

Dematiaceous fungi as etiological agents of superficial mycosis in North India

Bharti Sharma and Skarma Nonzom*

Department of Botany, University of Jammu, Jammu-180006, Jammu and Kashmir, India

*Corresponding author Email: skarmanunzom@yahoo.com

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ABSTRACT

Superficial mycosis are common fungal infections of the skin, hair and nails which are responsible for causing morbidity and cosmetic disfigurement, affecting the patient's quality of life. In addition to dermatophytes, non-dermatophyte molds, which are usually regarded as contaminants are emerging as causal agents of such infections. In the present study, four dematiaceous fungi viz., *Alternaria alternata*, *A. tenuissima*, *Curvularia brachyspora* and *Stemphylium sarciniforme* were recovered from the human skin specimens. Direct microscopy, cultural, morphological and microscopic characteristics were taken into account for the identification of the recovered fungal causal agents. Of the recovered etiological agents of superficial skin mycosis, *Stemphylium sarciniforme* represents a new global report, *A. tenuissima* and *Curvularia brachyspora* new reports from India and *A. alternata* is a new report from Jammu and Kashmir.

Keywords: Superficial mycosis, dematiaceous fungi, *Alternaria alternata*, *A. tenuissima*, *Curvularia brachyspora*, *Stemphylium sarciniforme*

INTRODUCTION

Superficial mycosis are the fungal infections limited to the skin, hair and nails which affect millions of individuals globally, with the prevalence rate of 20-25% (Havlickova *et al.*, 2008). The fungal agents responsible for causing these infections include species of dermatophytes, non-dermatophytes and yeasts (Abu-Elteen and Hamad, 2012). In the recent years, in addition to dermatophytes, non-dermatophytes, including dematiaceous fungi are emerging as important pathogens responsible for causing superficial mycosis (Hazarika *et al.*, 2019; Sharma and Nonzom, 2021a, b). These infections show geographical variations with respect to epidemiology, prevalence, etiological agents and are influenced by various factors viz., lifestyle, migration of people, type of population, cultural background, associated morbidities and climate (Tom *et al.*, 2019).

Dematiaceous fungi are darkly pigmented fungi responsible for infections ranging from superficial, brain abscess, pulmonary to allergic diseases (Arcobello and Revankar, 2020). The uniqueness of these fungi lies in the presence of melanin in their cell wall which results in dark pigmentation of their hyphae and spores and also has role in pathogenicity of fungi (Wong and Revankar, 2016). Though these infections hardly cause mortality, their significance lies in recurrence, morbidity and cosmetic disfigurement. These fungi act as opportunistic human pathogens with the ability to invade tissues and eventually causing infection, taking benefit from the predisposing factors. Survey of literature showed that several studies on superficial fungal infection by dermatophytes are reported from different countries worldwide and from different parts of India (Grover and Roy, 2003; Biswas *et al.*, 2020; Wang *et al.*, 2020). However, reports on non-dermatophytes as causal agents of superficial skin mycosis are comparatively rare especially from the Union Territory of Jammu and Kashmir (J&K). The present study was carried out with the objective to investigate the prevalence of superficial mycosis in the residents of District Jammu, J&K.

MATERIALS AND METHODS

Ethical approval

In order to carry out the present study, ethical approval was

granted by the Institutional Ethical Committee (IEC), University of Jammu (No. RA/19/3118 dated 23/08/2019) which follows the guidelines issued by the Medical Council of India, India.

Specimen collection and processing

The present study was conducted during the period of December 2019 to December 2020, wherein the clinical specimens (skin/hair) were collected from the suspected individuals of District Jammu, J&K. Before proceeding with the collection of samples, the affected or suspected site of the skin was cleaned with 70% alcohol to remove bacterial contamination or other contaminants from the surface of the skin. With the help of sterilized surgical blade or forceps, skin scrapings were taken from the active periphery of the lesions carefully in order to avoid any additional injury to the skin surface. Direct microscopy was done using 10% potassium hydroxide (KOH). It was followed by inoculation of the samples on three Petri dishes of Sabouraud dextrose agar (SDA) supplemented with chloramphenicol 0.05mg/ml. Petri plates were then incubated at a temperature of $28 \pm 2^\circ\text{C}$ for 8-14 days. The above steps were repeated three times by collecting skin samples after an interval of 15 days for the confirmation of the pathogenicity of the recovered fungus. If the culture yielded the same fungal species repeatedly, then only it was followed by other microscopic examinations, otherwise the cultures were discarded.

In-vitro antifungal susceptibility test

For *in-vitro* antifungal susceptibility test, four antimycotic drugs namely amphotericin B, fluconazole, itraconazole and ketoconazole, each 10mcg/disc were used by following disc diffusion method (Gould and Bowie, 1952). Antifungal activity was calculated by measuring the diameter of the inhibition zone formed around the disc.

RESULTS

Cultural (size and colour of colony, growth rate, pigmentation or exudates if any), diagnostic morphological and microscopic characteristics were taken into consideration for the identification of the isolated fungal species. Also, relevant

literature and mycological taxonomic keys (Ellis, 1971; Subramanian, 1971) were consulted for the same. The details of the patients investigated for superficial mycosis are shown in **table 1**.

(Revankar and Sutton, 2010; Arcobello and Revankar, 2020). Some of the common dematiaceous fungi causing infections in humans include *Alternaria*, *Exophiala*, *Bipolaris*, *Scedosporium*, *Madurella*, *Curvularia*, *Scytalidium*, and

Table 1: Details of the investigated patients for superficial mycosis

S. No.	Sex/Age (years)	Occupation	Duration of Infection	Clinical Type	Clinical Symptoms
1.	M/61	Farmer	3 years	Tinea pedis	Black coalescing lesions, itching, scaling of skin (Fig. 1a)
2.	F/62	Housemaker	3 years	Tinea pedis	Dry scaly skin, crusting, small black-coloured lesions (Fig. 1b)
3.	M/16	Student	5 months	Tinea corporis	Itching, black coloured lesion, crusting and peeling of skin (Fig. 1c)
4.	M/12	Student	11 months	Tinea mannum	The colour of the infected skin was darkened, peeling and crusting of skin (Fig. 1d)
5.	M/14	Student	10 months	Tinea mannum	Off white to grey-coloured lesions were present on fingers along with itching (Fig. 2a)
6.	F/27	Farmer	2 years	Tinea mannum	Scaling, peeling, hyperkeratosis was observed (Fig. 2b)
7.	M/15	Student	1 year	Tinea faciei	A black lesion was present below the lips on right side. Other clinical symptoms include, scaling, crusting and fissures (Fig. 3a)
8.	M/54	Farmer	7-8 years	Tinea pedis	Dark brown to black colored coalescing lesions were observed at the site of infection. Other clinical symptoms include rashes, crusting, scaling and itching (Fig. 4a-b)

M=Male; F=Female

Cultural and microscopic examination

Direct microscopic examination of the clinical specimens showed the presence of fungal elements and revealed positive results. Culturing of the suspected skin scales or hair sample on SDA medium yielded the non-dermatophytic fungi consistently. The details of cultural and microscopic characteristics are shown in **table 2**.

Of the recovered etiological agents, *Alternaria alternata* was found to cause superficial infection in 4 investigated patients (**Case 1-4**), therefore *in-vitro* antifungal susceptibility test was performed for this species. The results revealed fluconazole to be the most effective drug which completely inhibited the growth of the fungi, followed by ketoconazole (zone diameter = 19 ± 0.17 mm), itraconazole (zone diameter = 18 ± 0.17 mm) whereas no growth inhibition of fungi was observed for amphotericin B (**Fig. 5a-d**).

DISCUSSION

Superficial mycosis are common public infections caused by fungi which are pathogenic and are capable of parasitizing keratin rich tissues by fulfilling four criteria viz., ability to grow at high temperature, penetration or invasion of host tissue barrier, digestion along with absorption of host tissue components and withstanding the immune system of host (Kohler *et al.*, 2015). Non-dermatophytes including dematiaceous fungi are distributed worldwide as saprophytes residing in the soil and on decaying plant material. Around 70 genera with more than 150 species of dematiaceous fungi have been implicated in causing diseases in humans

Cladophiala (Brandt and Warnock, 2003; Sharma and Nonzom, 2021c). These fungal infections are prevalent in tropical and subtropical countries, such as, India where hot and humid climate is appropriate for the acquisition of fungal infections (Singh and Beena, 2003).

In the present investigation four non-dermatophyte species namely *Alternaria alternata*, *A. tenuissima*, *Curvularia brachyspora* and *Stemphylium sarciniforme* were recovered from the clinical specimens. *Alternaria*, a very large and complex dematiaceous genus with worldwide distribution shows frequent involvement in human disease (Sood *et al.*, 2007; Paster and Guarro, 2008). Few studies have reported *A. alternata* to be an etiological agent of cutaneous mycosis (Gurcan *et al.*, 2009; Kaur, 2015). On the other hand, few other similar studies have reported *A. tenuissima* involved in causing human infections (Viviani *et al.*, 1986; Romano *et al.*, 1997).

Similarly, the genus *Stemphylium* is comprised of 150 species and is distributed widely as phytopathogen affecting more than 43 plant genera (Das *et al.*, 2019). One of the studies has reported opportunistic cutaneous infection (phaeohyphomycosis) in cat caused by several fungal species including *Stemphylium* (Lloret *et al.*, 2013). Very few studies have reported the association of the genus *Stemphylium* with human infections including allergic reactions, lung and cutaneous infection (Sutton *et al.*, 1998; Wu *et al.*, 2004; Hotta *et al.*, 2017). So far, *Stemphylium sarciniforme*, a species recovered in present study has not been reported as an etiological agent of superficial mycosis. Thus, it constitutes a new addition to the category of non-dermatophytes capable of

Table 2: Details of cultural and microscopic characteristics of the recovered etiological agents

S. No.	Case studies	Cultural and Microscopic characteristics	Recovered Etiological Agents
1	Case 1,2,3,4	Cultural: On SDA medium colonies were 38-46mm in diameter, olivaceous black, velvety to light floccose, effuse, reverse black (Fig 1e) Microscopic: Mycelium brown, septate, smooth; hyphae 2.4-3.6mm in width. Conidiophores simple, arise singly, straight or branched, golden brown, septate. Conidia formed in long chains, pale brown to mid golden brown pyriform, obclavate, obpyriform, ovoid to ellipsoidal with short conical pale beak, with 1 - 6 transverse and few longitudinal and oblique septa, smooth when young, verruculose with age, $14.4 - 51.2 \times 7.2-18.4\mu\text{m}$ in size (Fig. 1f-h).	<i>Alternaria alternata</i> (Fr.) Keissl.
2.	Case 5, 6	Cultural: On SDA medium, colonies were 35-42mm in diameter, olivaceous green, plane, floccose to fluffy, hairy, reverse black (Fig. 2c) Microscopic: Mycelium brown, septate; hyphae measuring $2.5-4.3\mu\text{m}$ in width. Conidiophores solitary or in groups, simple or branched, pale to mid pale brown prominent with conidial scars, measuring $30.4-115.2 \times 3.2-5.6\mu\text{m}$. Conidia solitary or present in short chains, straight or curved, obclavate to ellipsoidal gradually tapering to a cylindrical beak, pale to dark olive buff, smooth or verruculose, generally with 3-7 transverse or several longitudinal or oblique septa, slightly or not constricted at the septa, $19.2-67.2 \times 5.2-11.5\mu\text{m}$ in size (Fig. 2d-f).	<i>Alternaria tenuissima</i> (Kunze) Wiltshire
3.	Case 7	Cultural: On SDA medium, colonies were 3-34mm in diameter, greyish brown to black, floccose, reverse black (Fig. 3b), Microscopic: Conidiophores light brown, smooth, aseptate, $64-152 \times 2.56-4.16\mu\text{m}$, flexous at the apex bearing conidia in clusters or groups (Fig. 3c, e). Conidia light brown, smooth walled, ellipsoidal to obovoid with a small scar at the basal end, asymmetrical with truly median septum, three septate, $12-27.2 \times 6.4-11.2\mu\text{m}$ in size (Fig. 3d, f).	<i>Curvularia brachyspora</i> Boedijn M.A. Curt
4.	Case 8	Cultural: On SDA medium, colonies were 19-25 mm in diameter. Colonies woolly, white when young and on maturity become greyish black, reverse black (Fig 4c) Microscopic: Mycelium septate, pigmented (brown); Conidiophores pigmented, golden brown, smooth walled, $38.4-56 \times 6.4-9.6\mu\text{m}$, having vesicular swellings with diameter $11.2-14.4\mu\text{m}$ (Fig 4d); Conidia golden brown, subspherical to broadly ellipsoidal with usually 3 transverse and several longitudinal septa, constricted at the median transverse septum, $30.4-51.2 \times 19.2-33.6\mu\text{m}$ in size (Fig 4e-h).	<i>Stemphylium sarciniforme</i> (Cavara) Wiltshire

causing superficial mycosis.

Another recovered genus i.e., *Curvularia* was firstly reported as human pathogen causing corneal infection in the year 1959 (Wilhelmus and Jones, 2001). Though less common, *Curvularia* species are also known to cause typical skin lesions, dialysis related peritonitis and infections of nervous system (Silveria and Marci, 2001; Revanker, 2006). There have been some reports on *Curvularia brachyspora*, causing cutaneous infections in humans in the 1990's (Berg *et al.*, 1995; Tordo and Jones, 1997). However, no similar association has been reported thereafter.

In the 8 case reports presented, the age of the patients varied from 12-62 years and duration of infection from 5 months to 8 years. In our study male preponderance was observed that corroborate with similar other findings (Sen and Rasul, 2006; Budak *et al.*, 2013). The prevalence of these infections is governed by environmental conditions, occupation,

individual's susceptibility and economical factors (like overcrowding, poverty and personal hygiene). Of the eight cases studied, 3 were farmers, 1 housemaker while rest were students. In farmers, the source of fungal infection could be their frequent contact with soil, vegetation, cowdung, cattle etc. Moreover, the nature of their job is such that they are further involved in more physical activity in the field which lead to sweat and perspiration and present conducive environment for growth of fungal agent. Similarly, in housemaker, their frequent engagement in wet works, such as cleaning, washing etc., might have contributed in superficial fungal infection. As all the students were male, their tendency to play outdoor games, helping their parents in the field and cleaning cowshed could have resulted in acquiring fungal infection. Some of the other predisposing factors probably responsible for superficial fungal infections include frequent contact with domestic animals, low socio-economic



Fig. 1: a-b: Clinical symptoms on feet of Case 1 and 2, scalp of Case 3 and hands of Case 4. c: Colony of *Alternaria alternata* on SDA medium. d: Microphotographs showing variously septate conidia. e-h: Camera lucida drawings of conidiophore and conidia (Bars, f-h=10 μ m).

condition, sharing clothes, towel and other things with their family members and lack of awareness regarding personal hygiene.

The clinical appearance or clinical pattern caused by one fungal species is indistinguishable from that caused by other fungal species (Singh and Beena, 2003). Therefore, for the identification and characterization of a fungal species, *in-vitro* culture is crucial for its proper diagnosis followed by appropriate treatment. As all the recovered dematiaceous fungal species are saprophytes commonly residing in soil and vegetation where the probability of their exposure is certainly high resulting in human infections. Therefore, repeated sample collection followed by culturing on SDA media yielding consistently the same non-dermatophyte in the pure state confirmed their role in causing superficial infections as seen in the present study.



Fig. 2: a-b: Clinical symptoms on right hand fingers of Case 5 and 6. c: Colony of *Alternaria tenuissima* on SDA medium. d: Microphotographs showing conidia. e-f: Camera lucida drawing of conidiophore and conidia (Bars, d-f=10 μ m).

Correct identification of fungal causal agents is crucial for appropriate treatment, for which culture is a vital adjunct to direct microscopy. The choice of treatment/therapy, in many cases is dependent upon the specific identification of etiological agent. Therefore, it becomes more important especially when such infections are caused by non-dermatophytic fungi. In such cases, treatment is usually resistant to the dosage of the therapy used for infections caused by dermatophytes (Rathod *et al.*, 2019).

The mechanisms of action of antimycotic drugs i.e., azole (fluconazole, itraconazole and ketoconazole) is fungistatic. They inhibit cytochrome P-450 dependent 14 α sterol demethylase which is responsible for conversion of lanosterol to ergosterol, the main sterol in fungal cell membrane (Sanati *et al.*, 1997). Earlier, the antifungal susceptibility have been evaluated for the species of dermatophytes and non-

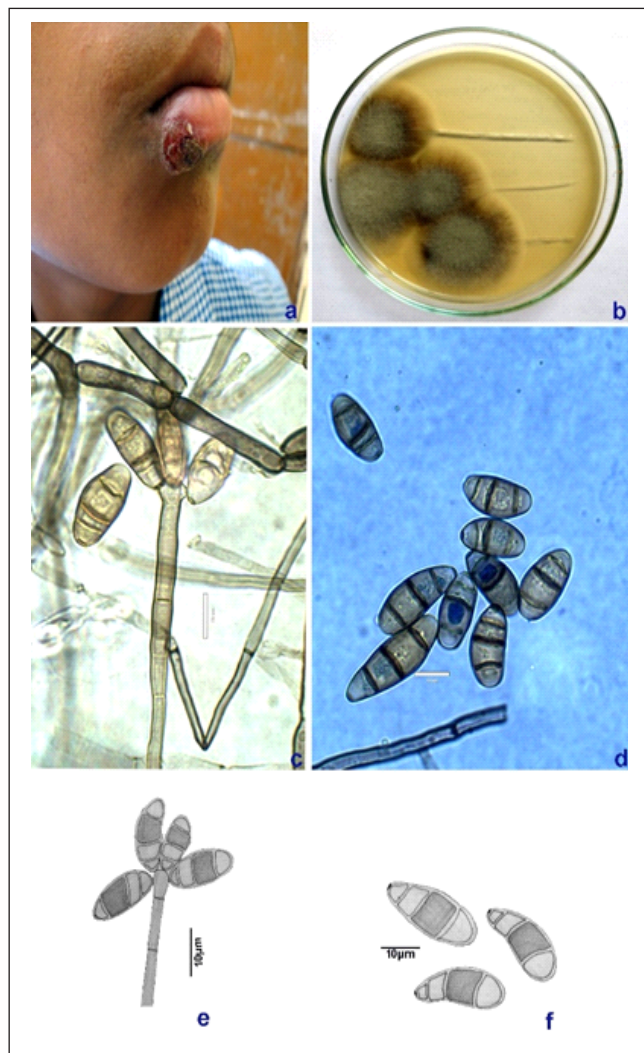


Fig. 3: a: Clinical symptoms on near lower lip of face of Case 7. b: Colony of *Curvularia brachyspora* on SDA medium. c-d: Microphotographs showing conidiophore and conidia. e-f: Camera lucida drawing of conidiophore and conidia (Bars, c-f=10 µm).



Fig. 4: a-b: Clinical symptoms on foot and portion of leg of Case 8. c: Colony of *Stemphylium sarciniforme* on SDA medium. d-f: Microphotographs showing conidiophore, conidia and germinating conidia. g-h: Camera lucida drawing of conidiophore and conidia (Bars, d-h=10 µm).

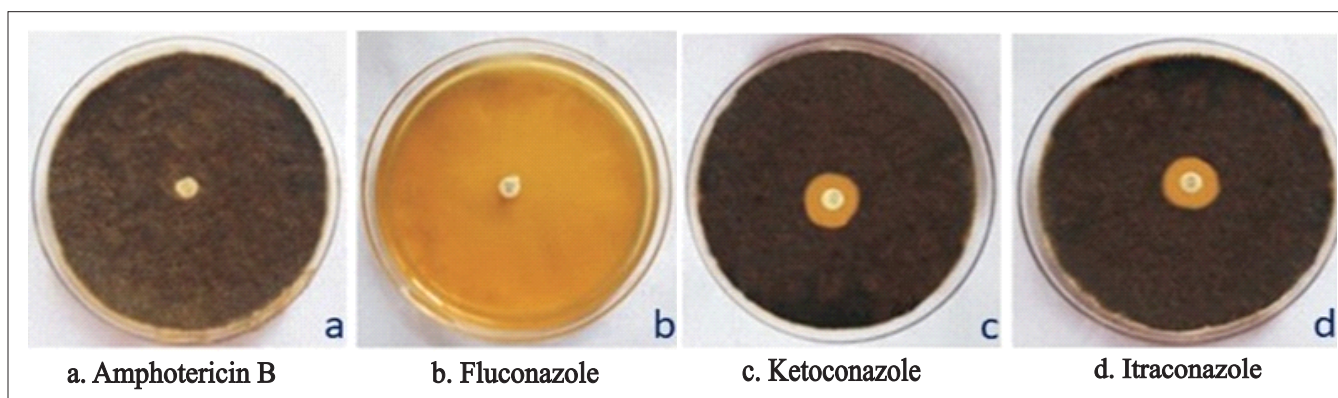


Fig. 5: *In-vitro* activity of antimycotic drugs against *Alternaria alternata*

dermatophytes by following disc diffusion method (Gupta *et al.*, 2015; Desam *et al.*, 2019). Similarly, Isabel (2000) and

Paster and Garro (2008) have also reported that the drug ketoconazole to be effective against species of *Alternaria*.

CONCLUSION

The present study reflects the changing trend of superficial mycosis with rise in the isolation of non-dermatophytes from affected skin area. For the establishment of accurate antifungal therapies, a proper and precise identification of fungal isolates at species level is much needed. Since superficial mycosis have public health importance, correct diagnosis and assessment of risk factors or source of infection can play a crucial role in the effective treatment of such infections. To the best of our knowledge, there is no published reports of *Stemphylium sarciniforme* worldwide, *A. tenuissima* and *Curvularia brachyspora* from India and *A. alternata* from J&K as an etiological agents of superficial skin fungal infections. These results indicate their potential to shift from saprophytic form to pathogenic which is further influenced by many factors including the immuno-suppressed status of the individual.

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