Occurrence of keratinophilic fungi with special *reference* to *Chrysosporium* species in soils of India

N. Nigam & R. K. S. Kushwaha

Department of Botany , Christ Church College, Kanpur, 208001, India

Nigam, N. & R. K. S. Kushwaha (1990). Occurrence of keratinophilic fungi with special reference to *Chrysosporium* species in soils of India. – Sydowia 42: 200–208.

During a survey for keratinophilic fungi from 7 habitats, 28 fungal species were isolated from 91 house dust and outdoor soil samples. Fourteen species of *Chrysosporium*, three of *Microsporum*, two each of *Myceliophtora, Aphanoascus, Arthroderma, Trichophyton* and one each of *Ctenomyces, Myxotrichum* and *Onygena* were isolated. House dust was found to be rich in *Chrysosporium* species. The vertical distribution of keratinophilic fungi in forest and garden soils of India is discussed.

Since Randhawa & Sandhu's (1965) initial comprehensive survey of Indian soils, more detailed studies on the occurrence of keratinophilic fungi and other dermatophytes have been conducted. All have shown that hyphomycetes of the genus *Chrysosporium*, which is related to the dermatophytes (Carmichael, 1962; van Oorschot, 1980) were commonly isolated (Deshmukh & Agrawal, 1983; Garg, 1966; Jain & Agrawal, 1977; Jain & al., 1985; Padhye & al., 1966; Randhawa & Sandhu, 1965; Sur & Ghosh, 1980; Verma & al., 1982). *Chrysosporium* spp. were also isolated from house dust in Southern California (Kozak & al., 1980). However, there has been little emphasis on the ecological significance and geographic distribution of the species of this genus as compared to other keratinophilic fungi (Ajello, 1960). The factors which favour their wide distribution are not known. The purpose of the present investigation was to study the occurrence of keratinophilic fungi in house dust and outdoor soils of India with special reference to *Chrysosporium* spp.

Material and methods

Sample collection was made from seven sites in Kanpur, India, an old industrial city with a population of over two millions situated on the right bank of the Ganges River at 26° 26′ N and 80° 20′ E. The soil is grey and brown, unpigmented, with salt, and the climate is semiarid. Dry, hot summers with temperature over 45 °C maximum change into warm, humid seasons (90% maximum relative humidity) during mansoon. The winter is severe with temperatures reaching

-20 °C. Dust storms are common in the summer and due to the dry climate dust gets lifted up in the atmosphere.

House floor sweepings were collected from houses of Kanpur, India in the morning at 5–6 AM with a sterile nylon broom and put in sterilized polythene bags. The soil samples were collected with a sterile plastic spoon from the superficial layer of soil (1–2 inches), and put in sterilized polythene bags. The house dust was also collected in summer, mansoon, and winter seasons. The daily samples of each season in 1982–83 (July – Sept.; Oct. – Feb.; March – June) were pooled and analysed.

The soil samples from different depths of forest and grassland were spread on agar containing sterilized soil. The indoor soil is referred to as house dust, while other soils types are designated outdoor soils throughout the study. The samples were brought to the laboratory and stored at room temperature until processed.

Ten to fifteen grams of house dust or outdoor soil from each sample was put into sterilized 90 mm Petri dishes and moistened with 5–10 ml of sterile distilled water. Human hairs, nails and buffalo horns, and bird feathers were used as baits (Benedek, 1962). After rinsing in water the baits were dried, cut into small pieces, and sterilized by autoclaving at 120 °C for 15 minutes. Fifty baits pieces were scattered over the soil in the Petri dishes and incubated at 28 ± 2 °C in an incubator for a period of 6–8 weeks. If no growth was observed after this incubation period the samples were considered to contain no keratinophilic fungi.

Isolations were done by direct transfer of fungal mycelia from the invaded bait to Sabouraud dextrose agar (dextrose 40.0 g, peptone 10.0 g, agar 15.0 g, 1000 ml distilled water) supplemented with streptomycin sulphate (40 i.U./ml). If more than one species grew on the bait the material was suspended in sterile normal saline and streaked on plates of the above mentioned medium for the purification of cultures.

The percentage of occurrence and frequency were defined as follows:

$$\% \text{ occurrence} = \frac{\text{No. of positive samples}}{\text{Total number of samples}} \times 100$$

$$\% \text{ frequency} = \frac{\text{No. of isolates of a fungus}}{\text{Total no. of isolates}} \times 100$$

Results

Of the 91 soil samples collected, 87% contained keratinophilic fungi. Nine genera were isolated by using bird feathers, human hair

and animal horn as baits (Tab. 1). *Chrysosporium* was represented by 14 species, with *Chrysosporium evolceanui* occurring at low frequency (Fig. 1).

Tab. 1. Recovery of keratinophilic fungi from different baits.

Genera	No. of species	% frequency feathers	baits hair	horn
Aphanoascus	2	3	4	4
Arthroderma	2	3	4	4
Chrysosporium	14	13	26	8
Ctenomyces	1	1	0	0
Microsporum	3	0	5	0
Myceliophthora	2	0	0	2
Myxotrichum	1	0	0	1
Onygena	1	0	0	1
Trichophyton	2	4	5	4

% frequency based on 100 baits.

Five isolates of *C. tropicum*, 2 of *C. queenslandicum* and 3 of *Microsporum* sp. differed from their respective type specimens in spore size range. The maximum frequency of *Chrysosporium* species was observed in house dust and that of the other fungi in forest soil (Fig. 2). Soil from cultivated fields was poor in *Chrysosporium* species.

Microsporum, Myceliophthora, Myxotrichum and *Onygena* were not isolated from feathers (Tab. 1). Hair baits did not yield *Ctenomyces, Myceliophthora, Myxotrichum* and *Onygena*. *Ctenomyces* and *Microsporum* could not be isolated from horn.

A seasonal periodicity of keratinophilic fungi was observed only in the samples of house dust (Fig. 3). *C. tropicum* and an unidentified species of *Chrysosporium* were most frequently isolated during the rainy season, summer and winter. *C. indicum* was recorded at a very low frequency during the rainy season and the summer. These fungi were most frequent in house dust during the winter and rainy season. The data revealed the prevalence of *Chrysosporium* sp. in house dust (Tab. 2). *C. tropicum* was the second most common species (25% in house dust and 52.5% in outdoor soil samples). *C. indicum, C. evolceanui* and *C. pannicola* were also common in both house dust and outdoor soil samples. *C. carmichaeli, C. farinicola, C. lucknowense* and *C. sulfureum* were rare and *C. tuberculatum* very common in outdoor soil only. *C. carmichaeli, C. merdarium, C. queenslandicum* were uncommon in house dust.

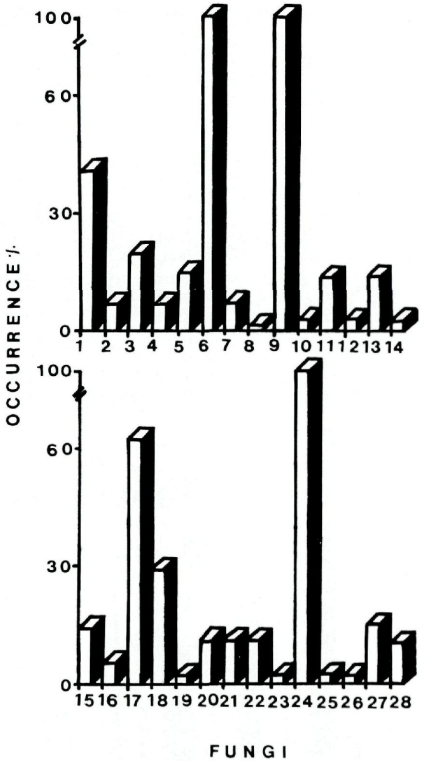

Fig. 1. – Occurrence of keratinophilic fungi in house dust, forest and grassland soil (in % of samples examined). – 1. *Aphanoascus* sp. – 2. *A. terreus* (Randhawa & Sandhu) Apinis. – 3. *Arthroderma flavescens* Rees. – 4. *A. gertleri* Bohme. – 5. *Chrysosporium* sp. – 6. *C. carmichaeli* van Oorschot. – 7. *C. crassitunicatum* Kushwaha & Agrawal. – 8. *C. evolceanui* (Randhawa & Sandhu) Garg. – 9. *C. farinicola* (Burnside) Skou. – 10. *C. indicum* (Randhawa & Sandhu) Garg. – 11. *C. keratinophilum* Frey ex Carmichael. – 12. *C. lucknowense* Garg. – 13. *C. merdarium* (Link ex Grev.) Carmichael. – 14. *C. pannicola* (Corda) van Oorschot & Stalpers. – 15. *C. queenslandicum* Apinis & Rees. – 16. *C. sulfureum* (Fiedl) van Oorschot & Samson. – 17. *C. tropicum* Carmichael. – 18. *C. tuberculatum* Kuehn. – 19. *Ctenomyces serratus* Eidem. – 20. *Microsporum* sp. – 21. *M. fulvum* Uriburu. – 22. *M. gypseum* (Bodin) Guiart & Grigorakis. – 23. *Myceliophtora* anamorph of *Arthroderma tuberculatum* Kuehn. – 24. *Myceliophtora* anamorph of *Corynascus novoguineensis* (Udagawa & Horie) von Arx. – 25. *Myxotrichum* sp. – 26. *Onygena* sp. – 27. *Trichophyton flavescens* Padhye & Carmichael. – 28. *T. vanbreuseghemii* (Rioux) Jarry & Juminer.

Tab. 2. Prevalence of *Chrysosporium* species in house dust and outdoor soils.

	house dust	outdoor soil
No. of samples examined	32	59
No. of samples positive	27 (84.3%)	52 (88.1%)
Chrysosporium sp.	13 (40.6%)	7 (11.8%)
C. carmichaeli	1 (3.1%)	0 (0%)
C. crassitunicatum	0 (0%)	3 (5.0%)
C. evolceanui	1 (3.1%)	2 (3.3%)
C. farinicola	0 (0%)	1 (1.6%)
C. indicum	2 (6.2%)	8 (13.5%)
C. keratinophilum	1 (3.1%)	0 (0%)
C. lucknowense	0 (0%)	1 (1.6%)
C. merdarium	1 (3.1%)	0 (0%)
C. pannicola	2 (6.2%)	3 (5.0%)
C. queenslandicum	2 (6.2%)	0 (0%)
C. sulfureum	0 (0%)	2 (3.3%)
C. tuberculatum	0 (0%)	12 (20%)
C. tropicum	8 (25.0%)	31 (52.5%)

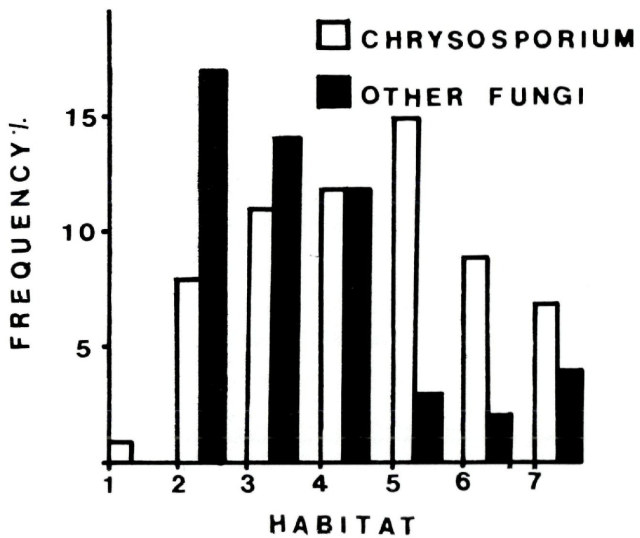

Fig. 2. – Frequency (%) of *Chrysosporium* spp. and other fungi in seven habitats. 1. Cultivated field. – 2. Forest. – 3. Gardens. – 4. Grassland. – 5. Houses. – 6. Playgrounds. – 7. Roadside.

During the study on the vertical distribution of keratinophilic fungi in forest and grassland soil, 7 genera representing 16 species

Tab. 3. Vertical distribution of keratinophilic fungi in forest and grassland soils.

Group	Forest soil	Grassland soil
Group A		
Fungi present throughout the soil profile	*Arthroderma flavescens* *A. gertleri* *Aphanoascus terreus* *Chrysosporium tuberculatum* *C. indicum* *C. tropicum* *Trichophyton flavescens* *T. vanbreuseghemii*	*A. flavescens* *Aphanoascus* sp. *C. tuberculatum* *Chrysosporium* sp. *C. crassitunicatum* *C. tropicum* *T. flavescens*
Group B		
Fungi present in upper profile (0–10 inches only)	*Aphanoascus* sp. *Myxotrichum* sp.	No fungal record
Group C		
Fungi present in lower profile (10–18 inches only)	*C. evolceanui* *Ctenomyces serratus* *Onygena* sp.	*A. terreus* *C. indicum* *C. lucknowense*

were isolated (Tab. 3). Thirteen and eleven species were isolated from forest and grassland soils respectively. *Arthroderma flavescens, Aphanoascus* sp., *Aphanoascus terreus, C. tuberculatum. C. indicum, C. tropicum* and *Trichophyton flavescens* occurred in both types of soil. *A. flavescens, C. tuberculatum, C. tropicum* and *T. flavescens* were particularly common in the profiles of the forest as well as in those of grassland soils while *Aphanoascus* sp. and *Myxotrichum* sp. were present only in the upper profile of the forest soils. In contrast, no keratinophilic fungi were found in the upper profile of the grassland soils. *C. evolceanui, C. serratus* and *Onygena* sp. occurred only in the lower profile of the forest soils. *A. terreus, C. indicum* and *C. lucknowense* were found only in the lower profile of the grassland soils. The former two species were recorded also from both profiles of the forest soils.

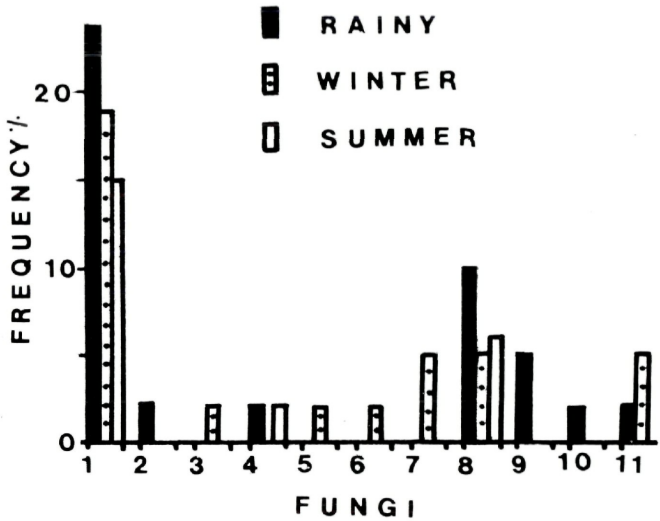

Fig. 3. – Seasonal variation in the keratinophilic flora of house dust. – 1. *Chrysosporium* sp. – 2. *C. carmichaeli*. – 3. *C. evolceanui*. – 4. *C. indicum*. – 5. *C. keratinophilum*. – 6. *C. merdarium*. – 7. *C. queenslandicum*. – 8. *C. tropicum*. – 9. *Microsporum* sp. – 10. *M. fulvum*. – 11. *M. gypseum*.

Discussion

The frequency of fungi in house dust is noteworthy. *Keratinophyton terreum* and *Microsporum gypseum* had been reported earlier from house dust by RANDHAWA & SANDHU (1965). *A. flavescens, A. gertleri, C. carmichaeli, C. queenslandicum, C. sulfureum, Myceliophtora* anamorph of *Arthroderma tuberculatum* and *Myceliophtora* anamorph of *Corynascus novoguineensis* were isolated for the first time in India by NIGAM & KUSHWAHA (1985a).

The prevalence of *Chrysosporium* sp. and other keratinophilic fungi with a definite seasonality had not been previously recorded in India. There are at least some clues that *Trichophyton ajelloi* survives well in the hills of the Himalayas where temperature remains low and that *C. indicum* is more frequent in the plains than in the hills (DESHMUKH, 1985; GARG, 1966; RANDHAWA & SANDHU, 1965). In the present study different frequencies were recorded in each season. The exact nature of the physiological mechanism that regulates the seasonality of keratinophilic fungi remains to be explored.

The prevalence of five species of *Chrysosporium* in both house and outdoor soils had previously been demonstrated by GARG (1966) and JAIN & al. (1985). *C. tropicum* and *Chrysosporium* sp. were not abundant in the outdoor soil and in house dust. The regular disturbance in indoor soil creates difficulty in assessing the definite prevalence of fungal species.

On the basis of vertical distribution in grassland and forest soil, keratinophilic fungi were categorised in three groups (Tab. 3). In both types of soil the upper profiles contained the most diverse populations. However, the number of isolates from the deeper layers decreased as the distance from the upper surface increased. VARDAVAKIS (1986) reported the occurrence of some plant pathogenic and cellulolytic fungi in a depth of 30 cm. In soil, CHEML & VLACILIKOVA (1975) found keratinophilic fungi in a depth of 55 cm. The differences in species number in soil profiles may be associated with adaptation to development at low partial pressure of oxygen and high concentrations of carbon dioxide in deep sites (ALEXANDER, 1961). The vertical distribution also seems to be associated with the concentration of keratinic substrates and with the composition of the soil atmosphere. The failure to isolate some of these fungi in previous surveys (NIGAM & KUSHWAHA, 1985b) may be due to the influence of vertical distribution. Further investigations will provide a better understanding of the keratinophilic mycoflora in Indian soils and house dust.

Acknowledgments

The research project on *Chrysosporium* was financed by the Council of Scientific and Industrial Research, New Dehli, India. We thank Professor L. AJELLO for his valuable comments and for reading the manuscript, Drs. A. A. PADHYE and L. SIGLER for their help in the identification of some of the isolates and Drs. J. PATTERSON and D. E. REUBEN for providing research facilities.

References

AJELLO, L. (1960). Geographic distribution and prevalence of the dermatophytes. – Annals New York Acad. Sci. 89: 30–38.

ALEXANDER, M. (1961). Introduction to soil microbiology. – John Wiley and Sons. Inc., New York, 472 p.

BENEDEK, T. (1962). Fragmenta mycologica. I. Some historical remarks on the development of 'hair baiting' of Tom-Karling Vanbreuseghem (The To Ka Va hair baiting method). – Mycopath. Mycol. Appl. 16: 104–106.

CARMICHAEL, J. W. (1962). *Chrysosporium* and some other aleurosporic hyphomycetes. – Can. J. Bot. 40: 1137–1173.

CHEML, L. & V. VLACILIKOVA (1975). Distribution of keratinophilic fungi in different depths of soil. – Sabouraudia 13: 185–191.

DESHMUKH, S. K. (1985). Isolation of dermatophytes and other keratinophilic fungi from soil of Mussoorie (India). – Mykosen 28: 98–101.

DESHMUKH, S. K. & S. C. AGRAWAL (1983). Prevalence of dermatophytes and other keratinophilic fungi in soils of Madhya Pradesh (India). – Mykosen 26: 574–577.

Garg, A. K. (1966). Isolation of dermatophytes and other keratinophilic fungi from soil of India. – Sabouraudia 4: 259–264.

Jain, P. C. & S. C. Agrawal (1977). Keratinophilic fungi from the soils of Mount Abu (India). – Geobios 4: 136–138.

Jain, M., P. K. Shukla & O. P. Shrivastava (1985). Keratinophilic fungi and dermatophytes in Lucknow soils and their global distribution. – Mykosen 28: 148–153.

Kushwaha, R. K. S. & S. C. Agrawal (1976). Some keratinophilic fungi and related dermatophytes from soil. – Proc. Ind. Natl. Sci. Acad. 42: 102–110.

Kozak, P. R., Jr., J. Gallup, L. H. Commins & S. A. Gilman (1980). Currently available methods for some mold surveys. II. Examples of problem homes surveyed. – Annals Allergy 45: 167–176.

Nigam, N. & R. K. S. Kushwaha (1985a). Eight new keratinophilic fungal records from India. – Proc. Natl. Acad. Sci. 55: 26.

Nigam, N. & R. K. S. Kushwaha (1985b). *Chrysosporium* and related fungi in different profiles of soil. – Proc. VIII Ind. Bot. Soc. 64:18.

Padhye, A. A., S. P. Misra & M. J. Thirumalachar (1966). Occurrence of soil inhabiting dermatophytes and other keratinophilic fungi from soil in Poona. – Hind. Antibiot. Bull. 9: 90–93.

Randhawa, H. S. & R. S. Sandhu (1965). A survey of soil inhabiting dermatophytes and keratinophilic fungi of India. – Sabouraudia 4: 71–79.

Sur, B. & G. R. Ghosh (1980) Keratinophilic fungi from Orisa, India I. Isolation from soils. – Sabouraudia 18: 269–274.

Vardavakis, E. (1986). Vertical distribution and seasonal fluctuations of cellulolytic fungi in a typic calcixeroll soil in Greece. – Trans. Br. Mycol. Soc. 86: 668–672.

Van Oorschot, C. A. N. (1980). A Revision of *Chrysosporium* and allied genera. – Stud. in Mycol. 20: 1–89.

Verma, T. N., B. K. Sinha & U. L. Das (1982). Isolation of keratinophilic fungi from soil in Bihar. – Mykosen 25: 449–452.