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Original Article

Microbiology Section

Effectiveness of Sabouraud's Dextrose Agar and Dermatophyte Test Medium in Detection of Candidiasis and Dermatophytosis in Superficial Skin Lesion

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ABSTRACT

Introduction: As time evolves fungal infections have increased its prevalence. Among the fungal infections, superficial fungal infections are the most common type. They can be either chronic or recurrent, therefore simple incidence figures are not the most useful means of understanding the burden of disease.

Aim: Isolation and identification of pathogenic fungi from clinically suspected cases of dermatophytosis and candidiasis of skin, also to compare two media used in isolation of the fungus.

Materials and Methods: A cross-sectional study was conducted over a period of one year, clinically suspected cases of dermatophyte infection and candidiasis who attended the Outpatient Department of Dermatology and Venereology Government Medical College, Thrissur, Kerala, India, were included randomly in the study. Sample size was taken as 150. Samples were collected from clinically suspected cases of dermatophytic infections and candidiasis of skin and was transported to the Microbiology Department in sterile bottles. Direct examination under KOH (Potassium hydroxide) solution was done. Culture of these samples on Sabouraud's Dextrose Agar (SDA) with chloramphenicol/gentamycin and Dermatophyte Test Medium (DTM) was analysed. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) software.

Results: *Trichophyton mentagrophytes* was the commonest isolate 57%, followed by *Trichophyton rubrum* 27%. Out of six clinically suspected cases of candidiasis, no organism was isolated in the cultures. Almost all dermatophytes isolated were grown in DTM within one week of incubation except *Trichophyton rubrum* which appeared in the second week, while in SDA only 31% of isolates were grown. Direct smear positivity was found in 95% of the cases, while culture positivity was 45%. All isolates were grown in DTM while 31% were grown in SDA.

Conclusion: *Trichophyton mentagrophytes* was the commonest species isolated. The next common isolate was *Trichophyton rubrum*. DTM was more useful as a screening medium as opposed SDA as identification medium and the isolation is more rapid.

Keywords: *Microsporum canis, Trichophyton mentagrophytes, Trichophyton soudanense, Trichophyton rubrum, Trichophyton verrucosum*

INTRODUCTION

Fungal infections have been affecting mankind from the time immemorial. They are quite widespread and have affected growing number of people in recent years. Dermatophytosis is one of the earliest known fungal infections and is very common throughout the world [1]. The term dermatophyte literally means "skin plants" [2]. It is estimated that superficial fungal infections affect roughly 20-25% of the world population [3]. Evolutionary development towards an accommodating host parasite relationship is present among dermatophytes which is absent among other fungal agents causing human disease [4]. The clinical importance of identifying species of dermatophyte is to find out the probable source of infection. Also, there are some prognostic considerations as well. The anthrapophilic group causes chronic infection which may be difficult to cure. The zoophilic and geophilic dermatophytes cause inflammatory lesions which easily respond to therapy and occasionally spontaneously heal [5]. The dermatophytes have distinct clinical manifestation in different parts of the body. Each focus of infection is due to local inoculation. The inflammation is seen maximum at the advancing margins leaving central area with some clearing. The diagnosis of dermatophytosis is based on combination of clinical observation supplemented by laboratory investigation. The history of patient is essential regarding age, sex, occupation, duration of illness, history of any treatment taken and any associated disease. In the laboratory, diagnosis depends on demonstration of causative pathogens in tissue by microscopy and isolation of the fungus in culture [6]. In this study, the culture was done in SDA and DTM for a better isolation of the pathogen.

The present study was done to isolate and identify most common dermatophyte species from the clinical isolates obtained from Thrissur Medical College dermatology department 2017-2018 and to compare the effectiveness of two culture media used for isolation.

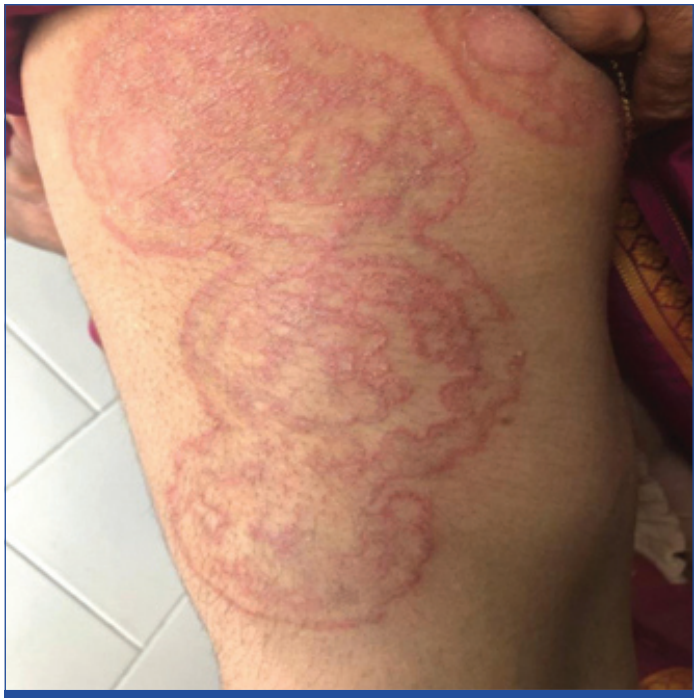
MATERIALS AND METHODS

A cross-sectional study was conducted over a period of one year (June 2017-June 2018). Clinically, suspected cases of dermatophyte infection and candidiasis who attended the Outpatient Department of Dermatology and Venereology Government Medical College, Thrissur, Kerala, India, were included randomly in the study. An Institutional Review Board (IRB) clearance was obtained for the study, informed consent was taken before sample collection from the patient.

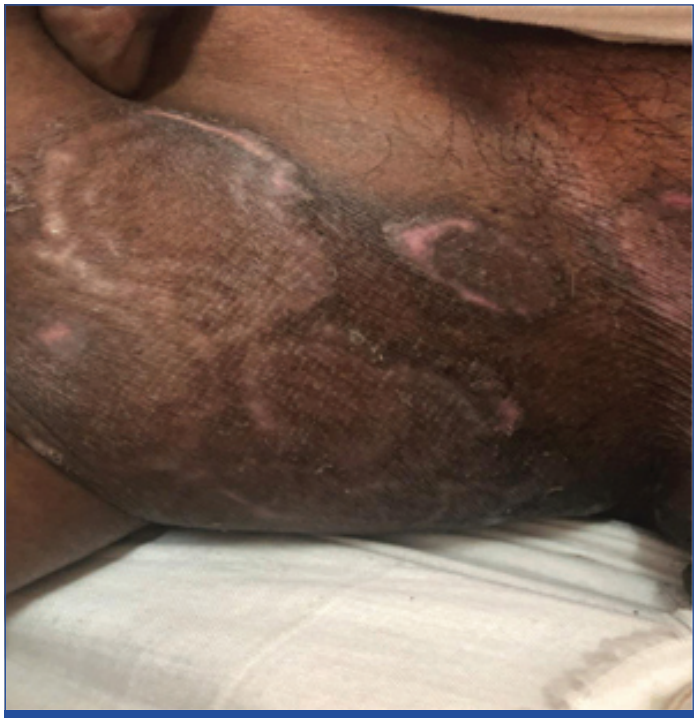
Sample size calculation: Sample size was calculated on the formula $Z\alpha^2p\ (100-p)/d^2$ {d=10-20% of p}, and it was obtained as 150.

Inclusion criteria: Patients with clinical diagnosis of superficial dermatophytic infections and candidiasis of skin and patients with tinea versicolor and fungal infections of hair and nails [Table/Fig-1,2]. Only those with clinical findings at the outpatient department were included in the study and those who were not within the inclusion criteria were excluded.

A detailed history was taken from all 150 patients using a preset proforma which included age, sex, occupation, duration of illness, history of any treatment taken and any associated disease.

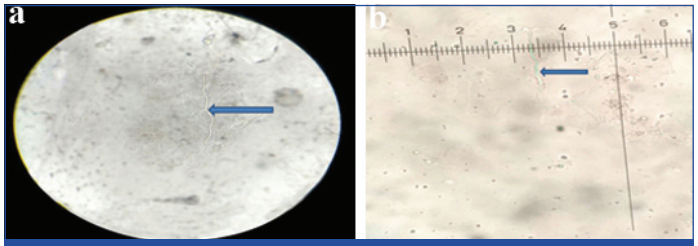


[Table/Fig-1]: Tinea corporis.



[Table/Fig-2]: Tinea cruris.

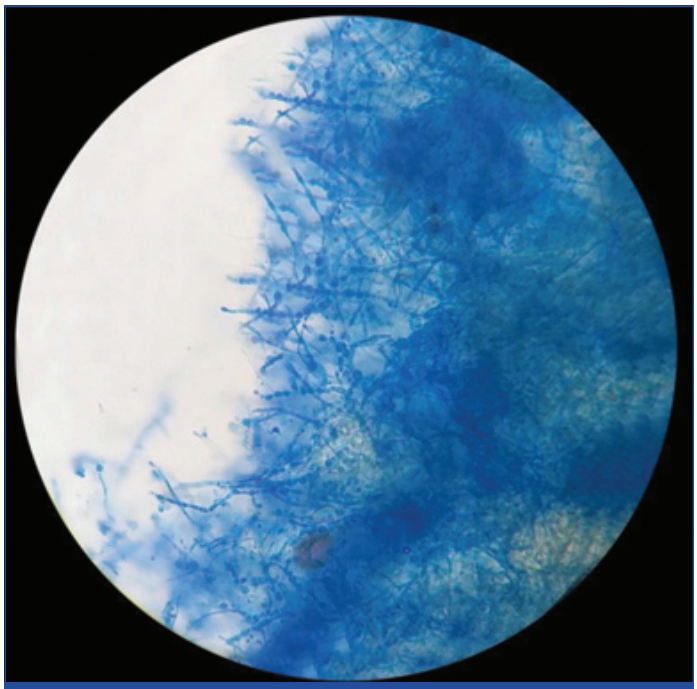
The infected part was cleaned with 70% alcohol to remove the contaminants on the surface. The specimens were collected in a sterile container. The skin scrapings were obtained from the active edge of the lesion. Direct microscopy [7,8] was done by adding 10% KOH to a small portion of skin scraping on a clean glass slide [Table/Fig-3]. The rest of the specimen was inoculated in SDA and DTM.



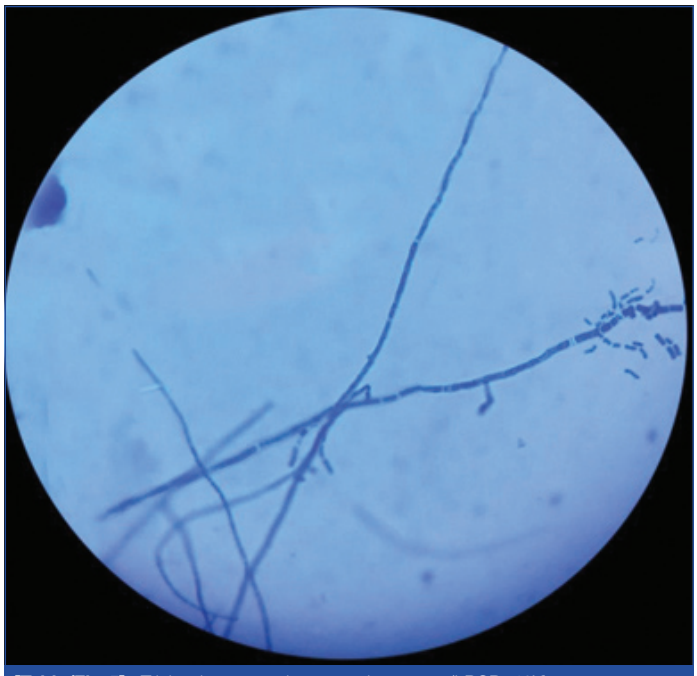
[Table/Fig-3a-b]: KOH smear showing fungal filaments (a) and measuring thickness of fungal fiament (b) (40X).

1. Sabouraud's Dextrose Agar (SDA) with Chloramphenicol and Cycloheximide (Actidione)

The slopes and agar plates were inoculated using a flame sterilised bent wire. SDA culture tubes were incubated at room temperature for four weeks. Daily observation of all the inoculated culture tubes were made and tubes were considered negative if there was no fungal growth even after four weeks of incubation [7]. Colonies were studied and details regarding the morphology of the colony, rate of growth and pigment production were recorded. Microscopic characteristics were studied by examining Lactophenol Cotton Blue (LPCB) preparation after teasing a tiny bit of the growth with teasing needle and putting a coverslip [Table/Fig-4-6].



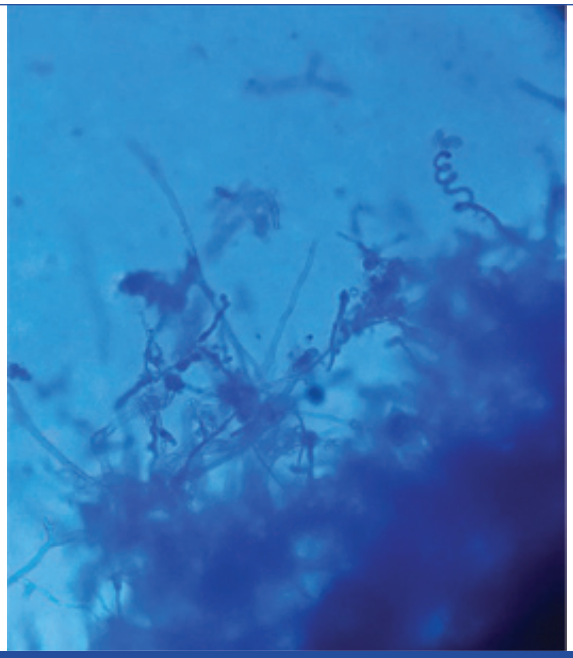
[Table/Fig-4]: *Trichophyton verrucosum* microscopy (LPCB, 40X).



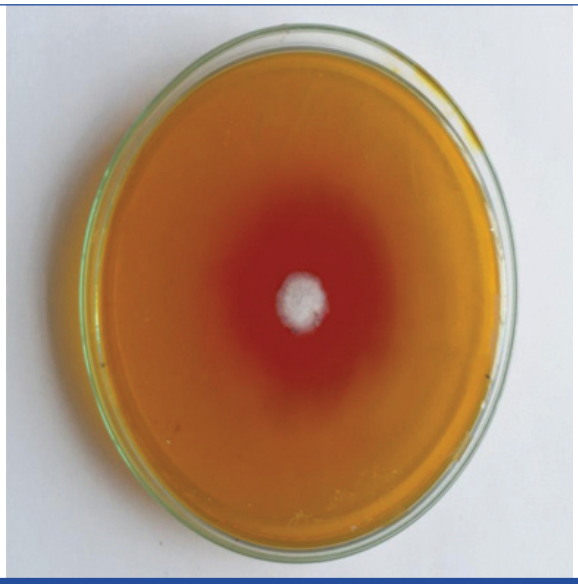
[Table/Fig-5]: *Trichophyton soudanense* microscopy (LPCB, 40X).

2. Dermatophyte Test Medium (DTM) for Dermatophytes

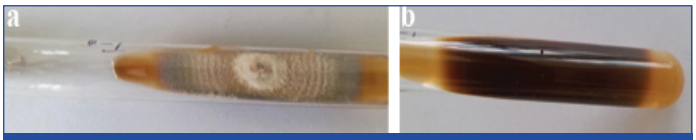
Specimen was inoculated in the DTM [Table/Fig-7,8a,b]. Growth of a dermatophyte in DTM was indicated by a change in colour, yellow to red [9]. The species of the dermatophytes were identified by noting the characteristic features. Urease test was done to identify *Trichophyton* spp. For morphological identification of all isolates, slide culture technique was performed.



[Table/Fig-6]: *Trichophyton mentagrophytes* microscopy (LPCB, 40X).



[Table/Fig-7]: Growth on dermatophytosis test medium.



[Table/Fig-8a-b]: *Trichophyton rubrum* melanocyte variant obverse (a) and reverse (b).

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS software. Result were obtained on following categories i.e.,; predominant type, age group, gender difference, type of organism, comparison of growth on two media.

RESULTS

Out of 150 samples studied the following results were obtained. Direct smear positivity was found in 143 (95%) of the cases, while culture positivity in 67 (45%) cases. There was a female preponderance in this study with female to male ratio being 1.3:1. Maximum incidence was in the age group 21-40 years, (31.3) and the least incidence was seen in age group above 61 years, 21 (14%) [Table/Fig-9].

Occupational profile of all the patients showed that the largest group in this study consisted of housewives, 42 (28%) followed by manual labourers, 39 (26%). The next larger group consisted of students, 35 (23%). In other groups, 10 (7%) were constituting retired persons, old age people [Table/Fig-9].

Variables	Number (%)
Gender	
Males	64 (43)
Females	86 (57)
Age group (years)	
0-20	36 (24)
21-40	47 (31.3)
41-60	46 (30.7)
>61	21 (14)
Occupation	
Housewife	42 (28)
Labour work	39 (26)
Student	35 (23)
Office work	15 (10)
Unemployed	9 (6)
Others	10 (7)

[Table/Fig-9]: Age and gender distribution in patients (N=150).
Others: retired persons and old age persons

Tinea corporis, 86 (57%) was the most common clinical type observed in the study. The second most common was the Tinea cruris with an incidence of about 44 (29%). Tinea corporis was more common in females 57 (38%) while tinea cruris was in males 25 (16%) [Table/Fig-10].

Diagnosis	Number of cases (male+female)	Percentage (%)
T.corporis	86 (29+57)	57
T.cruris	44 (25+19)	29
T.barabe	4 (4+0)	3
T.pedis	6 (4+2)	4
T.mannum	4 (0+4)	3
Candidiasis	6 (3+3)	4

[Table/Fig-10]: Distribution based on clinical type of infection (N=150).

Out of 150 cases, only 41 cases were recurrent (27%) of the study population. Major bulk of the study population consisted of new cases 109 (73%). Out of the total, 50% (n=75) were on treatment which included recurrent cases.

Out of 67 positive samples grown, *Trichophyton mentagrophytes* was the commonest isolate 38 (57%) followed by *Trichophyton rubrum* 18 (27%). Out of six clinically suspected cases of candidiasis no organism was isolated in the cultures. Out of the 38 *T. mentagrophytes* isolates there were seven isolates of *T. mentagrophytes* var interdigitale and out of 18 isolates of *T. rubrum*, two were melanocyte variant which produced black pigment rather than the red pigment of *T. rubrum*. No organism was isolated in tinea barbae. Out of the two isolates from tinea mannum, one was *Trichophyton mentagrophytes* and the other isolate was *Trichophyton tonsurans* [Table/Fig-11].

Diagnosis / Dermatophyte isolated	T.corporis	T.cruris	T.barbae	T.pedis	T.mannum	Total
T. mentagrophytes	25	9	0	3	1	38
T. rubrum	11	7	0	0	0	18
T. tonsurans	4	2	0	0	1	7
T. violaceum	0	1	0	0	0	1
Trichophyton soudanense	1	0	0	0	0	1
T. verrucosum	0	1	0	0	0	1
M. canis	1	0	0	0	0	1

[Table/Fig-11]: Concordance between clinical and mycological types of Dermatophytosis.

Out of 150 samples, 66 samples (44%) yielded positive in both direct microscopy and culture, 77 (51.3%) yielded positive in direct microscopy alone. One case was culture positive alone and 6 (4%) were negative for both direct microscopy and culture. Almost all dermatophytes isolated were grown in DTM within one week of incubation except *Trichophyton rubrum* which appeared in the second week. In SDA only, 31% of isolates were grown taking a minimum of 3-4 weeks. DTM was superior to SDA in isolation of dermatophytes from clinical samples in view of early isolation of the organism as 38 isolates *T. mentagrophytes,* all were grown in DTM and only 27 isolates were positive in SDA. Colony characteristics were better appreciated in SDA cultures [Table/Fig-12].

Isolates	Total	DTM (%)	SDA (%)
T. mentagrophytes	38	38 (100)	27 (71.1)
T. rubrum	18	18 (100)	8 (44.4)
T. tonsurans	7	7	5
T. violaceum	1	1	0
T. soudanense	1	1	1
T. verrucosum	1	1	0
M. canis	1	1	1

[Table/Fig-12]: Analysis of isolation of fungus with SDA and DTM.

DISCUSSION

The diagnosis of dermatophytosis is based on combination of clinical observation supplemented by laboratory investigation. In the laboratory, diagnosis depends on demonstration of causative pathogens in tissue by microscopy and isolation of the fungus in culture. Routine culture media used is SDA. DTM is a selective medium used in medical mycology for isolation of dermatophytes. Both media was used in the present study for better isolation of species from clinical samples. In the present study, maximum incidence was in the age group 21-40 years which constitutes about 47 (31.3%). This is closely followed by the age group 41-60 which constitutes about 46 (30%). In all age groups, the result shows a female predominance. These were in accordance with the results of the studies conducted by Hanumanthappa H and Patel P et al., [10,11].

The largest group in this study consisted of housewives 42 (28%) followed by manual labourers and students. Interestingly, it was not in accordance with the previous study, which showed a predominance of infection in manual labourers [10]. This may be because of a higher incidence in female population than male population obtained in this study.

Tinea corporis 86 (57%) was the most common clinical type observed in the study. Candidiasis constituted 6 (4%) of the total cases. This was in accordance with the studies of Hanumanthappa H et al., in which tinea corporis (33.3%) was the commonest clinical type [10]. In a cross-sectional study in Chennai by Kumar KA et al., tinea corporis accounted for 70.8% which formed the majority of clinical presentations [12]. The studies by Sahia S et al., Sen SS and Rasul ES, had reported 45%, and 51% of cases of tinea corporis respectively [13,14]. Tinea cruris 44 (29%) was the next common clinical type. This was in accordance with above studies [12-14]. Tinea pedis was among the least common clinical type according to the present study. This may be because the incidence of tinea pedis was higher in any population wearing occlusive shoes. Infected patients were from civilised, urban areas. Permanent retention of fungus laden scales in the socks is an important cause of tinea pedis under hot and humid climate. The high incidence of Tinea corporis and Tinea cruris as concluded from this study was probably due to its symptomatic nature (pruritis) which leads the patient to seek medical advice.

Out of 150 samples, 66 samples (44%) yielded positive in both direct microscopy and culture, 77 (51%) yielded positive result in direct microscopy alone. This was comparable with previous studies [10,11,15]. All these studies highlighted the importance of both direct microscopy and culture in definitive diagnosis of fungal infection [Table/Fig-13] [10,11,15].

Direct smear positive	Culture	Patel P et al., (2012) [11]	Hanumanthappa H et al., (2012) [10]	Putta SD et al., (2016) [15]	Present study (2021) with %
+	+	26.77%	36%	72%	66 (44%)
+	-	35.35%	30.6%	12%	77 (51.3%)
-	+	2.53%	12.6%	6.25%	1 (0.6%)
-	-	35.35%	20.6%	6.25%	6 (4%)

[Table/Fig-13]: Comparison of smear and culture with previous studies [10,11,15].

Trichophyton mentagrophytes was the commonest isolate 38 (57%) followed by *Trichophyton rubrum* 18 (27%). This was in accordance with the studies by Putta SD et al., Kolhapur in which isolated dermatophytes *T.mentagrophyes* was the most common isolate contributing 37.74%, followed by *T.tonsurans* 28.30% and *T.rubrum* 24.53% [15]. Bhatia VK and Sharma PC, had similar finding, *T.mentagrophytes* was most common isolate though they found different isolation rate [16]. Present study observed other isolates like *Trichophyton soudanense, T.verrucosum* and *M.canis* though rate of isolation was very less and thus not included in the study. Also, there was no *Epidermophyton* spp. grown.

Most remarkable observation in this study was *T.mentagrophytes* as the most common aetiological agent among dermatophytes. In this study, it could be noted that all dermatophytes isolated were grown in DTM within one week of incubation except *Trichophyton rubrum* which appeared in the second week, while in SDA only 31% of isolates were grown. The maximum incubation period was more than a week for SDA whereas DTM gave positive results on culture within a week of inoculation. SDA requires to be incubated at least for four weeks before reporting as negative. Several studies have included both the media for isolation [10,17].

Advantage of DTM is that positive results are available within seven days of incubation. In the case of SDA the colony characteristics can be well made out.

Limitation(s)

The disadvantage of DTM is that the colony characteristics such as pigmentation cannot be made out in the media, also some contaminants were grown showing a colour change. In SDA the colony takes more than a week to grow than DTM.

CONCLUSION(S)

Present study found out that DTM was more useful as a general screening medium as opposed to an identification medium and the isolation of dermatophytes was rapid when compared to SDA. However it is recommended that, tests to be performed on growth from SDA for complete identification.

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