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# Isolation and Characterization of a Dermatophyte, *Microsporum gypseum* from Poultry Farm Soils of Rewa (Madhya Pradesh), India

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Abstract: The study was aimed at assessing the frequency of *Microsporum gypseum*, a potentially pathogenic dermatophyte in various samples collected from poultry farm of Rewa, Madhya. Pradesh, India. The fungal flora was analysed in different samples for the presence of *Microsporum gypseum* by Hair Baiting Technique as recommended by Vanbreuseghem. The isolated strains of *M. gypseum* were cultured on Sabouraud's Dextrose Agar supplemented with cycloheximide and chloramphenicol for at least 3 weeks and then identified on the basis of their microscopic and macroscopic morphology. A total of 37 samples were observed for the presence of this fungi and out of them, 25 were found positive for the occurrence of *M. gypseum* (67.56%). The highest frequency of *M. gypseum* was observed in the 3rd type of samples which were taken between the months of August and February when the temperature and relative humidity is almost favourable. On the other hand, the rest of the samples i.e., 1st 2nd and 4th type were taken and observed during April, May and March, respectively showing less frequency of *M. gypseum*. This shows clearly that the frequency of *Microsporum gypseum* is related with the infection caused by this fungi in Rewa (M.P.), India.

**Key words:** Dermatophyte, *Microsporum gypseum*, keratinophilic fungi, poultry farm, frequency

### Introduction

The Keratinophilic fungi are of prime importance in regard to various skin diseases prevalent in various areas. These fungi are able to utilize keratin; a fibrous protein as sole carbon and nitrogen source and survive saprophytically in nature (English, 1963; Frey and Durie, 1956). The impact of keratinophilic fungi on human health seems unexplored part of various studies. We know that various keratinophilic fungi along with some dermatophytes are responsible for various skin infections. To date, little epidemiological data on fungal skin diseases in this area is available. The isolation of *Microsporum gypseum* was made for the

first time by Dey and Kakoti (1955) from the vicinity of animal house in Dibrugarh District of Assam, India. Later on Randhawa et al. (1965) and Garg (1966) have given comprehensive accounts of distribution of keratinophilic fungi in Indian soils. From the epidemiological point of view dermatophytes are responsible for a number of fungal infections i.e. mycoses (Spiewak and Szostak, 2000; Spiewak, 1998; Nooruddin and Singh, 1987; Ajello, 1960). These are filamentous fungi, which have the ability to invade the epidermis and keratinized structures derived from it such as hair or nails. Isolation of Microsporum gypseum from poultry farm soils in Rewa (M.P.), is an

effort for knowing more about the distribution of *Microsporum gypseum* which causes the infections in this area.

## **Materials and Methods**

There are various habitats in which these fungi are found frequently during this survey. Out of these sites only one site i.e., poultry farm was selected for this study. Five types of samples were taken from Poultry Farm Bicchhiya, Rewa, M.P., India during August 1999 to May 2000. The general method involves collection of soil samples i.e. from soils of poultry farm; preparation of keratin baits and isolation of the fungus (Hair Baiting Technique; Vanbreuseghem, 1952). Special emphasis was given on the collection of fresh samples of soil from that area. Pieces of hair were sprinkled on the surface of double sterilized soil. The soil was moistened with sterilized distilled water and remoistened whenever necessary and incubated at room temperature for up to four weeks. The moulds which appeared on the baits were transferred onto Sabouraud's dextrose agar (SDA) medium supplemented with antibiotics e.g. Chloramphenicol (50 ppm) and cycloheximide (500 ppm). [All chemicals used for preparation of media were of analytical grade].

Plates were incubated at 28°C for 10-21 days and the cultures were examined periodically for fungal growth. After purification isolates were again cultured on SDA for identification. The isolates were identified on the basis of their colonial and morphological characters using monographic descriptions and other available literature (Sarkar, 1962; El Ani and Arif, 1969; Spiewak, 1998; Stockdale, 1961, 1963; Orr and Kuehn, 1972).

### Results

Microsporum gypseum was recovered from 25 samples out of 37 samples observed (67.56%). Out of these, the highest frequency of this fungi was recovered with the 3rd type of sample whereas the considerable frequencies were obtained in 1st and 3rd type of samples i.e. 60 and 66.66% respectively. The lowest frequency of this fungus was seen in 2nd type of sample (57.14%) (Table 1 and Fig. 1). It was observed that Microsporum gypseum was recorded from August 1999 to July 2000. The colonies of Microsporum gypseum as clearly seen on Sabouraud's dextrose agar medium are flat, finely and densely

Table 1: Distribution of soil samples examined

No. of samples	No. of samples with microsporum	Frequency (%)
exammeu	Gypseum	M. gypseum
5	3	60.00%
7	4	57.14%
10	8	80.00%
15	10	66.66%
37	25	67.56%
	5 7 10 15	No. of samples examined         with microsporum Gypseum           5         3           7         4           10         8           15         10

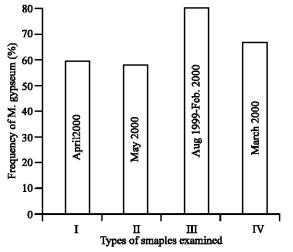


Fig. 1: Graph representing the seasonal variation in the distribution of *M. gyoseum* 

granular, with an arachnoid edge, pale buff, submerged mycelium strongly radiating Thallus colour is tan to cinnamon brown with cottony white centre. Mycelium (Fig. 2). Hyphae are branched, septate and hyaline;



Fig. 2: Colony of *M. gypseum* on SDA showing graunlar to powdery flat rextures



Fig. 3: May rough and thin walled macroconidia and pyriform microconidia of *M. gypseum* (40X)

macroconidia are thick walled whereas microconidia are rough walled as seen in Fig. 3. In the present study isolation and characterization of *Microsporum gypseum* was done to reveal many details of pathological importance (Weitzmann and Summerbell, 1995). To correlate the health problems (skin infections) in this area with the occurrence of dermatophytes, it is very important to investigate the occurring fungus and try to find out it's characteristic features in detail.

# Discussion

All these data clearly indicates that impact of environmental conditions viz. temperature, humidity and rainfall as an important parameter for the growth and survival of *Microsporum gypseum*. It is also observed that the IIIrd type of samples were taken in the months of August, September, October, November, December, January and February of the year 1999 and 2000

respectively i.e., the season with moderate temperature and humidity in this area of India so there is high frequency of M. gypseum (80%) in these samples. On the other hand, 1st, 2nd and 4th type of samples were observed for the presence of M. gypseum in the months of April, May and March, so they are observed with relatively low frequencies of distribution of this fungi i.e. 60, 57.14 and 66.66% respectively because of increasing temperature and decreasing humidity leading to give less favourable conditions for the growth of M. gypseum in comparison to 3rd type of samples. These studies also clearly indicate that under changing atmospheric conditions, microorganisms are capable to grow. Garg (1966) in his study has shown somewhat the same type of distribution of the above genera in the plains of Rajasthan and hilly areas of Kashmir. McAleer (1980) in his study pointed out that winter season favours the growth of keratinophilic fungi. Kaplan (1967) and Thakur et al. (1982) have also reported the higher incidence of ringworm infections in goats during the months of November to March. Some reports published also support these data. So, it can be concluded that change in the season is apparently important in the occurrence of Microsporum gypseum in soil. Moreover, temperature also plays an important role as a major physiological factor in controlling the physiology and metabolic activities of these fungi. Temperature has a remarkable effects on the rate of any chemical reactions involved in the cell metabolism and hence on the growth rate. In the case of many mesophilic fungi, the growth retardation with increase in temperature above the optimum, results from enzyme inactivation due to the heat denaturation of proteins. In a nutshell all these indicates that the environmental factors are the major cause for the variation in the frequencies as described here. The data revealed many facts of fundamental importance.

Comparison with the previous results: Several reports are present in literature showing related facts about the distribution of *Microsporum gypseum(Pugh and* Mathison, 1962; Kishimoto and Baker, 1969; Ajello and Alpart, 1972. After Dey and Kakoti (1955), reports about the isolation of this fungi were also published by many workers (Randhawa *et al.*, 1959; Padhye, 1961; Puri, 1961; Randhawa and Sandhu, 1965; Garg, 1966; Padhye *et al.*, 1967, 1966a). Studies performed in other countries (Youssef *et al.*, 1989; Me Aleer, 1980) which clearly satisfies the essence of climatic conditions, environmental factors for the growth of *M. gypseum* are almost similar in results but the variation in frequency is the main factor for reorganizing our data.

In the recent years this fact is well established that keratinophilic fungi (including dermatophytes) are most pathogenic to men and animals. Studies on these soilinhabiting molds from various habitats are receiving considerable attention in recent years. The present research was undertaken to investigate the distribution of Microsporum gypseum with special reference to areas where biotic factors, which provide a keratin source for these fungi are present. The aim of this contribution is to consider what is known about distribution of M. gypseum in relation to various types of soil samples collected from poultry farms of this area in India. Here M. gypseum is considered in most detail because in recent years the ecological aspects of soil mycology have attracted much attention with a view to alleviate human sufferings. Hence it seems essential to undergo a systematic survey of different types of soils collected under different environmental conditions in order to see the distribution pattern of Microsporum gypseum to determine their pathogenicity in men and animals. Because of this, it is important to determine more about the distribution of this fungus, which is common in occurrence and pathogenic too. Possibly this will help in the further studies on epidemiology of dermatophyte infections in this area. It is evident from the present study that the presence of Microsporum gypseum in poultry farm soils is common and has a health risk. During the examination of samples, the data revealed the fact that abundant numbers of samples were found with Microsporum gypseum. It was revealed that out of 37 samples, examined from various poultry farm soils of this area, 25 samples were seen positive. This shows an average of 67.56% samples were found positive for the presence of Microsporum gypseum. On the other hand a total of four types of samples were taken from different poultry farms. From these the highest frequency of Microsporum gypseum was found with 3rd type of sample i.e. eight out of ten samples examined (80%).

Moreover, the lowest frequency of Microsporum gypseum was encountered with the IInd type of sample i.e., four out of seven samples examined (57.14%). There is no doubt that the studies performed in other countries (Bagy and Mallek, 1991; Aho, 1983; Moubasher et al., 1993, Hasan et al., 993; Mahmoud, 1993; Della Farnca and Caretta, 1984) and in India (Sarkar, 1962; El Ani and Arif, 1969; Spiewak, 1998; Stockdale, 1961, 1963; Orr and Kuehn, 1972) have reported nearly same findings but in Rewa, M.P., India the amount of research that have been performed on dermatophytes is scanty and there is a wide possibility to investigate the fact that more the number of dermatophytes higher is the incidence of infection. Since these poultry farms are the favourable sites for the occurrence of a wide variety of fungi specially M. gypseum, they are therefore expected to play an important role in the epidemiology of human mycoses in this area.

### References

- Aho, R., 1983. Saprophytic fungi isolated from the hair of domestic and laboratory animals with suspected dermatophytes. Mycopathologia, 83: 65-73.
- Ajello, L., 1960. Geographic distribution and prevalence of the dermatophytes. Ann. NY Acad. Sci., 89: 30-38.
- Ajello, L. and E.M. Alpert, 1972. Survey of Easter Islands soils for keratinophilic fungi. Mycologia, 64: 161-166
- Bagy, M.M.K. and A.Y. Abdel-Mallek, 1991. Fungi on the hair of small mammals in Egypt. Cryptogamie Mycol., 12: 63-9.
- Delia Franca, P. and G. Caretta, 1984. Keratinophilic fungi isolated from the air at Pavia. Mycopathologia, 85: 65-8.
- Dey, N.C. and L.M. Kakoti, 1955. *Microsporum gypseum* in India. J. Indian Med. Assoc., 25: 160-164.
- EI-Ani and S. Arif, 1969. The macroconidium appendages in *Microsporum gypseum*. Mycologia, 69: 510-517.
- English, M.P., 1963. The saprophytic growth of keratinophilic fungi on Keratin. Sabouraudia, 2: 115-130.
- Frey, D. and E.B. Durie, 1956. The isolation of Keratinophilic fungi including *Microsporum gypseum* from Australian soil Aust. J. Exp. Biol. Med. Sci., 24: 199-204.
- Garg, AX., 1966. Isolation of dermatophytes and other Keratinophilic fungi from soils in India, Sabouraudia, 4:259-264.
- Hasan, H.A.H., M.M.K. Bagy and A.Y. Abdel-Mallek, 1993. The incidence of fungi in human. axillary hair and their toxigenic potentialities. Cryptogomie Mycol., 14: 297-306.
- Kaplan, W., 1967. Epidemiology and public health significance of ringworm in animals. Arch. Derm., 96: 403.
- Kishimoto, R.A. and G.E. Baker, 1969. Pathogenic and potentially pathogenic fungi isolated from beach sands and selected soils of Oahu, Hawaii. Mycologia, 61: 537-548.
- Mahmoud, A.L.E., 1993. Dermatophytes and other associated fungi isolated from ringworm lesions of camels. Folia Microbiol., 38: 505-8.
- Me Aleer, R., 1980. Fungal infections of the scalp in the Western Australia. Sabouraudia, 18: 185-190.
- Moubasher, A.H., A.M. Elnaghy, S.M. Maghazy and Z. El-Gendy, 1993. Dermatophyte and cycloheximide resistant fungi isolated from patients with tinea capitis and from air in hospitals in Minia, Egypt. The Korean J. Mycol., 21: 77-84.
- Nooruddin, M. and B. Singh, 1987. Dermatophytosis in buffaloes, cattle and their attendants. Mykosen, 30: 594-600.

- Orr, G.F. and H.H. Kuehn, 1972. Notes on Gymnoascaceae II. Some Gymnoascaceae and keratinophilic fungi from Utall. Mycologia, 64: 55-72.
- Padhye, A.A., 1961. Microsporum gypseum from soil in Poona, Prof Agharkar Commenwealth Volume, pp: 117-119.
- Padhye, A.A., S.P. Mishra and M.J. Thirumalachar, 1966a.

  Occurrence of soil inhabiting dermatophytes and other keratinophilic fungi from soil in Poona. Hindustan Antibiotics Bull., 9: 90-93
- Padhye, A.A., V.H. Powar, R.S. Sukapure and M.J. Thirumalachar, 1967. Keratinophilic fungi from marine soils of Bombay, India, Part I. Hindustan Antibiotics Bull., 10: 138-141.
- Pugh, G.J.F. and G.E. Mathison, 1962. Studies on fungi in coastal soils III. An ecological survey of keratinophilic fungi. Trans. Brit. Mycol. Soc., 49: 567-572.
- Puri, H.R., 1961 Isolation of dermatophytes from Indian soils. Bull. Calcutta. Sch. Trop. Med. Hyg., 8: 121-122
- Randhawa, H.S. and R.S. Sandhu, 1965. A survey of soil inhabiting dermatophytes and related keratinophilic fungi of India. Sabouraudia, 4: 71-79.
- Randhawa. H.S., A. Nath and R. Vishwanathan, 1959. Isolation of Microsporum gypseum (bodin) guiart and Grigorakis from soils in Delhi. Sci. and Culture, 25: 326-327.
- Sarkar, A., 1962. Perfect state of fructification of *Microsporum gypseum* in unsterile soil. Bull. Calcutta Sch. Trop. Med. Hyg., 10: 76-77.
- Spiewak, R., 1998. Zoophilic and geophilic fungi as a cause of skin diseases in farmers. Ann. Agric. Environ. Med., 5: 97-102.
- Spiewak, R. and W. Szostak, 2000. Zoophilic and geophilic dermatophytoses among farmers and non-farmers in eastern Poland. Ann. Agric. Environ. Med., 7: 125-129.
- Stockdale, P.M., 1963. The *Microsporum gypseum* complex .Sabouraudia, 3: 114-126.
- Stockdale, P.M., 1961. Nannizzia incurvata gen. nov. sp. nov. a perfect state of *Microsporum gypseum* Bodin Guiart et. Grigorakis. Sabouraudia, 1: 41-48.
- Thakur, D.K., S.K. Mishra and P.C. Choudhary, 1982.
  Dermatophytosis in goats in India. Mykosen, 25: 442-448.
- Vanbreuseghem, R., 1952. Biological technique for the isolation of dermatophytes from soil. Ann. Soc. Beige. Med. Trop., 32: 173-178.
- Weitzmann, I. and R.C. Summerbell, 1995. The dermatophytes. Clin. Microbiol. Rev., 8: 240-259.
- Youssef, Y.A., A.A. Karam, El-Din and A. Mohamed, 1989. Survey of soil for human pathogenic fungi from Ismailia Governorate. Egypt. 1. Isolation of Keratinophilic Fungi Mans Sci. Bull., 16: 153-163.