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Does competition for existence pushed the evolution of the 'once' saprophytic fungi to parasitic life?

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ABSTRACT

Aims: The current study was planned to understand how the 'once' saprophytic fungi would have adapted/equipped themselves to be pathogens in human environment and what environmental events that would have compelled/ facilitated certain fungi to become human pathogens.
Methodology and results: Antibiosis of soil fungi such as *Chrysosporium keratinophillum*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium* sp., *Rhizopus oryzae* and *Curvularia lunata* on different test fungi viz. different species of dermatophytes, *Malassezia furfur*, *Cryptococcus neoformans* and *Histoplasma capsulatum* was studied. *In vitro* susceptibility testing of secretory and intracellular substances of soil fungi was also tested on the test fungi of clinical significance. The ability of the test fungi for saprophytic survivability was tested on sterile and un-sterile soil. *M. furfur* and the anthrophilic dermatophytes were susceptible to the secretory substance and intracellular substances of the soil fungi.
Conclusion, significance and impact study: The anthropophilic dermatophytes are not capable of existing in soil as saprophytes. The antagonistic effect of *C. keratinophillum* (sharing the same nutritional preference viz keratinophilic nature and ecological niche) could be one among the possible early events that formed the basis for the evolution of obligate parasitism in certain dermatophytes. Other obligate anthropophilic fungi, *M. furfur* is also not capable of existing even in sterile soil as a saprophyte. Most of the soil fungi tested show inhibition on *M. furfur in vitro*. The ability of fungi to cause disease in humans appears to be an accidental phenomenon With the exception of a few dermatophytes, pathogenicity among the molds is not necessary for the maintenance of dissemination of the species (Rippon, 1988). Further, the fungi that are able to cause disease seem to do so because of some peculiar trait of their metabolism that is not shared by taxonomically similar species. The survival and growth of fungi at the elevated temperature of the body, the reduced oxidation-reduction environment of tissue and the ability to overcome the host's defence mechanisms clearly sets 'mere' saprophytic and pathogenic fungi apart from each other. It is always of greater interest to know how these organisms would have adapted/equipped themselves to be pathogens in human environment and what environmental events that would have compelled/facilitated certain fungi to become human pathogens. This study reports the possible fungus-fungus interaction which would have been one of the driving forces of fungal evolution resulting in obligate anthropization of certain species.

Keywords: Fungal interaction, dermatophytes, evolution

INTRODUCTION

Fungi are often found existing in the ecosystem as heterotrophs. Based on the nature of their heterotrophic existence fungi can be further classified as either 'saprobes' or as 'parasites'. But such a classification is always uncertain as there is always not a sharp distinction between the parasites and saprophytes as the natural habitats of most of the fungi that cause systemic infection are in organic wastes or debris, or in soils enriched by organic wastes (Rippon, 1988). Parasitism is truly severe and hence these fungi are termed as true pathogenic fungi which includes dimorphic organisms such *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis* and *Paracoccidioides brasiliensis*. (Crum *et al.*, 2004; Kauffman, 2006). These dimorphic

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fungi are present as saprophytes in the soil, guano of bats and pellets of various birds (Rippon, 1988), but as they encounter the human habitat (usually by chance) they exhibit true pathogenic potential irrespective of the immune status of the host. It is really amazing to know how these fungi evolved the 'super specialty' of existing in the saprophytic form and yet cause life threatening diseases in 'immunocompetent' people.

Opportunistic pathogens are organisms, which cause infections under certain host conditions such as immuno-suppression. These fungi are also commonly prevalent in soil and or in bird guano etc. as saprophytes. When host immunity is compromised/suppressed these fungi cause moderate to life threatening diseases. An example of such a pathogen is *Cryptococcus neoformans*. Studies have shown that cryptococcal infection poses a major threat to the life of AIDS patients all over the world (Drouhet, 1997). It would be interesting to unravel how these geophilic fungi can spontaneously specialize themselves to colonize, invade and cause disease in human beings. Serious doubts exist that whether the lack in immune barrier is the major cause for these infections or the evolving adaptations in the fungi that contribute to the parasitism as such adaptations viz. melanin and capsule production, mannitol utilization etc. (Chaturvedi *et al*., 1996) in *C. neoformans* are unique to certain geophilic group among the whole of the fungal community. Decoding of the 'adaptive ability' of these fungi would definitely provide a better management strategy for containing the diseases caused by them. But what early events where responsible for the evolution of the saprophytes in to parasites is always a million dollar question. Though, when in soil the fungus needs to interact with a lot of micro and macro-organisms, the role of fungus–fungus interaction as an evolutionary factor cannot be undermined. Our understanding of this complex and dynamic system of fungus-fungus interactions in the environment is limited and therefore forms a new avenue of investigation to get a clearer picture on whether such interactions appropriated the fungal evolution.

The ecological status of some of the above organisms is never limited to its original habit. Besides the ability to survive freely as saprobes, these organisms cause infections in human beings at varying degree from mild to severe and opportunistic to true pathogenic-life threatening infections as seen in case of *C. neoformans* and *H. capsulatum* respectively (Rippon, 1988). While, in case of the dermatophytes, the anthropophilic and zoophilic group are not able to exist in soil for a prolonged period while their geophilic counterparts like *Microsporum gyseum* and *Microsporum nanum* despite their ability to survive in nature also can cause skin lesions of severe nature.

Several keratinophilic fungi biologically closely related to dermatophytes have been isolated from soil (Rippon, 1988). However among the dermatophytes known from clinical sources, only *M. gypseum* is reported to be prevalent in soil throughout the world. Other species of dermatophytes, which have been isolated from soil such as *Microsporum cookei*, *Trichophyton ajelloi*, *Trichophyton terrestre*, *Microsporum distortum* etc. are not known to be pathogenic (Novak and Galgoczy, 1966). The saprophytic existence of obligate parasitic dermatophytes in soil is not exactly known. However, short-term survivability of some species cannot be ruled out (Ranganathan *et al*., 1996).

Unlike many other fungi, *Malassezia* yeasts are rarely found in the environment. Their habitat is primarily the skin and mucosae of mammals and birds (Midgley, 1989; Guillot and Bond, 1999). Lipid-dependent *Malassezia* organisms are frequently isolated from human skin.

The antibiosis/predation of other soil organisms on different groups of dermatophytes and *Malassezia furfur* should not been underestimated as possible reasons of the obligate anthropophization of these species.

Even though *C. neoformans* and *H. capsulatum* are able to survive as saprobes, their isolations are mostly documented only from soils which are enriched by excreta of birds or bats. The objectives of this study were to determine whether these fungi are able to survive the competition of other soil organisms in environments free from the fecal pellets / guano of birds / bats warrants a detailed study.

MATERIALS AND METHODS

Cultures used

Soil fungi

Chrysosporium keratinophillum, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium* sp., *Rhizopus oryzae*, *Curvularia lunata*.

Test fungi

Two isolates of *C. neoformans* were of clinical origin and the other two were non-pigment producing environmental isolates. The two isolates of *H. capsulatum* earlier collected from PGIMER, Chandigarh and maintained at Department of Microbiology, The New College, Chennai, India were used for the study. The test other organisms include dermatophytes [Geophilic: *Microsporum gypseum* (4 isolates); Zoophilic: *M. canis* (4 isolates); Anthropophilic: *Trichophyton rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. violaceum* and *Epidermophyton floccosum* (4 isolates each)] *Malassezia furfur* (4), *Histoplasma capsulatum* (2 isolates), *Cryptococcus neoformans* (4 isolates). All the dermatophytes and *C. neoformans* isolates used in the current study were obtained from the culture collection of Department of Microbiology The New College, Chennai.

Antibiosis of soil fungi on test fungi

Antibiosis of soil fungi such as *C. keratinophillum*, *A. niger*, *A. fumigatus*, *A. flavus*, *Penicillium* sp., *R. oryzae* and *C. lunata* on different of test fungi viz. different species of dermatophytes, *M. furfur*, *C. neoformans* and *H. capsulatum* was studied according to the method described by Prochacki and Engelhardt-Zasada (1972).

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The degree of antibiosis was recorded as per the below category:

I - Mutually intermingling growth
II - Partial inhibition
III - Dominance / Complete inhibition or over growth by antagonist
IV - Mutual slight inhibition (<10 cm)
V - Co-dominance / Mutual inhibition at a distance (>10 cm)

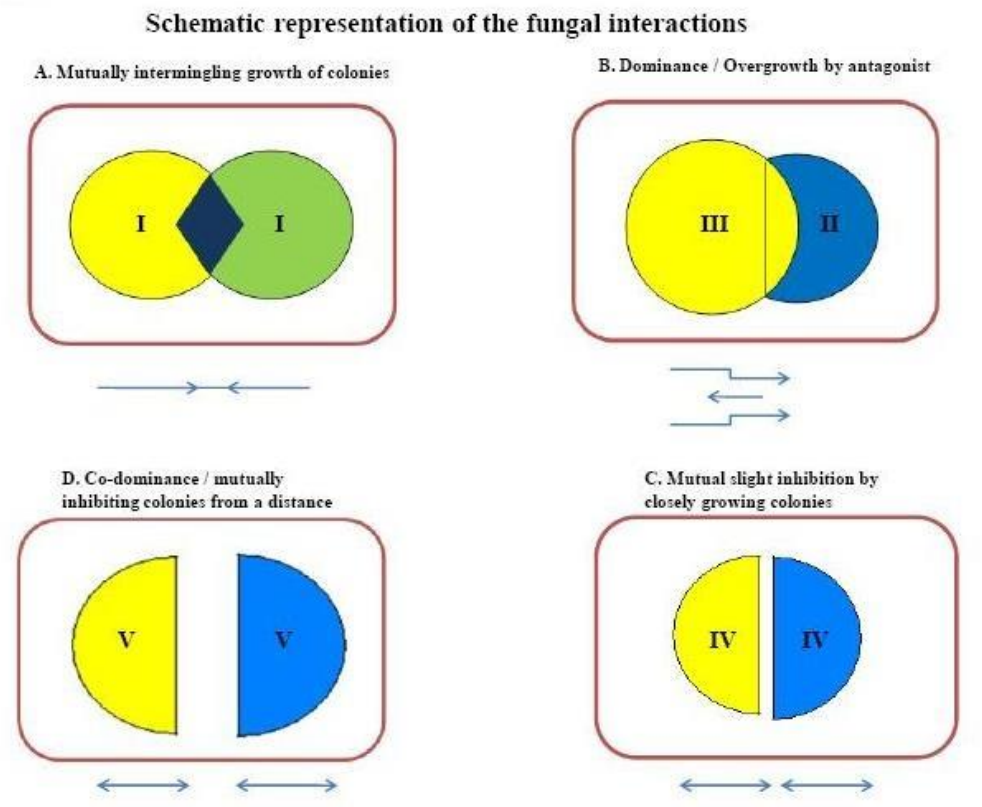

Figure 1: Schematic representation of the fungal interactions

***In vitro* susceptibility testing of secretory and intracellular substances of soil fungi on test fungi (Gokulshankar *et al.*, 2005)**

Twenty microliters of the fungal suspension of each of the soil fungi was prepared in distilled water and adjusted to an absorbance of 0.6 at 450 nm were inoculated into 50 mL of Sabouraud's dextrose broth (pH 7) and incubated at 26 °C. The fungal mat of each of the soil fungi was collected separately by filtration on the 5^{th}, 10^{th}, 15^{th}, 20^{th} and 25^{th} day of growth and the respective culture filtrates were filter sterilized separately. The filtrate was concentrated using a rotary vacuum system at 26 °C. The concentrate was weighed and dissolved in 5% dimethyl sulfoxide (DMSO) to a concentration of 4 mg/mL. This was used as a stock solution of the secretory substance (SS) of the soil fungi. Similarly the fungal mat of all the soil fungi were collected on the 5^{th}, 10^{th}, 15^{th}, 20^{th} and 25^{th} days of growth was washed three times and ground in distilled water using a mortar and pestle. The fungal homogenate was then sonicated to release the intracellular substances (ICS). After centrifugation at 10,000 g for 30 min the supernatant was concentrated and a stock of 4 mg/mL was prepared in 5% DMSO. This was used as the stock solution of ICS of the test fungi.

Broth dilution method was employed for susceptibility testing. Briefly, the stock solutions of SS and ICS were incorporated in 1 mL of Sabouraud's dextrose growth (pH 7) separately and serially diluted to achieve different concentrations of SS and ICS ranging from 2 mg/mL to 62.5 µg/mL. Approximately 20 µL of the fungal suspension of test isolates of each species of dermatophytes, *M. furfur*, *C. neoformans* and *H. capsulatum* (10 days old) adjusted to an absorbance of 0.6 at 450 nm were inoculated into each tube. Sabouraud's dextrose broth containing no SS and ICS, inoculated only with each of the test fungi separately was used as control. The assay was done in duplicates and the tubes were incubated at 26 °C for 21 days. The experiment was repeated thrice wherever necessary. The minimum inhibitory

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concentrations (MIC) of the SS and ICS of the soil fungi on the test fungi were determined using standard procedure.

Saprophytic survivability testing (Ranganathan, 1996)

Each of the test organisms viz. all the species of dermatophytes chosen for the study, *M. furfur*, *C. neoformans* and *H. capsulatum* were inoculated into 100 mL of Sabouraud's broth in a 250 mL conical flask, kept in a rotary shaker for 18 days at 26 °C. Garden soil was collected and was divided in to aliquots of 100 g each. Each 100 g aliquot was transferred in to 250 mL beaker and the pH of the soil was adjusted to 7.5. Approximately 10 g of the fungal mat was inoculated into the soil and the soil was mixed well. The soil was moistened with sterile distilled water. For each organism eight beakers in duplicate were maintained so as to study the survivability period of the organism on 10, 20, 30, 40, 50, 60, 90 and 120th days after inoculation. The viability of the organism on the 1st day after inoculation was confirmed. All the beakers were covered with aluminum foil and were incubated at 26 °C for 120 days. For each organism eight beakers in duplicate were maintained so as to study the survivability period of the organism on the 10th, 20th, 30th, 40th, 50th, 60th, 90th and 120th days after inoculation. The viability of the organism was confirmed on the first day after inoculation. Similarly each of the organisms was maintained in sterile soil for comparison. The soil was sterilized at 160 °C for 2 h/day for 7 days. The pH of the soil was adjusted before sterilization.

Attempts were made to recover the organisms from sterile and un-sterile soil at different time intervals by plating techniques in appropriate media. For dermatophytes hair-baiting technique was also employed in addition to plating technique for recovery of the organisms as per standard procedure (Ulfig and Ulfig, 1990).

RESULTS

Antibiosis of soil fungi on test fungi

No antibiosis of *C. keratinophillum, A. niger, A. fumigatus, A. flavus, Penicillium sp, R. oryzae* and *C. lunata* on different of test fungi was recorded with any species of dermatophytes viz. *M. gypseum*, *M. canis, T. rubrum, T. mentagrophytes, T. tonsurans and E. floccosum*. The interactions were of Type IV category, where mutual slight inhibition at a distance was found. The colony of *T. violaceum* was overgrown by *C. keratinophillum* (Type III interaction). The interaction of *Penicillium* sp. with *T. tonsurans* and *C. keratinophillum* with *H. capsulatum* were of type I category, where there was mutually intermingling growth of the two species. *C. neoformans* was not inhibited by any of the test fungi except *C. lunata*. The inhibition of *C. neoformans* by *C. lunata* was possible only for the environmental (non pigment producing) isolates and not for pigment producing isolates. The growth of *M. furfur* was inhibited by all the soil fungi except *C. keratinophillum*. (Table 1, Figure 2) .The colony of *M furfur* inhibited pigment production in *C.lunata.*. The growth of *T. rubrum, T. tonsurans* and *E. floccosum* near the colony of *C. keratinophillum* showed the presence of sterile hyphae.

***In vitro* susceptibility testing of secretory substances (SS) and intracellular substances (ICS) of soil fungi on test fungi**

Activity of C. keratinophillum on test fungi

The SS of *C. keratinophillum* released on 15th day inhibited all the isolates of *T. rubrum,* while the SS released on 10th day inhibited all the isolates of *T. tonsurans, T. violaceum, E. floccosum.*

The isolates of *T. mentagrophytes* and *M. furfur* were inhibited by the SS of *C. keratinophillum* released on 5th day. None of the isolates of *M. gypseum*, *M. canis*, *H. capsulatum* (Mold and yeast form) were inhibited by the SS of *C. keratinophillum* released even up to 25 days.

Three out of four isolates of *M. canis* were inhibited by the *C. keratinophillum* released on 20th day, while the other isolate was also susceptible to the SS of *C. keratinophillum* released on 25th day.

The ICS of *C.keratinophillum* failed to inhibit all the isolates of *M. gypseum*, *M. canis*, *Trichophyton rubrum*, *C. neoformans*, and *H. capsulatum*. All the isolates of *T. mentagrophytes* and *T. tonsurans* were inhibited by ICS of *C. keratinophillum* released on 25th and 20th day respectively. The ICS of *C. keratinophillum* released on 15th day totally inhibited the isolates of *T. violaceum* while that released on the 10th day inhibited the isolates of *E. floccosum.* All the isolates of *M. furfur* were susceptible to the ICS of *C. keratinophillum* released on the 5th day (Table 2).

Activity of A.niger on test fungi

None of the strains of *M. gypseum*, *M. canis*, *T. rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. violaceum*, *E. floccosum*, *C. neoformans* and *H. capsulatum* were inhibited by the SS and ICS of *A.niger* released even on 25th day, while all the 4 isolates of *M. furfur* were inhibited by SS and ICS of *A. niger* released on 15th day (Table 3).

Activity of A. fumigatus on test fungi

None of the strains of *M. gypseum*, *M. canis*, *T. rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. violaceum, E. floccosum*, *C. neoformans* and *H. capsulatum* were inhibited by the SS and ICS of *A. fumigatus* released even on 25th day. The SS and ICS of *A. fumigatus* released on 15th day and 20th day respectively inhibited all the isolates of *M. furfur* (Table 4).

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Table 1: Types of Interactions between Colonies -Test fungi versus Soil fungi.

Soil fungus	Test fungus									
	M. gypseum	*M. canis*	*T.rubrum*	*T. mentagrophytes*	*T. tonsurans*	*T. violaceum*	*E. floccosum*	*M. furfur*	**C. neoformans*	*H. capsulatum*
C. keratinophillum	IV	IV	IV	IV	IV	III	IV	IV	IV	I
A. niger	IV	IV	IV	IV	IV	IV	IV	III	IV	IV
A. fumigatus	IV	IV	IV	IV	IV	IV	IV	III	IV	IV
A. flavus	IV	IV	IV	IV	IV	IV	IV	III	IV	IV
Penicillium sp	IV	IV	IV	IV	IV	IV	IV	III	IV	IV
R. oryzae	IV	IV	IV	IV	IV	IV	IV	III	IV	IV
C. lunata	IV	IV	IV	IV	IV	IV	IV	II	III	IV

* Environmental non-pigment producing isolate of *C. neoformans*

I - Mutually intermingling growth
II - Partial inhibition
III - Dominance / Complete inhibition or over growth by antagonist
IV - Mutual slight inhibition
V - Co-dominance / Mutual inhibition at a distance

Table 2: Susceptibility pattern of test fungi to SS and ICS of *C. keratinophillum.*

Test fungus	No. of isolates tested	SS released at different growth intervals (Days) / Inhibition of test fungus (n)					ICS released at different growth intervals (Days) / Inhibition of test fungus (n)				
		5	10	15	20	25	5	10	15	20	25
M. gypseum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. canis	4	3+	2+	2+	1+	-	4+	4+	4+	4+	4+
T.rubrum	6	5+	1+	-	-	-	6+	6+	6+	6+	6+
T. mentagrophytes	6	-	-	-	-	-	6+	6+	6+	6+	-
T. tonsurans	6	1+	-	-	-	-	6+	6+	6+	-	-
T. violaceum	4	2+	-	-	-	-	4+	3+	-	-	-
E. floccosum	4	1+	-	-	-	-	3+	-	-	-	-
M. furfur	4	-	-	-	-	-	-	-	-	-	-
C. neoformans	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
H. capsulatum Yeast form	2	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
H. capsulatum Mold form	2	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+

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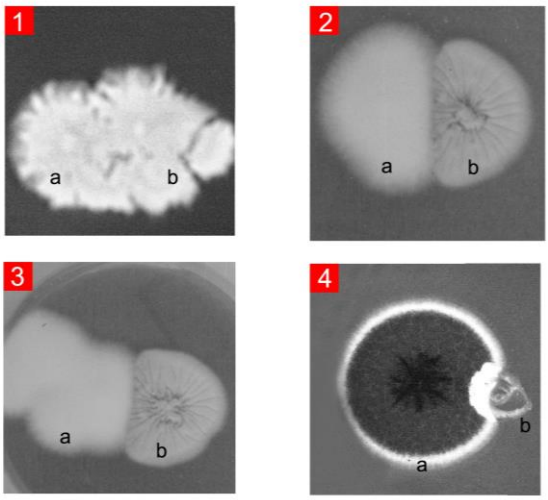
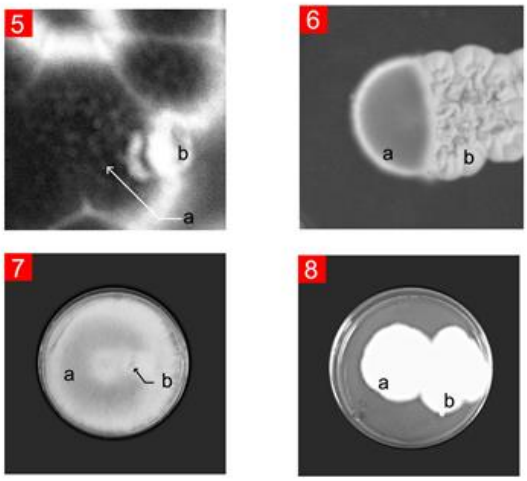


1 Mutually intermingling two colonies of *M.gypseum*

2 Mutual slight inhibition of *C.keratinophillum* (a) and *T.rubrum* (b)

3 Mutual slight inhibition of *C.keratinophillum* (a) and *T.mentagrophytes* (b)

4 Partial encirclement and lysis of *M.furfur* (b) by *C.lunata* (a)



5 Encirclement and inhibition of *C.neoformans* (a) by *C.lunata* (b)

6 Mutual inhibition by *Penicillium sp* (a) and *T.tonsurans* (b)

7 Complete overgrowth and inhibition of *M.furfur* (b) by *Rhizopus* (a)

8 Mutually intermingling colony of *H.capsulatum* (a) and *C.keratinophillum* (b)

Figure 2: Interactions between test fungi and soil fungi.

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Table 3: Susceptibility pattern of test fungi to SS and ICS of *A. niger.*

Test fungus	No. of isolates tested	SS released at different growth intervals (Days) / Inhibition of test fungus (n)					ICS released at different growth intervals (Days) / Inhibition of test fungus (n)				
		5	10	15	20	25	5	10	15	20	25
M. gypseum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. canis	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
T.rubrum	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. mentagrophytes	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. tonsurans	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. violaceum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
E. floccosum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. furfur	4	3+	2+	-	-	-	3+	2+	-	-	-
C. neoformans	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
H. capsulatum Yeast form	2	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
H. capsulatum Mold form	2	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+

Activity of A. flavus on test fungi

All the isolates of *M. gypseum*, *M. canis*, *T. rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. violaceum*, *E.floccosum*, *C. neoformans* and *H. capsulatum* were resistant to the SS and ICS of *A. flavus* released on different time intervals. i.e. 5^{th}-25^{th} days. The SS of *A. flavus* released on 5^{th} day inhibited all the 4 isolates of *M. furfur*, while the ICS of *A. flavus* released on the 10^{th} day inhibited all the isolates of *M. furfur* (Table 5).

Activity of Penicillium sp. on test fungi

The SS and ICS of *Penicillium* sp. released on 5^{th} day inhibited all the 4 isolates of *M. furfur*, while SS and ICS released on the 15^{th} day inhibited the mold form of *H. capsulatum*. The yeast form of *H. capsulatum* was not inhibited either by the SS and ICS of *Penicillium* sp. None of the isolates of *M. gypseum*, *M. canis*, *T. rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. violaceum*, *E. floccosum*, and *C. neoformans* were inhibited by the SS and ICS of *Penicillium* sp. (Tables 6).

Table 4: Susceptibility pattern of test fungi to SS and ICS of *A. fumigatus.*

Test fungus	No. of isolates tested	SS released at different growth intervals (Days) / Inhibition of test fungus (n)					ICS released at different growth intervals (Days) / Inhibition of test fungus (n)				
		5	10	15	20	25	5	10	15	20	25
M. gypseum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. canis	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
T.rubrum	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. mentagrophytes	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. tonsurans	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. violaceum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
E. floccosum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. furfur	4	3+	2+	-	-	-	3+	2+	1+	-	-
C. neoformans	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
H. capsulatum Yeast form	2	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
H. capsulatum Mold form	2	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+

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Table 5: Susceptibility pattern of test fungi to SS and ICS of *A. flavus.*

Test fungus	No. of isolates tested	SS released at different growth intervals (Days) / Inhibition of test fungus(n)					ICS released at different growth intervals (Days) / Inhibition of test fungus (n)				
		5	10	15	20	25	5	10	15	20	25
M. gypseum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. canis	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
T.rubrum	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. mentagrophytes	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. tonsurans	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. violaceum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
E. floccosum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. furfur	4	-	-	-	-	-	2+	-	-	-	-
C. neoformans	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
H. capsulatum Yeast form	2	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
H. capsulatum Mold form	2	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+

Activity of R. oryzae on test fungi

The SS of *R. oryzae* released on 10th day inhibited all the isolates of *M. furfur*, while the ICS released on the 15th day inhibited all the isolates of *M. furfur*. All other test fungi viz, *M. gypseum*, *M. canis*, *T.rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. violaceum*, *E. floccosum*, *C. neoformans* and *H. capsulatum* were resistant to the SS and ICS of *R. oryzae* (Tables 7).

The SS and ICS of *C. lunata* released on 20th day inhibited all the isolates of *M. canis* and *T. rubrum*. The SS released on 15th day inhibited all the isolates of *T. mentagrophytes* and *T. tonsurans* while that released on the 10th day inhibited all the isolates of *T. violaceum* and *E. floccosum.* The environmental isolates of *C. neoformans* were susceptible to SS released on 10th day while the clinical isolates were not inhibited by the SS of *C. lunata*. All the isolates of *M. furfur* were susceptible to the SS released on 5th day. The SS failed to inhibit yeast form of the *H. capsulatum* while the mold form was inhibited by SS released on 20th day

Table 6: Susceptibility pattern of test fungi to SS and ICS of *Penicillium* sp.

Test fungus	No. of isolates tested	SS released at different growth intervals (Days)/ Inhibition of test fungus (n)					ICS released at different growth intervals (Days)/ Inhibition of test fungus (n)				
		5	10	15	20	25	5	10	15	20	25
M. gypseum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. canis	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
T.rubrum	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. mentagrophytes	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. tonsurans	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. violaceum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
E. floccosum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. furfur	4	-	-	-	-	-	-	-	-	-	-
C. neoformans	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
H. capsulatum Yeast form	2	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
H. capsulatum Mold form	2	2+	1+	-	-	-	2+	2+	-	-	-

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Table 7: Susceptibility pattern of test fungi to SS and ICS of *R. oryzae.*

Test fungus	No. of isolates tested	SS released at different growth intervals (Days)/ Inhibition of test fungus (n)					ICS released at different growth intervals (Days)/ Inhibition of test fungus (n)				
		5	10	15	20	25	5	10	15	20	25
M. gypseum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. canis	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
T.rubrum	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. mentagrophytes	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. tonsurans	6	6+	6+	6+	6+	6+	6+	6+	6+	6+	6+
T. violaceum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
E. floccosum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. furfur	4	3+	-	-	-	-	3+	1+	-	-	-
C. neoformans	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
H. capsulatum Yeast form	2	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
H. capsulatum Mold form	2	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+

Table 8: Susceptibility pattern of test fungi to SS and ICS of *C. lunata.*

Test fungi	No. of isolates tested	SS released at different growth intervals (Days)/ Inhibition of test fungi (n)					ICS released at different growth intervals (Days)/ Inhibition of test fungi (n)				
		5	10	15	20	25	5	10	15	20	25
M. gypseum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. canis	4	3+	2+	1+	-	-	3+	2+	1+	-	-
T.rubrum	6	5+	2+	1+	-	-	6+	4+	2+	-	-
T. mentagrophytes	6	3+	1+	-	-	-	4+	3+	1+	-	-
T. tonsurans	6	2+	1+	-	-	-	2+	1+	-	-	-
T. violaceum	4	2+	-	-	-	-	2+	-	-	-	-
E. floccosum	4	1+	-	-	-	-	2+	-	-	-	-
M. furfur	4	-	-	-	-	-	-	-	-	-	-
C. neoformans	4*	4+	2+	2+	2+	2+	4+	2+	2+	2+	2+
H. capsulatum Yeast form	2	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
H. capsulatum Mold form	2	2+	2+	1+	-	-	2+	2+	1+	-	-

*2 environmental and 2 clinical isolates

Minimum Inhibitory Concentration testing

The SS of *C. keratinophillum* recorded MIC of 1000 µg/mL against *M. canis* and *T. rubrum*, 500 µg/mL against *T. mentagrophytes* and *T. tonsurans*, 250 µg/mL against *T. violaceum* and *E. floccosum* and 125 µg/mL against *M. furfur*. The ICS of *C. keratinophillum* recorded MIC of 2500 µg/mL against *M. canis*, *T. rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. violaceum* and *E. floccosum.*

The MIC of ICS of *C. keratinophillum* against *M. furfur* was at 250 µg/mL. Both SS and ICS of *C. keratinophillum* were not active up to a concentration of 2500 µg/mL against *M. gypseum*, *C. neoformans* and *H. capsulatum* (mold and yeast form).

The SS and ICS of *A. niger*, *A fumigatus*, *A. flavus, Penicillium* sp. and *R. oryzae* were not active up to a concentration of 2500 µg/mL against all the species of dermatophytes, *C. neoformans* and yeast form of *H. capsulatum*, *M. furfur* recorded MIC of 250 µg/mL for the SS of *A. niger*, *A. fumigatus*, *A. flavus*, *Penicillium* sp. and *R. oryzae,* while the MIC value was at 1000 µg/mL for the ICS released by the above fungi. The SS and ICS of *Penicillium* sp. recorded MIC value of 1000 µg/mL against the mold form of *H. capsulatum.*

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The SS of *C. lunata* recorded MIC of 1000 µg/mL against *M. canis*, *T.rubrum*, *C. neoformans* (environmental isolates) and mold form of *H. capsulatum*, 500 µg/mL against *T. mentagrophytes* and 250 µg/mL against *T. tonsurans*, *T. violaceum* and *E. floccosum* while it was 125 µg/mL against *M. furfur* ICS of *C. lunata* recorded MIC of 1000 µg/mL against *M. canis*, *T.rubrum*, *C. neoformans* (environmental isolates) and mold form of *H. capsulatum*, 500 µg/mL against *T. mentagrophytes*, *T. tonsurans*, *T. violaceum* and *E. floccosum*, 250 µg/mL against *M. furfur* (Table 9).

The clinical isolates of *C. neoformans* did not record any susceptibility to SS and ICS of *C. lunata* up to a concentration of 2500 µg/mL.

Saprophytic survivability testing of test fungi in unsterile soil

Only 2/4 strains of *T. rubrum*, *T. mentagrophytes*, and *C. neoformans* could be recovered on 10th day by plating technique. Recovery of *T. tonsurans*, *T. violaceum*, *E. floccosum*, *M. furfur* and yeast form of *H. capsulatum* was not possible on 10th day.

One out of two strains of mold form of *H. capsulatum* could be recovered on 10th day. On 20th day it was possible to recover only one isolate of *T. mentagrophytes*, while all other test organisms viz. *T. rubrum*, *T. tonsurans*, *T. violaceum*, *E. floccosum*, *M. furfur*, *C. neoformans* and *H. capsulatum* could not be recovered after 20 days. *H. capsulatum* (mold form) could be recovered up to 120th day. All the 4 isolates of *M. gypseum* could be recovered up to 120 days. Isolation of *M. canis* was not possible after 30 days. A similar pattern of results was obtained when hair-baiting technique was employed to recover the test dermatophytes from the unsterile soil.

Saprophytic survivability testing of test fungi in sterile soil

Recovery of 1/4 isolate of *T. rubrum* and 2/4 isolates of *T. mentagrophytes* was possible on the 30th day. Two isolates each of *T. tonsurans* and *E. floccosum* could be recovered on 10th day after which their recovery was not possible. All the 4 isolates of *T. violaceum* and *M. furfur* could not be isolated even on the 10th day. One isolate of *T. mentagrophytes* could be recovered on 40th day while recovery of *T. rubrum* after 30th day was not possible.

All the 4 isolates of *C. neoformans* could be recovered up to 120th day. Isolation of *H. capsulatum* in the mold form was possible up to 120 days, but the yeast form of *H. capsulatum* could not be recovered after 20 days by plating technique.

It was possible to isolate two strains of *T. rubrum* on 30th day and one strain of *T. mentagrophytes* on 40th day while a single isolate each of *T. tonsurans* and *E. floccosum* was possible on 10th day by hair baiting technique. *T. violaceum* could not be isolated even on the 10th day by hair baiting technique.

All the 4 isolates of *M. gypseum* could be recovered up to 120 days by both plating and baiting techniques. Isolation of *M. canis* after 60 days was not possible (Tables 10 and 11).

DISCUSSION

Fungal evolution and antibiosis

It has long been thought, and has been confirmed by modern phylogenetic studies (Makimura *et al.*, 1998; Makimura *et al.*, 1999; Gräser *et al.*, 1999; Summerbell *et al.*, 1999) that pathogenic dermatophytes probably arose from soil-borne, nonpathogenic ancestors that are likely to be similar in habitat to today's nonpathogenic dermatophytoids (eg. *T. ajelloi*, *T. terrestre*).

The present investigation revealed that the Secretory substances (SS) released by *C. keratinophillum* possess antidermatophytic activity against *T. rubrum*, *T. tonsurans*, *T. mentagrophytes*, *T. violaceum* and *E. floccosum*. The SS did not, however, inhibit the growth of *M. gypseum* and *M. canis*. *M. gypseum* is a geophilic fungi prevalent throughout the world (Rippon, 1988). The selective ability of *M. gypseum* to counter the antagonistic activity of the SS of *C. keratinophillum* may be one of the reasons for worldwide distribution of this fungus in soil (Gokulshankar *et al.*, 2005). The susceptibility of these anthropophilic dermatophytes to intra cellular substances (ICS) of *C. keratinophillum* is relatively less when compared to the SS secreted by the organism.

It is interesting to note that when *C. keratinophillum* and an anthropophilic dermatophyte were co-inoculated on Sabouraud's dextrose agar plate, *C. keratinophillum* did not inhibit the mycelial growth of *T. rubrum*, *T. ton*surans, *T. mentagrophytes* and *E*. *floccosum*. However conidia formation did not occur on hyphae of *T. rubrum*, *T. tonsurans* and *E. floccosum* grown near *C. keratinophillum*. It is presumed from the present study that the nature and quantity of the SS released by *C. keratinophillum* during its early growth phase might not by very active to inhibit the growth of these dermatophytes. Further, when both *C. keratinophillum* and an anthropophilic dermatophyte were inoculated simultaneously on Sabouraud's agar plate, the growth of dermatophyte may also occur before the release of SS by*C. keratinophillum*. The absence of conidia formation on the hyphae of *T. rubrum*, *T. tonsurans* and *E. floccosum* grown near the *C. keratinophillum* indicates that SS of the organism possess anti-dermatophytic substances. Further, the results of susceptibility testing also showed that the SS released on the 5th day was comparatively less active against dermatophytes compared the SS released on the 10th, 15th, 20th and 25th day of growth. The colony of *T. violaceum* was totally encircled by the colony of *C. keratinophillum* on co-inoculation, which is a strong indication of inhibition of the former by the later.

Earlier studies (Gokulshankar *et al.*, 2001; Gokulshankar *et al.*, 2005) on the co-inoculation of different species of dermatophytes and *C. keratinophillum* in sterilized soil revealed that none of the isolates of *T. rubrum*, *T. mentagrophytes* var. *interdigitale* and *E. floccosum* could be recovered from soil after 15th day

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either by baiting or plating technique. Whereas, these dermatophytes could be recovered from sterilized soil when inoculated alone up to 40 days by plating technique. Ranganathan (1996) have already recorded that none of these anthropophilic dermatophytes could exist in soil as saprophytes. However, the viability of these organisms at least in sterilized soil for a prolonged period cannot be ruled out. The inability to recover these dermatophytes even by plating technique from the sterilized soil when they were inoculated along with *C. keratinophillum* (Gokulshankar *et al.*, 2005) strongly suggests the possibility of SS released by *C. keratinophillum* has a definite role in the complete elimination of the dermatophytes. Interestingly, the growth of *M. gypseum* was not affected by SS produced by *C. keratinophillum*. This might be one of the reasons for the geophilic nature of the fungi. Why some species of dermatophytes have selected parasitic mode of existence while majority of dermatophytes species still live in soil as saprophytes is not known. The established homology between the obligate parasitic dermatophyte *T. rubrum* and strict saprophyte *T. ajelloi* (Rippon, 1988; Makimura *et al.*, 1998; Makimura *et al.*, 1999; Gräser *et al.*, 1999; Summerbell *et al.*, 1999) suggests the possibility of the past existence of the present day obligate parasitic dermatophytes in soil. Whenever, baiting technique is employed for isolation of dermatophytes from soil, *Chrysosporium* spp. is the predominant fungi to be isolated (Gokulshankar *et al.*, 2001). *Chrysosporium* and allied genera accounted for 53.8% distribution, with *C. indicum* being the dominant species among the keartinophylic fungi in soil (Deshmukh and Agarwal, 2004). Considering the global prevalence of *Chrysosporium* spp. and in combination with the results in the present study, it is speculated whether the anti-dermatophytic activity of these fungi might also be one of the early events for the evolutionary divergence of some geophilic archi-dermatophyte (saprophytic) to obligate parasitic dermatophyte species (Gokulshankar *et al*., 2005).

The antibiosis of other soil microbes on dermatophytes also cannot be underestimated for such a parasitic evolution of dermatophytes. In our present study several other soil inhabiting fungi such as *Aspergillus niger, A. fumigatus*, *A. flavus*, *Penicillium* sp., *R. oryzae* and *Curvularia lunata* were also tested for their anti-dermatophyte activity. Among these the SS and ICS of *C. lunata* was found to have a definite role in inhibition.

M. furfur was inhibited by *C. keratinophillum*, *A. niger*, *A. fumigatus*, *A. flavus*, *Penicillium* sp., *R. oryzae* and *C. lunata.* Further the SS and ICS of all the soil fungi tested were inhibitory to *M. furfur*. This suggests the inability of the organism to co-exist with any of these fungi. Since saprophytic existence of *M. furfur* is also not known, it makes one to contemplate whether the inhibition by other organisms would have forced *M. furfur* to adapt to an obligate commensal/parasitic existence in the course of evolution? (If only there had been a past existence of this group in soil).

But it is really surprising to note that when *C. lunata* and *M. furfur* were co-inoculated, despite the overgrowth and inhibition of *M. furfur* by *C. lunata*, there is inhibition of pigment production in *C. lunata* by *M. furfur.* It is presumed that this could be because of the production of azelaic acid, a metabolite that can affect pigment formation in the skin, the possible mechanism for the depigmentation seen in some patients of tinea versicolor (Nazzaro-Porro and Passi, 1978). *Curvularia* also produces a black pigment (similar to melanin in human skin ?) (Lanisnik Rizner and Wheeler, 2003), which may be inhibited by *M. furfur.*

Saprophytic survivability of obligate parasitic dermatophytes and *M. furfur* in soil

The role of animals and soil in disseminating human dermatophytosis is known (Georg 1960a, b). However, the most frequent mode of transmission of the disease is through various exogenous sources such as floor, combs, linen etc. (Otecenasek, 1978). The viability of the fungal elements in these sources is not known. Prochacki and Engelhardt-Zasada (1972) have reported the antibiotic effect of *T. megnini* on various species of dermatophytes. The antibiotic effect of the major keratinophilic fungi in soil, *Chrysosporium* sp., on various species of dermatophytes during their survival in soil is not known. In the present study, the SS of *C. keratinophillum* showed inhibitory effect on the growth of the anthropophilic dermatophytes in the *in vitro* studies. In light of the above findings, it is speculated that *Chrysosporium* species may pose a potential challenge to dermatophytes in soil, as both of these organisms are keratinophilic in nature. The role of other members of genus *Chrysosporium* coupled with the antibiosis of other soil bacteria/fungi could have compelled these dermatophytes to evolve parasitic adaptations. The reported low incidence of *T. rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. violaceum* and *E. floccosum* in soil may also be due to the well-defined anthropophization of these dermatophytes species.

The present study revealed that none of the strains of *T. tonsurans*, *T. violaceum* and *E. floccosum* could be recovered from unsterile soil on 10^{th} day. Further *T. rubrum* and *T. mentagrophytes* were not recovered on the 20^{th} and 30^{th} day respectively from unsterile soil. The inability to recover the organisms from unsterile soil suggests that the antibiotic effect of soil microbes might play a role in limiting their survivability in soil. However most of these organisms could not be recovered even from sterile soil after 30 days. This strongly suggests that, saprophytic survivability for these organisms in soil may not be possible due to their well-defined anthropophization. But the viability of the fungal elements especially arthroconidia and chalamydospores in soil for a shorter period cannot be ruled out. However, in our present investigation one isolate of *T. mentagrophytes* could be recovered from sterile soil on 40^{th} day by plating and baiting technique. This shows that these organisms can survive in soil for longer period and thereby proves

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Table 9: Minimum inhibitory concentration of SS and ICS of soil fungi on test fungi

Soil fungus	MIC of the SS of Soil fungi on test fungi (µg/mL)											MIC of the ICS of soil fungi on test fungi (µg/mL)										
	M. gypseum	*M. canis*	*T.rubrum*	*T .mentagrophytes*	*T .tonsurans*	*T .violaceum*	*E. floccosum*	*M .furfur*	*C .neoformans**	*H. capsulatum-yeast*	*H .capsulatum-mold*	*M. gypseum*	*M. canis*	*T.rubrum*	*T .mentagrophytes*	*T .tonsurans*	*T .violaceum*	*E. floccosum*	*M .furfur*	*C .neoformans**	*H. capsulatum-yeast*	*H .capsulatum-mold*
C. keratinophillum	N.A	1000	1000	500	500	250	250	125	N.A	N.A	N.A	N.A	2500	2500	2500	2500	2500	1000	250	N.A	N.A	N.A
A.niger	N.A	N.A	N.A	N.A	N.A	N.A	N.A	250	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	1000	N.A	N.A	N.A
A. fumigatus	N.A	N.A	N.A	N.A	N.A	N.A	N.A	250	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	1000	N.A	N.A	N.A
A. flavus	N.A	N.A	N.A	N.A	N.A	N.A	N.A	250	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	1000	N.A	N.A	N.A
Penicillium sp	N.A	N.A	N.A	N.A	N.A	N.A	N.A	250	N.A	N.A	1000	N.A	N.A	N.A	N.A	N.A	N.A	N.A	1000	N.A	N.A	1000
R oryzae	N.A	N.A	N.A	N.A	N.A	N.A	N.A	250	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	1000	N.A	N.A	N.A
C .lunata	N.A	1000	1000	500	250	250	250	125	1000	N.A	1000	N.A	1000	1000	500	500	500	500	250	1000	N.A	1000

* MIC of environmental isolates of *C. neoformans*
N.A: Not active up to a concentration of 2500 µg/mL

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Table 10: Saprophytic survivability of test organisms in unsterile and sterile soil - recovery study by soil plating technique.

Test organisms	No. of isolates	Recovery from unsterile soil in days/ No. of isolates								Recovery from sterile soil in days/ No. of isolates							
		10	20	30	40	50	60	90	120	10	20	30	40	50	60	90	120
T. rubrum	4	2+	-	-	-	-	-	-	-	4+	3+	1+	-	-	-	-	-
T. mentagrophytes	4	2+	-	-	-	-	-	-	-	4+	3+	1+	-	-	-	-	-
T. tonsurans	4	2+	1+	-	-	-	-	-	-	3+	2+	2+	1+	-	-	-	-
T. violaceum	4	-	-	-	-	-	-	-	-	2+	-	-	-	-	-	-	-
E. floccosum	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. gypseum	4	-	-	-	-	-	-	-	-	2+	-	--	-	-	-	-	-
M. canis	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. furfur	4	4+	4+	2+	-	-	-	-	-	4+	4+	3+	3+	2+	2+	-	-
C. neoformans	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H. capsulatum Yeast form	2	2+	-	-	-	-	-	-	-	4+	4+	4+	4+	4+	4+	4+	4+
H. capsulatum Mold form	2	-	-	-	-	-	-	-	-	2+	2+	-	-	-	-	-	-

Note: +, could be recovered; -, could not be recovered

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Table 11: Saprophytic survivability of test organisms in unsterile and sterile soil by hair baiting technique.

Test organisms	No. of isolates	Recovery from unsterile soil in days/ No. of isolates								Recovery from sterile soil in days/ No. of isolates							
		10	20	30	40	50	60	90	120	10	20	30	40	50	60	90	120
T. rubrum	4	2+	-	-	-	-	-	-	-	4+	3+	2+	-	-	-	-	-
T. mentagrophytes	4	2+	1+	-	-	-	-	-	-	3+	2+	2+	1+	-	-	-	-
T. tonsurans	4	-	-	-	-	-	-	-	-	1+	-	-	-	-	-	-	-
T. violaceum	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. floccosum	4	-	-	-	-	-	-	-	-	1+	-	-	-	-	-	-	-
M. gypseum	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. canis	4	4+	4+	3+	-	-	-	-	-	4+	4+	4+	4+	3+	3+	-	-

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the wide ecological niche of these organisms. Several workers have reported the isolation of *T. mentagrophytes* from soil from different parts of the world (Galgoczy, 1963; Grin and Ozegovic, 1963; Doss, 1992).

Similarly the recovery of zoophilic dermatophyte, *M. canis* was also not possible even in sterile soil after 60 days suggesting the inability of this organism for saprophytic existence. The organism could not be isolated either by hair baiting or plating technique. So the viability of the organism even as a spore in soil for prolonged period seemed impossible.

The recovery of *M. gypseum* from sterile and unsterile soil even up to 120 days by plating and baiting method substantiates the geophilic nature of this organism. A similar finding was reported earlier (Gokulshankar *et al.*, 2001; Gokulshankar *et al.*, 2005).

Hair baiting was found to be a superior method for isolating keratinophilic fungi especially dermatophytes from soil. The present study suggests that saprophytism may not be possible for *T. rubrum*, *T. tonsurans*, *T. violaceum* and *E. floccosum*. The ecological niche of different species of dermatophytes varies from species to species despite the fact that they are basically keratinophilic. No clear-cut answer has previously been given for how and why such divergence in their habitat preference has taken place. Probably the anti-dermatophytic activity of *C. keratinophillum*, the predominnt keratinophilic fungi in soil, could have played a role in the parasitic divergence of the anthropophilic and zoophilic dermatophytes.

M. furfur is not capable of saprophytic existence both in sterile and unsterile soil, even for a period of 10 days. This clearly indicates that the organism is capable of only obligate parasitic/commensalistic existence in human/animal hosts.

Saprophytic existence of *C. neoformans* and H. capsulatum in soil

Saprophytic survival of *C. neoformans* was possible up to 120 days in sterile soil but not in unsterile soil. The reason could be because of predation by soil organism like nematodes and amoebae as reported by earlier workers (Steenbergen and Casadevall, 2003). However, presence of soil predators was not determined in the present study but it is possible that they could have been present. The source of soil used for testing the survivability of the *C. neoformans* isolates in sterile soil also was the same and the elimination of predators would have occurred by the sterilization process adopted. That justifies the reason for the isolation of *C. neoformans* from sterile soil up to a period of 120 days. Both the melanin producing and non-melanin producing isolates could be isolated from sterile soil whereas the recovery of both of these isolates was not possible in unsterile soil.

Saprophytic survivability for *H. capsulatum* was possible up to 120 days for the mold suspension however; the yeast suspension was not able to survive both in sterile and unsterile soil even for 20 days. This clearly illustrates that yeast morphogenesis is an adaptation developed by the organism only for pathogenic intra-cellular state while a mold form is necessary for the saprobic existence.

Conclusion

The competition/anatoginsm/fungus-fungus interaction for existence in soil coupled with various biotic and abiotic factors could have played a definite role in the emergence parasitism in the once sapropobic fungi.

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