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Review on Dermatophytes Highlighting the Status at National and International Level: A Critical Appraisal.

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ABSTRACT

Dermatophytes as the name suggest are the fungus that feed on skin. The chief source of their growth is keratin which is widely available in skin, nails and hairs and causes disease in animals and humans due to their ability to obtain nutrients from keratinized material. The organisms colonize the keratin tissues and inflammation is caused by host response to metabolic byproducts. They are usually restricted to the nonliving cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host.

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INTRODUCTION

Dermatophytes are a common label for a group of fungus that commonly causes skin, hair and nails disease in animals and humans due to their ability to obtain nutrients from keratinized material [1]. Keratinases are proteolytic enzymes in nature secreted by dermatophytes to feed on keratin which is the main constituent of structures that grow from the skin [2]. The dermatophytes consist of three genera: Epidermophyton, Microsporum and Trichophyton [3]. Dermatophytic infections are probably the most common cause of skin disease in developing countries of tropical regions [4].

Cases of dermatophytosis have increased over the past few decades. These infections are often recalcitrant to therapy. In the last few years, several antifungal [5, 6] agents have become available for the treatment of these infections [7]. The objective focuses on an approach to bring the research work of various scientists working on dermatophytes in India and at International level at one place in this review article.

STATUS OF DERMATOPHYTES RESEARCH AT INTERNATIONAL LEVEL

PREFACE

Dermatophytes are highly specialized pathogenic fungi that exclusively infect the stratum corneum, nails or hair, and it is evident that secreted proteolytic activity is important for their virulence. Endo- and exoproteases-secreted by dermatophytes are similar to those of species of the genus *Aspergillus*. However, in contrast to *Aspergillus spp*., dermatophyte-secreted endoproteases are multiple and are members of two large protein families, the subtilisins (serine proteases) and the fungalysins (metalloproteases). In addition, dermatophytes excrete sulphite as a reducing agent. In the presence of sulphite, disulphide bounds of the keratin substrate are directly cleaved to cysteine and S-sulphocysteine, and reduced proteins become accessible for further digestion by various endo- and exoproteases secreted by the fungi. Sulphitolysis is likely to be an essential step in the digestion of compact keratinized tissues which precedes the action of all proteases [8].

The immune response to infection by dermatophytes ranges from a non-specific host mechanism to a humoral and cell-mediated immune response. The currently accepted view is that a cell-mediated immune response is responsible for the control of dermatophytosis. Indeed, some individuals develop a chronic or recurrent infection mediated by the suppression of a cell-mediated immune response. The immune response to *Trichophyton* is unusual in that this fungus can elicit both immediate hypersensitivity (IH) and delayed-type hypersensitivity (DTH) in different individuals when they are submitted to a skin test reaction. Understanding the nature and function of the immune response to dermatophytes is an exciting challenge that might lead to novel approaches in the treatment and immunological prophylaxis of dermatophytosis [9].

A wide variety of dermatophytes have been isolated from animals, but a few zoophilic species are responsible for the majority of the cases, viz. *Microsporum canis, Trichophyton mentagrophytes, Trichophyton equinum* and *Trichophyton verrucosum*, as also the geophilic species *Microsporum gypseum*. According to the host and the fungal species involved, the typical aspect of dermatophytic lesions may be modified. As a consequence, an accurate clinical examination, a good differential diagnosis and laboratory analyses are required for a correct identification. Few antifungal agents are available and licensed for use in veterinary practice, and the use of systemic drugs is limited in livestock due to the problems of residues in products intended for human consumption. The high resistance of the dermatophyte arthroconidia in the environment, the multiplicity of host species, and the confinement of animals in breedings are cause of an enzootic situation in many cases. Prevention is difficult, but research development on the immune response to dermatophytes and the use of vaccination, especially in cattle, has brought some interesting results [10].

GROWTH MEDIUM

Culture conditions were examined for *Trichophyton mentagrophytes* and *Trichophyton rubrum*, which are major pathogens involved in dermatophytosis. They grew well in Sabouraud's dextrose broth or RPMI 1640. Growth in phosphate-buffered yeast nitrogen base supplemented with glucose was very slow, although growth improved significantly with the addition of amino acids or proteins to the medium. The fungi could also grow using human nail fragments as the only source of nutrition. Examination of proteases by substrate gel electrophoresis indicated that distinct sets of proteases are secreted from the dermatophytes in two different media, Sabouraud's dextrose broth and nail fragments. A protease inhibitor, phenylmethanesulfonyl fluoride, inhibited the growth of the fungi on nail fragments, but it did not inhibit their growth in Sabouraud's dextrose broth [11].

A new medium (DBM) was compared with dermatophyte test medium (DTM) for the diagnosis of dermatophyte infection. The sensitivity was 103 cfu/mL (2 x 101 cfu/slant) for both DTM and DBM with a suspension of *Trichophyton rubrum*. In axenic cultures, all dermatophytes tested altered the color of both media. Although most non dermatophytic molds made a color change, it was at a slower rate. In nail samples of dermatophyte infection, all dermatophytes altered the color of both media. However, the time for discoloration was shorter with DBM than with DTM (5.83 +/- 0.39 days vs. 7.32 +/- 0.41

days, t = 2.63, P = 0.01). Most isolates of nondermatophyte also made a discoloration, but they could be distinguished from dermatophytes by their colonial diameters when the color began to change (> or = 5 mm). The results were in good agreement with a professional laboratory of medical mycology; however, the latter is regularly able to differentiate exactly the species of the growing dermatophyte. The DBM medium is more convenient, rapid, more accurate and economical to use than DTM [12].

Azure dye-impregnated sheep's wool keratin (keratin azure) was incorporated in a high pH medium and overlaid on a keratin-free basal medium. The release and diffusion of the azure dye into the lower layer indicated production of keratinase. Fifty-eight fungal taxa, including 49 members of the *Arthrodermataceae*, *Gymnoascaceae* and *Onygenaceae* (Order Onygenales), were assessed for keratin degradation using this method. The results were comparable to measures of keratin utilization reported in studies using tests based on the perforation or erosion of human hair in vitro [13].

A mycological study was carried out in 400 clinically suspected cases of dermatomycosis. Dermatophytes were found in 250 (62.5 percent) cases by direct microscopical examination and culture was positive in 325 (81.25 percent) cases. Five cases were found KOH Negative showed positive results on culture. The commonest clinical type found was tinea corporis (37.5 percent). This was followed by tinea cruris (25 percent), tinea capitis (22.5 percent), tinea pedis affected (11.25 percent) and tinea barbae (3.75 percent). The commonest etiological agent encountered was T*ricophyton rubrum* (67.38 percent) followed by *Trichophyton mentagraphytes* (23.01 percent), *Trichophyton violaceum* (7.69 percent) *Epidermophyton flocossum* (2.15 percent) and *Candida albicans* (1.84 percent). Males were found to be more susceptible than females [14]

***IN VITRO* STUDY**

The biodiversity and richness of keratinophilic fungal communities including dermatophytes were assessed in three stream sites and three swimming pools in the Nablus district in Palestine, using hair baiting (HBT) and surface dilution plate (SDP) techniques, over 8- and 6-month periods, respectively. The effect of wastewater effluent and selected ecological factors on these fungi in relation to species diversity and population densities was also considered. Fifty keratinophilic fungal species were recovered from the aquatic habitats studied, of which 42 were recovered from stream sites and 22 from swimming pools. Of these fungi 6 were either dermatophytes (*Microsporum gypseum*, and *Trichophyton mentagrophytes*) or dermatophyte related species (*Chrysosporium merdarium*, *Ch. tropicum, Ch. keratinophilum and T. terrestre*). The most frequently isolated species in the three pools were *Acremonium strictum* and *Cladosporium cladosporioides*, using Sabouraud dextrose agar medium (SDA). The most abundant species were *Acr. strictum*, and *Aspergillus flavus*. However, only 4 species were isolated using the SDA medium amended with 5-flurocytosine (5-FC). The most frequent and abundant species in the three stream sites using SDA medium were *Geotricum candidum*, and *Penicillium chrysogenum*. The most frequent species in the three sites using the 5-FC medium was *Paecilomyces lilacinus*. Using HBT, the most abundant and frequent species in the three stream sites were *G. candidum*, and *Pa. lilacinus*, on SDA medium, and *Pa. lilacinus*, and *Gliocladium nigrovirens* on the 5-FC medium. The 5-FC medium was more suitable for the isolation of dermatophytes and closely related species than the SDA medium; 6 were recovered on 5-FC, whereas only one on the SDA medium. Variation in the levels of keratinophilic fungal populations from the three stream sites sampled 5 times over an 8-month period, followed comparable fluctuation patterns. Wastewater affected fungal population densities with the highest levels in the un-polluted stream sites, and lowest in the heavily polluted sites. Swimming pools, polluted and un-polluted stream sites were found to be rich in pathogenic and potentially pathogenic fungi [15].

The keratinolytic activity of five species of the dermatophytes which include *Trichophyton rubrum*, *T. mentagrophytes, T. tonsurans, Microsporum audouinii* and *M. gypseum* isolated from school children were tested using human hair as the substrate. *M. gypseum* was found to possess the highest keratinolytic activity with a net value of released protein being 78.8 ug/ml after five weeks of incubation. Also the net value of released protein for *T. tonsurans, T. rubrum, T. mentagrophytes* and *M. audouinii* were 55.5 ug/ml, 52.5 ug/ml, 43.8 ug/ml and 26.3 ug/\ml respectively. Only *T. mentagrophytes* and *M. gypseum* were able to cause structural damage in form of perforations on the hair shaft. Also during the degradation of the hair, the pH of the basal medium for each dermatophyte increased. The increase in pH was highest in the medium with *M. gypseum* but lowest in that of M. audouinii [16].

Microsporum canis is the most prevalent dermatophyte of domestic animals. Several enzymes produced by dermatophytes, particularly keratinases, are considered to play a role in the virulence of this fungus. The relationship between keratinase, elastase, lipase and DNase levels produced in vitro by different isolates and virulence as expressed in a guinea pig model were studied. Samples isolated from symptomatic dogs and cats showed a statistically significantly ($P < 0.05$) higher keratinase activity than samples isolated from asymptomatic animals. Experimental infection of guinea pigs showed that a strain with high in vitro keratinase activity induced acute infection, which resolved clinically and mycologically faster than the infection induced by a strain with low keratinase activity. This suggested a strong correlation between high keratinase activity and the development of symptoms. The same correlation was not observed for other enzymes tested [17].

CASE REPORTS

Uslu et al [18] determine the colonisation of causative agents for fungal skin infections on the tools and surfaces of barbershops. A total of 357 samples from tools and surfaces of 32 barbershops in Erzurum, Turkey were collected and examined for fungal pathogens. From the combs, *Trichophyton rubrum* (1), non-dermatophytic moulds (35) and *Candida albicans* (1); from the hairbrushes, *T. rubrum* (3), *T. mentagrophytes* (1), non-dermatophytic moulds (21) and yeast (1); from the shaving brushes, non-dermatophytic moulds (2) and *C. albicans* (2); from the headrest of barber chairs, *T. rubrum* (1), non-dermatophytic moulds (19) were isolated. No fungi were isolated from towels. In conclusion, this study showed that shared tools and contacted surfaces in barbershops are important sources for fungal colonization and may play an important role in spreading mycotic infections among people.

Buzina W et al [19] reported the case of a 28-year-old immunocompetent male suffering from otitis externa. The right external auditory meatus was filled with cerumen and detritus, the tympanic membrane covered wallpaper-like with layers of fungi. Mycological analysis revealed *Trichophyton rubrum*. With further examination tinea pedis of plantar and interdigital type and concomitant onychomycosis of the toenails due to *T. rubrum* could be detected. The auditory meatus was cleaned and treated topically with clotrimazole. Two weeks later the auditory meatus and the tympanic membrane were bare of fungi and the inflammation was resolved. Treatment of tinea pedis and onychomycosis with terbinafine (systemically and topically) is still lasting.

The prevalence of onychomycosis has been estimated at approximately 6.48% (95% confidence interval 6.09-6.88%) within the Canadian population. Dermatophytes are the most commonly cultured organisms, appearing in approximately 75 to 91% of nails with fungal involvement, with *Trichophyton rubrum* and *Tricophyton mentagrophytes* most commonly isolated. However, *Candida spp* and nondermatophyte molds are also sometimes cultured. The most common presentation is distal and lateral subungual onychomycosis (DLSO), which can involve 75% of patients with pedal onychomycosis. The distribution of DLSO, superficial white onychomycosis, and proximal subungual onychomycosis (PSO) has been reported to be 360:59:1 in patients with mycologic confirmation of onychomycosis; however, some reported that the incidence of PSO is slightly higher in immunocom-promised individuals. Age, gender, family history, and the presence of tinea pedis are all elements associated with a nail fungal infection. In addition, many conditions, including diabetes mellitus, immune disorders, and vascular disease, have been associated with the presence of onychomycosis. When choosing the best treatment regimen for individuals with onychomycosis, it is very important to consider all of the factors involved, including the infecting species, the presentation of the disease, the level of disease progression, and its predisposing factors [20].

Onychomycosis, most commonly caused by two species of dermatophyte fungi--*Trichophyton rubrum* and *Trichophyton mentagrophytes*--is primarily treated with regimens of topical and systemic antifungal medications. This study was undertaken to evaluate in vitro the efficacy of low-voltage direct current as an antifungal agent for treating onychomycosis. Agar plate cultures of *T rubrum* and *T mentagrophytes* were subjected to low-voltage direct current electrostimulation, and antifungal effects were observed as zones in the agar around the electrodes lacking fungal growth. Zones devoid of fungal growth were observed for *T rubrum* and *T mentagrophytes* around anodes and cathodes in a dose-dependent manner in the current range of 500 microA to 3 mA. Low-voltage direct current electrostimulation has great clinical potential for the treatment of onychomycosis and perhaps other superficial maladies of fungal etiology [21].

ANTIDERMATOPHYTIC SCREENING

The chemical composition of the essential oils obtained by hydrodistillation from the aerial parts of *Mentha cervina* collected during the flowering and vegetative phases of the plants were investigated by GC and GC-MS. Quantitative differences were observed in the compositions, particularly in the amounts of pulegone (12.9-79.6%) and isomenthone (8.7-77.0%). Antifungal activity of the oils was evaluated by minimal inhibitory concentrations (MIC) and minimal lethal concentrations (MLC) against *Candida, Apergillus* and dermatophyte strains. Antifungal activity of the sample containing lower amounts of pulegone was the highest for dermatophytes, particularly for *Epidermophyton floccosum* with MIC and MLC values of 0.63 microL mL(- 1). *Mentha cervina* oils with low content of pulegone, may be an alternative as antifungal agents in dermatophytosis [22].

The chemical profile of each essential oil of *Mentha aquatica L., Mentha longifolia L.,* and *Mentha piperita L*. was determined by GC-MS and TLC. All essential oils exhibited very strong antibacterial activity, in particularly against *Esherichia coli* strains. The most powerful was *M. piperita* essential oil, especially towards multiresistant strain of *Shigella sonei* and *Micrococcus flavus* ATTC 10,240. All tested oils showed significant fungistatic and fungicidal activity [expressed as minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) values, respectively], that were considerably higher than those of the commercial fungicide bifonazole. The essential oils of *M. piperita* and *M. longifolia* were found to be more active than the essential oil of *M. aquatica*. Especially low MIC (4 microL/mL) and MFC (4

microL/mL) were found with *M. piperita* oil against *Trichophyton tonsurans* and *Candida albicans* (both 8 microL/mL). The RSC was evaluated by measuring the scavenging activity of the essential oils on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and OH radicals. All examined essential oils were able to reduce DPPH radicals into the neutral DPPH-H form, and this activity was dose-dependent. However, only the *M. piperita* oil reduced DPPH to 50 % (IC50 = 2.53 microg/mL). The *M. piperita* essential oil also exhibited the highest OH radical scavenging activity, reducing OH radical generation in the Fenton reaction by 24 % (pure oil). According to GC-MS and TLC (dot-blot techniques), the most powerful scavenging compounds were monoterpene ketones (menthone and isomenthone) in the essential oils of *M. longifolia* and *M. piperita* and 1,8-cineole in the oil of M. aquatic [23].

The essential oils (EO) of *Mentha suaveolens*, a wild Labiatae, which grows in several regions in Morocco, were characterized and their antimicrobial activity assessed. The main aromatic constituents of this plant, as characterized by IR, NMR and MS studies, were pulegone, piperitenone oxide (PEO) and piperitone oxide (PO) occurring in different amounts depending on the subspecies. These constituents as well as a series of other aromatic products such as carvone, limonene and menthone, were tested for their antimicrobial activity against 19 bacteria including Gram-positive and Gram-negative and against three fungi, using solid phase and microtitration assays. Pulegone-rich essential oil inhibited efficiently all the micro-organisms tested with MICs ranging between 0.69 and 2.77 ppm. Among the components from *Mentha suaveolens* EO, pulegone was the most effective against the tested microorganisms, followed by PEO and PO. The structure-activity relationship is discussed on the basis of the activity of the other aromatic derivatives tested such as carvone, limonene, menthone and the profile of the essential oils of *Mentha suaveolens* was compared with other Mentha species [24].

Hofbauer et al [25] investigated the in vitro activity of terbinafine against fresh veterinary isolates of *Microsporum canis* and the potential of this organism to develop resistance in vivo during oral therapy. Dermatophyte cultures (n = 300) were obtained from naturally infected cats and dogs undergoing oral therapy with terbinafine or griseofulvin. *M. canis* comprised 92% of isolates; other species included *Microsporum gypseum* and *Trichophyton mentagrophytes*. Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of terbinafine and griseofulvin were determined by broth macrodilution assay. Terbinafine was highly active against all three species with MIC90< or =0.03 microg ml(-1), in agreement with published data. However, terbinafine exhibited primary cidal activity against 66% of *Microsporum* isolates (n = 275) in contrast to the almost complete cidal effect in *Trichophyton* (n = 18). Griseofulvin was significantly less active than terbinafine (MIC90 = 4 microg ml(-1)) but had a primary cidal action on about 40% of the isolates. The data were analysed for changes in MIC and MFC during the course of therapy, which could be indicative for development of acquired resistance. Oral treatment of 37 animals with terbinafine for up to 39 weeks caused no increase in MIC or MFC of terbinafine, either in individual patients or in the whole group.

Fernández-Torres et al [26] have evaluated a disk diffusion method to determine the activities of five drugs against 50 strains of dermatophytes and to assess the influence of the culture medium (antibiotic medium 3, high-resolution medium, and RPMI) on the inhibition zone diameters (IZD). There were no differences among the medium/drug combinations, except for itraconazole-RPMI, which showed the narrowest IZD.

Hossein et al [27] finds the effectiveness of 4 different antifungal agents in an *in vitro* model with some similarities to *in vivo* conditions. Methods: Strains of *Trichophyton rubrum* I-III, *Trichophyton mentagrophytes* (usual form), *Trichophyton mentagrophytes* 73, *Epidermophyton Flucosom, Microsporum Canis,* and *Trichophyton schoenleini* which were isolated from the nails of patients, were hired. Inocula suspensions were prepared from 7 to 14 day-old cultures of dermatophytes. Antifungal agents including fluconazole, ketoconazole, terbinafine, and griseofulvin were obtained as standard powders. For each antifungal agent, initial MIC was calculated by registering the optical density for 10 two-fold serially diluted forms which was incubated with diluted fungal suspensions with RPMI 1640. Human nail powder inoculated with different strains and incubated in RPMI 1640 and different concentrations of antifungal drugs for 4 weeks. Final MIC at different steps of 1, 2, 3 and 4 weeks were investigated. The final MIC that resulted from the incubation of dermatophytes with nail powder was much more than the initial which was concluded from conventional MIC assay. Terbinafine had the lowest rate of initial and final MICs.

STATUS OF DERMATOPHYTES RESEARCH AT NATIONAL LEVEL

PREFACE

Dermatophytosis due to *Trichophyton rubrum* is a chronic non-inflammatory “ring-worm” skin infection generally restricted to stratum corneum. Pathogenecity of *Trichophyton rubrum* depends upon a variety of local and systemic factors that affect the natural host defense. In patients with compromised immunity the prevalence of dermatophytosis can be extremely high and the clinical presentation very unusual. Deep tissue invasion, mutivisceral dissemination and even death may occur in patients with immune suppression due to disease or drug therapy. Local radiation therapy, long term topical steroid application and trauma to epithelial barrier are some of the local factors predisposing to deep follicular invasion by *T. rubrum* in an otherwise immunocompetent individual [28].

CLINICAL STUDY

Different strains of common dermatophytes including different mating strain, collected from clinical, animal and soil sources as also some tester strains, were studied by Ranganathan et al [29] for their proteolytic enzyme activity during vegetative and sporulation phases. Sabourauds dextrose broth and Takashio broth were used to induce vegetative and sporulation phases respectively in these dermatophytes. All the strains of *T. rubrum* showed very low enzyme activity during sporulation when compared vegetative growth phase. In other species of dermatophytes the enzyme activity was found to be almost similar during both the growth phases. The enzyme activity of clinical isolates of the non-anthropophilic species such *Trichophyton simii*, *Microsporum nanum* and *Microsporum gypseum* were relatively low when compared to *Trichophyton mentagrophytes* var. *interdigitale* and *Trichophyton tonsurans*. The severity of the lesion produced by these established the fact that protease in dermatophytes has a define role in pathogenesis. Protease production during sporulation in *Trichophyton rubrum* may be one of the selective advantages of this species. General protease production was found to be independent of mating type in most of the dermatophyte species.

Kalla et al [30] conducted clinicomycological study was on 200 cases of Tinea capitis in Jodhpur. Incidence of tinea capitis among superficial mycoses was 4.43 and male to female ratio being 1.8:1. Majority of patients were from urban area (88%) and positive family history of dermatophytoses was present in 29% of cases. Majority of patients attended hospital OPD from July to October (39%) and January to April (49%). Persons using mustard oil as hair applicant had single or less lesions as compared to individual using other oil. Endothrix involvement of hair was seen in 78% cases and *Trichophyton violaceum* was predominant fungus (88.5%) recovered on culture.

Singh and Beena [31] evaluate the usefulness of two different microscopic techniques and three different culture media for the identification and isolation of dermatophytes from clinical samples. Skin, hair and nail samples from 260 clinically suspected cases of dermatophytosis were screened by direct microscopic examination using 10% potassium hydroxide (KOH) with and without 40% dimethyl sulphoxide (DMSO) mounts. All the samples were inoculated for culture in Sabouraud dextrose agar (SDA), dermatophyte test medium (DTM) and ready to use enriched dermatophyte medium (EDM). Fungal elements were detected in 157 samples by both the methods but faster and better visualization was noted with 40% DMSO added to 10% KOH. Fungi were recovered from SDA, DTM and EDM in 96.5%, 98.3% and 85.3% of the cases respectively. CONCLUSIONS: When performing direct microscopic examination of skin, hair and nail for dermatophytes, addition of 40% DMSO to the KOH mount gives better and faster results. The efficiency of SDA and DTM was found almost equal and slightly better than EDM. The EDM, although quite efficient with 85.3% isolation rate, requires further evaluation as its ready to use format makes the application and microscopy much easier and faster.

Even though dermatophytes, especially *Trichophyton rubrum*, are most frequently implicated as the causative agents in onychomycosis, yeasts and moulds are increasingly recognized as causative pathogens. A study to analyze the morphological variants and mycological and cultural positivity of onychomycosis was carried out in 35 patients attending the Dermatology outpatient department of Command Hospital, Air Force, Bangalore [32].

Singh and Beena [33] made an attempt to find the species prevalence of various dermatophytes in patients with dermatophytosis was made in hospital in Baroda. Two hundred and sixty clinically suspected cases of dermatophytosis were subjected to mycological studies. One hundred and fifty seven cases (60.38%) were positive for fungus in direct microscopy while 116 (44.62%) were culture positive. Tinea corporis was the most common clinical presentation followed by tinea cruris. Young adults in the age group of 16-30 yrs were mainly affected. The male to female ratio was 1.57:1. *Trichophyton rubrum* (73.27%) was the most common isolate, followed by *Trichophyton mentagrophytes* (17.24%), *Epidermophyton floccosum* (7.75%) and *Trichophyton violaceum* (1.72%).

HOST RANGE

Diversity of keratinophilic mycoflora in the soil of Agra was under observation for 1 year (July 2001-June 2002) and isolation of keratinophilic fungi was followed by the hair-baiting method. The frequency of occurrence of keratinophilic fungi in 284 soil samples collected from various hospitals, cattle yards, poultry farms, crop fields and playgrounds was determined, 204 samples (72%) having been found to be positive. A total of 33 species classified into 11 genera (*Acremonium, Aspergillus, Chrysosporium, Emmonsia, Geomyces, Keratinophyton, Microsporum, Myceliophthora, Penicillium, Sporotrichum, Trichophyton*) were encountered from the soil samples. *Sporotrichum spp*. was found to be the most dominant species followed by *Trichophyton simii*. The parameter of keratinophilic fungi found in the samples studied ranged from 62 to 80% where playgrounds yielded the maximum number of species (80%) while the least dominating soil was hospital soil (62%). Among all the baits used maximum fungi occurred on human hairs (82%) followed by chicken feather (74%), wool (61%) and the least on horns (45%). The spectrum of keratinophilic fungi isolated from different sites differed considerably according to the frequency of use by humans [34].

Clinico-mycological study of 2743 clinically diagnosed cases of superficial mycoses attending skin and VD OPD of VSS Medical College during the year 1995 - 96 was conducted. Male predominance was observed. Highest incidence of tinea versicolor was found. *T. rubrum* was the commonest fungus isolated [35].

Dermatophyte infection is a very common disease, but that of the male genitalia is said to be rare. Two patients gave history of application of steroid-containing preparations and another had diabetes mellitus. Culture of the scraping of the lesional skin yielded *Trichophyton rubrum* in two cases and *Epidermophyton floccosum* in the other two. All cases resolved completely with topical terbinafine with or without oral antifungals [36]. Jagtap et al [37] had done hair penetration tests on human scalp hair (rough black and soft brown) of adults of both sexes. It appeared that T. *violaceum*, T. schoenleinii, *T. rubrum* and *M. audouinii* were non penetrators of human scalp hair.

Clinico-mycological study of 250 cases of dermatophytoses was undertaken in a desert district of Western Rajasthan. Incidence of dermatophytoses in this area was 8.60% with tinea cruris (34.4%) as the major clinical type followed by tinea corporis (24.0%) Incidence of tinea capitis was 16.8% and 90% of those affected were in the age group of 0-10 years. Male preponderance was observed (M:F=2:1). There were 15 cases of tinea faciei (6%), majority belonging to 0-10 year's age group. *Trichophyton violaceum*was isolated In majority (55.76%) from all clinical types followed by *Trichophyton rubrum*(42.3%) [38].

Singh et al [39] isolated dermatophytes, related keratinophilic and opportunistic fungi from indoor dust samples of 46 hospitals and 47 houses in Kanpur. A total of 19 fungi represented by 11 genera were isolated by the hair-baiting technique from 230 and 235 samples from hospitals and houses respectively. The isolated fungi were *Acremonium implicatum* (Indian Type Culture Collection) ITCC 5266 *, A. strictum* (Germplasm Centre for Keratinophilic Fungi) GPCK 1137 *, Aphanoascus fulvescens* GPCK 1081 *, Arthroderma simii* GPCK 1275 *, Chrysosporium queenslandicum* ITCC 5270 *, C. indicum* ITCC 5269 *, C. pannicola* GPCK 1022 *, C. tropicum* GPCK 1269 *, Ctenomyces serratus* ITCC 5267 *, Gymnoascus reessii* ITCC 5265 *, Malbranchea fulva* GPCK 1075 *, Malbranchea pulchella* ITCC 5268 *, Micosporum gypseum* GPCK 1038 *, Microsporum cookei* GPCK 2001, *M. fulvum* GPCK 2002 *, Paecilomyces lilacinum* GPCK 1080 *, Penicillium expansum* GPCK 1082, *Trichophyton mentagrophytes* GPCK 2003 and *T. terrestre* GPCK 2004. In hospitals, the minimum frequency was of *Ctenomyces serratus* ITCC 5267 while the maximum frequency was of *Arthroderma simii* GPCK 1275. In houses, *Chrysosporium queenslandicum* ITCC 5270 and *C. tropicum* GPCK 1269 were with minimum and maximum frequencies respectively. This makes the first report of these fungi with keratinolytic ability in the indoor dust of hospitals and houses.

Soil samples from twenty salt pans and their vicinity around Mumbai and Thane were screened for the occurrence of keratinophilic fungi and related dermatophytes. Ten species classified in six genera were recovered using horse hair as bait. The isolated species were reported in the following order of dominance: *Chrysosporium indicum* (12.0%), *Microsporum gypseum* complex (7.2%), *C. tropicum* (5.6%), C. state of *Ctenomyces serratus* (4.0%), *Trichophyton* terrestre (3.2%), *Malbranchea aurantiaca* (2.4%), *C. fluviale* (1.6%), *Uncinocarpus reesii* (1.6%), *Malbranchea sp.* (0.8%), and *T. mentagrophytes* (0.8%) [40].

Jain and Sharma [41] has done screening of 217 soil samples of different habitats, such as PG study centre, garden, farmhouse, nursery, roadside, hostel, animal habitat, bird habitat, marriage garden, temple, vegetable market and house dust, was carried out for the presence of dermatophytes and related fungi in relation to soil pH. A total of 461 isolates belonging to 26 genera and 34 species were recorded. Soil pH values vary from 3 to 10.5. *Trichophyton* verrucosum, *Microsporum audouinii* and *M. canis* were isolated for the first time in Jaipur from pH range 7.0 to 9.0. *Chrysosporium tropicum* (46.08%) was the most predominant fungus isolated from pH range 6.5 to 9.5. *Trichophyton mentagrophytes* (24.88%) was the second most common fungal species isolated from pH 6.5 to 9.5. Most of the keratinophilic fungi were isolated from pH 6.5 to 8.5. Only one isolate of *Fusarium moniliforme* was reported from a highly acidic site at pH 3. Roadside and garden soils were found to be the most suitable sites for almost all keratinophilic fungi.

Nucleic acid-based identification techniques may also be valuable when diagnosing onychomycosis; however, multiple steps may be necessary to determine the causative species. Confocal microscopy may also be a fast and reliable method of diagnosing onychomycosis, though it has very limited ability to distinguish between dermatophyte and mold infections. Prior to treatment an accurate diagnosis can provide guidance about the choice of antifungal agent, especially since the causative organism may vary in its response to the antifungal therapies available [42].

ANTIFUNCAL AGENTS

Natarajan et al [43] determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) for the extracts of the leaves and seeds of the plant *Azadirachta indica* against various dermatophytes. Clinical isolates of dermatophytes (*Trichophyton rubrum, Trichophyton mentagrophytes* and *Microsporum nanum*) were treated with extracts of leaves and seeds of the plant *Azadirachta indica* (neem) for antifungal activity by *in vitro* tube dilution technique. The MIC of neem seed extracts was 31µg/mL for all the dermatophytes tested. The neem seed extract at 15µg/mL concentration (below MIC) was observed to be sufficient for distorting the growth pattern of the organisms tested.

Different extracts and oils from 13 plant materials were tested in vitro for antifungal activity against *Trichophyton verrucosum*, *Trichophyton mentagrophytes* and *Trichophyton* simii by cup plate method. Eight extracts/oils from 6 plants showing zone of inhibition more than 10 mm in radius against *T. mentagrophytes* and *T. simil* in the preliminary screening were further tested to determine the MIC against *T. mentagrophytes*. Ether and chloroform extracts of seeds of *Mucuna pruriens* and stem of *Curcuma longa* were found to be fungistatic. Ether extract of the resin of *Shores robusta*, chloroform extract of *Moringa pterlgosperma* and oils of *Azadirachts Indica* and *Pongamia gbbrs* were found to be fungicidal [44].

The antibiotic effect of the active ingredients in Meijer medicated chest rub (eucalyptus oil, camphor and menthol) as well as the inactive ingredients (thymol, oil of turpentine, oil of nutmeg and oil of cedar leaf) were studied in vitro using the fungal pathogens responsible for onychomycosis, such as the dermatophytes Tricophyton rubrum, *Trichophyton mentagrophytes*, *Microsporum canis*, *Epidermophyton floccosum* and *Epidermophyton stockdale*. The zones of inhibition data revealed that camphor (1). menthol (2). thymol (3). and oil of Eucalyptus citriodora were the most efficacious components against the test organisms. The MIC (100) for mixtures of these four components in various carrier solvents revealed that formulations consisting of 5 mg/mL concentrations of each have a potential to be effective in controlling onychomycosis [45].

Sixteen essential oils were screened in vitro for their fungitoxicity against the two dermatophytes *Trichophyton rubrum* and *Microsporum gypseum*. Five oils (from *Artemisia nelagrica, Caesulia axillaris, Chenopodium ambrosioides, Cymbopogon citratus* and *Mentha arvensis*) showed strong activity and were assessed for their fungitoxicity against eight other dermatophytes as well as against *Aspergillus fumigatus* and *Cladosporium trichoides*. These five essential oils by formulation of ointments were able to cure experimental ringworm in guinea pigs within 7 to 12 days. Artemisia oil was found to be the most effective essential oil [46].

During screening of 12 essential oils of higher plants against two ringworm fungi *Trichophyton mentagrophytes* and *Microsporum audounil* by poisoned food technique, the oils of plants viz. *Cinnamomum tamala, Citrus maxima, Cymbopogon citratus, Eucalyptus citriodora, Eupatorium cannabinum, Nepeta hindostana Ocimum canum* showed absolute toxicity against both the test fungi. The minimum inhibitory concentration of the oils of *Cinnamomum tamala* and *Citrus maxima* was 500 ppm against both the test fungi and these oils showed superiority in efficacy over some synthetic antifungal agents. The oils exhibited fungicidal or fungistatic nature of toxicity [47].

CONCLUSION

Dermatophytoses are one of the most frequent skin diseases of pets and livestock. Contagiousness among animal communities, high cost of treatment, difficulty of control measures, and the public health consequences of animal ringworm explain their great importance. Onychomycosis is a common infection of the nail predominantly caused by anthropophilic dermatophytes, and to a lesser extent by yeasts (Candida species) and non-dermatophyte molds. The treatment of onychomycosis is dependent on several variables, including the type of onychomycosis and the causative organism. Various techniques have been used to accurately diagnose onychomycosis, with microscopy and culture being used most frequently. Histological examination can aid in confirming the presence of invasive disease. This review put a light on various aspects of research on dermatophytes around the globe.

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Author's biography with Photo

Avinash Marwal **Surendra Meena** **Dr Subhash Chandra** **Dr Anima Sharma**




Avinash Marwal and Surendra Meena has successfully completed their Masters with good academic record. Both has completed the DST student project with remarkable results under the guidance of Dr Anima Sharma and Dr Subhash Chandra on "In-vitro study of antidermatophytic activity of Brittle gum (Eucalyptus mannifera), Mint (Menthapiperita) and Onion (Allium cepa) against *Trichophyton rubrum* and *Microsporum canis*", funded by Department of Science & Technology, Govt of Rajasthan. Both has command over various molecular, biotechnological and bioinformatics tools and techniques. Their core interests are Microbiology, Immunology and Plant Molecular Biology. Dr Anima Sharma is presently working as associate professor in JECRC University. She did her masters from Banasthali Vidyapeeth University, Rajasthan and later completed her PhD from University of Rajasthan on Dermatphytes. She is actively working on dermatophytes in screening of various antifugal agents. Dr Subhash Chandra is presently working as associate professor and Head of Department in Jyoti Vidyapeeth womens University, Jaipur, Rajasthn. He completed his masters from University of Rajasthan and later successfully completed his PhD. He has made significant contributions in microbiology and published number of papers in national/international journals and presented good sum of articles in national and international conferences.