# ORIGINAL RESEARCH PAPER

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# DIVERSITY OF DERMATOPHYTIC FUNGI ISOLATED FROM PATIENTS

# **Botany**

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# ABSTRACT

Dermatophytes are fungi that can cause infections of the skin, hair and nails, in part because of their ability to utilize keratin. The cutaneous infections they cause (also called tinea) are among the most common infections in humans worldwide. In the USA, 10% of the population has cutaneous fungal infections at any given time, and at least 40% will acquire this skin condition at some time in their life. The World Health Organization estimates global prevalence of dermatomycoses to be approaching 20%. The dermatophytes include three genera of molds in the class Euascomycetes: *Trichophyton, Microsporum*, and *Epidermophyton*. In the present investigation totally 41 fungal species belonging to 20 genera (Deuteromycetes -16 genus and 32 species, Phycomycetes - 4 genus and 9 species) were identified from the dermatophytic patients at Rajamirasudhar Government Hospital, Thanjavur, Tamil Nadu, India, during August 2006 - July 2008. In general, among the 20 genera recorded, the genus *Aspergillus* (10 species) was dominant genera followed by *Mucor* (4 species), *Penicillium, Fusarium, Rhizopus* and *Trichophyton* (3 species). *Microsporum gypseum* and *Trichophyton rubrum* were the common one, which showed 100% frequency in two years study period.

# **KEYWORDS**

Dermatophytes; fung; infections; Microsporum gypseum and Trichophyton rubrum

## INTRODUCTION

Human disease is an abnormal medical conditions caused by external factors such as infection, diseases or internal disinfections such as autoimmune diseases. There are four types of diseases, pathogeneses, deficiencies, hereditary and physiological. Over 9.5 million people die each year due to infectious diseases and nearly all these deaths are in developing countries. Tuberculosis, Gonorrhea, Malaria and Childhood ear infections are just a few of the common and widespread diseases that have become more difficult to treat due to the emergence of drug resistant pathogen.

Superficial fungal infections are common skin diseases, affecting millions of people worldwide (Pierard and Arrese, 1996). These infections occur in both healthy and immune compromised patients and etiologic agents consist of dermatophytes are responsible for most superficial fungal infections and the estimate life time risk of acquiring a dermatophyte infection is between 10-20% (Drake *et al.*, 1996).

Dermatophytes are the most common cause of fungal infections worldwide, resulting in treatment costs of close to half a billion dollars annually in the USA (Smith *et al.*, 1998; Drake *et al.*, 1996). The World Health Organization estimates global prevalence of dermatomycoses to be approaching 20% (Marques *et al.*, 2000). Despite this, researchers lack a sophisticated understanding of dermatophyte pathogenesis (White *et al.*, 2008).

# MATERIALS AND METHODS

## **Collection of samples**

Samples were collected from the patients who outpatients of dermatology sections in Raja Mirasudhar Government Hospital, Thanjavur, Tamil Nadu (Fig. - 1). Suspected lesions from infected human skins were cleaned with 70% alcohol to remove dirt and contaminants (Pl. - 1). Well sterilized cotton swabs were used to collect with sterile iso saline and these were rubbed on to the patient's skin. They were kept under sterile vessels and brought to the laboratory.



## Isolation of dermatophytic fungi

The Potato Dextrose Agar Medium was poured into two conical flasks and cotton plugged and sterilized in pressure cooker for 20 minutes. Streptomycin sulphate (100mg<sup>-1</sup>) was added to the medium to prevent the bacterial growth. The medium was transferred to the sterile petridishes. Samples were streaked on the PDA plates for the cultivation of fungal strains. The plates were incubated at  $25\pm 2^{\circ}$ C for the five days and the fungi appearing on the medium were recorded. Fungi were isolated through the simple streak methods and pure cultures were maintained for further studies.

## Identification of dermatophytic fungi

The fungal cultures were identified by using lactophenol cotton blue staining technique. The stain contains four constituents namely phenol, which serves as a fungicides, lactic acid, which acts as a clearing agent, cotton blue which strain the cytoplasm of the fungus and glycerin which gives a semi-permanent slide preparation. The slides were observed under microscope (400X). Identification of the fungi was carried out by referring the standard manuals of Raper and Thom (1949), Raper and Fenell (1965), Ellis (1976), Kohlmeyer and Kohlmeyer (1979) and Gillman (1957) and it photographed using Nikon Photomicroscope (Japan).

### **Presentation of data**

Frequency occurrence was calculated as follows in order to identify their existence in the dermatophytic samples collected from different months.

Number of samples in which a particular fungus occurred % frequency = \_\_\_\_\_\_ x 100

Total number of samples examined

Based on the frequency occurrences the fungi were grouped as rare (0-25% frequency), Occasional (26-50%. frequency), Frequent (51-75% frequency) and common (76-100% frequency) species.

# **RESULTS AND DISCUSSION**

Dermatophytes are the most common agents of fungal infections worldwide (Robert *et al.*, 2004 and Yuanwu *et al.*, 2009). Dermatophytic infections have been considered to be a major public health problem in many parts of the world. The infections are common in the developing countries, and are of particular concern in the tropics, especially in infants (Guest and Sam, 1998). The infections are caused by 40 species of fungi which are grouped into three genera; *Trichophyton, Microsporum and Epidermophyton* (David *et al.*, 2010). The mode of spread is either by direct or indirect contact with an infected particle which is usually a fragment of keratin containing viable fungus. Indirect transfer may occur via the floor of swimming pools, bath rooms or on brushes, combs, towels and animal grooming implements (Nweze, 2001). Dermatophytes infections are hardly fatal but mostly debilitating and disfiguring diseases that can give rise to permanent deformations if untreated (Yuanwu *et al.*, 2009).

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Superficial fungal infections are common skin diseases, affecting millions of people worldwide (Pierard et al., 1996). These infections occur in both healthy and immune compromised patients and etiologic agents consist of dermatophytes, yeasts and nondermatophyte molds. Dermatophytes are responsible for most superficial fungal infections (Aly, 1994) and the estimated lifetime risk of acquiring a dermatophyte infection is between 10 to 20% (Drake et al., 1996).

The prevalence of superficial mycoses had been studied in different part of the world (Hay et al., 2001; Akploat et al., 2005). The relative occurrence of the etiologic agents of these infections varied from country to country and from one climatic region to another (Ayadi et al., 1993; Korstanje and Staats, 1995) In tropical countries, a warm and humid climate, crowded living and poor sanitary conditions all promoted the spread of these infections (Abdel Rahman and Nahata, 1997).

In the present investigation totally 41 fungal species belonging to 20 genera (Deuteromycetes -16 genus and 32 species, Phycomycetes - 4 genus and 9 species) were identified from the dermatophytic patients at Rajamirasudhar Government Hospital, Thanjavur, Tamil Nadu, India, during August 2006 - July 2008. In general, among the 20 genera recorded, the genus Aspergillus (10 species) was dominant genera followed by Mucor (4 species), Penicillium, Fusarium, Rhizopus and Trichophyton (3 species each) and Microsporum (2 species). All other genera were represented by one species each (Table-1). Similarly Bakheshwain et al. (2011) isolated 19 fungal species from dermatophytic samples belonging to the fungal genera Alternaria, Aspergillus, Bipolaris, Cladosporium, Exophiala, Fusarium, Graphium, Malassezia, Prototheca, Rhizopus, Rhodotorula, Trichosporon and Ulocladium. Aspergillus was the leading genus represented by six species. Evidently, Mbata and Nwajagu, (2007) reported Microsporum gypseum and Trichophyton species were the main etiologic agents in a total of 2,117 nursery and primary school children aged 1-13 years for hair scalp infection in Awka, Nigeria. Other non dermatophytic fungi species isolated were Alternaria alternata 14 (17%), Aspergillus fumigatus 10 (12%), Fusarium solani 7 (8%), Penicillium sp. 3 (4%) and Candida albicans 3 (4%).

Dion and Kapica, (1975) studied 10057 specimens of scrapings from skin, nails and scalp examined for dermatophytes, yeasts, pityriasis versicolor and systemic mycoses between 1963 and 1973, 30.4 percent were positive for fungi. Among the isolates, Trichophyton rubrum was the predominant species (23.6 percent); of lesser prevalence were Microsporum canis (9.3 percent), T. mentagrophytes (8.4 percent) and Epidermophyton floccosum (4.8 percent).

#### Table - 1: List of fungi isolated from Dermatophytic Patients

	8	1 0
S.No	Name of	the fungal isolates
1.	Deuteromycetes	
2.	Acrophialophora fusispord	a (S.B.Saksena) Samson, 1970

- Alternaria alternata (Fr) Keissl, 1912 3.
- Aspergillus candidus Link, 1809 4. 5.
- A. clavatus Desm, 1834
- 6. A. flavus Johann Heinrich Friedrich Link, 1809
- 7. A. fumigatus Fresenius, 1863
- 8 A. luchuensis Lnui,
- 9 A. niger Van Tieghem, 1867
- 10. A. ochraceous Wilhelm
- 11. A. oryzae (Ahiburg) E.cohn
- 12. *A. terreus* Thom
- 13. A. versicolor Thom and Raper
- 14. Blastomyces dermatitidis Gilchrist & Stokes, 1898
- 15. Candida albicans (C.P.Robin) Berkhout 1923
- 16. Cladosporium carrionii, Trejos, 1954
- 17. Curvularia lunata (Wakker) Boedijn
- 18. Fusarium moniliforme Seld 1904
- 19. *F. oxysporum* schlecht emerded snyder and Hansen
- 20. F. solani f.pisi (Jones) Snyder and Hansen 1941
- 21. Geotrichum sp. Link
- 22. Helminthosporium solani Durieu&Mont., 1849
- 23. Madurella sp. Brumpt in 1905
- 24. Malassezia furfur (Robin) Baill, 1889
- 25. Microsporum canis Var. distortum
- 26. M. gypseum Guiart and Grigorakis in 1928
- 27. Penicillium chrysogenum Them
- 28. P. citrinum Them
- 29. P. restrictum J.C.Gilman & E.V.Abbott 1927
- 30. Trichophyton mentagrophytes Var. erinacei
- T. rubrum Var.rodhaini 31.
- T. schoenleinii (Lebert) Langeron Milochevitch 32. Phycomycetes
- 33 Absidia sp. Van Tieghem, 1878
- 34. Mucor ambiguus Vuill. 1887
- 35. M. flavus Bainier, 1903
- 36 M. hiemalis wehmer 1903
- 37. M. indicus Lendn. 1930
- 38. Rhizomucor sp. (Lucet & cost) Wehmer, 1907.
- 39. Rhizopus nigricans Ehrenberg.
- 40. R. oryzae Went & prins, Geerl, 1895.
- 41. Rhizopus sp. Ehrenb, 1820.

The fungal species diversity was recorded in the month of August 2006 to January 2007 was 20 species belonged to 14 genera which showed variations. Among the 14 genera, the genus Aspergillus was represented by the maximum number of five species followed by Mucor and Rhizopus (2 species each). All other genera were represented by one species each (Tab. - 2).

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S. No	Name of the fungi		Fungi availability				
		Aug	Sep	Oct	Nov	Dec	Jan
1.	Acrophialophora fusispora	-	+	-	-	-	+
2.	Alternaria alternata	-	-	+	-	+	+
3.	Aspergillus flavus	+	+	+	-	+	+
4.	A. fumigates	+	+	+	+	+	+
5.	A. niger	+	+	+	+	-	+
6.	A. ochraceous	-	-	-	+	-	-
7.	A. oryzae	+	-	+	-	-	-
8.	Candida albicans	-	-	-	-	+	+
9.	Curvularia lunata	-	-	+	-	-	+
10.	Fusarium solani	+	+	-	+	-	+
11.	Geotrichum sp.	+	-	-	-	-	+
12.	Helminthosporium solani	+	-	+	-	-	-
13.	Microsporum gypseum	+	+	+	+	+	+
14.	Mucor hiemalis	-	+	-	-	-	-
15.	Mucor indicus	-	-	+	-	+	-
16.	Penicillium citrinum	+	-	+	-	-	+
17.	Rhizopus nigricans	+	-	+	-	+	-
18.	R. oryzae	-	-	+	-	-	-
19.	Trichophyton rubrum	+	+	+	+	+	+
20.	Verticillium sp.	-	+	-	-	-	-
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(+) Present (-) Absent

Totally 24 fungal species belonged to 15 genera were recorded during February 2007 to July 2007. Among the 15 genera recorded the genus

Aspergillus and Mucor were constituted by the maximum number of species (3 species each) followed by Fusarium, Penicillium and Rhizopus (2 species each) and all other genera were represented one species each (Tab. - 3).

## Tab. - 3: Isolation and identification of fungi from dermatophytic patients (February 2007 - July 2007)

S.No	Name of the fungi	Fungi availability					
		Feb	Mar	April	May	Jun	Jul
1.	Acrophialophora fusispora	+	-	-	+	+	-
2.	Alternaria alternate	-	-	-	-	+	-
3.	Aspergillus candidus	-	-	-	-	+	-
4.	A. fumigates	+	+	+	-	-	+
5.	A. luchuensis	+	+	+	-	+	+
6.	A niger	+	+	-	-	-	-
7.	A. versicolor	-	+	+	+	-	+
8.	Candida albicans	-	-	+	+	-	+
9.	Cladosporium carrionii	+	-	+	-	-	-
10.	Curvularia lunata	+	+	-	+	+	-
11.	Fusarium moniliforme	+	+	+	+	+	+
12.	F. oxysporum	-	+	+	+	+	+
13.	Geotrichum sp.	-	+	+	+	-	-
14.	Helminthosporium solani	-	-	-	-	+	+
15.	Microsporum gypsum	+	+	+	+	+	+
16.	Mucor flavus	+	-	+	+	-	+
17.	M. hiemalis	-	+	+	+	+	-
18.	M. indicus	-	+	-	+	-	+
19.	Penicillium chrysogenum	+	+	-	-	-	-
20.	P. citrinum	+	+	+	+	+	+
21.	Rhizopus nigricans	+	-	+	+	-	+
22.	R. oryzae	-	+	+	+	+	-
23.	Trichophyton rubrum	+	+	+	+	+	+
24.	Verticillium sp.	+	+	+	+	+	-

(+) Present (-) Absent

In the month of August 2007 to January 2008, a total number of 27 fungal species belonged to 17 genera were recorded. Among the 17 genera, the genus Aspergillus by the maximum number of 5 species followed by Trichophyton and Rhizopus (3 species each), Mucor and Penicillium (2 species each) and all other genera were represented one species each (Tab. - 4).

# Tab. - 4: Isolation and identification of fungi from dermatophytic patients (August 2007 - January 2008)

S.No	Name of the fungi		Fungi availability				
		Aug	Sep	Oct	Nov	Dec	Jan
1.	Acrophialophora fusispora	+	+	-	+	+	-
2.	Alternaria alternate	+	+	+	+	+	+
3.	Aspergillus candidus	-	+	-	+	+	+
4.	A. flavus	+	-	+	-	-	-
5.	A. fumigates	-	-	-	-	-	+
6.	A. luchuensis	+	+	-	+	-	-
7.	A. niger	-		-	-	+	+
8.	Blastomyces dermatitidis	-	-	+	-	+	-
9.	Candida albicans	-	+	-	-	-	-
10.	Cladosporium carrionii	-	+	-	+	-	-
11.	Curvularia lunata	+	-	-	+	-	+
12.	Fusarium solani	-	+	+	-	-	-
13.	Geotrichum sp.	-	-	-	-	-	+
14.	Helminthosporium solani	+	+	-	-	+	+
15.	Microsporum gypseum	+	+	+	+	+	+
16.	Mucor hiemalis	-	+	+	-	-	-
17.	M. indicus	+	+	+	+	+	+
18.	Penicillium chrysogenum	-	+	-	-	+	-
19.	P. citrinum	+	+	+	+	+	+
20.	Rhizomucor sp.	+	-	+	-	-	-
21.	R. nigricans	+	+	+	+	-	+
22.	R. oryzae	-	+	-	-	+	+
23.	Rhizopus sp.	-	+	-	+	+	+
24.	Trichophyton mentagrophytes	-	+	+	+	+	+
25.	T. rubrum	+	+	+	+	+	+
26.	T. schoenleinii	+	+	-	+	+	+
27.	Verticillium sp.	+	+	+	-	+	+
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(+) Present (-) Absent

the 20 genera recorded, the genus Aspergillus constituted by the maximum number of 6 species followed by Mucor, Trichophyton (3 species each), Fusarium and Penicillium (2 species each). All other genera were represented one species each (Tab. - 5).

were recorded during the month of February 2008 to July 2008. Among - 5: Isolation and identification of fungi from dermatonhytic tionts (Februs Tab

The maximum number of 33 fungal species belonged to 20 genera

ab. – 5: Isc	lation and identification of fungi from dermatop	phytic patients (February 2008 - July 2008)

S.No	Name of the fungi	Fungi availability					
		Feb	Mar	April	May	Jun	Jul
1.	Absidia sp.	-	-	-	-	-	+
2.	Acrophialophora fusispora	-	-	-	+	+	-
3.	Alternaria alternata	+	+	+	+	+	+
4.	Aspergillus clavatus	+	-	+	+	+	-
5.	A. flavus	+	+	-	-	+	-
6.	A. fumigates	+	+	-	-	+	+
7.	A. luchuensis	-	-	+	-	-	-
8.	A. niger	-	+	-	+	+	-
9.	A. terreus	+	+	+	+	+	+
10.	Blastomyces dermatitidis	+	+	+	+	+	+
11.	Candida albicans	-	-	-	-	-	+
12.	Cladosporium carrionii	+	-	+	-	-	-
13.	Curvularia lunata	+	-	+	-	+	-
14.	Fusarium oxysporum	-	-	+	+	-	+
15.	F. solani	-	-	+	+	+	+
16.	Geotrichum sp.	-	-	-	+	-	-
17.	Helminthosporium solani	+	+	-	-	+	+
18.	Madurella sp.	+	+	-	-	-	+
19.	Malassezia furfur	+	+	+	+	+	+
20.	Microsporum canis	+	+	+	+	+	-
21.	M. gypseum	+	+	+	+	+	+
22.	Mucor ambiguous	-	-	+	-	-	-
23.	M. hiemalis	-	-	-	+	-	-
24.	M. indicus	-	+	+	-	+	-
25.	Penicillium chrysogenum	+	+	+	+	+	+
26.	P. restrictum	-	+	-	+	-	+
27.	Rhizomucor sp.	+	-	+	+	-	-
28.	Rhizopus nigricans	+	-	-	+	-	+
29.	R. oryzae	-	+	-	-	+	+
30.	Trichophyton mentagrophytes	-	+	+	+	-	+
31.	T. rubrum	+	+	+	+	+	+
32.	T. schoenleinii	+	+	-	+	-	+
33.	Verticillium sp.	-	+	-	-	-	-

(+) Present (-) Absent

## **Percentage frequency**

Microsporum gypseum and Trichophyton rubrum were the common one, which showed 100% frequency in two years study period (Tab. -8). M. indicus (68.3%), Alternaria alternata (66.7%), Aspergillus fumigatus, P. citrinum, Rhizopus nigericans (62.5%) were frequent. A.

niger, Curvularia lunata, Helminthosporium solani, Verticillium sp. (50%), Acrophialophora fusispora, R. oryzae (45.8%), F. solani, A. flavus, Penicillium chrysogenum (41.7%), A. luchuensis, Trichophyton mentagrophytes, T. schoenleinii (37.5%), Blastomyces dermatitidis, F. oxysporum, M. hiemalis (33.3%), Candida albicans, Geotrichum sp. (29.2%) were occasional while rest of others were rare in occurrence (Tab.-6).

### Tab. - 6: Percentage frequency and frequency class of different species of fungi recorded at August 2006 to July 2008 (n=24)

S.No	Fungal isolates	No. of months in which the	Percentage frequency	Frequency class
		fungus occurred		
1.	Absidia sp.	1	4.1	R
2.	Acrophialophora fusispora	11	45.8	0
3.	Alternaria alternate	16	66.7	F
4.	Aspergillus candidus	5	20.8	R
5.	A. clavatus	4	16.7	R
6.	A. flavus	10	41.7	0
7.	A. fumigates	15	62.5	F
8.	A. luchuensis	9	37.5	0
9.	A. niger	12	50.0	0
10.	A. ochraceous	1	4.2	R
11.	A. oryzae	2	8.3	R
12.	A. terreus	6	25	R
13.	A. versicolor	4	16.7	R
14.	Blastomyces dermatitidis	8	33.3	0
15.	Candida albicans	7	29.2	0
16.	Cladosporium carrionii	6	25	R
17.	Curvularia lunata	12	50	0
18.	Fusarium moniliforme	6	25	R
19.	F. oxysporum	8	33.3	0
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20.	F. solani	10	41.7	0
21.	Geotrichum sp.	7	29.2	0
22.	Helminthosporium solani	12	50	0
23.	Madurella sp.	3	12.5	R
24.	Malassezia furfur	5	20.8	R
25.	Microsporum canis	5	20.8	R
26.	M. gypseum	24	100	С
27.	Mucor ambiguous	1	4.2	R
28.	M. flavus	4	16.7	R
29.	M. hiemalis	8	33.3	0
30.	M. indicus	14	68.3	F
31.	Penicillium chrysogenum	10	41.7	0
32.	P. citrinum	15	62.5	F
33.	P. restrictum	3	12.5	R
34.	Rhizomucor sp.	5	20.8	R
35.	Rhizopus nigricans	15	62.5	F
36.	R. oryzae	11	45.8	0
37.	<i>Rhizopus</i> sp.	4	16.7	R
38.	Trichophyton mentagrophytes	9	37.5	0
39.	T. rubrum	24	100	С
40.	T. schoenleinii	9	37.5	0
41.	Verticillium sp.	12	50	0

R-Rare (0-25%); O-Occasional (26-50%); F-Frequent (51-75%); C-Common (76-100%)

### CONCLUSION

The human body is covered with a vast amount and diverse range of germs. These germs live harmlessly within the body and on the skin. However, certain types of fungus can build up on the skin and cause infections. A fungal infection usually appears on the skin, as the organisms live on a protein called keratin. This protein makes up the nails, skin and hair. The various symptoms of a fungal infection depend on the type of the fungus that has caused the infection. Emergence of new diseases, re-emergence of old, development of resistant strains, side effects of some currently available drugs including toxicity and other undesirable effects in allergic patients are a few major problems which require immediate attention to combat these diseases with effective drugs of high therapeutic index. Furthermore, effective drugs are also needed for immune compromised patients who are at great risk by opportunistic pathogens that normally do not pose any major threat in the normal population. Therefore, there is an immense need for the development; and discovery of new and safer bioactive compounds.

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