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EVALUATION OF THE ANTIFUNGAL ACTIVITY OF ENILCONAZOLE AGAINST PATHOGENIC FUNGI

BY

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INTRODUCTION

During the last few years more attention has been directed to fungus infections in man and animals in Egypt. The fungi commonly isolated belonged to dermatophytes, yeasts and moulds. The dermatophytes recovered from man were Trichophyton violaceum, T. rubrum, T. schoenleini, T. mentagrophytes, T. tonsurans, Microsporum canis and Epidermophyton floccosum (Refai, 1967, Abdel-Fattah et al., 1967, El-Mazny et al., 1973, Abdel-Aziz et al., 1989 and Abdel Aal et al., 1989). In animals, T. verrucosum is the most common cause of cattle sheep and goat ringworm (Soliman et al., 1977, Refai et al., 1983, and Abdel-Halim et al., 1988), M. canis and T. mentagrophytes were recovered from cats and dogs (Refai et al., 1986).

Yeasts, particularly Candida albicans and Cryptococcus neoformans were isolated from diseased man and animals (Refai and Amer, 1974, Refai et al., 1975, Refai et al., 1986 and 1990). These 2 species were also recovered from flies (Merdan et al., 1974 and Hafez et al., 1976), from the soil and bird droppings (Refai et al., 1983).

Moulds, particularly Aspergillus species have been incriminated in diseases of the respiratory system in man, animals and birds (El-Batrawi, 1980, Youssef and Refai, 1986).

Beside treatment of diseased man and animals, control of spreading and transmission of fungal diseases is of great importance. This is achieved by application of hygienic measures with particular emphasis on the use of effective antifungal disinfectants. Various antimycotic agents have been tested by El-Bahay et al. (1968), Saif and Refai (1977).

The present work is dealing with the evaluation of the efficacy of Enilconazole (Imaverol), a newly introduced antifungal drug in Egypt, against representatives of dermatophytes, yeasts and moulds recovered from pathologic cases in Egypt.

MATERIALS AND METHODS

1. Test organisms:

Dermatophytes : Microsporum canis(Fig.1 and2), M. gypseum(Fig.3and4) and Trichophyton mentagrophytes (Fig. 5).

Yeasts : Candida albicans.

Moulds : Aspergillus fumigatus

2. Antifungal agent: Imaverol (janssen Pharmaceutical) A viscous liquid containing 150 mg/ml Enilconazole as an active principle (1- [2- (2,4-dichlorophenyl) -2-(2 propenylloxy)ethyl 1-1H-imidazole)].
The drug was tested in concentrations of 1, 10, 100 µg, 1,2 and 3 mg/ml dist, water.

Fig. (1): Culture of Microsporum canis on SDA medium.

Fig. (2): A slide culture of Microsporum canis

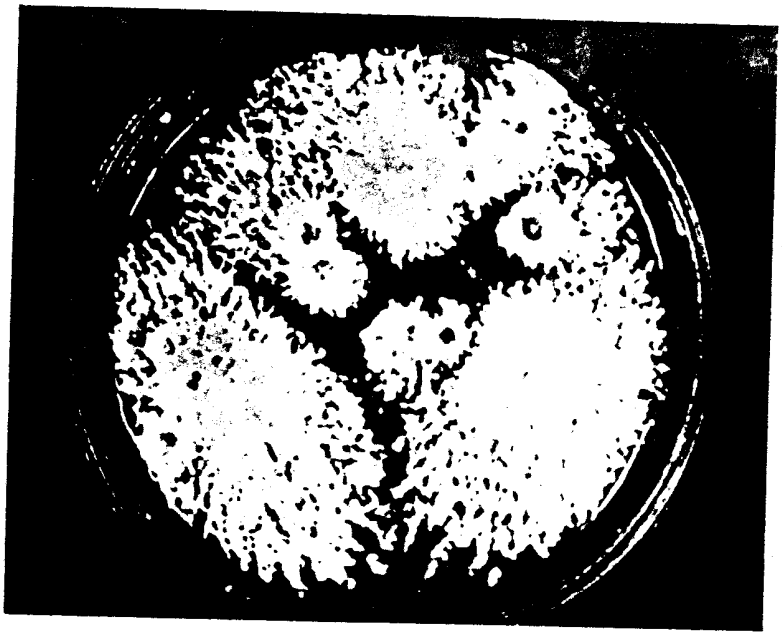

Fig. (3): A culture of Microsporum gypseium on SDA medium.

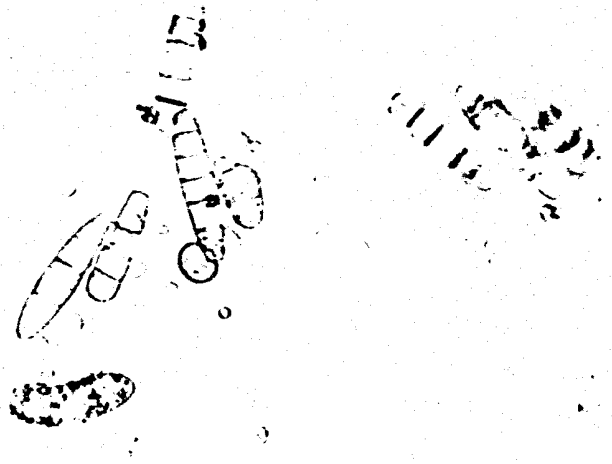

Fig. (4): A slide culture of Microsporum gypseum showing Macrocandida. X 400

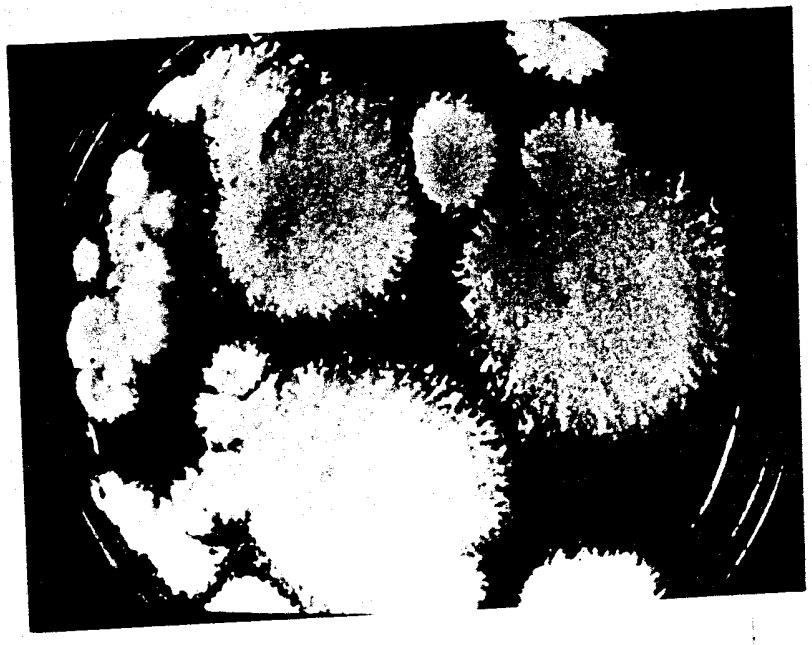

Fig. (5): A culture of Trichophyton mentogrophytes on SDA medium.

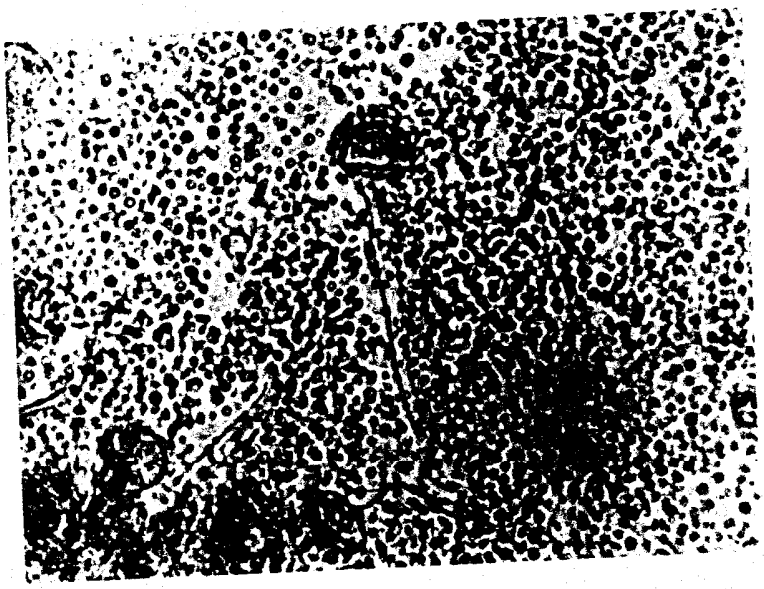

Fig. (6): A culture slide of Aspergillus fumigatus showing the conidial head.

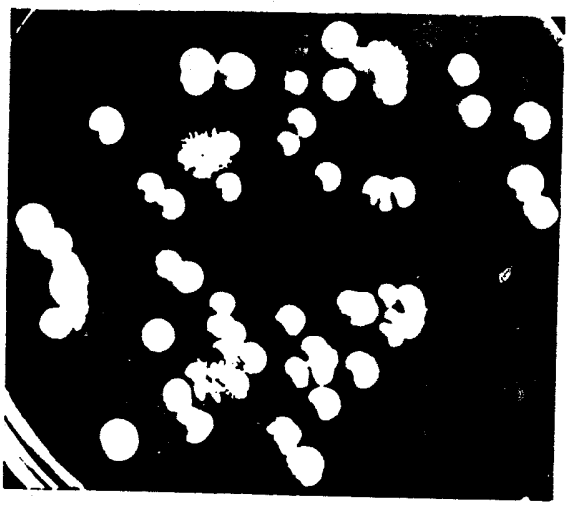

Fig. (7): Candida albicans colonies on SDA medium.

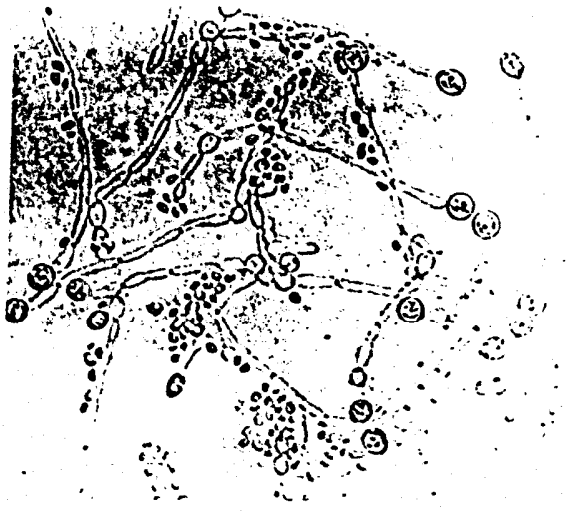

Fig. (8): A slide culture of candida albicans showing pseudomycelium and chlamydo-spores X 400.

Methods of testing:

1. **Exposure of entire culture:** (Oster and Golden, 1949): Plates of sabouraud dextrose agar were inoculated with 0.2 ml of cell suspensions of the test fungi ($5x10^6$ cells/ml) and incubated for 1-3 weeks at 25-30°C. The growing colonies were then flooded with the decimal dilutions of Imaverol and subcultures on fresh sabouraud plates were made from treated colonies after 5 minutes, 2, 8 and 24 hours. The pieces of the colony is immersed in sterile dextrose agar before inoculation to remove any traces of the drug.

2. **Exposure of spore suspension:** (AOAC, 1975): Decimal dilutions of the drug were inoculated with 0.5 ml of the spore suspension ($5x10^6$/ml). After 5, 10 and 15 minutes a loopful (4mm) was removed and placed in 10 ml 2% dextrose broth then subcultured into fresh tubes of dextrose broth. The latter were then incubated at 25-30°C for 1-3 weeks and examined for growth.

RESULTS

The exposure of growing fungal cultures to imaverol inhibited the growth of all tested fungi after an exposure time of 2 hours at concentration of 3 mg/ml **(table, 1)**. Aspergillus fumigatus was the most sensitive as 100 µg/ml inhibited its growth after 2 hours. Next came Trichophyton mentagrophytes and Microsporum gypseum which could not grow after exposure to 1 mg/ml drug for 2 hours. The most resistant organism was candida albicans which was sencitive only to the drug at a concentration of 3 mg/ml.

The AOAC method revealed the sensitivity of the test fungi in the following order: A. fumigatus, T. mentagrophytes, M. gypseum and C. albicans at much lower concentration than the above mentioned method **(Table 2)**.

Table (1): The effect of Enilconazole as a fungicidal disinfectent (Exposing entire culture method)

Test organism	Concentration																			
	1 µg				10 µg				100 µg				1 mg				2 mg				3 mg			
	Time of exposure																							
	5 m	2h	8h	24h	5	2h	8h	24h	5	2h	8h	24h	5	2h	8h	24h	5	2h	8h	24h	5	2h	8h	24h
Trichophytom mentagrophytes	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	-	-	No growth			
Microsporum gypseum	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	-	-	-	No growth			
Microsporum canis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	-
Aspergillus fumigatus	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+	-	-	-	No growth			
Candida albicans	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-

Table (2): The effect of Enilconazole as a fungicidal disinfectant (A.O.A.C. Method)

Test organism	Conc./ml	1 µg			10 µg			100 µg			1 mg			2 mg			3 mg		
	Time of exp. in minute	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15
Trichophyton mentogrophytes	+ + +	+	+	+	+	-	-				No growth								
Microsporum gypseum	+ + +	+	+	+	+	+	+	+	+	+	No growth								
Microsporum canis	+ + +	+	+	+	+	+	-	+	-	-	No growth								
Aspergillus fumigatus	+ + +	+	-	-	-	-	-	+	-	-	No growth								
Candida albicans	+ + +	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-

DISCUSSION

From the results obtained in the present work it is clear that imaverol is an effective antifungal, specially against Aspergillus fumigatus and dermatophytes. A. fumigatus is of particular interest as it is known to cause great losses in poultry (Refai and Rieth , 1966 and Refai et al., 1990). The fungicidal activity of Enilconazole against A. fumigatus has been already described by Van Cutsem et al. (1988). They reported a complete inhibition of the growth of A. fumigatus at µg. ml. In comparison with the data obtained by Saif and Refai (1977) it is clear that the inhibitory concentration of imaverol towards A. fumigatus is much lower (1 µg. ml) than that of thiabendazole (20 µg/ml). The in vitro testing is a useful means of judgement on the efficiency of the drug in the field. The AOAC (1970) considered that the highest dilution which kills the spores in vitro within 10 minutes could be expected to disinfect inanimate surfaces contaminated with pathogenic fungi. The fact that imaverol kills the fungal spores at such low concentration allows the dilution economic. It may however, be necessary to carry out field trials to be able to judge its efficacy under field conditions.

SUMMARY

Imaverol (Enilconazol) was proved to kill spores of Aspergillus fumigatus after 10 minutes to 1 µg/ml (AOAC method). A concentration of 1 mg/ml killed dermatophytes after 5 minutes and 3 mg/ml killed Candida albicans after 10 minutes.

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