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Occurrence of keratinophilic fungi in paddy fields and infestation of field workers of Chhattisgarh, Central India

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Prevalence of keratinophilic fungi was observed from different paddy field soils in Bilaspur district of Chhattisgarh state in Central India. Out of 70 soil samples 90% samples were found to be positive for the keratinophilic fungi. The species of *Microsporum* and *Trichophyton* were the commonest dermatophytes isolated from most of the soil samples collected from paddy fields. The non-dermatophytic viz. *Aspergillus*, *Chrysosporium*, *Fusarium* together with other opportunistic fungi also obtained invading keratin substrates. The geophilic fungi *Trichophyton* and *Chrysosporium tropicum* were found most prevalent due to their resistance tendency against supra-optimal temperature prevailing in the state. The field workers who spent a large period of their time in paddy fields remained wet in rainy season were found to be exposed to more pathogenic fungi causing dermatosis.

Key words: Paddy fields, keratinophilic fungi, dermatophytic fungi, mycosis

INTRODUCTION

Keratinophilic fungi an ecologically important group recently has attracted the attention throughout the world. These fungi present variable tendency in occurrence and distribution pattern according to the availability of keratinous substances (Marples 1965; Mantovani, 1978; Devroey, 1984). The non-dermatophyte keratinophilic fungi are most frequently isolated as contaminants from the environment, while zoophilic dermatophytes of keratinophilic species occurring in living tissue are found on their hosts, and some of them occur in soil as saprophytes termed geophilic dermatophytes. The *Microsporum gypseum*, *Trichophyton rubrum* and *T. mentagrophyte* are basically animal pathogens which can develop infection just after few hours (Rippon 1982, 1985) and they rarely grow actively as saprophytes, but survive in a dormant state on

contaminated material of animal origin. The *Aspergillus*, *Chrysosporium* and *Fusarium* are closely related with dermatophytes and tend to be found remain associated with skin and nails.

The composition of keratinolytic fungi shows at least two such characteristics, i.e., the restricted number and a set of similar predominating species in different habitats and the possibility of fungal growth indices either of living or dead tissues is concern under any environmental conditions. For example the *Aspergillus*, *Chrysosporium*, *Microsporum*, *Trichophyton* are the commonest fungi of this group which are found in variable numbers of habitats, because of their craving tendency for using keratin as a substrate to obtain the nutrients necessary for their development.

The keratin waste most often found in association

with keratinophilic fungi is a better source to know how distribution and occurrence of dermatophytes and other fungi have been a great risk to human beings. The availability of keratin waste in soil explains the incidences of dermal infections in man and animal. This is so important natural material available in the form of hair, wools, feathers, horn, hooves, nails, skin and other appendages that constituting natural baits for growth and dissemination of keratinophilic fungi (Deshmukh and Shukla, 2001; Khanam and Jain, 2002). Since the soil serves as a natural reservoir for both the pathogenic and saprophytic groups, therefore more attention has been shown by researchers towards the soil mycoflora with respect to keratinophilic fungi (Al-Musallam, 1998; Al-Musallam *et al*, 1995; Ogbonna and Pugh, 1987, Soon 1991; Ulfing and Koroz, 1995; Caglar and Akgun, 2006).

The prevalence of keratinophilic fungi is directly related with the amount of keratin waste. The fungi usually use the keratin of dead animals or hairs, feathers, and skin that have been shed in the soil and they develop high numbers of spores in the soil to form the potential inoculums as saprobes. Padhye *et al*, (1966) have found that soils rich in keratinous matter such as cattle and poultry farm soils showed higher occurrence of keratinophilic fungi. Azello (1974) and De Vroey (1984) have studied the spores of dermatophytic fungi and narrated that macro and micro-conidia, ascospores, and other propogules, biotypes, (bird's nests) form the potential inoculums in the soil which disseminate through man and animal groups.

The soil is a huge reservoir of the mycoflora, where keratinophilic fungi represent a huge biodiversity of form, habitat and substrates. In Central-India a very little information is available on occurrence of keratinophilic fungi from the paddy fields (Sunderam, 1987). In Chhattisgarh, a preliminary work has been conducted to detect keratinophilic fungi from different soil habitats i.e. coal mines, veterinary and poultry farm (Deshmukh and Shukla, 2001; Pahare and Shukla, 2014). Many of the workers are in opinion that frequency of occurrence of keratinophilic fungi is higher in the alkaline soil (Sharma and Rajak, 2003) however *Trichophyton ajelloi* fungus has been reported to be more often associated with acidic soil rather than alkaline soil (Roberts, 1982).

The exposure of the individual in occupational con-

ditions, habit and habitats, and his customs accounts for the fungal invasion. The tight bearing dhoti of loin cloth worn by men and women with tight-fitting in waist, the chemical fertilizers, pesticides, in paddy fields, and synthetic detergents, used in house job are the conditions imposing fungal infection in waist, foot and hand. The poor farmers, painters, barbers, house wives, in contact with solvents and caustic chemical products often suffer with dermatitis. The cattle breeders, cleaning employees, building construction and mining work-



Fig. 1 : A man suffering fungal dermatophytes



Fig. 2 : Farmer with thickened, discolored fingernails



Fig. 3 : Women suffering from *Tinea pedis*

ers, constantly exposed to warm and wet conditions tend to have maceration of skin and nails for mycotic infection. Besides paddy production, the sonjhar group seeking precise metal particles (silver and gold) from river (Mahanadi and Irb) current, the fisherman, remain engaged partly in their occupational work mostly found suffering with dermatitis (Figure 1 and 2).

The keratinophilic dermatophytes affect a large proportion of rural women that remain engaged in domestic and agricultural works. The women group has to suffer at the most as they are constantly exposed in their own house and daily wages of other houses. Owing to poor education they are being identified hired labour within and outside the state. The women of poor family working as maid servant engaged in kitchen work and washing cloth of such family cannot afford adequate gloves tend to have their foot and hands wet for long periods of time and this causes a maceration of the skin and nails. In addition this condition is further prejudiced by the use of detergents and caustic substances that act chemically to potentiate the physical effect. Therefore, in view of awareness on hygienic and ecological condition an extensive and intensive survey was conducted to isolate the fungi from living and dead keratinous tissues.

MATERIALS AND METHODS

The soil samples from Bilaspur, Bilha, Kota, Gourela-Pendra, Marwahi, Takhatpur, Masturi-blocks within the Bilaspur district were collected in sterilized polythene bags from the different paddy fields, which were studied for the occurrence of keratinophilic fungi. The keratin colonizing fungi were isolated using "Hair Baiting Technique". The hair and feathers used as keratin baits were sterilized and spread over the moist soil samples in sterilized Petri plates. These plates were kept undisturbed at room temperature for 15-20 days until visible fungal mycelium appeared on baits. The samples, on which no fungal growth visualized after 8 weeks incubation were discarded.

The fungal nail infections are diagnosed by taking a sample of the debris under the nail. The most fungal elements are found under the nail and closest to the skin, therefore the nail should be trimmed before a sample is taken. The infected toe skin were also taken from certain cases which were gently washed and transferred in to sterilized Petri

plates containing Sabouraud's dextrose sugar (SDA) medium (dextrose 40 g. peptone 10 g. agar 20 g. and distilled water 1 liter). The cultures were identified on the basis of their morphological characteristic features.

RESULTS AND DISCUSSION

The red soil a common type of soil which has lesser water retention capacity is found in major portion of Chhattisgarh and parts of many other neighbouring states in Central-India. The soil in paddy field is generally gray-brown with natural to acidic-pH, and rich in heavy metals particularly with higher percentage of ferrous-oxide. The soil samples of paddy fields collected from 7 blocks of Bilaspur district yielded 118 fungi. Maximum numbers belonging to zoophilic group of keratinophilic fungi obtained from the Bilaspur, Bilha, Kota. The samples of Gourela, Marwahi, Takhatpur also yielded a considerable number of zoophilic species of *Microsporum* and *Trichophyton*, while Masturi block was found lag behind for yielding this group of fungi. *Chrysosporium. tropicum*, *Microsporum gypseum*, *Trichophyton* and the species of *Aspergillus*, were isolated from more than 80 percent of the samples collected from seven blocks. The genus *Chrysosporium* is a common saprophyte of which *Chrysosporium indicum*, *C. tropicum*, *C. zonatum*, were recovered from the paddy fields. *Chrysosporium zonatum*, predominantly obtained in similar way of its findings from poultry form (Deshmukh and Shukla, 2001). The occurrence of *C. tropicum* which has strong keratinolytic activity and capable to utilize keratin (Richardson and Aliabre, 1993) in many more samples might be due to acidic soil type of the state.

Microsporum gypseum referred as parasitic saprobe (De Vroey, 1984, 1985) is a common geophilic fungus widely distributed in soil (Mohammed and Rana, 2000). The conidia of the fungus produced during the saprophytic growth on keratin substances in the soil as potential inoculum are transmitted either directly or indirectly. The *Trichophyton rubrum* isolated from the samples reported more often to occurred in acid soils than alkaline soils Marples, 1965). Both *Trichophyton rubrum*, *Trichophyton mentagrophytes* constitute main infectious element in the soil and just within few hours they undergo to produce mycelium between keratinous cell or even inside the cell. The

Table 1 : Isolation of keratinophilic fungi from paddy fields soil of Bilaspur (Chhattisgarh)

| Name of the Fungi | | | | | | | | Total | % |
|---|----------|-------|------|---------|---------|-----------|---------|-------|-----|
| | Bilaspur | Bilha | Kota | Gaurela | Marwahi | Takhatpur | Masturi | | |
| <i>Aspergillus fumigatus</i> | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 9 | 9 |
| <i>A. flavus</i> | 1 | 1 | 1 | - | - | 1 | - | 4 | 4 |
| <i>A. niger</i> | 1 | 1 | - | - | 1 | - | 1 | 4 | 4 |
| <i>A. terreus</i> | 1 | - | 1 | - | - | - | - | 2 | 2 |
| <i>Acremonium</i> sp. | 1 | - | - | - | - | - | - | 1 | 1 |
| <i>Alternaria alternata</i> | - | 1 | 1 | 1 | - | - | - | 3 | 3 |
| <i>Curvularia lunata</i> | - | 1 | - | - | - | 1 | - | 2 | 2 |
| <i>C. clavata</i> | - | 1 | - | 1 | - | 1 | - | 3 | 3 |
| <i>Chrysosporium indicum</i> | 2 | 1 | 1 | 2 | 3 | - | 1 | 10 | 10 |
| <i>C. tropicum</i> | 3 | 1 | 2 | 1 | - | 2 | 2 | 11 | 11 |
| <i>C. zonatum</i> | 3 | 3 | 2 | - | - | 1 | 1 | 10 | 10 |
| <i>Fusarium oxysporium</i> | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 | 7 |
| <i>Geotrichum aurantia</i> | - | 1 | - | - | - | - | - | 1 | 1 |
| <i>Humicola insolense</i> | 1 | 1 | - | - | - | - | - | 2 | 2 |
| <i>Microsporium gypseum</i> | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 10 | 10 |
| <i>M. fulvum</i> | 1 | 1 | 1 | 1 | 1 | 1 | - | 6 | 6 |
| <i>Malbranchea pulchella</i> | - | 1 | - | - | - | - | - | 1 | 1 |
| <i>Paecilomyces</i> sp. | - | 1 | - | - | - | - | - | 1 | 1 |
| <i>Penicillium</i> sp. | 1 | - | - | - | - | - | - | 1 | 1 |
| <i>Rhizetonia</i> sp. | - | - | - | - | - | 1 | - | 1 | 1 |
| <i>Theilavia</i> sp. | - | - | 1 | - | - | - | - | 1 | 1 |
| <i>Trichophyton mentagrophyte</i> | 2 | 3 | 2 | 1 | 2 | 2 | 1 | 13 | 13 |
| <i>T. rubrum</i> | 3 | 2 | 3 | 1 | 1 | 2 | 1 | 13 | 13 |
| <i>Scytalidium</i> sp. | 1 | - | - | - | - | - | 1 | 2 | 2 |
| Fungi sp. recorded from paddy fields of each Blocks | 26 | 24 | 18 | 11 | 11 | 17 | 11 | 118 | 118 |

(where, + present, - absent)

The blocks (1-7) Bilaspur-Masturi representing paddy fields in Bilaspur district.

arthoconidia of *Trichophyton mentagrophytes* attached to human develop infection just after few hours. Since the species are adopted to grow and survive on human keratin, so are transmitted from person to person.

The non-dermatophytic group of keatinophilic species viz. *Aspergillus*, *Acremonium*, *Alternaria*, *Fusarium*, *Geotrichum*, *Humicola*, *Penicillium*, *Scytalidium*, found as per the habitats. *Aspergillus*, *Acremonium*, *Alternaria* and *Fusarium* were obtained more samples while *Geotrichum*, *Humicola*, *Penicillium*, *Scytalidium* are subjected

to more selective in their presence and obtained less than 50 percent of soil samples. Many of them have often associated with living tissues of skin, feathers, but it is not always possible to show whether they are transient or occasionally quests of weather they are responsible for leasions (Rippon, 1982). Therefore pathogenic role of these fungi remain as an open question.

The species of *Aspergillus* namely *A. fumigatus*, *A. flavus*, *A. niger* and *A. terreus* are the most dominating fungi found in 60-80% in the samples. These are common species found in greater prevalence

which may cause infection in a similar proportion to men and the women (Howard, 1983). *Aspergillus niger*, *Fusarium solani*, *F. oxysporum*, *Acremonium*, saprobic fungi are potent causative agents for the nail infections. The *Aspergillus niger*, *Acremonium*, *Alternaria*, *Chrysosporium* could be erroneous and correspond to contamination of nail was considered causing agents criteria used to certify these mould usually can be categorised as environmental pollution.

Curvularia lunata and *C. clavata* occasionally recovered from soil by species are ubiquitous species present in soil and on plant material. They are occasionally recovered and rarely cause superficial infections of skin and nails in man. *Penicillium*, *Paecilomyces* and *Geotrichum* are another fungi, which were obtained from most of the samples. The fungi also develop on keratinous tissues. *Penicillium* from soil by hair-baiting technique are reported as human pathogenic fungi (Cole and Samson, 1979) obtained a case of onychomycosis caused by *C. lunata* while Gugnani *et al.*, 2000, reported *C. clavata* as an etiological agent of human skin infection (Gordon *et al.*, 1967).

In tropics, the forest land used for agricultural activities, farm-yards by the people and presence of birds and animal introduce keratin waste that serve substrata for keratinophilic fungi. This is in reference to the area having nutrient rich soil to attract much more livelihood of man and animals groups. Although, many blocks near by Bilaspur town exhibit the limits of nutrient lateritic soil type which cannot retain appropriate soil moisture in support of winter crops and grazing yards to put and attraction for much more human and animal population.

The drought prone crop-land grazing yards representing cereal crop failure have become more critical particularly in Masturi blocks, where most of paddy field soil in weathered and lack good fertility. Since the majority of farmers keep them away from agriculture crop production, therefore the block area can be related for scant population of domestic animal and lower in-put of keratin waste in to the dry paddy fields, where keratinophilic fungi do not find suitable environment. This is an indication that living organic contents directly influence the occurrence and distribution of keratinophilic fungi acquire the ability to invade in keratinized human and animal tissue (Rippon, 1982). However, numbers of poultry form nearby Bilaspur town,

and on way to Bilha and Kota town, attributing higher occurrence of keratinophilic fungi in paddy fields. The higher influx of dropping, feather, claws, in poultry forms in soil of poultry form and other areas attributing keratin substance in vast area that helps for distribution and development of keratinophilic fungi particularly *Aspergillus* and *Chrysosporium* species (Table 1).

Obviously, the occurrence of keratinophilic fungi is mainly influenced by the human and animal population and the amount of keratinized material available in the soil. Many of the keratinophilic fungi frequently parasitize keratinous tissue, viz. skin, nails and hairs in man and animals constituting a special group called dermatophytes causing mycoses in human and animal (Ajello, 1962). De Vroey (1985) studied epidemiology of *Microsporium. canis* commonly infect pet animals especially cat and dogs, which sheds infective particles in to the environment.

The species *Trichophyton rubrum*, followed by *T. mentagrophytes* that are most frequently invade the nail. Both the species form part of the nail the so called anthropophilic dermatophytoses as they are adapted to their development on human keratin and so are transmitted person to person. *Trichophyton rubrum* has been found more prevalent among the urban population (Philpot, 1977) due to modern way of life; using rubber shoe, excessive use of detergent, cosmetics, facial, synthetic wearing etc. The fungus *T. mentagrophytes* is reported transmitting from wild rodents causing infections in tribal population living in the forest vicinity (Georg, 1956; Kaben, 1967). Beside the above group of tribal often tend to have wet feet most of them some using rubber boots that hinder the elimination of sweat and moisture a causative factor of dermal infection. Nevertheless, the infections of the nails caused by fungi have not been considered a health problem rather than cosmetic problem for minor importance. More over the human being and domestic animals, supporting the growth of keratinophilic fungi causing mycoses have drawn the attention of medical and veterinary epidemiologists (Ajello, 1962).

In rainy season, both the higher temperature ranges and forest cover that maintains high humidity level in the state are the most important factors to support the fungi to become potentially pathogenic to human and animal life. The highly

acidic mining water in coal mines and calcium oxide content of the cement help to erode the feet for aiding fungal invasion in the skin (Ajello, 1974; Rippon, 1985; Pahare and Shukla, 2014). In paddy fields the men and women spent more time working in wet condition found more susceptible for fungal infection in toe nails. The fungi being a zoophilic, causing skin of the foot usually in inter digital clefts locally called 'kandwa', sometimes spreading to the soles Figure, ankles, leg, toe nails (Baxter and Rush-Munro, 1980). These are more common fungus transmitted from domestic animals and rodents causing infections in poor farmers and tribal population. The sole and foot of paddy growers in contact with chemical fertilizers, pesticides, in paddy fields found burnt during ploughing and transplantation process. The wearing of rubber shoes, for as long as 8-10 hours a day, by the occupational groups provide a warm and wet condition favourable for the growth of *Trichophyton rubrum* and *T. mentagrophyte*.

The micro and macroconidia of many dermatophytic fungi resistant to warmer conditions survive for long periods in various environmental conditions where they naturally occur. *Trichophyton* were has been found to frequently invade the nails, especially *T. rubrum* followed by *T. mentagrophytes*. It is also able to adapt to its environment in way other species cannot emulate. The species *T. ajelloi* commonly found in colder climate but is also sporadic in hot climate (Marples, 1965). The genus *Scytalidium* an etiological agent of superficial skin and nail infection more common in tropical conditions (Gupta *et al*, 1998; Stevens, 1989). Numerous cases of human infections in coalmines and cement factory workers are most probably suspected by the species of *Scytalidium* due to its thermophilic tendency (Ajello, 1974; Rippon, 1985).

The study demonstrates that dermatophytes do in fact produce the great majority of fungal infections of the toe-nails and the skin in majority of working groups, but non-dermatophytes keatinophilic species of fungi viz. *Aspergillus*, *Acremonium*, *Alternaria*, *Fusarium*, *Geotrichum*, *Humicola*, *Penicillium*, *Scytalidium*, which varied in their presence in relation to the geographical area are equally important where the study is conducted. Although many of these isolated from the patients suffering with mycoses, but they seems to be only secondary contaminants, because they had no influence

on the evolution of the disease nor the response to the treatment.

Virtually, the study on distribution and natural occurrence of these keratinophilic fungi in the environment which are unfavourable for the diffusion of keratinophilic fungi has not been studied so far. Therefore, it necessitates to identifying both the environments and fungi, where people are exposed to them. The hot and humid environment responding occurrence of pathogenic fungi for dermal infection is a major health concern particularly in lowland paddy growing areas. Keratinophilic fungi have certain universal characteristics indicating environmental pollution in different ecological, including climatic conditions. Therefore selection of certain keratinophilic fungal species and their epidemiological study could have been useful in mapping the polluted habitats.

Although present study is based on local survey conducted on the prevalence of mycoses through visual observation and questionnaire with working groups. The study based on patients is quite subjective and it may have a very limited value. However, it has been noted that mycoses is more frequent in the working groups that can be related with the factors such as diabetes, blood deficiency, arthritis and traumatism. More than 10 per cent women of the state suffering with anemic conditions are in favour of mycotic complications worsen the foot the loss of sensitivity foot distorting foot nails resulting ulceration, bacterial infection followed by inflammation and necrosis.

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