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ENZYMATIC AND ANTIBIOTIC ACTIVITIES OF MICROSPORUM GYPSEUM, CHRYSOSPORIUM KERATINOPHILUM AND CHRYSOSPORIUM INDICUM.

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SUMMARY : 60 *Microsporum gypseum*, 50 *Chrysosporium keratinophilum* and 15 *Chrysosporium indicum* were tested for their ability to hydrolyse urea, starch and fat, to liquefy gelatin, to haemolyse red blood cells and to produce antibiotics. All isolates could hydrolyse urea but not starch or fat. 60 % of the isolates liquefied gelatin. The haemolytic activity varied from 33.3 % to 60 %. The mycelia of the 3 species produced antibiotic substances that inhibited the growth of *Corynebacterium ovis*, *Staphylococcus aureus* and *Escherichia coli* but not *Pseudomonas aeruginosa*.

INTRODUCTION

The study of the biological activities of fungi may be helpful in the identification and may disclose the nature of pathogenicity of such microorganisms. This is of particular interest in case of geophilic dermatophytes, which are not well - studied. *Microsporum gypseum*, *Chrysosporium keratinophilum* and *Chrysosporium indicum* were found to be the most common geophilic fungi in Egypt (Refai et al, 1984). In the present work, 125 isolates of these 3 species were therefore studied for their biological activities.

MATERIAL AND METHODS

60 *Microsporum gypseum*, 50 *Chrysosporium keratinophilum* and 15 *Chrysosporium indicum* were examined for their ability to hydrolyse urea, starch and fat, to liquefy gelatin, to haemolyse red blood cells and to produce antibiotics.

All isolates were inoculated on urea, starch, lipase and gelatin media (Larone, 1976, Hellgren and Vincent, 1980). For

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the detection of haemolysin the isolates were cultured on Sabouraud dextrose agar enriched with5 % defibrinated sheep blood. Ten siolates of each species were tested for antibiotic production. The isolates were grown in Sabouraud dextrose browth for 3 weeks. The mycelial mat of the wellgrown fungi was removed by a sterile forceps and placed on the surface of blood agar inoculated with *Corynebacterium ovis* ,*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* . Moreover, sterile filter paper discs (Whatmann no. I) were soaked into the culture filtrate and placed likewise on the surface of blood agar inoculated with the same bacteria.

RESULTS

Urea hydrolysis

From **Table 1** it is clear that all isolates of the 3 species developed the enzyme urease, but the rate and amount of the enzyme varied from one species to another. There were also variations among the isolates of the same species with regard to the time needed for giving positive reaction.

It is clear that *Chr.keratinophilum* was the most active in hydrolysis of urea as 90 % of the isolates gave positive rection within 10 days, followed by *chr. indicum* (86.6 %), while *M.gypseum* was the slowest as more than one half of the isolates gave positive reaction after 15 days.

Gelatin liquefaction

Only 60 % of the isolates liquefied gelatin **(Table 2).** The most active was *Chr.. indicum* , where all 15 tested isolates gave positive test. On the other hand, *M . gypseum* was the least active, as 50 % of the isolates were weak positive and 41.7 % were negative.

Starch and fat hydrolysis

All isolates were unable to hydrolyse starch or fat.

Table 1: Urea hydrolysis

Species	No. examined	No.of positive isolates Time needed for hydrolysis in days		
		5	10	15
M. gypseum	60	12 (20 %)	13 (21.7 %)	35 (58.3 %)
Chr.keratinophilum	50	21 (42 %)	24 (48 %)	5 (10 %)
Chr. indicum	15	5 (33.3%)	8 (53.3 %)	2 (13.3 %)
Total	125	38 (38.4%)	45 (36 %)	42 (33.6 %)

Chr. = Chrysosporium

Table 2: Gelatin liquefaction

Species	No.of examined isolates	Degree of reaction							
		+++		++		+		-	
		No.	%	No.	%	No.	%	No.	%
M.gypseum	60	-	-	5	8.3	30	50	25	41.7
Chr.keratinophilum	50	-	-	17	34	18	36	15	30
Chr.indicum	15	10	66.65	33.3	-	-	-	-	-

+++ = Complete liquefaction, ++= moderate liquefaction,

+ = slight liquefaction, - = no liquefaction

Enzymatic and antibiotic activities of M.gypseum Chr.keratinophilum and Chr.indicum.

Table 3: Haemolysis of sheep red blood cells.

Species	No.of examined isolates	Positive haemolytic isolates	
		No.	%
M. gypseum	60	24	40.0
Chr. keratinophilum	50	30	60.0
Chr. indicum	15	5	33.3
Total	125	59	48.5

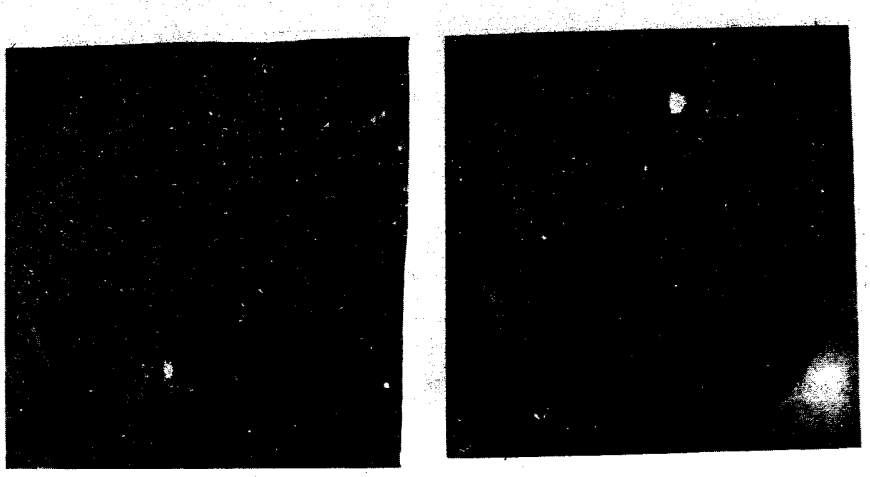

Fig.1 : Haemolysis of sheep red blood cells by *M. gypseum* (left) and *Chr. keratinophilum* (right).

Haemolysis of sheep red blood cells

Chr. keratinophilum was the most active as 60 % of its isolates produced clear zones of haemolysis around the colonies. The percentage of haemolytic isolates was 40 % in case of *M. gypseum* and 33.3 % in case of *Chr. indicum* . The zones of haemolysis were particularly clear in case of *M. gypseum* and *Chr. . keratinophilum* (Fig. 1 and Table 3).

Antibiotic production

The culture filtrate of *M . gypseum* (3 out of 10 isolates) inhibited the growth of *C . ovis* only. The culture filtrate of *Chr . keratinophium* (8 isolates) inhibited the growth of *C. ovis* and *Staph .aureus* .

The culture filtrate of *Chr. indicum* (5 isolates) inhibited the growth of *E.coli* only (Table 4 and Fig.2 and 3).

The mycelial mat of all species was more effective. *M. gypseum* inhibited markedly *C . ovis* while *Staph . aureus* and *E.coli* were slightly inhibited. *Chr.kerntinophilum* and *Chr. indicum* inhibited the growth of the three aforementioned bacteria (Table 5). None of the tested isolates could inhibit *P.aeruginosa* . Fig.4 shows inhibition of *C . ovis* and *S. aureus* by *Chr . keratinophilum* .

DISCUSSION

The results obtained in this study with regard to urea hydrolysis is in complete agreement with that reported by Abd El-Hamid (1982), as all isolates were positive. In case of starch and fat hydrolysis there is a complete disagreement with the result of this author as none of our isolate showed positive reaction, whereas all isolates of dermatophytes tested by Abd El-Hamid (1982) were positive in starch and 6 out of the 23 isolates had lipolytic effect. The same controversy was observed with regard to gelatin liquefaction.

The 3 species tested in this work haemolysed sheep red blood cells to a varying extent. Gip and Palsson (1970) reported the haemolytic activity of *M . gypseum* . Refai and

Enzymatic and antibiotic activities of M.gypseum, Chr.keratinophilum and Chr.indicum.

Table 4: Antibiotic production in Sabouraud dextrose broth

Species	Bacteria tested			
	C. ovis	*S.aureus*	*E.coli*	*P.aeruginosa*
M. gypseum	++	-	-	-
Chr. keratinophilum	+++	++	-	-
Chr. indicum	-	-	++	-

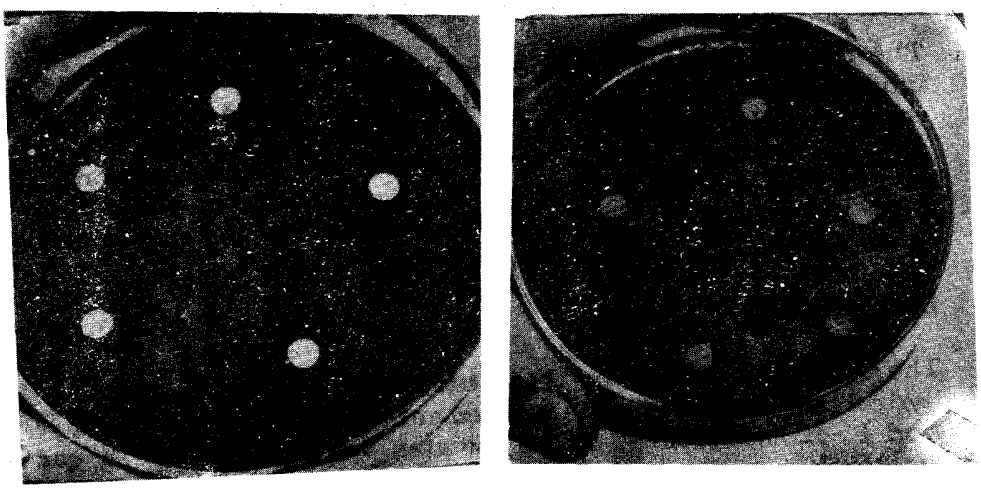

Fig.2:Inhibition of *C. ovis* (left) and *Staph aureus* (right by culture filtrate of *Chr. keratinophilum*.

Table 5: Antibiotic production by mycelia of *M. gypseum* and *Chrysosporium* species.

Species			Bacteria tested	
M. gypseum	+++	+	+	-
Chr. keratinophilum	+++	+	++	-
Chr. indicum	+++	+	++	-

Fig. 3: Inhibition of *E. coli* by culture filtrate of *Chr. indicum*.

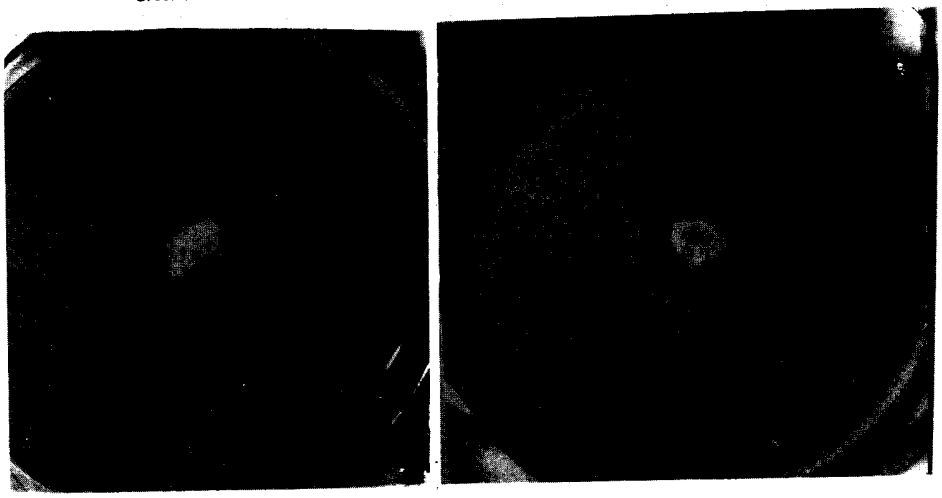

Fig.4: Inhibition of *C. ovis* (left) and *Staph. aureus* (right) by mycelium of *Chr. keratinophilum*.

Soliman (1976) found that *T. verrucosum* and *T. violaceum* but not *T.schoenleinii* could haemolyse red blood cells of guinea pigs, cows, buffaloes, horses and sheep.

Of interest was the finding that the three species produced antibacterial antibiotics. The fact that the tested mycelial mat of all isolates inhibited the growth of *C. ovis*, *Staph. aureus* and *E. coli* indicates that the antibiotics are broadspectrum. The results of *C. ovis* should attract our attention as it seems that this organism is highly sensitive to the metabolites of the three species of fungi.

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