DETERMINATION OF PERCENT FREQUENCY OF KERATINOPHILIC FUNGI ISOLATED FROM CATTLE SHEDS AND BARBERS SHOP WASTE SOIL OF GORAKHPUR REGION

Sandeep Chaudhary and C. O. Samuel*

Natural Fungicide laboratory, Department of Botany, St. Andrew's P.G. College, Gorakhpur, U.P. (India)

Abstract: The present study deals with the isolation and distribution analysis of keratinophilic fungi of Gorakhpur region. Two sites were chosen: three cattle sheds and three waste soil of barbers' shop. A total of thirty six samples were taken. Thirty samples out of thirty six examined were found positive for fungal presence. Two baits i.e. human and buffalo hair were used. The fungi grown were isolated and identified. Their percent frequency was determined and a comparative study was made. There is a marked difference in the nature, percent occurrence and distribution of fungi of the two sites. Total 378 occurrences of fungi were recorded. 229 fungal isolates were found in cattle shed soil, whereas in barbers shop soil the no was 149. Most dominant fungi were *Aspergillus, Chrysosporium, Fusarium* and *Trichophyton.*

Keywords: Keratinophilic fungi, waste land, plant and human pathogenic fungi, soil sample, dermatophytes.

Introduction:

For the first time French Dermatologist Sabouraud stated that dermatophytes are primary soil saprophytes (1893). Soils that are rich in keratinous matters are most suited for the growth and occurrence of keratinophilic fungi. So much so that it may be comfortably said that percentage occurrence of fungi is directly proportional to soil organic matter especially keratin.

Vanbreusegham (1952) was the pioneer in this field to discover keratinophilic fungi. He was followed by Gordan (1953). Keratinofers occur in many natural and manmade habitats and exhibit affinity to keratinous substrates while degrading them in natural conditions. The ubiquity of these fungi in soil and various other environments is well known.

The fungi are also reported from other habitats viz. rice fields : Sundaram (1977) and Singh *et al.* (1994), lake side soil : Ghosh & Bhatt (2000), muddy soil : Zaki *et al.* (2005) and forest and farm soil: Moallaei *et al.* (2006).By and large keratinophylic fungi were paid special attention by Deshmukh (1983), Deshmukh and Agrawal (2003), Sharma and Rajak (2003), Singh *et al.* (2012), Agrawal and Khanum (2013), Sarkar *et al.* (2014), Bisen & Tiwari (2015) .The present investigation was carried out to have an idea of keratinophilic fungi in Gorakhpur region.

Abundance of keratinophilic microbes in the habitats which are having keratin remnants of human and animal origin is well expected. Some of those fungi display pathogenic properties also. Therefore, studies on their presence in agricultural and wasteland environments become epidemiologically significant.

Gorakhpur being a part of Terai belt of Himalayas experiences a very wide range of variation in temperature, rainfall and humidity across the year. On top of everything it is densely populated. These conditions help in the proliferation of a large number of microbes round the year. They belong to various categories; keratinophilic fungi are one of them. Some most common ones are: A*spergillus, Curvularia, Fusarium, Chrysosporium, Trichophyton, Alternaria* and *Penicillium* etc. They are common saprophytes in soil and plant debris, while some of them are often recorded as contaminants.

For the present study soils from cattle sheds and barbers' shops (where hair is discarded after hair cutting and is settled there for longer periods) were collected. Six localities from each were used taking into consideration that there may be difference in the mycoflora of the cattle shed and barber' shop as one is related to animals and plants (cattle feed) and one is to human beings.

Materials and Method:

Thirty six soil samples were collected from twelve localities (three each from six cattle sheds and six barbers' shop sites) in sterilized polythene bags in the month of September 2017. A sterile spatula was used to dig the soil from the specified layer (2-3 cm deep).The bags were labeled properly and closed tightly by rubber band.

The soil was brought to the laboratory, filled in sterilized Petri plates. Two baits - Human and Buffalo hair were used. Hairs were collected, cut in to pieces, washed thoroughly with distilled water (4-5 times) and surface sterilized with chloroform. Again washed with distilled water to remove chloroform, dried in oven .These baits were spread over the soil in the Petri plates under aseptic conditions, left for 35 days at about 25^0C, in incubator. The soil was moistened periodically to maintain appropriate moisture with distilled water. When optimum fungal growth was observed the mycelial fragments were taken and inoculated on Sabouraud Dextrose Agar (SDA) medium. They were kept in refrigerator for preservation for further study when pure fungal colonies were well developed.

The fungi were observed and assigned to their systematic position with the help of different available literature.

Observation:

S. N.	Name of Fungus	Cattle Shed Soil					Barbers' Shop Soil				
		Site I	Site II	Site III	Total	%	Site I	Site II	Site III	Total	%
1	*Absidia glauca*	1	2	5	8	4.0	2	2	1	5	3.0
2	*Alternaria alternata*	2	2	3	7	3.5	1	1	--	2	1.2
3	*Aphanoascus terreus*	--	--	--	--		2	1	1	4	2.4
4	*Acremonium sp.*	--	--	--	--		1	2	--	3	1.8
5	*Aspergillus flavus*	3	2	2	7	3.5	2	3	1	6	6.6
6	*A.fumigatus*	4	2	2	8	4.0	1	2	--	3	1.8
7	*A.nidulans*	5	2	2	9	4.5	2	--	2	4	6.6
8	*A.niger*	3	2	1	6	3.0	2	1	2	5	3.0
9	*Candida albicans*	4	5	2	11	5.5	1	2	--	3	1.8
10	*Cladosporium herbarum*	3	3	2	8	4.0	1	1	--	2	1.2
11	*Chrysoaporium indicum*	4	3	--	7	3.5	5	6	4	15	9.0
12	*C.tropicum*	3	3	2	8	4.0	2	--	2	4	2.4
13	*C.keratinophyllum*	5	5	8	18	9.0	5	4	2	11	6.6
14	*Curvularia lunata*	2	1	--	3	1.5	--	--	1	1	0.6
15	*Drechslera oryzae*	3	3	2	8	4.0	---	1	--	1	0.6
16	*Fusarium oxysporum*	6	7	1	14	7.0	2	2	1	5	3.0
17	*F.solani*	3	3	2	8	4.0	2	2	1	5	3.0
18	*Geomyces destructans*	3	3	--	6	3.0	2	2	4	8	4.8
19	*Gymnoascus reesii*	1	2	--	3	1.5	1	1	1	3	1.8
20	*Geotrichum candidum*	3	3	1	7	3.5	--	2	2	4	2.4
21	*Mycosporium gypseum*	1	2	--	3	1.5	--	--	--	--	
22	*Mucor mucedo*	2	2	3	7	3.5	2	1	2	5	3.0
23	*Myceliophthora thermophila*	1	1	1	3	1.5	--	--	1	1	0.6
24	*Nocardia sp*	2	2	--	4	2.0	3	--	--	3	1.8
25	*Paecilomyces sp*	2	1	1	4	2.0	---	1	--	1	0.6
26	*Penicillium chrysogenum*	2	4	--	6	3.0	--	2	2	4	2.4
27	*P.notatum*	3	2	2	7	3.5		1	1	2	1.8
28	*Rhizopus globosus*	3	3	2	8	4.0	--	--	1	1	0.6
29	*R.nigricans*	1	2	--	3	1.5	2	--	2	4	2.4
30	*R.stolonifer*	2	2	1	5	2.5	2	3	--	5	3.0
31	*Torula*	2	--	2	4	2.0	1	--	1	2	1.2
32	*Trichophyton mentagrophytes*	4	--	4	8	4.0	3	2	3	8	4.8
33	*T.simii*	3	3	1	7	3.5	2	--	1	3	1.8
34	*T.terrestre*	3	4	--	7	3.5	2	2	2	6	6.6
35	*Verticillium*	3	4	--	7	3.5	2	4	--	6	6.6
Total Number of Fungi		**92**	**85**	**52**	**229**		**53**	**51**	**42**	**149**	

$$\text{Percent frequency} = \frac{\text{No of occurrence of fungal species}}{\text{Total No of all the Fungi}} \times 100$$

Result and Discussion:

A total of 378 colonies of different keratinophilic fungi were isolated from 36 soil samples. The isolated keratinophilic fungi were classified into 35 species belonging to 24 genera. The cattle shed showed more no of encounters (229) in comparison to the waste soil of barbers shop (149). It is noteworthy that three sp each of *Chrysporium* and *Trichophyton* were observed which are pathogenic in nature. The isolated keratinophilic fungi were in the following order of dominance: *Aspergillus* sp ; 32% (C14+B18), *Chrysosporium;*31.5% (C13.5+B18) and *Trichophyton* 24.2 % (C11+ B13.2). Followed by *Fusariun* sp 17.4 % (C11.4+B6), *Rhizopus* 13% (C7+B6). The presence of keratinophilic fungi in different soil has been reported worldwide (Deshmukh SK. 2004; Shadzi S, *et al.* 2002; Saxena P, *et al.* 2004, Zarei MA & Zarrin M. 2008; Shrivastava JN, *et al*. 2008).

The table shows the presence of *Aphanoascus* and *Acremonium* only in B soil .It may be due to their human pathogenicity. As *Aphanoascus* attacks hair cuticle first, its presence in B soil is significant. *Curvularia* and *Drechslera* are saprophytes as well as cause plant pathogenic diseases ,their presence in cattle shed may be due to cattle feed plants. Most of the sp. as *Absidia glauca*, *Aspergillus nidulans*, *Candida albicans*, *Chrysosporium keratinophylum and Fusarium oxysporum* etc. are much in higher number in C soil rather than B soil comparatively. On the contrary only *C.indicum* is more in C soil than in B soil.

Keratinolytic activity of fungi is important ecologically and has attracted the attention of researchers throughout the world (Fillipello *et al.* 2000; Zarrin M, Haghgoo R. 2011). These fungi are associated with human and animal mycoses. Although the fungi isolated are commonly of nondermatophytic in nature, but some of the isolates are found to be pathogenic to humans. Study showed that the genus *A. niger*, one of the dominant fungi in the waste soils, is pathogenic to humans and causes aspergillosis and may also cause pulmonary disease in immunocompromised (Nakagawa *et al.* 1999.*). A. flavus*, also isolated during the present study, is reported to have keratinase activity. This possibly describes the recovery of fungus from the sterile hair bait. Several reports have indicated that *Aspergillus s*pecies are among the most prevalent keratinophilic fungi in the soils (Avasn *et al.* 2012; Mini KD, *et al.* 2012;Maruthi YA, *et al.* 2012; Mini KD *et al.* 2012 and Maruthi *et al.* 2012 Maruthi *et al.* 2012**)**. Presence of *Rhizopus*, *Mucor*, and *Curvularia* species in various soil samples have also been reported by various workers. The occurrence of *Chrysosporium* sp. in waste soils is an important finding of present study as pathogenic potential of this fungus was confirmed in several studies in different countries. For instance, *C. zonatum* was showed causing systemic infection in a person with a chronic granulomatous disease (Ulfig K. 2006). Various species of *Chrysosporium* have been

reported from Indian soils. Therefore hygiene protocol should be taken care of to prevent the spread of pathogenic fungi in these environments as there is a risk of fungal infections of human. These findings should be taken into consideration and necessary treatment methods should be taken up periodically.

Acknowledgements:

Authors are grateful to Rev. Prof. J. K. Lal, The Principal, St. Andrew's College, Gorakhpur for providing infrastructure and facilities. One of the authors Sandeep Chaudhary is thankful to University Grant Commission - Rajiv Gandhi National Fellowship (UGC-RGNF) scheme for providing financial assistance.

References:

Agarwal, S.C. and Khanam, S.J.P. 2014, Isolation of Keratinophilic Fungi from Soils of Poultry Farms; In. J. of Inno. in Eng. R. & M.; Volume: 01 (05); page:1-4

Avasn YM, Hossain K, Priya DH, Tejaswi B. 2012, *Ad. in Appl. Sc. Res*., 3(1):605-610.

Bisen Pratima and Tiwari Shashi. 2015, A Review on Keratinophilic Fungi of Madhya Pradesh, Journal of Pharmacy and Biological Sciences. 10, 6 Ver. IV (Nov - Dec. 2015), 18-22.

Deshmukh, S.K. 1983; Soil inhabiting Keratinophilic fungi and their distribution in Madhya Pradesh; Shodhganga on line jounral: 12-32

Deshmukh SK & agrawal SC. 2003, Isolation of dermatophytes and other keratinophilic fungi from Jammu ,India .Mycoses 46 (5-60) 226-228.

Deshmukh SK. 2004, *Mycopathologia*, 157: 265-7.

Ghosh GR &Bhatt S. 2000, Keratinophilic fungi from Chilka lake Side soil Orissa (India) .Indian Journal of Microbial 40 247-254 .

Gorden MA. 1953, The occurrence of the dermatophyte *Micosporium gypseum* as a saprophyte in soil.J Invest Derm., 20, 201-206.

Fillipello MV. In Kushawaha RKS, Guarri J, (Eds), 2000. Biology of dermatophytes and other keratinophilic fungi. (Revista Iberoamericana de Micologia), Spain. 77-85.

Mini KD, Mathew J, Mini SS, Paul K. 2012. *Euro. J. Exp. Bio.*,2 (4):1261-1264.

Maruthi YA, Chaitanya DA, Hossain K, Sravani A, Jagadish S. 2012. *Euro. J. Exp. Bio.*, 2 (1):13-16.

Mini KD, Paul MK, and Mathew J. 2012, *Adv. Appl. Sci. Res.*, 3(4):2073-2077.

Maruthi YA, Hossain K, Chaitanya DA. 2012, *Asian J. Plant Sci. Res.*, 2 (4):534-538.

Maruthi YA, Hossain K, Priya DH, Tejaswi B. 2012, *Adv. Appl. Sci. Res.*, **,** 3(1):605-610.

Moallaei H ,Zaini F ,Pihet M,mahmoudi M& Hashemi J. 2006, Isolation of keratinophilic fungi from soil samples of forests and farm yards. Iranian J Publ Health, 35, 94062-69.

Nakagawa Y, Shimazu K, Ebihara M, Nakagawa K. 1999. *J. Infect. Chemother*., 5:97-100..

Sabouraud R. 1893, Note sur1'hypothese d'une existence saprophyte des Trichophytens.An Dermatol et Syphild, 3, 561-566.

Saxena P, Kumar A, Shrivastava JN. 2004, *Folia Microbiol (Praha)*, 49: 430-4.

Singh,S., Shrivastava,A.R., Gupta,A., Singh,A.K., Gopalan,N. and Chaudhary,H.S. 2012, Keratinloytic Actinomycetes Isolated from Poultry Waste; Journal of Chemical and Pharmaceutical Research:4(9); :4107-4111.

Singh CJ, Geetha SB, Singh BS. 1994, *Ad. in Plant Sc.*, **7**: 280-291.

Sharma R. & Rajak R.C. 2003; Keratinophilic Fungi: Nature's Keratin Degrading Machines! Their Isolation, Identification, and Ecological Role; Resonace; 28-40.

Shrivastava JN, Satsangi GP, Kumar A. 2008, *J. Environ. Biol*., 29: 125-6.

Ashis Kumar Sarkar ,Vibhuti Rai and Ashwini Kumar Gupta. 2014 : Incidence of keratinophilic fungi in areas of Raipur City, Chhattisgarh region, India African journal of microbiology research 8(3):264-269, January.

Zarei MA, Zarrin M. 2008, *Jundishapur J. Microbiol*., 1: 20-3.

Zarrin M, Haghgoo R. 2011. *Jundishapur J. Microbiol*., 4(3): 191-194.

Ulfig K. 2006, *Polish J. of Env. Studies.*, 15(2):341-346.

Vanbreusegham R. 1952, A Keratin digestion by dermatophytes :a specific diagnostic method Mycologia, 44, 176-182.