



## Isolation and Characterization of Dermatophytes in a Tertiary Care Hospital in Thiruvananthapuram, South India

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### ABSTRACT

**Background and aim:** The study was done to identify and characterize the fungal species causing the various clinical types of Dermatophytosis by microscopy, culture and biochemical tests. It also compared the efficacy of the culture media for isolating the dermatophytes and observed the current trend of the etiological fungal species in the locality.

**Material and methods:** The study included 82 patients exhibiting clinical signs of Dermatophytosis. Skin and nail scrapings and nail clippings were examined by direct microscopy; cultures using DTM (Dermatophytes test medium) and SDA (Sabouraud dextrose agar) with cycloheximide were performed; and biochemical tests, such as the urease test and the hair perforation test, were performed.

**Results:** Tinea corporis was the most common type [55 (67.07%)]. Three-quarters of the patients had no co-morbidities, and diabetes was not a significant association. Direct microscopy was positive for fungus in 71 (86.58%) samples. In comparison, only 57 (69.51%) were positive by culture, but one sample was positive by culture, although negative in direct microscopy, indicating the need for culture if skin scraping is negative. Interestingly, Trichophyton mentagrophytes (44, 77.19%) was the most common species and was conforming to the current trend, which makes one wonder if that could be contributory to Dermatophytosis occurring even in those without co-morbidities. Also, DTM was more useful for faster primary isolation of fungus compared to SDA with cycloheximide.

**Conclusions:** The current mycological profile reveals that Trichophyton mentagrophytes are the emerging fungal pathogen, and DTM is a more effective medium than SDA with cycloheximide.

### 1. Introduction

Dermatophytoses or tinea infection is the most common superficial cutaneous fungal infection affecting the skin, hair and nails. It is caused by keratinophilic filamentous fungi of the three genera, Trichophyton, Microsporum, and Epidermophyton, broadly called the dermatophytes. The Trichophyton species usually infect skin, hair and nails; Microsporum infects skin and hair; and Epidermophyton infects skin and nails. Direct microscopy using 10–20% KOH (potassium hydroxide) is the common screening technique for detecting the fungus. Species identification is done by culture, using media like Sabouraud Dextrose Agar (SDA) with added antibiotics

(chloramphenicol, cycloheximide) and Dermatophytes Test Medium (DTM) where the topography texture pigmentation on the obverse and reverse sides will be different for different species. DTM has been observed to be better in isolation of fungus. The positivity rates by KOH smears and culture have been variable in different locations. Biochemical tests like urease and hair perforation tests help confirm fungal species. Trichophyton mentagrophytes, M.gypseum and Epidermophyton floccosum are urease positive, whereas Trichophyton rubrum is urease negative. Trichophyton mentagrophytes and M.gypseum give positive hair perforation test, whereas Epidermophyton floccosum and Trichophyton rubrum give a negative test.<sup>[1]</sup>

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## 2. Material and methods

This study was a hospital-based cross-sectional study. The institutional ethics committee obtained human ethical clearance (HEC.No.07/14/2018/MCT). All patients clinically diagnosed with dermatophytosis attending the outpatient wing of the Department of Dermatology and Venereology, Government Medical College, Thiruvananthapuram, Kerala, from May 2018 to October 2018, were included in the study. After obtaining informed consent, the 82 enrolled patients were interrogated for the details and examined clinically, and the details were entered in a proforma, after which the samples were collected. Skin scrapings were taken from the active edge of the lesion after cleaning with 70% alcohol. Nail specimens were taken from the nail plate and subungual region of the nail using a sterile blade and nail cutter. Hair samples would be plucked with epilator forceps in case of hair involvement. All specimens were subjected to KOH wet mount examination (10% KOH in case of skin and hair, 20% KOH in case of nails). For culture, samples were collected in a presterilized black paper packet with proper labeling. Duplicate tubes of DTM and SDA with cycloheximide were inoculated and incubated at 37°C for four weeks. Daily observation of all the inoculated culture tubes was made, and tubes were considered negative if there was no fungal growth even after four weeks of incubation. Colonies were studied in terms of morphology, rate of growth, and pigment production. The growth in DTM is indicated by a change in color from yellow to red due to the production of alkaline byproducts.[1] In SDA, growth is initially powdery or cottony, velvety or waxy, which later turn into flat, folded or suede-like colonies with obverse and reverse pigmentation.[1] Microscopic characteristics were studied by examining the teased Lacto Phenol Cotton Blue (LPCB) preparation. LPCB is a fungal stain containing lactic acid, phenol, glycerol, and cotton blue. It is used to study fungal

morphology. Cotton blue stains the fungal elements blue. Morphology of the dermatophytes, like the presence or absence of micro and macroconidia, their arrangements, and types of hyphae, were studied by slide culture technique with Potato Dextrose agar. A comparison was made between SDA and DTM in the primary isolation of dermatophytes. The urease test and hair perforation test further confirmed isolates. Christensen's urease agar medium is used for the urease test. The urease-enzyme-producing-organisms split the urea in the medium to ammonia, thereby altering the pH of the medium and the color changes from yellow to red in the presence of phenol red indicator. A hair perforation test is done by placing 1cm long sterilized infant hair on a filter paper strip (soaked with sterile distilled water and 10% yeast extract) in a sterile Petri dish, directly inoculating the fungal colony to the hair shaft and incubating at room temperature for four weeks. The hair perforation test is positive if wedge-shaped perforations occur on the hair shaft. Fungal stocks were maintained by subculturing on neutral SDA (Emmon's modification) for further tests if required.

## 3. Results

### Demographic characteristics

Gender-wise, there was a slight female preponderance, with 45 females (54.87%) and 37 males (45.12%) (Male:Female=1:1.2). Table 1 shows the gender-wise distribution of the different clinical types of dermatophytoses. The highest number of patients were in the age group 10-20 years (22 patients, 26.82%), and the lowest was in the 61-70 years age group with only 7 (8.53%) patients. The distribution according to age group is shown in Fig. 1. Manual laborers and homemakers were the dominant group, with 24 (29.26%) patients each, followed by 22 students (26.82%). Fig. 2.

Table 1. Gender-wise Distribution and Clinical Types of Dermatophytoses.

Clinical Type	Total	%	Male	%	Female	%	Total Type	%
Tinea corporis	36	43.9%	14	38.88%	22	61.11%	55	67.06%
Tinea cruris	11	13.41%	7	63.63%	4	36.36%	30	36.58%
Tinea faciei	5	6.09%	3	60%	2	40%	11	13.41%
Tinea incognito	6	7.31%	1	16.66%	5	83.33%	9	10.97%
Tinea unguium	4	4.87%	3	75%	1	25%	4	4.87%
Tinea manuum	1	1.21%	0	0	1	100%	1	1.21%
T.corporis / cruris	10	12.19%	7	70%	3	30%	---	---
T.corporis / cruris / faciei	6	7.31%	2	33.33%	4	66.66%	---	---
T.corporis / cruris / incognito	3	3.65%	0	0	3	100%	---	---
Total	82	100	37	45.12%	45	54.87%	---	---

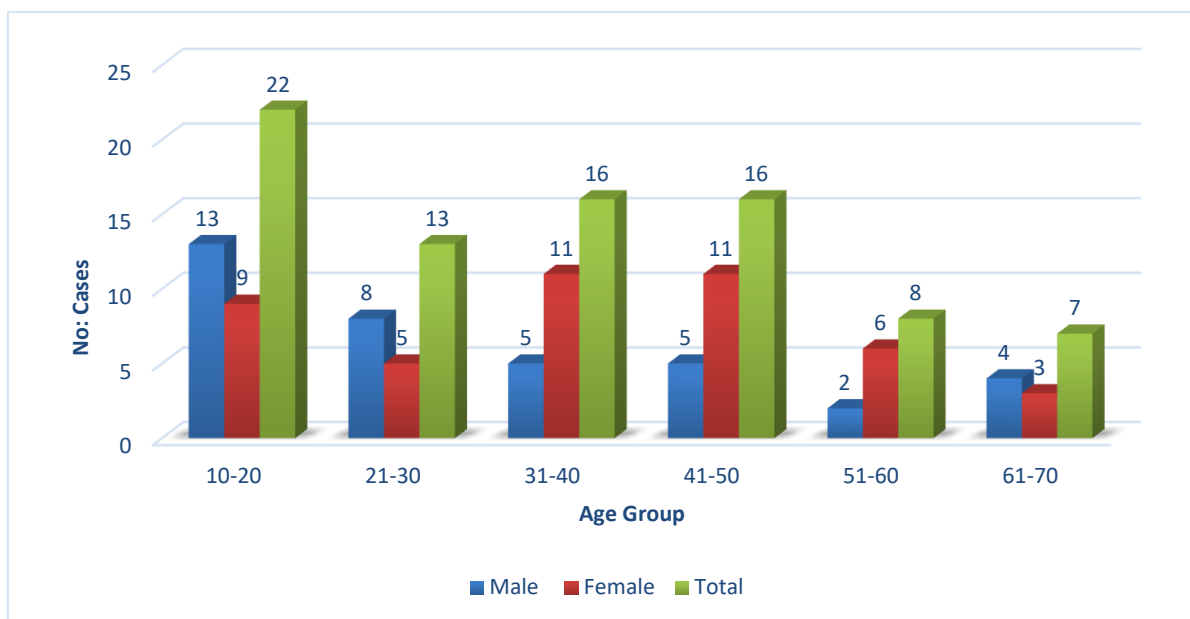


Fig. 1. The gender distribution of dermatophytoses according to age group.

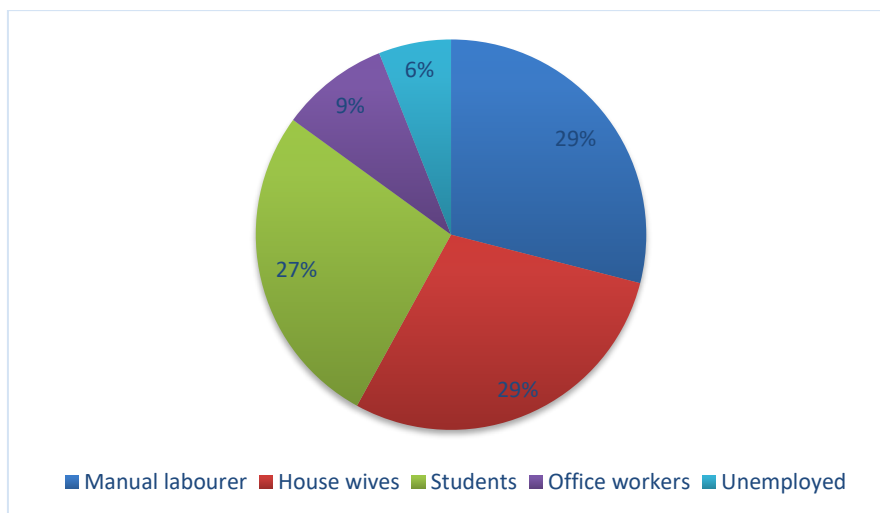


Fig. 2. Distribution of Dermatophytoses according to Occupation.

In an attempt to find the origin of the infection, it was noted that the majority of patients (60, 73.17%) did not have any past history of fungal infections, while 22 patients (26.83%) had similar episodes in the past. A History of similar family lesions or contact of cases was present in 24 (29.26%) cases. Contact with animals was reported in 9 (10.97%) patients. Co-morbidities like diabetes mellitus were present in only 8.53% of patients, while 61 patients (74.4%) had no associated diseases. Arthritis was present in 6.1%; 3.65% had skin diseases and kidney diseases, and 1.21% had asthma, heart disease and thyroid issues.

**Distribution of clinical types**

A total of 55 of the 82 (67.07%) cases had T.corporis; 30 (36.58%) had Tinea Cruris; 11 (13.41%) had T.faciei and 9 had (10.97%) T.incognito either as the only type or in combination with other types. The commonest single type of dermatophytosis was T.corporis accounting for 36 (43.9%) among 82 patients with M: F=0.63:1, followed by Tinea Cruris (11, 13.41%, M:

F=1.75:1); T.incognito (6, 7.31%, M: F=0.2:1); T.faciei (5, 6.1%, M: F=1.5:1) and T.unguium (4, 4.87%). The least common clinical type was T.manuum (1, 1.21% (M: F= 1). Mixed (multiple) site infections were also encountered in our study. The distribution of cases according to gender and clinical types is shown in Table 1.

**Direct smear positivity v/s culture positivity**

Direct microscopy for fungus was positive in 71 (86.58%) of 82 patients, whereas culture positivity was seen in only 57 (69.51%). Both direct microscopy and culture were positive in 56 (68.29%) of 82 samples, while in 15 (18.29%), only direct microscopy yielded positive results. Both direct microscopy and culture were negative in 10 (12.19%) clinically diagnosed patients. Culture was positive in one case (1.21%), while skin scrapings were negative on direct microscopy. The relationship between direct smear positivity and culture positivity is shown in Table 2. Culture positivity was slightly higher in females, 30 (52.63%) versus 27 (47.36%) males.

Table 2. Relationship between direct smear positivity and culture positivity.

Direct Smear (KOH Mount)	Culture Positivity	Number	Percentage
Positive	Positive	56	68.29%
Positive	Negative	15	18.29%
Negative	Positive	1	1.21%
Negative	Negative	10	12.19%
Total		82	100

#### Types of specimen and culture positivity

The skin was involved in 78 (95.12%) patients, and maximum isolation by culture was obtained from skin scrapings in 57 (73.07%) patients. Nail involvement was seen in only 4 (4.87%), but the fungus was not detected by microscopy or culture. There were no cases of hair involvement in this study.

#### Culture positivity v/s clinical types

Tinea corporis was the most common type with maximum culture positivity (45 positive among 55 cases = 81.81%). T.corporis was also the most common single clinical presentation, with maximum culture positivity (27 positive among 36 cases = 75%) accounting for 47.36% of the 57 culture positives, followed by Tinea Cruris (7, 12.3%). Characteristics findings of the

culture and LPCB preparations, as well as the urease tests and hair perforation tests done on the positive cultures, showed that out of 27 isolates of solely Tinea corporis, 21 were Trichophyton mentagrophytes (77.77%) 4 were Trichophyton rubrum (14.81%), and 2 were Epidermophyton floccosum (7.40%). In the seven isolates of solely Tinea Cruris, Trichophyton mentagrophytes was isolated in 4 cases (57.14%), Trichophyton rubrum in 1 (14.28%) and Microsporum gypseum in 2 (28.5%). The correlation between clinical and mycological types of dermatophytosis is shown in Table 3. Out of 9 cases that had a history of contact with animals, six isolates (66.66%) were Trichophyton mentagrophytes, 2 (22.22%) were Trichophyton rubrum, and 1 (11.11%) were Epidermophyton floccosum.

Table 3. Correlation between clinical and mycological types of dermatophytosis.

Clinical Type	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	<i>M. gypseum</i>	<i>E. floccosum</i>	Total Isolate
Tinea corporis	21	4	0	2	27
Tinea cruris	4	1	2	0	7
Tinea faciei	2	0	0	0	2
Tinea incognito	3	0	0	0	3
Tinea unguium	0	0	0	0	0
Tinea manuum	0	0	0	0	0
T.corporis / cruris	7	2	1	0	10
T.corporis / cruris / faciei	6	0	0	0	6
T.corporis / cruris / incognito	1	0	1	0	2
Total	44 (77.19)	7 (12.28)	4 (7.01)	2 (3.50%)	57

#### Species of dermatophytes isolated

Trichophyton mentagrophytes (44, 77.19%) was the commonest species isolated by culture. (Figs. 3a, b, c, d). The others in descending order were Trichophyton rubrum (7, 12.28%) (Figs. 4a, b, c, d); M.gypseum (4, 7.01%)

(Figs. 5a, b). and Epidermophyton floccosum (2, 3.50%) (Figs. 6a, b). A positive hair perforation test, which confirmed the species, is shown in Fig. 7.

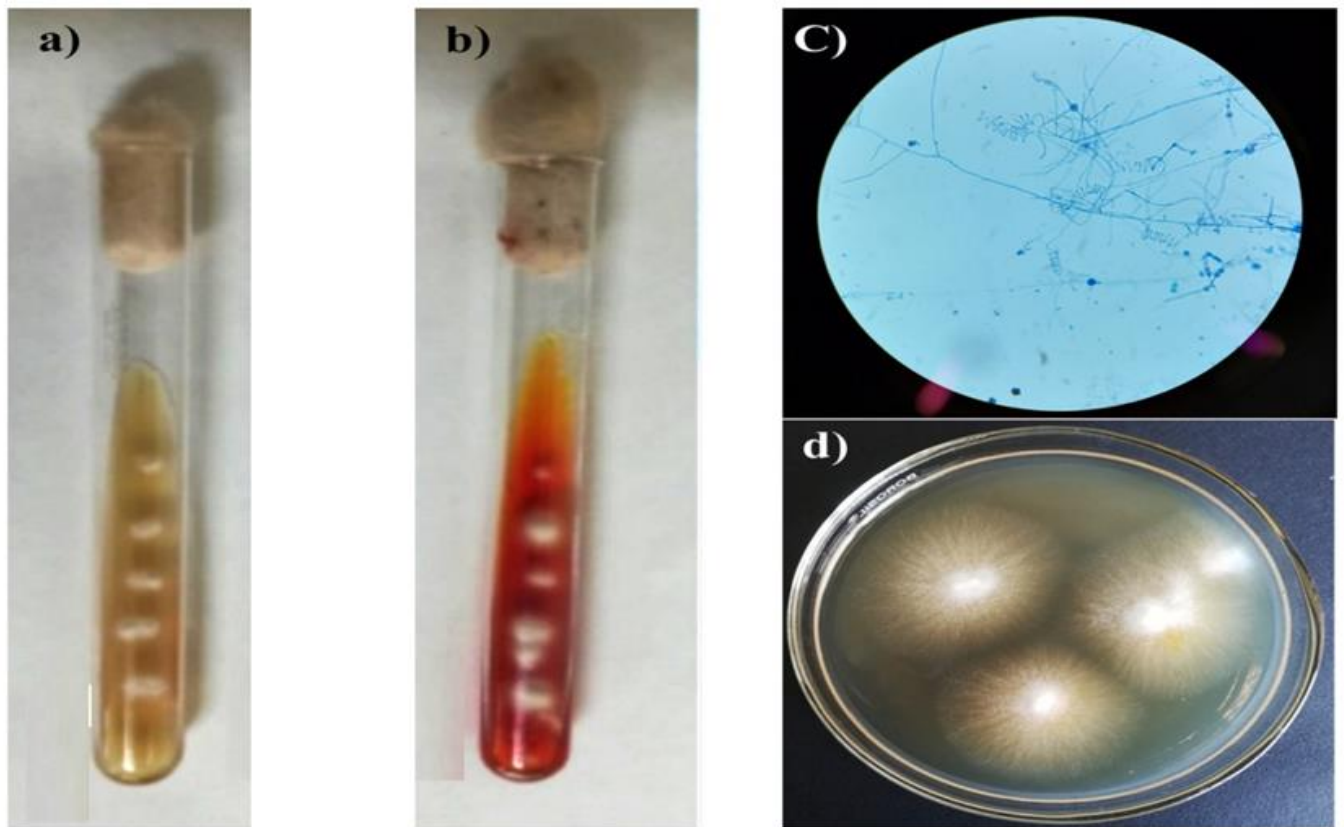


Fig. 3. *Trichophyton mentagrophytes*. a) Growth on SDA with cycloheximide . b) Growth on DTM. c) LPCB mount showing spiral hyphae (400x) e) Growth on SDA with cycloheximide plate.

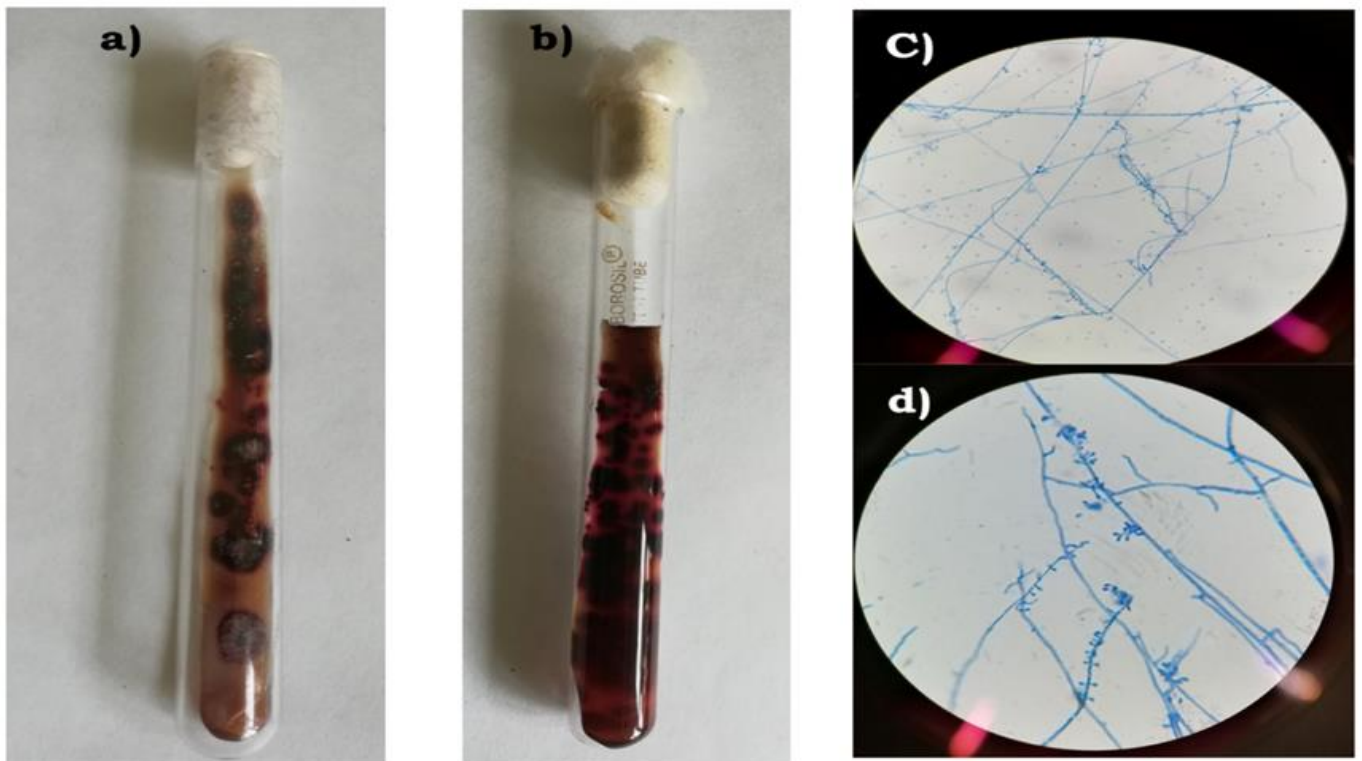


Fig. 4. *Trichophyton rubrum*. a) Growth on SDA with cycloheximide . b) Growth on DTM. c and d) LPCB mount showing tear drop shaped microconidia (400x, 1000x).

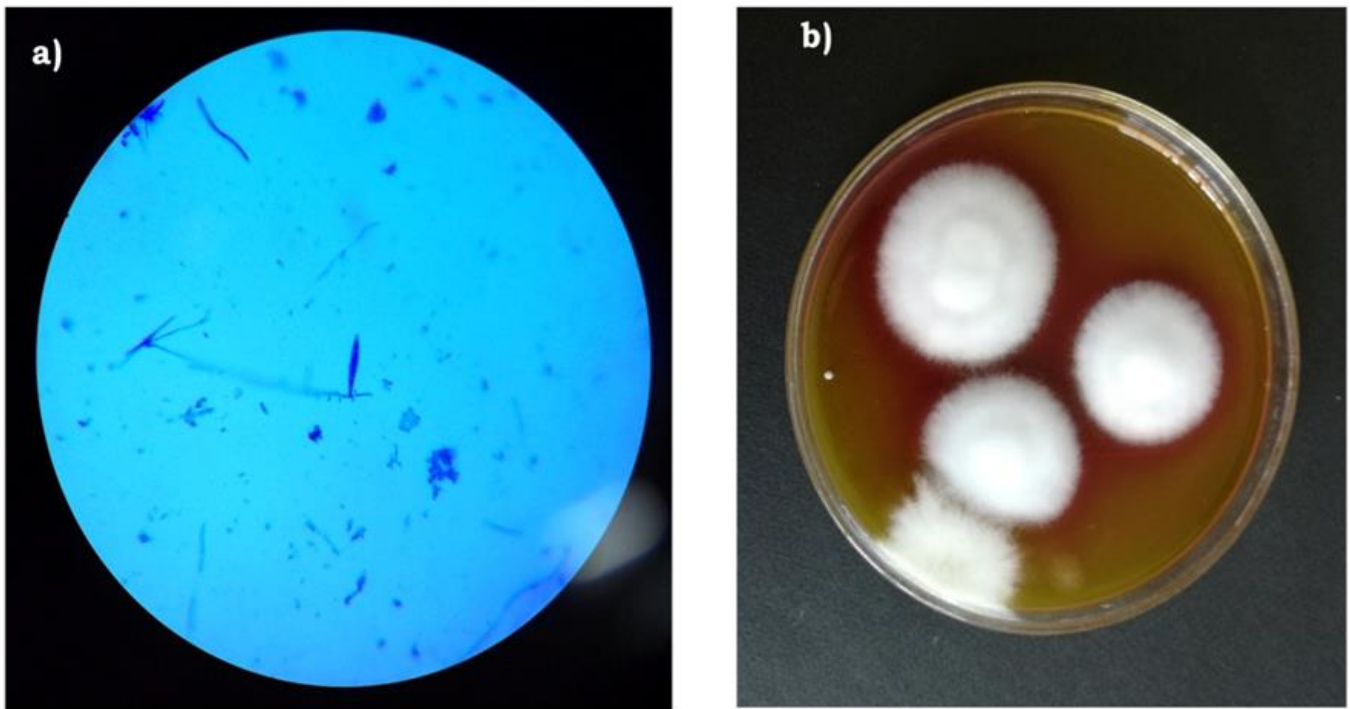


Fig. 5. *Microsporium gypseum*. a) LPCB mount showing macroconidia (400x). b) Growth on DTM plate.

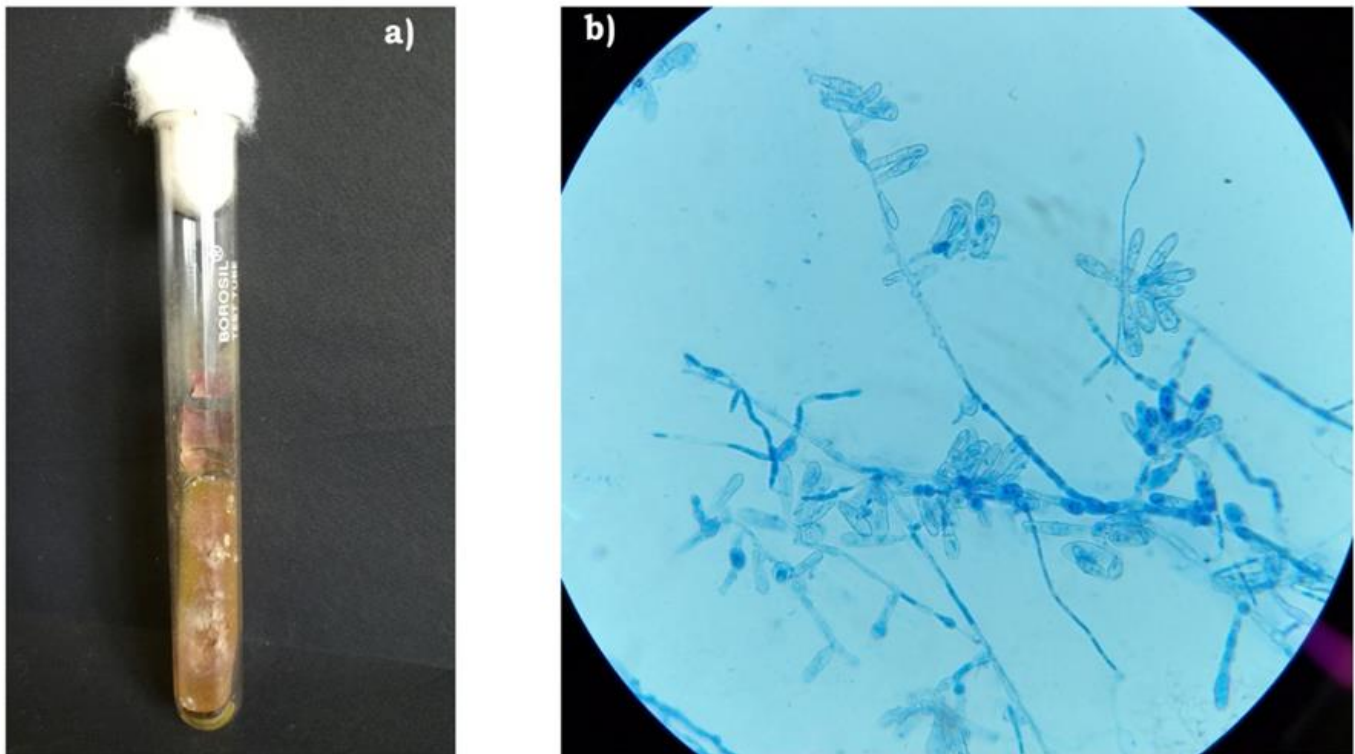


Fig. 6. *Epidermophyton floccosum*. a) Growth on SDA with cycloheximide. b) LPCB preparation showing large clavate macroconidia with rounded apical end.

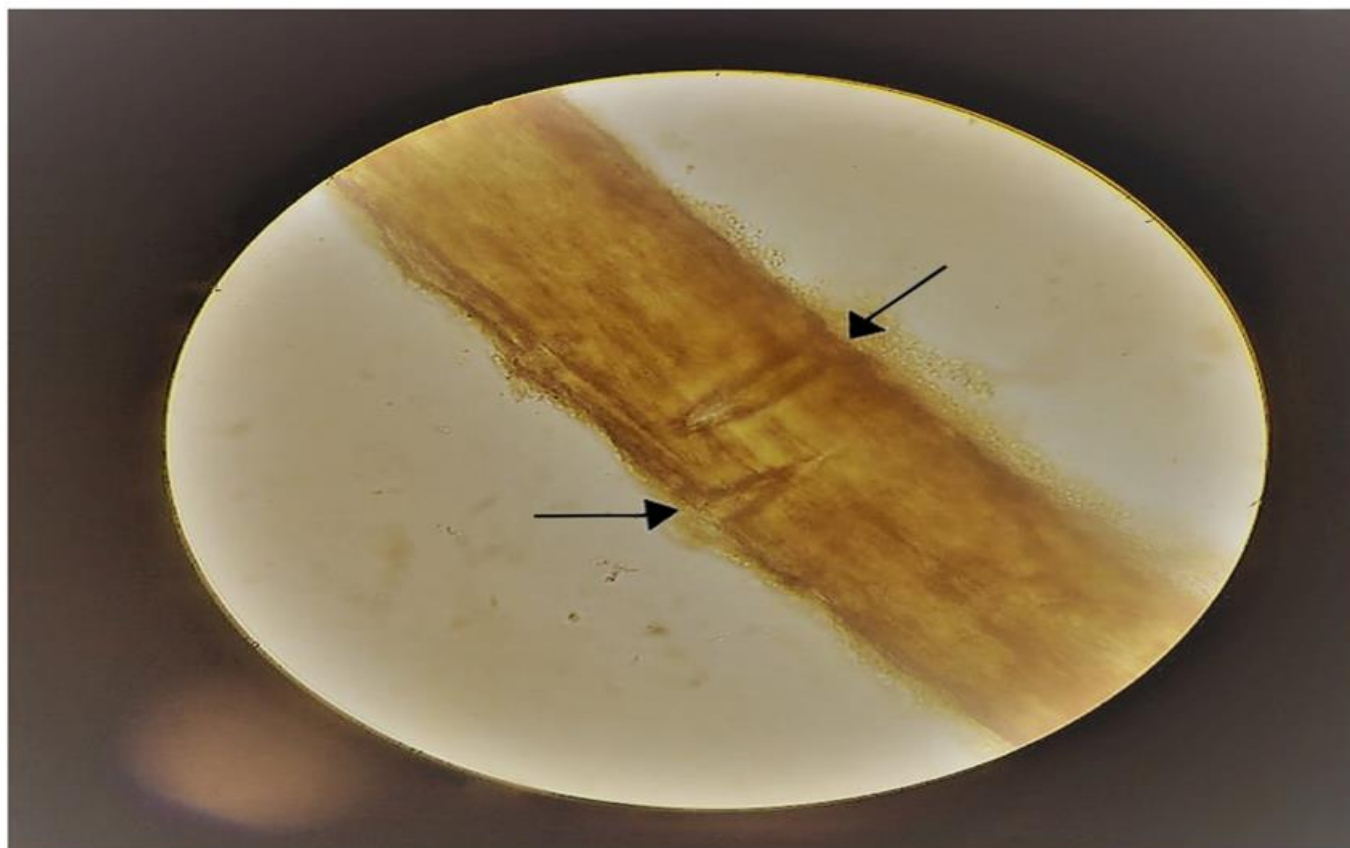


Fig. 7. Hair perforation Test-Wedge shaped perforations indicat positive test.

#### Fungal isolation in SDA v/s DTM

It was noted that among the 57 positive culture isolates, 56 (98.24%) were isolated on DTM and 52 (91.22%) on SDA with cycloheximide. Also, 50 (89.28%) of the dermatophytes were isolated on DTM within 5-10 days after inoculation, while only 38 (73.07%) were isolated on SDA with

cycloheximide within 5-10 days. Fungal growth in SDA occurred more after ten days. A comparison of the growth rate of DTM and SDA with cycloheximide is shown in Table 4.

Table 4. Comparison of growth rate on DTM and SDA with cycloheximide.

Time Duration (Days)	Growth on DTM	Growth of SDA with Cycloheximide
5 – 10	50 (89.28)	38 (73.07)
11 – 15	4 (7.14)	10 (19.23)
>15	2 (3.57)	4 (7.69)
Total	56	52

#### 4. Discussion

This study observed that females outnumbered males (M: F=1:1.2). This was in contrast to the study by Gadadavar et al.,<sup>[2]</sup> where 79.54% were males and 20.45% were females.<sup>[2]</sup> Among the culture-positive cases also in this study, 52.63% were female patients, and 47.36 % were males. This study showed a higher prevalence (26.82%) of dermatophytoses in the age group 10-20 years, similar to other studies.<sup>[3, 4]</sup> The probable reason for this age predilection may be excessive sweating because of higher physical activity. However, a maximum clustering of cases in the 30-40 age group has also been reported.<sup>[5, 6]</sup> As in other studies,<sup>[7]</sup> manual laborers and homemakers constituted the majority of patients in this study (29.26% each), followed by

students (26.82%). Only 29.26% had a history of contact with affected people, and 10.97% had contact with animals. Similar episodes in the past were present in 26.83% of patients. Chandanakonda et al.<sup>[8]</sup> concluded from their studies that 23% of cases gave a history of contact with possible sources of infection, like affected family members. There was no associated disease in 74.4% of patients. Diabetes mellitus was associated with only 8.53% of patients. Lakshmi Vasantha Polari et al.<sup>[4]</sup> had diabetic mellitus in 8.06%, anemia in 6.45%, atopy in 3.23% and HIV infection in 1.61% of cases. Similar to other studies, the commonest clinical types were *T. corporis* and *Tinea Cruris*.<sup>[7, 9, 10]</sup> The high incidence of *T.corporis* and *Tinea Cruris* is probably due to its symptomatic nature, which prompts the patient to seek

medical advice. The increased male predilection in *Tinea Cruris* may be because men wear more occlusive clothing and are more physically active than women, and the groin may remain warm and moist for longer periods. In this study, a male predominance in *T. unguium* may be attributed to increased outdoor activity, a higher chance of trauma, and the use of concomitant occlusive footwear. 95.12% of patients had skin involvement; culture was positive in 73.07%. Only 4 (4.87%) had nail infections, but the fungus was not demonstrated, and none had hair involvement. In this study, 86.58% were positive by direct microscopy, and 69.51% of patients were culture-positive. This correlates with other studies.<sup>[10,11]</sup> whereas, Poyyamozi et al.<sup>[7]</sup> reported a direct microscopic positivity of 65.2% and culture positivity of 45.2%. In one (1.21%) case in the current study, culture was positive, while direct microscopy was negative. Therefore, although about 60-70% may be positive by both direct microscopy and culture, skin scraping being negative for fungus does not rule out dermatophytosis. Both direct microscopy and culture were negative in 12.19% of the clinically diagnosed patients. In such cases, response to treatment with purely antifungal agents should be monitored to make a probable diagnosis of dermatophytosis. *Trichophyton mentagrophytes* formed 77.19% of the species isolated in this study. The others were *Trichophyton rubrum* (12.28%), *M. gypseum* (7.01%), and *Epidermophyton floccosum* (3.50%). Jegadeesan et al.<sup>[12]</sup> reported that the principal dermatophyte was *Trichophyton mentagrophytes* (64%) followed by *Trichophyton tonsurans* (20%), *Trichophyton rubrum* (12%) and *Epidermophyton floccosum* (4%), while others<sup>[13]</sup> reported *Trichophyton mentagrophytes* (38.75%) followed by *Trichophyton rubrum* (27.13%) and *Epidermophyton floccosum* (6.9%). However, *Trichophyton rubrum* was reported as the dominant isolate by others.<sup>[6,14-16]</sup> Out of 9 cases who had a history of contact with animals in this study, 6 (66.66%) isolates were *Trichophyton mentagrophytes*, 2 (22.22%) were *Trichophyton rubrum*, and 1 (11.11%) were *Epidermophyton floccosum*. Therefore, the possibility of *Trichophyton mentagrophytes* being a zoophilic species may be considered and has been recovered from dogs and cats.<sup>[17]</sup> Considering the sole clinical presentations, maximum culture positivity was obtained from *T. corporis* (47.36%), followed by *Tinea Cruris* (12.3%). The least cultural positivity was for *T. faciei*. This correlates with the proportion of clinical types of dermatophytoses in this study and is comparable with others.<sup>[15]</sup> Out of 27 isolates of solely *Tinea corporis*, 77.77% were *Trichophyton mentagrophytes*, 14.81% were *Trichophyton rubrum*, and 7.40% were *Epidermophyton floccosum*. These findings are by Jegadeesan et al.,<sup>[12]</sup> where *Trichophyton mentagrophytes* infected tinea at multiple sites. The effectiveness of DTM for the isolation of dermatophytes was 98.24%, whereas, in the case of SDA with cycloheximide, it was 91.22%. 89.28% of dermatophytes were isolated on DTM within 5-10 days after inoculation, while only 73.07% of the dermatophytes were isolated on SDA with cycloheximide. Majeed et al.<sup>[6]</sup> also showed that 86.1% of dermatophytes were isolated on DTM within 5-10 days of inoculation. In contrast, only 47.05% were isolated on SDA within ten days but showed more positive growth after ten days. Therefore, if SDA is used with cycloheximide as the culture medium, it must be incubated for at least four weeks before being reported as negative. DTM was found to be superior to SDA by Katay et al. and Rahman et al.<sup>[18,19]</sup> The advantage of DTM is that it is more useful for higher rate and faster isolation (after seven days of incubation) and fungus identification compared with SDA with cycloheximide. However, the disadvantage is that colony characteristics such as pigmentation cannot be made out. In the case of SDA with cycloheximide, although the colony's growth takes more than a week, the colony characteristics can be well made out.

## 5. Conclusion

This study highlights the mycological profile of patients with dermatophytoses in and around Thiruvananthapuram in South India. *Tinea corporis* was the common clinical presentation. *Trichophyton mentagrophytes* was found to be the most common aetiological agent. DTM is a good screening media for the faster primary isolation of dermatophytes, but SDA with cycloheximide would be preferable for species-level identification. However, it needs to be incubated for longer before reporting as negative.

## Conflict of Interest

The authors declared that there is no conflict of interest.

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