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Mycological and Physiological Studies on Fungi, Isolated from Skin Diseases

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Abstract: Fifty cases of dermatomycoses were recorded from adult males and females at Qena Gvernorate and these included tinea capitis (62 % of total cases), tinea corporis (20 %), tinea versicolor (12 %) and tinea unguium (6 %). Males are more susceptible to tinea capitis, tinea corporis, tinea versicolor and tinea unguium than females.
Thirty -one species and 2 varieties belonging to 16 genera were collected, of which 14 were recovered from ringworms and dermatophytes and closely related fungi in the examined cases. These were represented by *Aphanoascus fulvescens, A. terreus, Nannizzia fulva, N. obtusa, Trichophyton rubrum* and *T. soudanense*. Also, several saprophytes were found but with different incidence such *as: Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Cochliobolus lunatus, Mycosphaerella tassiana, Penicillium chrysogenum* and *P. citrinum*.
Twenty -one fungal isolates of dermatophytes and other moulds were able to hydrolyze gelatin but with variable capabilities. *Trichophyton rubrum* was the most active protease producer and the maximum production were 8 days after incubation at 30 °C with the incorporation of maltose as a carbon source and peptone as a nitrogen source in Sabouraud's basal medium initially adjusted at pH 6.

Key words: Dermatophytes, diseases and protease production

Introduction

Dermatophytes and other pathogenic fungi have been isolated from keratinized materials of animals, birds and human in many parts of the world (Gugnani *et al.*, 1975; Hubalek & Hornick, 1977; Abdel-Hafez, 1987; Ali-Shtayeh *et al.*, 1988a, b; Nichoils & Midgley, 1989; Lee *et al.*, 1990; Abdel-Hafez *et al.*, 1995; and others).
The diseases caused by dermatophytes have been named according to their anatomical location and these were tinea capitis (tinea means "ringworm", capitis means" head or scalp"), tinea corporis (body), tinea cruris (groin), tinea pedis (foot), tinea versicolor and tinea unguium or onychomycosis (nails) (Ogbonna *et al.*, 1985).
Because protease has an important role in the pathogenicity of a number of microorganisms (Meevootisom & Niederpruem, 1979), the ability of dermatophytes and other moulds to produce this enzyme was investigated by many researchers (Drucker, 1972; Cohen, 1973 ; Takiuchi *et al.*, 1982 ; Sanyal *et al.*, 1985 ; Abdel-Hafez *et al.*, 1995 and other). The optimum conditions for protease activity were studied by others (Mohamed & Turner, 1983 ; Afzal & Chadhary, 1991; Abdel-Hafez *et al.*, 1995).
The present investigation is aimed at study of the prevalence of dermatophytic diseases and associated fungi in adult males and females in Qena Governorates (Egypt) and protease production by dermatophytes and some other moulds.

Materials and Methods

Collection of dermatophytic specimens: Fifty specimens were collected from various locations of patient bodies (adult males and females) during March to July 2000, from dermatology hospital at Qena Governorates . The patients were clinically examined and diagnosed diseases were tinea capitis, tinea corporis (tinea circinata), tinea versicolor and tinea unguium (onychomycosis). Skin scraping was collected using sterile scalpel or glass slides. Each sample was placed in sterile labeled petri dishes (5 cm diameter).

Examination and culturing of dermatophytic specimens:
Direct microscopic examination: Scrapings of the stratum corneum obtained from the suspected area were mounted in 20 % potassium hydroxide on a glass slide and covered with a slip. The preparation was warmed gently and left for 20 min before examination (Rhode & Hartmann, 1980). One drop of lactophenol cotton blue was then added and the specimens were examined using a light microscope. In positive cases the fungal hyphae, arthrospores or budding yeast cells were seen.

Isolation and identification of fungi: When the direct microscopic examination was positive, the dermatophytic specimen was deposited on the surface of Sabouraud's dextrose agar medium. Plates were incubated at 25 °C for 2-3 weeks and the developing fungi were identified by baiting technique.

Proteolytic activity of dermatophytes and other moulds
Screening of fungal isolates for protease production: Twenty-one isolates representing 14 species of dermatophytes and other moulds recovered from tinea capitis (14 isolates), tinea corporis (5) and tinea unguium (onychomycosis) (2) were screened for their ability to produce protease enzymes. Using a sterile cork borer (9 mm. diameter), the inoculum (agar disc bearing spores and mycelium from the agar culture) was obtained. Also, one cavity was made in the center of plate containing solid medium of gelatin peptone agar (pH 6. 8). Three plates were used for each organism. The inoculum of each culture was put in the cavities of gelatin peptone agar medium . Plates were incubated for 8 days at 25 °C.

Plates were flooded with mercuric chloride solution as a reagent ($HgCl_2$, 15g ; conc. HCl, 20 ml. ; dist. water, 100 ml) and the uncoloured zone indicated the production of protease. In case of positive strains, the average diameter of clear zones (in mm.) of the triplicates for each isolate was recorded.

Factors affecting protease production: The effect of different ecological and nutritional factors on protease production by *Trichophyton rubrum* were studied. This isolate was obtained from tinea corporis and found to be the most active dermatophyte to produce protease.

Effect of temperature and time course: Three flasks containing 30 ml Sabouraud liquid medium (pH 6. 8), were incubated at 20, 25, 30 and 40 °C. Three flasks at intervals 2, 4, 6, 8, 10, 12 and 14 days were removed and the mycelia were harvested by filtration in Whatmann filter paper (10 cm). Filtrates from triplicate samples were combined and the resulting solution was assayed for protease activity.

Effect of pH values: The test organism was cultured in the

same medium which was initially adjusted to different pH values ranging from 2 to 12. Adjustment of pH was made by 0. 1 N HCl or NaOH. After incubation at 30 °C for 8 days (proper temperature and incubation period for protease production), cultures were filtered and the clear filtrates were tested for enzyme activity.

Effect of different carbon sources: The culture medium was supplemented with 1 % each of the following carbon sources: cellulose, glucose, maltose, sucrose, wheat bran and wheat straw. Cultures were incubated at 30 °C for 8 days followed by filtration and the enzyme was assayed in filtrates.

Effect of various nitrogen sources: $NaNO_3$, KNO_3, NH_4NO_3, casein, gelatin, peptone, urea and yeast extract were used as sole nitrogen sources in the culture medium. Cultures containing peptone served as control. After incubation at 30 °C for 8 days cultures were filtered and the clear filtrate was assayed for crud enzyme.

Assay for protease activity: Protease activity was determined according to the method of Rick (1963). This method is based on hydrolytic action. The release of soluble tyrosine was estimated by Folin reagent. The activity was calculated from the difference between these control and experimental titration value. Enzyme activity was expressed by units of L-tyrosine liberated during the reaction. The units were determined by a standard curve early constructed using different concentrations of L - tyrosine.

Results and Discussion

Tinea capitis: Tinea capitis was the most common disease and was emerged in 62 % of total cases (Table 1). This is almost in agreement with the results recorded in many parts of the world (Chadegani *et al.*, 1987; Ekanem & Gugnani, 1987; El-Gendy, 1988 ; Zohdi *et al.*, 1988 ; Mahmoud, 1991; El-Shanawany, 1993 and Abdel-Hafez *et al.*, 1995). Males were more affected by tinea capitis (64. 5 % of total cases) than females (35. 5) (Table 1). This result is in harmony with the finding of Zaini & Chagari (1989). *Nannizzia* (anamorph: *Microsporum*) and *Trichophyton* were the two main dermatophytes found in tinea capitis, comprising 29. 03 % and 22. 58 % of cases, respectively. Of the above two genera the most prevalent species were *Nannizza obtusa* (anamorph :*Microsporum nanum*, 22. 58 % of cases) and *Trichophyton soudanense* (16. 13%). *Nannizzia fulva* (anamorph: *Microsporum gypseum*) and *Trichophyton rubrum* were less common (6. 45 % and 6. 45%, respectively) (Table 2). These dermatophytes were isolated previously from cases of tinea capitis in Egypt (El-Gendy, 1988 ; Mahmoud, 1991 ; El-Shanawany, 1993 and Abdel-Hafez *et al.*, 1995), as well as in some parts of the world (Al-Sogair *et al.*, 1989; El-Benhawi *et al.*, 1991). Several saprophytes were associated with cases of tinea capitis and these were *Aphanoascus fulvescens, A. terreus, Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Cochliobolus luntus, Mycosphaerella tassiana, Penicillium chrysogenum* and *P. funiculosum* (Table 2) .

Tinea corporis: Tinea corporis (tinea circinata) ranked second in incidence and distribution among patients of dermatomycoses and was recovered from 20 % of total cases (Table 1). This is almost in harmony with the results of Chadegani *et al.* (1987), Ekanem & Gugnani (1987), El-Gendy (1988) and Abdel-Hafez *et al.*, (1995). Results revealed that males (70 % of total cases) are more susceptible to this disease than females (30 %). This is almost in accord with the results obtained from patients in Egypt (El-Shanawany, 1993 and Abdel-Hafez *et al.*, 1995), as well as in cases from Libya (Elghoul *et al.*, 1989). *Nannizza obtusa* and *Trichophyton rubrum* were the main causative species of tinea corporis and were recovered from 50 % and 20% cases, respectively (Table 2). The above dermatophytes were encountered previously in several cases of tinea corporis from patients in some Egyptian Governorates (Zohdi *et al.*, 1988 ; Mahmoud, 1991; El-Shanawany, 1993 and Abdel-Hafez *et al.*, 1995) as well as from cases in many parts of the world (Shekelakov *et al.*, 1984; El Ghoul *et al.*, 1989; Katoh *et al.*, 1991 and others) . *Aspergillus flavus, A. fumigatus* and *Mycosphaerella tassiana* were rarely recovered in cases of tinea corporis. Abdel-Hafez *et al.* (1995) found that *Aspergillus flavus* and *A. fumigatus* were with low incidences in cases of tinea corporis.

Tinea versicolor: Tinea versicolor occurred in 6 cases out of 50 (12 %) (Table 1). This is almost in agreement with the results obtained previously (El-Gendy, 1988; Imwidthaya *et al.*, 1989; Mahmoud, 1991 ; El-Shanawany, 1993 and Abdel-Hafez *et al.*, 1995). Males were more susceptible than females (83. 3 % and 16. 7% of cases, respectively). Almost similar findings were recorded in patients by (El-Shanawany, 1993 and Abdel-Hafez *et al.*, 1995). *Malassezia furfur* the causative fungus of tinea versicolor, was sufficient to be detected by direct microscopic examination and was recovered in about 66. 7 % cases of tinea versicolor (Table 1) .

Tinea unguium (onychomycosis): Three cases of tinea unguium were found (6% of total cases) (Table 1). Abdel-Hafez *et al.* (1995) found that this type of infection of the skin was the least common (4. 3% of total cases). In Egypt, onychomycosis was infrequently among patients of dermatophytic disease El-Gendy, 1988 ; Mahmoud, 1991; El-Shanawany, 1993 and Abdel-Hafez *et al.*, 1995). One dermatophytic fungus (*Trichophyton soudanense*) was recovered from 33. 3 % of total cases of tinea unguium. *Cochliobolus lunatus* emerged in 66. 7 % cases of tinea unguium. Some cases of onychomycosis were caused by a well known saprophytes such as members of *Alternaria, Aspergillus, Fusarium, Penicillium, Scopulariopsis* and some unidentified dermatiaceous species (Velez & Diaz, 1985 and Wadhwani & Srvastaval, 1985). On the other hand, several of the above fungi were frequently recovered from skin (Abdel-Hafez *et al.*, 1995).

Proteolytic activity of dermatophytes and some other moulds: Dermatophytes and some other moulds recovered from cases of ringworms were screened for their ability to produce protease enzyme. Most of tested fungi had the ability to produce protease enzyme, but with different degrees ranging from high to weak (Table 3). High proteolytic activity was exhibited by *Aspergillus flavus* and *Trichophyton rubrum*. Isolates of *Aspergillus niger, Nannizzia fulva, N. obtusa, Trichophyton soudanense* and *Penicillium chrysogenum* showed moderate proteolytic activity. The remaining isolates were of weak or non-proteolytic activity. Abdel-Hafez *et al.* (1995) found that some isolates of *Aspergillus, Nannizzia, Penicillium, Scopulariopsis* and *Trichophyton* could be realized as good producers of protease.

Trichophyton rubrum was chosen to study the effects of different ecological and nutritional factors on protease production, since it was the most active dermatophytic producer (Table 3). The maximum protease production by *T. rubrum* was recorded after 8 days of incubation at 30 °C (Fig. 1). Abdel-Hafez *et al.* (1995) found maximum protease production by *Trichophyton soudanense* after 8 days of incubation at 30 °C.

Table 1: Distribution of dermatophytic diseases according to sex, number and percentage of positive cases shown by direct microscopic examination.

Clinical diagnosis	Number of cases	% of total cases	Sex				**	
			Male		Female		+ ve cases	% of +ve cases
			No.	%	No.	%		
Tinea capitis	31	62	20	64. 5	11	35. 5	28	90. 3
Tinea corporis	10	20	7	70	3	30	7	70
Tinea versicolor	6	12	5	83. 3	1	16. 7	4	66. 7
Tinea unguium	3	6	2	66. 7	1	33. 3	3	100
Total number of cases	50	100	34	68	16	32	42	84

** Direct microscopic examination

Table 2 :Incidence (I) and percentage incidence (I) of dermatophytes, related fungi and other fungi associated with the different mycotic diseases

Clinical diagnosis	Tinea capitis		Tinea corporis		Tinea unguium		Total cases	
Genera and species	I	%	I	%	I	%	I	%
Dermatophytes and related fungi								
Aphanoascus	3	9. 7					3	6. 82
A. fulvescens (Cook) Apinis	1	3. 2					1	2. 27
A. terreus (Randhawa & Sandhu) Apinis	2	6. 5					2	4. 55
Nannizzia	9	29. 03	5	50			14	31. 82
N. fulva Stockdale	2	6. 45					2	4. 55
N. obtusa Dawson &Gentles	7	22. 58	5	50			12	27. 27
Trichophyton	7	22. 58	2	20	1	33. 3	10	22. 73
T. rubrum (Castellani) Sabouraud	2	6. 45	2	20	-		4	9. 09
T. soudanense Joyeux	5	16. 13		1	33. 3	6	13. 64	
Other fungi								
Aspergillus	8	25. 81	2	20			10	22. 72
A. flavus Link	4	12. 90	1	10			5	11. 36
A. fumigatus Fresenius	2	6. 45	1	10			3	6. 82
A. niger Van Tieghem	1	3. 23				1	2. 27	
A. terreus Thom	1	3. 23				1	2. 27	
Cochliobolus lunatus Nelson & Haasis	1	3. 23			2	66. 7	3	6. 82
Mycosphaerella tassiana(de Not.) Johanson	1	3. 23	1	10			2	4. 55
Penicillium	2	6. 44					2	4. 54
P. chrysogenum Thom	1	3. 22					1	2. 27
P. funiculosum Thom	1	3. 22					1	2. 27
Total isolates	31	100	10	100	3	100	44	100
Number of genera	7	-	4	-	2	-	7	-
Number of species	14	-	5	-	2	-	14	-

Table 3: Proteolytic activities (diameter (mm). of clear zones hydrolyzed) and degree of proteolysis* of fungal isolates recovered from ringworm.

Organisms	Tinea capitis	Tinea corporis	Tinea unguium
I-Dermatophytes and related fungi			
Aphanoascus			
A. fulvescens (Cook) Apinis	19 W	-	-
A. terreus (Randhawa&Sandhu) Apinis	24 W	-	-
Nannizzia			
N. fulva Stockdale	35 M	-	-
N. obtusa Dawson &Gentles	32 M	37 M	-
Trichophyton			
T. rubrum (Castellani) Sabouraud	-	50 H	-
T. soudanense Joyeux	-	54 M	19 W
II-Other fungi			
Aspergillus			
A. flavus Link	58 H	27 W	-
A. fumigatus Fresenius	22 W	18 W	-
A. niger Van Tieghem	33 M	-	-
A. terreus Thom	28 W	-	-
Cochliobolus lunatus Nelson & Haasis	-ve	-ve	-
Mycosphaerella tassiana(de Not.) Johanson	-ve	-ve	-
Penicillium			
P. chrysogenum Thom	44 M	-	-
P. funiculosum Thom	-	-	20 W

Figures between parentheses refer to the number of isolates.
*Degree of proteolysis: H=high proteolysis, 50-70; M=moderate proteolysis, 30-49; W=weak proteolysis, less than 30 mm. ; -ve = nonproteolytic isolates.

The best pH for protease production by *T. rubrum* was recorded within a pH 6 (Fig. 1). Groninger & Eklund (1966), Lenney & Dalbec (1967) and Olutiola & Nwaogwugwu (1982) found that protease from *Trichosporon* sp., *Saccharomyces cerevisiae* and *Aspergillus aculeatus* were highly active at pH 7. Also, Abdel-Hafez *et al.* (1995) found that the maximum protease production by *Trichophyton soudanense* within a pH range of 6-8.

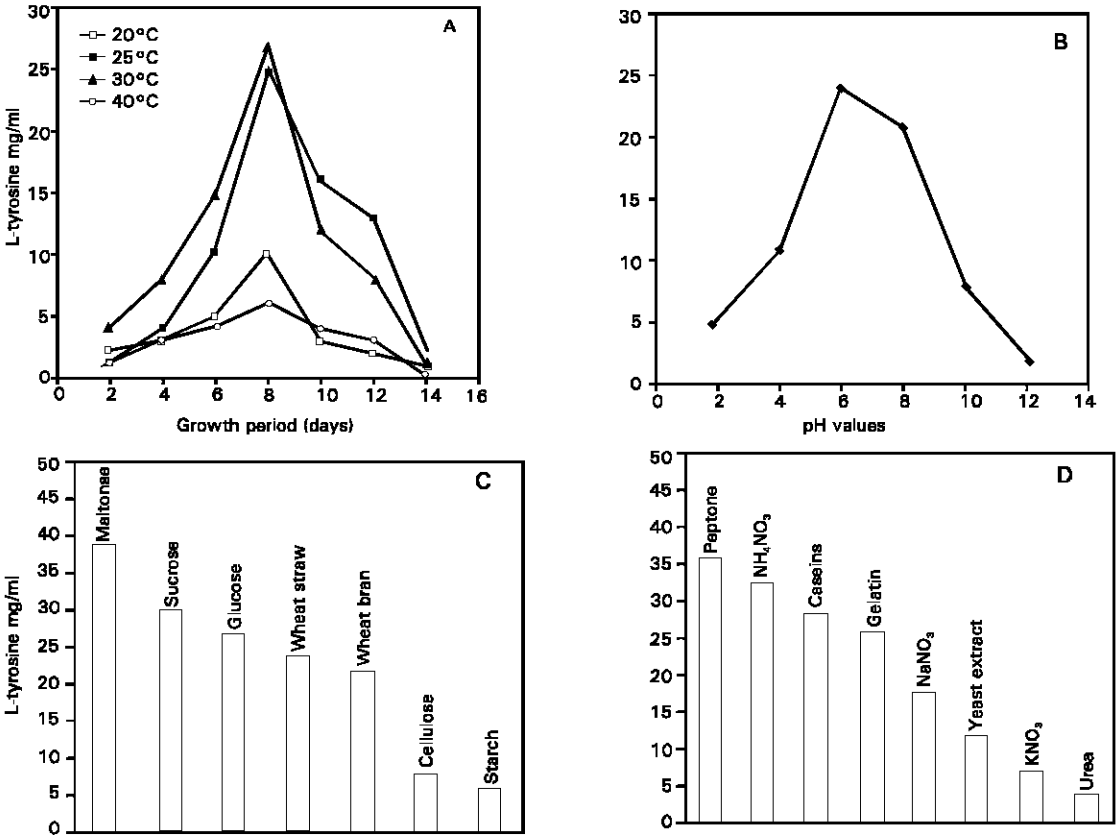

Fig. 1: Effect of time course and temperature (A), pH values (B), different carbons © and nitrogen sources (D) on production of protease by *Trichophyton rubrum*.

Maltose was the best carbon source for proteolytic activity of *T. rubrum* (Fig. 1). Maximum protease production was detected with the incorporation of sucrose as carbon source in the growth medium of *Aspergillus aculeatus* (Olutiola & Nwaogwugwu, 1982) *Aspergillus niger* (Chopra & Mehta, 1985), *Penicillium chrysogenum* (Mahmoud, 1988). Also, Abdel-Hafez *et al.* (1995) found that maltose followed by sucrose were the best carbon sources for proteolytic activity by *Trichophyton soudanense*.

Peptone followed by ammonium nitrate and casein were the best nitrogen sources for protease production by *T. rubrum* (Fig. 1). Abdel-Hafez *et al.* (1995) found that casein followed by gelatin and ammonium nitrate were the best nitrogen sources for protease production by *Trichophyton soudanense*.

In conclusion, Dermatomycoses were recorded from adult males and females in this investigation were tinea capitis, tinea corporis, tinea versicolor and tinea unguium. Males are more susceptible to tinea than females. Fungal isolates of dermatophytes and other moulds were able to produce protease enzyme but with variable capabilities. So, it is important to take in consideration the various methods for protection from diseases which caused by dermatophytes.

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References

Abdel-Hafez, A. I. I., 1987. Survey on the mycoflora of goat and sheep hairs from Gaza Strip. Bull. Fac. Sci. Assiut Univ., 16: 16-21

Abdel-Hafez, S.I.I., A. H. M. El-Said and T. A. Maghraby, 1995. Studies on fungi isolated from skin diseases and associated fungi of students in Qena and Red Sea Governorates, Egypt. Bull. Fac. Sci., Assiut Univ., 24:181-209.

Afzal, M. M. and M.I. Cgaudhary, 1991. Effect of carbon sources on the production of alkaline protease by *Aspergillus oryzae*. Pak J. Sci. Ind Res., 34: 91-94.

Ali - Shtayeh, M. S., H. M. Arda, M. Hassouna and S. F. Shaheen, 1988a. Keratinophilic fungi on the hair of goats from the West Bank of Jordan. Mycopathologia, 104: 103-108.

Ali - Shtayeh, M. S., H. M. Arda, M. Hassouna and S. F. Shaheen, 1988b. Keratinophilic fungi on the hair of cows, donkeys, rabbits, cats and dogs from the West Bank of Jordan. Mycopathologia, 104: 109-121

Al-Sogair, S. M., Y. M. Al-Humaidan and M. K. Moawad, 1989. Scalp fungus infection in the eastern province of Saudi Arabia. Annals of Saudi Medicine, 9: 259-262.

Chadegani, M., A. Moment, S. Shadzi and M. A. Javaheri, 1987. A study of dermatophytoses in Esfahan (Iran). Mycopathologia, 98: 101-104.

Chopra, S. and P. Mehta, 1985. Influence of various nitrogen and carbon sources on the production of pectolytic, cellulytic and proteolytic enzymes by *Aspergillus niger*. Folia Microbiol., 30: 117-125.

Cohen, B. L., 1973. Regulation of intracellular and extra cellular neutral and acid protease in *Aspergillus nidulans*. J. Gen. Microbiol., 79: 311-320.

Drucker, H., 1972. Regulation of exocellular protease in *Neurospora crassa*: Induction and repression of enzyme synthesis. J. Bacteriol., 110: 1041-1049.

Ekanem, L. S. and H. C. Gugnani, 1987. Etiology of dermatophytoses among school children in Cross River State of Nigeria. Mykosen 30: 493-498.

El-Benhawi, M. O., S. Fathy, A. H. Moubasher and N. S. Alem, 1991. Mycologic study of tinea capitis in Qater. Int. J. Dermatol., 30: 204-205.

El-Gendy, Z. K. A., 1988. Studies on dermatophytes in Minia Governorate, M. Sc. Thesis. Bot. Dept. Fac. Sci. Minia Univ., Egypt.

Elghoul, M. T., M. Safi, R. M. Joshi and H. Mashina, 1989. Frequency of dermatophytes causing tinea corporis in Tripoli, Libya. Trans. royal Soc. Trop. Med. and Hygien 83: 418.

El-Shanawany, A. A., 1993. Human dermatophytes in Assiut and New Valley Governorates, Ph . D . Thesis, Bot Dept., Fac. Sci., Assiut Univ., Egypt. (pp. 151).

Groninger, H. S. and M. W. Eklund, 1966. Characteristics of a proteinase of a Trichosporon species isolated from Dungeness crab meat. Appl. Microbiol., 14 :110-114.

Gugnani, H. C., B. L. Wattal and R. S. Sandhu, 1975. Dermatophytes and other keratinophilic fungi recovered from small mammals in India. Mykosen, 18: 529-538.

Hubalek, Z. and M. Hornick, 1977. Experimental infection of white mouse with *Chrysosporium* and *Paecilomyces*. Mycopathol. Mycol. Appl., 62: 173-178.

Imwidthaya, S., M. Tianprasit and S. Srimuang, 1989. A study of pityriasis versicolor in Bangkok (Thailand). Mycopathologia, 105: 157-161.

Katoh, T., K. Nishioka and T. Sano, 1991. Isolation of *Microsporum canis* from clinically normal skin. Japanese J. Med. Mycol., 32: 127-131.

Lee, M. M., D. G. Diven, E. B. Smith and E. B. Pupo, 1990. Onychomycosis . Archives of Dermatology, 126: 402.

Lenney, J. R. and J. M. Dabec, 1967. Purification and properties of two proteinase from *Saccharomyces cerevisiae*. Archives of Biochem. Biophys., 120: 42-48.

Mahmoud, A. L. E., 1988. Some physiological studies on fungi isolated from Poultry feedstuffs. M. Sc. Thesis, Bot. Dept. Fac. Sci., Assiut Univ., Egypt (pp: 161).

Mahmoud, A. L. E., 1991. Some physiological and biochemical studies of fungi isolated from skin of mammals. Ph. D. Thesis. Bot Dept. Fac. Sci., Assiut Univ., Egypt. (pp. 173).

Meevootisom, V. and D. J. Niederpruem, 1979. Control of exocellular protease in dermatophytes and especially in *Trichophyton rubrum*. Sabouraudia, 17: 91-106.

Mohamed, A. K. A. and A. G. Turner, 1983. Proteolytic activity of *Nomuraea rileyi* and host insect cuticle. Mycopathologia 82:13-15.

Nichoils, D. S. H. and G. Midgley, 1989. Onychomycosis caused by *Trichophyton equinum*. Clinic. Exp. Dermatol., 14: 464-465.

Ogbonna, C. I. C., R. O. Robinson and J. M. Abubakar, 1985. The distribution of ringworm infections among primary school children in Jos, Plateau State of Nigeria. Mycopathol., 89:101-106.

Olutiola, P. O. and R. I. Nwaogwu, 1982 . Growth, Sporulation and production of maltase and proteolytic enzymes in *Aspergillus aculeatus*. Trans. Br. Mycol. Soc., 78 :105-113.

Rhode, B. and G. Hartmann, 1980. Introducing mycology by examples. Hamburg, presented by Schering Aktienges-Sellschaft.

Rick, W., 1963. Methods of enzymatic analysis. Academic Press Inc., New York. Trypsin P. 807-818. In H. U. Bergmeyer (ed.).

Sanyal, A. K., S. K. Das and A. B. Banerjee, 1985. Purification and partial characterization of an extracellular protease from *T. rubrum*. Sabouraudia, 20: 281-288.

Shekelakov, N. D., Z. V. Stepanova, I. Y. Klimova and N. A. Mosaleva, 1984. Role of humans as the source of infection in Zooanthropanotic, microsriosis caused by *M. canis*. Vestnik Dermatologii. I. Venerologii, 12: 29-30.

Takiuchi, I., D. Higuchi, Y. Sei and M. Koga, 1982. Isolation of an extracellular proteinase, Kerationase from *M. canis*. Sabouraudia, 20: 281-288.

Velez, H. and F. Diaz, 1985. Onychomycosis due to saprophytic fungi. Mycopathol., 91:87-92.

Wadhwani, K. and A. K. Srivastava, 1985. Some cases of onychomycosis from North India indifferent working environments. Mycopathol., 92: 149-155.

Zaini, F. and A. Ghagari, 1989. Epidemiological and mycological studies on tinea capitis at nurseries and schools of Bander Chabahar. Iranian Pub. Health, 18 :39.

Zohdi, H. A. A., Y.A. Youssef, M. M. A. Abdel-Moneim, M. S. Farghaly, F.M. Emam and M.A. Abdallah, 1988. Study of dermatophytes and dermatophytosis. Egypt. J. Derm. Ven., 8: 41-50.