long) were plated directly on the surface of Sabouraud's dextrose agar medium (16) supplemented with 40 μg ml^{-1} streptomycin. The plates were incubated at 28 ± 1°C and the growing moulds were examined at weekly intervals for 5 weeks.

(b) The soil plating technique

The bottom of Petri-dish was covered with double sterilized soil (autoclaved at 121°C for 30 minute). Sterilized distilled water was added for moistening the soil and pieces of each sample (about 1 cm long) were placed on the soil surface. The plates were incubated at room temperature for about 3 months, and examined at periodic interval (22). When growth occurred, the moulds were transferred to the surface of Sabouraud's dextrose agar medium supplemented with 40 μg ml^{-1} streptomycin and 0.05% cycloheximide.

Degradation of keratin

The method described by Kunert (11) was mainly used. The experiments were carried out with *Alternaria alternata, Chrysosporium indicum, C. keratinophilum* and *C. tropicum*. These isolates were collected from licid-free human hair samples during the present study. The cultures were maintained on Sabouraud's agar in Petri dishes at 28°C. For inoculation, a suspension of spores was obtained by agitating the surface growth of 10 d old colonies with glass beads in sterile physiological saline and filtering through four layers of guaze. The suspension was diluted so as to have an absorbance of 0.3 at 580 nm when measured on the spectrophotometer in 1 cm cuvettes. The keratinous substrate was human scalp hair, washed in warm water with detergent, warm and cold distilled water and dried in the air. It was cut into pieces of about 1 cm in length and added to the culture medium in a final concentration 2.6 g/L. 50 ml portions of the basal culture medium which contained: glucose, 0.5 g; $MgSo_4.7H_2O$, 0.6; inositol, 0.05 g; thiamine - HCl, 0.01 g and pyridoxine - HCl, 0.01 in 1.000 ml of 28 mM phosphate buffer, pH 7.8 were dispensed into each of 100 ml Erlenmeyer flasks. The flasks were sterilized at 1.5 atmosphere for 20 minute and inoculated with 1 ml of spore suspension of the test fungus. Licid was added to the culture medium to obtain four concentrations: 0, 500, 1000 and 1500 μg ml^{-1}. Four flasks were prepared for each concentration for each fungus, and incubated at 28 ± 1°C for 60 days on shaker (80 r.p.m.). After the incubation period amino-N and protein-N were determined quantitatively according to the method described by (12) and Lowry *et. al.* (13), respectively.

RESULTS

A total of twenty eight species and one variety belonging to 18 genera were isolated from untreated and licid-treated hair samples using two techniques of isolation (Table 1).

Direct plating technique

From the genus *Aspergillus,* thirteen species and one variety of *A. flavus* were collected of which *A niger* was the most prevalent one. The number of cases of isolation of *A. niger* was increased in licid-treated hair samples (isolated from 55 out of 96 samples) than in untreated ones (isolated from 29 samples only). *A. flavus* was recovered in a moderate frequency of occurrence, and emerged in 25 and 29 of untreated and treated samples, respectively. The remaining *Aspergillus* species were rare.

Penicillium funiculosum came in the third position. It occurred in 20 and 21 of untreated and licid-soaked samples, respectively.

Alternaria alternata was isolated from 16 samples of licid-free hair and from 13 samples of treated hair.

Table 1: Number of cases of isolation and occurrence remarks of fungal species isolated from untreated and licid-treated human hair samples using two techniques of isolation.

	Direct plating technique		Soil plating technique	
SPECIES	Untreated Hair NCI and OR	Licid-Treated Hair NCI and OR	Untrated Hair NCI and OR	Licid-Treated Hair NCI and OR
Acremonium strictum W. Gams	1 R	-	1 R	-
Alternaria alternata (Fries) Keissler	16 L	13 L	11 R	3 R
Aspergillus flavus Link	35 M	29 M	36 M	3 R
A. candidus Link	1R	-	-	-
A. flavus var columnaris Raper and Fennell	-	1 R	9 R	5 R
A. flavipes (Bain. and Sart.) Thom and Church	-	2 R	-	-
A. fumigatus Fres.	2 R	-	-	1 R
A. japonicus Saito	-	2 R	-	-
A. niger van Tighem	29 M	55 H	27 M	3 R
A. ochraceus Wilhelm	2 R	3 R	2 R	-
A. parasiticus Speare	-	-	1 R	-
A. sydowii (Bain. and Sart.) Thom and Church	1 R	-	-	-
A. tamarii Kita	-	2 R	1R	-
A. terreus Thom	-	2 R	-	-
A. ustus (Bain.) Thom and Church	-	-	1 R	-
A. versicolor (Vuill.) Trab.	-	-	1 R	-
Candida albicans (Robin) Berkhout	1 R	-	-	-
Chrysosporium indicum (Rand. et Sand.) Gary	-	-	3 R	-
C. keratinophilum (Frey) Carmichael	1 R	-	18 L	3 R
C. queenslandicum Apinis and Ress	-	-	1 R	-
C. tropicum Carmichael	2 R	1 R	22 L	4 R
Cladosporium cladosporioides (Fresh.) de Vries	10 R	9 R	2 R	-
Cunninghamella echinulata (Thaxt.) Thaxt. ex Btak	-	1 R	-	-
C. elegans Leandner	-	-	1 R	-
Curvularia lunata (Wolker) Boed	-	1 R	-	-
C. ovoidae (Hiroe and Watan.) Muntanola	-	-	1 R	-
Drechslera spicifera (Bain.) Von Arx	-	2 R	1 R	-
Emercilla nidulans (Eldam) Vuillemin	2 R	1 R	1 R	-
Emmonsia parva (Emmons and Ashbum) Cif. Montemartini	-	1 R	2 R	-
Microsporum nanum Fuentes	-	-	1 R	-
Mucor hiemalis Wehm.	-	-	2 R	1 R
M. racemosus Fresenius	1 R	-	1 R	-
Penicillium chrysogenum Thom	6 R	5 R	1 R	-
P. corylophilum Dierekx	-	1 R	-	-
P. duclauxi Delacroix	2 R	2 R	1 R	-
P. funiculosum Thom	20 L	21 L	6 R	4 R
P. janczewski Zaleski	2 R	2 R	-	-
P. jenseni Zaleski	5 R	4 R	-	-
P. purpurogenum Stoll	4 R	2 R	-	-
P. stoloniferum Thom	-	-	-	1 R
Penicillium sp.	-	1 N	-	-
Red Yeast	1 R	2 R	-	-
Rhizopus stolonifer (Ehrenb. ex Fr.) Lind	1 R	8 R	3 R	1 R
Scopulariopsis brumptii Salvanet-Duval	1 R	-	-	-
Sporthrix schenckii Hektoen and Perkins	1 R	-	1 R	-
Sterile mycelia (dark)	1 R	-	-	-
Syncephalastrum racemosum (Cohn) Schroater	-	-	1 R	-
Number of isolated species	24	26	28	11

*NCI = number of cases of isolation (out of 96 samples)

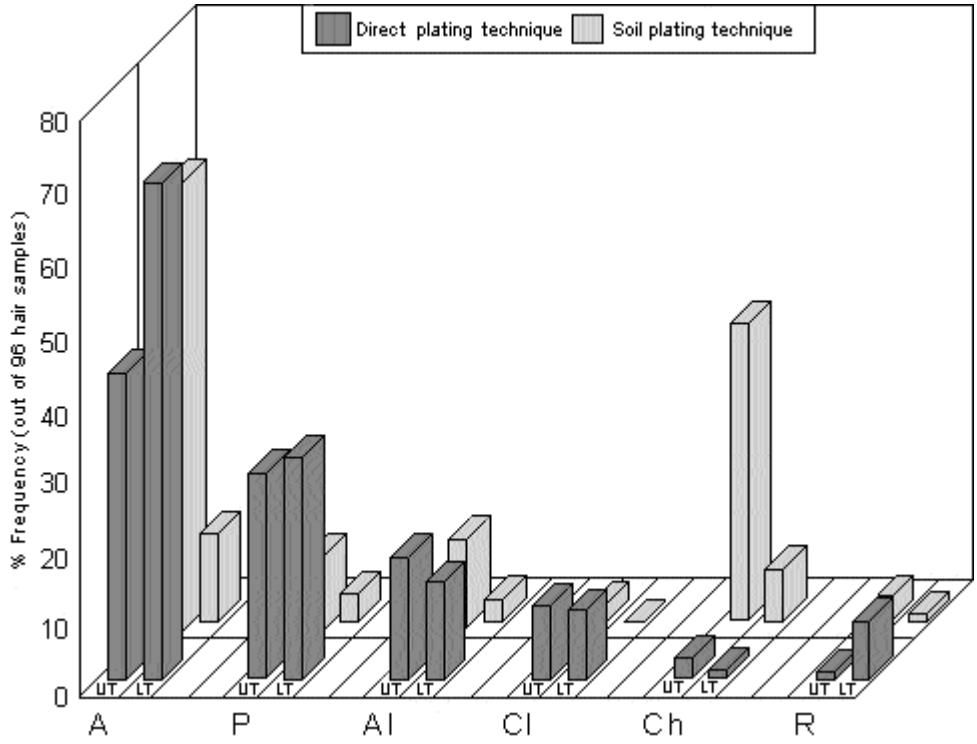
OR = occurrence remarks; H= high occurrence, between 48 - 96 samples

M = moderate occurrence, between 24-47

L = low occurrence, between 12-23 samples

R= rare occurrence less than 12 samples

Figure 1: Frequency of occurrence of the most common fungus genera (A = Aspergillus; P = Penicillium; AI = Alternaria; CI = Cladosporium; Ch = Chrysosporium; R = Rhizopus) in untreated (UT) and Licid treated (LT) hair samples using two techniques of isolation.



The frequency of occurrence of Rhizopus stolonifer was promoted in licid treated samples (isolated from 8 samples) compared with untreated ones (isolated from one sample only).

Soil plating technique

The number of cases of isolation of all fungal species recovered from untreated hair samples was retarded in licid-treated samples (Table 1).

A. flavus and *A. niger* were the most prevalent species. They emerged from 36 (out of 96) and 27 of untreated samples, respectively. In licid-treated samples they encountered from 3 samples only.

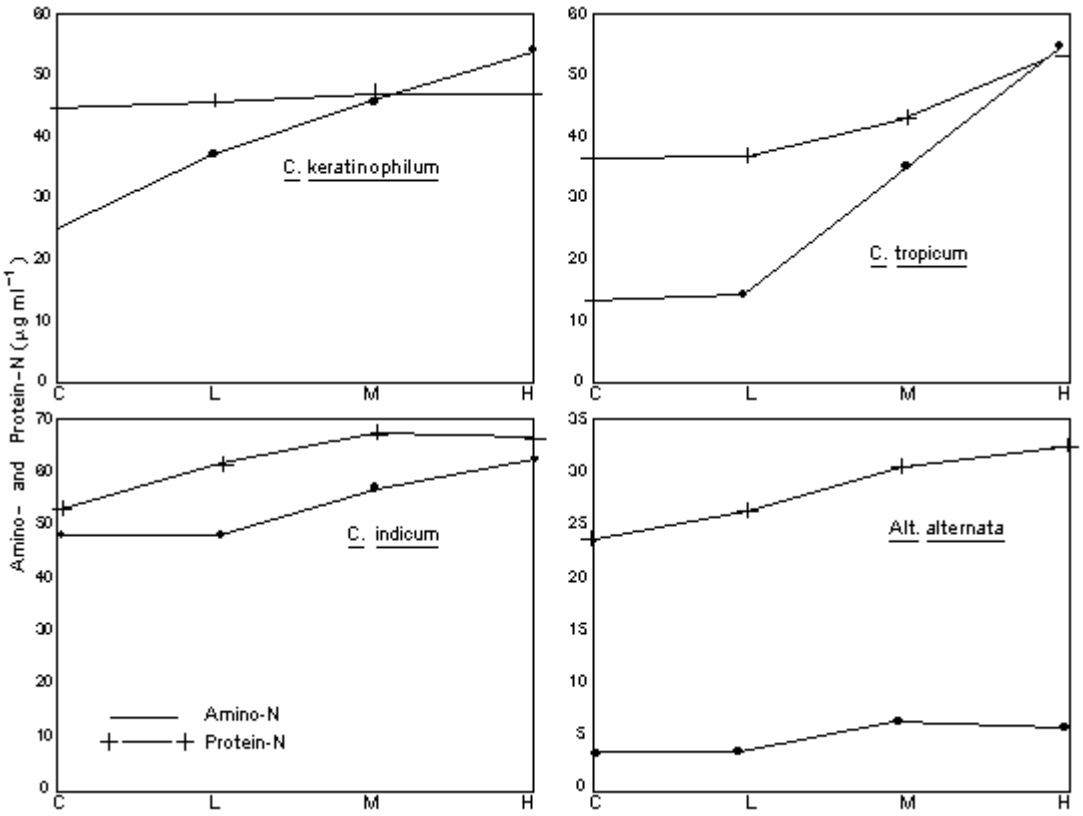
Chrysosporium tropicum came next to *A flavus* and *A. niger*. It emerged from 18 and 3 of untreated and licid-treated samples, respectively.

C. keratinophilum was the fourth and re-covered from 18 of untreated samples, whereas in treated ones it emerged in 3 samples only.

The remaining fungal species listed in Table 1 were isolated in low frequency and recovered from 1-10.4% of the samples tested.

The results in Figure 1 show the frequency of occurrence of the most commonly encountered fungal genera in untreated and licid-treated hair samples using the two mentioned techniques. Using direct plating technique, the most commonly fungal genera recovered from untreated samples were arranged according to their incidence (in relation to the total samples) as follows: *Aspergillus* (42.7%), *Penicillium* (29.2%), *Alternaria* (16.7%), *Cladosporium* (10.4%), *Chrysosporium* (3.1%) and *Rhizopus* (1%). The frequency of occurrence of each of *Aspergillus*, *Penicillium* and *Rhizopus* was promoted

Figure 2: Effect of licid when incorporated into culture medium on keratin degradation by four fungal species.
C= Control; L= 500 μg ml^{-1}; M= 1000 μg ml^{-1}; H= 1500 μg ml^{-1}



in licid-treated samples compared with the control ones, while the other three genera were retarded. Using soil plating technique, the frequency of occurrence of all fungal genera recovered from licid-treated hair samples were retarded. Using soil plating technique, the frequency of occurrence of all fungal genera recovered from licid-treated hair samples were retarded. *Chrysosporium* was found to be the second most frequent genus came behind *Aspergillus* and represented in 41.7% of untreated samples.

The results in Figure 2 show the effect of licid when incorporated into liquid medium using four concentrations (0, 500, 1000 and 1500 μg ml^{-1}) on the degradation of keratin (human scalp hair) by each of *Alternaria alternata, Chrysosporium indicum, C. keratinophilum* and *C. tropicum* and incubated at 28°C ± 1 for 60 days on shaker. The degradation was detected by measuring amount (μg ml^{-1}) of amino-N and protein-N in culture filtrates.

The highest levels of amin-N and protein-N were detected in culture filtrate of *Chrysosporium indicum* supplemented with licid at concentrations of 1500 and 1000 μg ml^{-1}, respectively. On the other hand, the lowest level of amino- and protein - N was recorded in culture filtrate of *Alternaria alternata* in the control treatment (without licid). With all test fungi, the amount of amino - and Protein - N accumulated in larger quantity in licid-treated medium than in medium free from licid.

DISCUSSION

The results obtained from the present investigation show that a total of 28 species and one variety

belonging to 18 genera were isolated and identified from 96 of untreated and licid-treated human hair samples using direct plating technique on Sabouraud's dextrose agar and soil plating technique. All of these species had been isolated previously from human scalp hair (15) and human axillary hair (10), and are already known as colonizers of bait hairs (3,4,7,8).

Both *Aspergillus flavus* and *A. niger* were isolated in moderate frequency of occurrence (26%, 30%) from the untreated samples using the two mentioned techniques. *Penicillium funiculosum* (21%) came next to *A. flavus* and *A. niger* in untreated hair samples using direct plating technique, whereas its frequency (6%) put it in 7th position using soil plating technique. In this respect, Moharram *et al.* (15) isolated *A. flavus* (16%), *A. niger* (14%) and *P. funiculosum* (14%) in low frequency of occurrence from 100 human hair samples examined.

Our results show that *Chrysosporium* was the second most frequent genus using soil plating technique, and represented by four species namely: *C. indicum, C. keratinophilum, C. queenslandicum* and *C. tropicum.* Both *C. keratinophilum* and *C. tropicum* showed low frequency and they emerged from 18 and 22 (out of 96) of untreated hair samples, respectively. The remaining two *Chrysosporium* species in addition to three other dermatophytes namely: *Candida albicans, Emmonsia parva* and *Microsporum nanum* were rare. De Vroey (5) reported that *Chrysosporium* species are occasionally isolated in the clinical laboratory from skin, hair or nails. *C. tropicum* and *C. keratinophilum* were isolated from 21% and 5% of human hair samples examined by Moharram *et al.* (15). Dermatophytes isolated from human axillary hair were represented by five species belonging to *Chrysosporium*, *Trichophyton* and *Candida* (10).

Alternaria alternata was isolated in low frequency of occurrence (17%) using direct plating technique. It's incidence in licid-treated hair samples were retarded. *Alternaria* species was found to colonize hair (9) and able to grow on keratinized substrates (7).

The results of the present investigation indicate that licid caused reduction in number of cases of isolation of all fungal species recovered from treated hair samples using soil plating technique. The degree of inhibition was about 82.5% in *Chrysosporium* spp., 79.6% in *Aspergillus* spp., 72.7% in Alternaria alternata, 66.6% in *Rhizopus stolonifer* and 55.5% in *Penicillium* spp. However, the frequency of occurrence of each of *A. flavus, A. niger* and *R. stolonifer* was promoted in treated samples using direct plating technique. Licid contains 0.6 % of one of synthetic pyrethroid insecticide which has a quickest knock down activity and a strong killing effect on lice and nits, these effects are accelerated by the addition of synergist 2.4% 3,4 methylen dioxy - 6 - propyl benzyl - 2 - butyl diethylene glycol ether which enhances the efficiency of tetramethrin to obtain a prolong insecticidal action, furthermore the preparation is presented in special oil base. The inhibitory effect of licid on frequency of several fungal species might be due to one of its components. Several investigations have been done on the inhibitory effect of fungistations (natural or manufactred ones), fungicides and insecticides on fungal growth. Reports of the American Pharmaceutical Association (2) showed that some agents in over - the - counter antidandruff products possess antibacterial and antifungal activity. Pugh and Agrawal (17) tested the sensitivity of Trichophyton ajelloi to some common agrochemicals, and they reported that verdasan (organomercury compound) was very effective and prevented the growth of the test organism. Moharram *et al.* (15) tested the antifungal activities of 12 types of shampoos and oils which are commonly applied to human hair for treatment of dry skin and dandruff on 42 strains of keratinophilic and thermophilic or thermotolerant fungi isolated from 100 human hair samples. Three out of four types of shampoos proved to be highly

effective against all the test fungi, and *Chrysosporium* isolates were the most sensitive fungi to shampoos. Efficacy of 4 fungicides, i.e. dithane M-45, vegoll-6, difolatan and captan having chemically different active ingredients was tested against the spore germination (19) and the growth (20) of some keratinophilic fungi. He found that the effect of these fungicides was inhibitory to the test fungi and the highest inhibitory effect was caused by dithane M-45 against Nannizzia incurvata (+) strain. Hasan *et al.* (10) found that Bac (sweet deodorant) at 1% concentration inhibited the mycelial dry weight of *A. flavus*, A. fumigatus, *A. niger*, *C. tropicum* and *P. funiculosum* which were isolated from human axillary hair. Also, the inhibitory effect of different plant oils against dermatophytes and other fungi had been investigated (1,6).

The results of the present investigation show that keratinolytic abilities (detected by measuring the quantities of amino-N and protein-N accumulated in liquid medium) of four test fungal species were generally promoted with the different concentrations used of licid. This indicate that licid did not inhibit the production of enzymes responsible for keratin degradation. Proteolytic activity of dermatophytes have been studied by several authors (18,21). Protease synthesis by *F. compactum* was found (14) to be greatly reduced by citral, citronellal, nerol, cinnamaldehyde and caproic acid, while carvone and thymol accelerated protease production by this fungus.

From the preceding results and discussion it can be said that licid is a safe treatment when used as recommended by professionals and applied only to healthy persons. Owing to its stimulatory effect on keratin degradation by test fungi, licid must be contraindicated in individuals who have dermatophytosis.

REFERENCES

1. Abdel-Aal EMM : Mycoflora and mycotoxins of some spices. M Sc Thesis, Bot Dept, Fac of Sci, Assiut Univ. 1990.

2. American Pharmaceutical Association : Handbook of Nonprescription Drugs. American Pharmaceutical Association (The National Professional Society of Pharmacists), Washington (DC) 202:628-4410, 1967.

3. Bagy MMK and AY Abdel-Mallek : Fungi on the hair of small mammals in Egypt. Cryptogamie, Mycol. 12:63-69, 1991.

4. Bagy MMK and AY Abdel-Mallek : Saprophytic and keratinolytic fungi associated with animals hair from Riyadh, Saudi Arabia: Zentralb Mikrobiol, 146:305-310, 1991.

5. De Vroey C : Sur quelques ascomycetes isole's de lesions cutanees chez l'homme. Bull Soc Fr Mycol Med 5:161-162, 1976.

6. El-Gendy Z Kh A : Studies on dermatophytes in Minia governorate. M Sc Thesis, Bot Dept, Fac of Sci, Minia Univ. 1988.

7. English MP : The saprophytic growth of non-keratino-philic fungi on keratinized substrate and a comparison with keratinolytic fungi. Trans Brit Mycol Soc. 48:219-235, 1965.

8. Filipello-Marchisio V : Keratinolytic and keratinophilic fungi of children's sandpits in the city of Turan, Mycopathologia 94:163-172, 1986.

9. Griffin DM : Fungal colonization of sterile hair incontact with soil. Trans Brit Mycol Soc, 43:583-596, 1960.

10. Hasan HAH, MMK Bagy, AY Abdel-Mallek : The incidence of fungi in human axillary hair and their toxigenic potentialities. Cryptogamie, Mycol, 14:297-306, 1993.

11. Kunert J : Biochemical mechanism of keratin degredation by the actinomycete Streptomyces fradiae and the fungus Microsporum gypseum: A comparison, J Basic Microbiol. 29:597-604, 1989.

12. Lee YP, T Takahashi : An improved colorimetric determination of amino acids with the use of ninhydrin. Analytical Biochem. 14, 71-77, 1966.

13. Lowry OH, NJ Rosebrough, AL Farr and RJ Randall : Protein measurement with the Folin-Phenol reagent. J Biol Chem 193, 256-275, 1951.

14. Mahmoud ALE : Some physiological and biochemical studies on fungi isolated from skin of mammals. Ph D Thesis, Bot Dept, Fac of Sci, Assiut Univ, Egypt, 1991.

15. Moharram AM, KM Abdel-Gawad and SS Mohamed El-Maraghy : Ecological and physiological studies on fungi as sociated with human hair. Folia Microbiol. 33:363-371, 1988.

16. Moss ES and AL McQuown : Atlas of Medical Mycology (3rd edition). The Williams and Wilkins Company. Baltimore, 1969.

17. Pugh GJF and SC Agrawal : Sensitivity of Trichophyton ajelloi to some common agrochemicals. Mycopathologia 81, 117-121, 1983.

18. Sanyal AK, SK Das and AB Banerjee : Purification and partial characterization of an extracellular proteinase from T rubrum Sabouraudia, 20:281-288, 1985.

19. Singh BG : Effect of fungicides in relation to spore germination of some keratinophilic fungi. Acta Bot Indica 16:115-116, 1988.

20. Singh BG : Fungicides in relation to the growth of certain keratinophilic fungi. Adv Plant Sci, 3:110-116, 1990.

21. Takiuchi I, D Highuchi, Y Sei and M Koga : Isolation of an extracellular proteinase (Keratinase) from M canis Sabouraudia 20:281-288, 1982.

22. Vanbreuseghem R : Technique biologique pour le isolement des dermatophytes du sol. Ann Soc Belge Med Trop, 32:173, 1952.

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