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ISOLATION AND IDENTIFICATION OF KERATINOPHILIC FUNGI FROM DIFFERENT SOIL SAMPLE IN SEHORE OF MADHYA PRADESH CITY (INDIA)

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ABSTRACT

The soil samples were collected from districts (Sehore) of Madhya Pradesh, India during August 2016 to February 2017. The soil samples were collected from roadside, public parks, poultry farm, slaughterhouses and barber shop dump area. During the course of the present study 310 fungal isolates were isolated. The isolation technique adopted was Vanbreuseghem's hair baiting method (1952). 100 soil samples were collected randomly from 20 different sites of Sehore district of Madhya Pradesh. Keratin rich baits included human hair, animal hair, human nails and chicken feathers. a total of 303 colonies of keratinophilic fungi were isolated. The fungal isolates belonged to 14 genera and 17 species. *Trichophyton* (10.89%) genera were the most predominant in this study. The 17 genera are as follows *Cephalotheca foveolata* (6.61%), *Trichophyton spp* (10.89%), *Penicillium* (10.2%), *Microsporum gypseum* (5.28%), *Trichothecium roseum* (7.26%), *Paecilomyces javanicus* (6.27%), *Fusarium spp.* (4.29%), *Aspergillus spp.* (2.3%), *Trichophyton equinum* (5.28%), *Acremonium* (7.59%), *Cladosporium cladosporoides* (4.62%), *Microsporum fulvum* (6.55%), *Arthroderma fulvum* (1.32%), *Chrysosporium spp* (4.62%), *porothrix schenickii* (6.23%), *Gymnoascus reessii* (3.96%), *Debaryomyces hansenii* (4.62%), and *Unidentified species* (2.31%) . Total of 100 keratinous substrates baiting plates were screened, in which 85 (85%) were positive for keratinophilic fungi and 15 (15%) found negative.

INTRODUCTION

The keratinous wastes accumulate in nature mostly in the form of feathers, hairs, hooves, horns and nail clippings. These keratinous substrates considered as environmental pollutants and generated mostly from the poultry farms, slaughter houses and leather industries [1]. Each year, globally 24 billion chickens are slaughtered and approximately 8.5 billion tons of feathers are produced. According to a present report, India's part alone is 350 million tons[2]. The leather industries throw out wide amount of waste products and considered as extremely polluting industries with negative environmental impact.The poultry feathers and other keratin-containing wastes are dumped, land filled and incinerated. These activities cause the soil, water and air pollution. Discarded feather furthermore causes various human ailments including, chlorosis and fowl cholera [3].

Keratin protein is major structural fibrous protein, providing an outer covering such as hair, wool, feathers and nails etc. Due to the strength and steadiness of keratin, only some microorganisms are able to break it down and degrade. Nature has provided the planet earth with an assortment of beneficial organisms. Keratinophilic fungi are one of the nature's gifts and the largest group of organisms, which have the capability to degrade the keratin and their substrates . Soils are rich in keratinous substrates and organic materials and the best candidate for the growth and occurrence of keratinophilic fungi [4]. The keratin-degradation ability of keratinophilic fungi has been credited with the production of the keratinase enzyme [5]. Fungal keratinase is the industrially significant enzyme offers bioconversion of keratinous wastes, utilization as animal feed supplements and dehairing agents in tannery in eco-friendly way [6].

MATERIAL AND METHOD

The Sehore district was divided into 20 zones and each zone five location were selected and each location soil samples were collected randomly from different sites viz, gardens, schools, poultry farms, rivers, hospitals and garbage dumping sites roots where, most of the microbial activity is concentrated. Soil samples (approximately 5g) were collected with clean dry and sterile polythene bags along with sterile spatula[7]. The collected samples brought to the laboratory and preserved for further studies.The soil samples were collected from the month of India during August 2016 to February 2017 in Sehore District (extends between the parallels of Latitude 22'31 to 23'40 North and between the meridians of Longitude 76'22 and 78'08 East) at various locations. The soil samples collected from twenty different zones of Sehore district mentioned in Table 1

Procedure for sample collection

Before collection of soil samples, superficial debris and other vegetative materials were removed from the soil surface. All the soil samples were collected from the superficial layer (depth not exceeding 3-5 cm) with the help of sterilized spoon in pre-sterilized polythene bags (10 x 20 cm). Each polythene bag was labeled indicating the date and site of collection[8]. These samples were then tightly closed to maintain the original moisture and were brought to the laboratory and stored at 4°C till further processing.

Collection and preparation of baits

Keratin rich baits were collected from different places; the baits included human hair, animal hair, human nails and chicken feathers. Defatting of the baits was done by soaking all the baits for 24 hrs in either diethyl ether or in a chloroform/methanol (1:1) mixture and later rinsed 4-5 times with distilled water and air dried[9]. After defatting, baits were sterilised by the process of tyndallisation which involve heating the baits at 80°C for 1 hour followed by incubation for 24 hours at 28°C.The process was repeated thrice for three consecutive days.

Baiting of soil samples

Hair baiting technique was used for isolating the keratinophilic fungi from the collected soil samples (Vanbreuseghem, 1952). The samples were processed rapidly after collection[10]. Each of the collected soil samples as homogenized thoroughly and a required amount of soil was

taken in sterilized petridishes under sterilized conditions and moistened with water. The plates were baited by keeping sterile human hair, animals hair, human nails and chicken feathers on the soil. These Petridishes were incubated 28°C±2°C and examined daily from the fifth day for fungal growth over a period of 4 weeks. Sterilized water was added to each plate to keep the soil moist. The plates were monitored regularly and sterilized distilled water was added as and when required to maintain the moisture level[11].

Screening the incubated plates for the presence of fungal hyphae

The incubated plates were monitored regularly to observe any fungal growth on them. Once the fungal growth was observed on any plate, a small portion of the fungal mycelia was picked up for fungal examination with the help of a sterilized needle, mounted on a slide under glass cover slip containing a drop of sterilized distilled water or lacto phenol cotton blue, and The purity of the isolated fungus was confirmed by examined under a microscope of the culture at 400X magnification using light microscope for the identification of the fungi[12]. The fungal mycelium was also inoculated on appropriate media for growth of the culture. The following morphological characteristics were evaluated: colony growth (length and width), presence or absence of aerial mycelium, colony color, presence of wrinkles and furrows, pigment production etc. The characteristics were compared with the standard descriptions.

Isolation of the pure fungal isolates from the samples

Sabouraud's Dextrose Agar (SDA) media supplemented with chloramphenicol (0.05mg/ml) and cycloheximide (0.5mg/ml) was used for culturing. Media was prepared and autoclaved at 121°C for 15 min and after a Sehore district preliminary examination of fungal growth on baits, the fungal mycelium was subsequently transferred to the petriplates of Sabouraud's dextrose agar (SDA). The petri dishes were incubated at 28±2°C for two weeks and routinely checked for fungal growth. The samples were sub cultured to obtain pure cultures[13]. Pure cultures thus obtained were maintained on SDA slants at 4°C.

SCREENING OF POTENTIAL FUNGAL STRAIN FOR KERATINASE ENZYME PRODUCTION

Enzymatic assay

The protease activity was measured as described by Meyers and Ahearn (1976) with some modifications. 0.5 mL of glycine NaOH buffer (pH 10, 0.2 M) was added to 0.5 mL of appropriately diluted enzyme and was incubated with 1 mL of 1% casein solution (prepared in glycine NaOH buffer, pH 10) for 15 min at 60 °C. The reaction was stopped by the addition of 4 mL of 5% (v/v) trichloroacetic acid. The contents were centrifuged after 1 h at 3000 x g for 10 min and the filtrate was used for measuring protease activity on the basis of color change[14]. 5 mL of 0.4 M sodium carbonate solutions was added to 1 ml of the filtrate and was kept for 10 min. Folin's Ciocalteau Phenol reagent of 1:1 dilution was added and kept in dark for 30 min. The color change was determined at 660 nm. One unit of protease was equivalent to the amount of enzyme required to release 1mg/mL/min of tyrosine under standard assay conditions.

RESULT AND DISCUSSION

The soil samples were collected from districts (Sehore) of Madhya Pradesh, India during August 2016 to February 2017. The soil samples were collected from roadside, public parks, poultry farm, slaughterhouses and barber shop dump area. During the course of the present study 310 fungal isolates were isolated. The isolation technique adopted was Vanbreuseghem's hair baiting method (1952)[15]. 100 soil samples were collected randomly from 20 different sites of Sehore district of Madhya Pradesh.

Table 1 : Soil sample collection areas of Jammu and Kashmir for isolation of keratinophilic fungi

S.No.	Site of soil collection	Types of soil	No. of soil samples examined	Location	Total No. of fungal isolated
1	Land filling site kasba sehore	Surface soil	5	Sehore	15
2	Krishi Upaj Mandi,	Surface soil	5	Jawar Ashta Sehore	7
3	Ayodhya Seeds Private Limited	Surface soil	5	Shyampur, Sehore	8
4	Nikky Bawa Ladies Salon, Sehore	Surface soil	5	Sehore	14
5	Observe Healthcare (OPC) Private Limited	Surface soil	5	Sehore	16
6	Crescent Spa And Resorts (Indore) Private Limited	Surface soil	5	Nasrulla ganj Sehore	14
7	Sisodia Poultry Farm Amlaha Sehore	Dumping site soil	5	Budhni, Sehore	19
8	Vardhman Fabrics (A unit of vardhman Textile	Dumping site soil	5	Sehore	6
9	Alfa Proteins (Pvt. Ltd) Krishi Upaj Mandi,	Main ground soil	5	Sehore	5
10	ABS Poultry farm, Astha	Main ground soil	5	Ichhawar, Sehore	12
11	Alfa Proteins Pvt. Ltd.	Dumping site soil	5	Ichhawar sehore	7
12	New kesh kalagents parlour, Sehore	Main ground soil	5	Pachama Sehore	15
13	Sisodia Poultry Farm, Sehore	Dumping site soil	5	Indore Naka Ashta, Sehore	18
14	Municipal landfill site, Ichhawar	Dumping site soil	5	Ichhawar, Sehore	20
15	New look hai saloon sehore	Main ground soil	5	Budhni,Sehore	12
16	Rural Agro Resource Development Private Limited	Dumping site soil	5	Shudha Oils Private Limited	8

17	Shudha Oils Private Limited	Dumping site soil	5	Budhwara Ashta Sehore	6
18	Sathvik Agro-Food Industries Private Limited	Dumping site soil	5	Budhwara Road Ashta Sehore	7
19	Dodi Foods Private Limited	Main ground soil	5	Kasera Patti Ichavar Sehore	8
20	Nivedita Handlooms Private Limited	Main ground soil	5	Astha Sehore	16

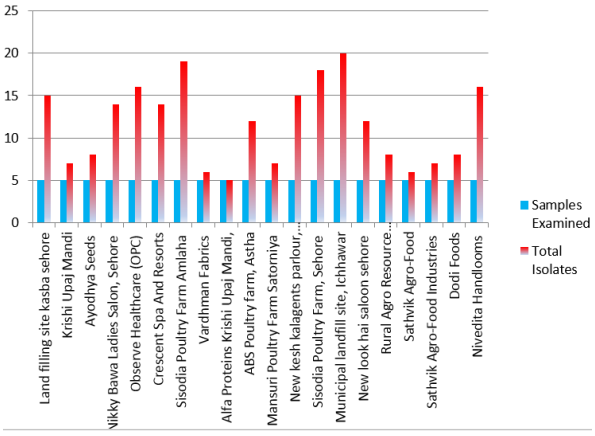

Figure 4.1 : Total number of keratinophilic fungi isolated from different soilsamples

pH Analysis of Soil Samples

The soil samples were processed in the Microbiology Research Laboratory, University, (India). The soil samples were shade dried and sieved for pH analysis[16]. The pH range of collected soil samples was founded in minimum 6.4 and maximum 10.0 pH.

Eight soil samples were found between 6.00-6.99 pH, forty two soil samples were found between 7.0-7.99 pH, forty two samples were found between 8.00-8.99 pH and Eight samples were found ≥9.00 pH (Table 2).

Table 2: pH of soil samples

No. of soil sample	pH of Soil Sample	No. of soil sample	pH of Soil Sample
Sd01	6.65	SD51	7.92
SD02	8.10	SD52	7.21
SD03	7.80	SD53	8.65
SD04	8.00	SD54	7.46
SD05	7.82	SD55	6.95
SD06	8.15	SD56	8.20
SD07	7.94	SD57	8.52
SD08	7.63	SD58	8.70
SD09	7.70	SD59	6.41
SD10	10.60	SD60	8.60
SS11	7.70	SD61	8.10
SD12	7.80	SD62	7.56
SD13	7.45	SD63	8.40
SD14	8.25	SD64	7.05
SD15	8.18	SD65	7.65
SD16	6.83	SD66	7.60
SD17	8.40	SD67	8.03
SD18	9.72	SD68	7.67
SD19	7.96	SD69	8.30
SD20	7.55	SD70	8.05
SD21	8.33	SD71	9.10
SD22	8.70	SD72	8.65
SD23	7.82	SD73	7.56
SD24	8.13	SD74	8.17
SD25	7.99	SD75	8.06
SD26	8.30	SD76	8.13
SD27	8.60	SD77	7.21
SD28	7.80	SD78	8.65
SD29	8.00	SD79	7.46
SD30	7.85	SD80	6.95
SD31	6.15	SD81	8.20
SD32	7.72	SD82	7.80
SD33	7.62	SD83	8.70
SD34	7.70	SD84	6.41
SD35	10.60	SD85	8.65
SD36	7.70	SD86	8.40
SD37	9.40	SD87	7.56
SD38	7.40	SD88	8.45
SD39	7.80	SD89	7.05
SD40	8.18	SD90	7.65
SD41	7.83	SD91	9.72
SD42	8.40	SD92	8.02
SD43	9.72	SD93	7.67
SD44	7.96	SD94	8.32
SD45	6.72	SD95	8.05
SD46	8.30	SD96	9.15
SD47	7.96	SD97	8.65
SD48	8.30	SD98	7.80
SD49	8.60	Sd99	8.17
SD50	7.80	SD100	7.42

Isolation of Keratinophilic Microflora

During the course of the present study, the isolation of keratinophilic fungi was completed by the well-known **Hair-Baiting Technique** of Vanbreuseghem (1952). Hundred soil samples were divided into four categories on the basis of keratinous substrates, e.g. human hair, animal hair, human nails and chicken feathers. A total of 100 keratinous substrates baiting plates were screened, in which 85 (85%) were positive for keratinophilic fungi and 15 (15%) found negative[17].

All the soil samples were processed for the isolation of keratinophilic fungi. Apart from hair, animal hair, human nail clipping and Checken feathers, used as bait for the isolation of keratinophilic fungi. Among the baits used for the isolation of keratinophilic fungi, feather bait was highly utilized by the fungus followed by nails, and hair. Feather as bait was found to be most suitable for the growth of keratinophilic fungi as compared to other baits[18]. This differential degradation of the keratin substrates can be attributed to the hardness of the keratin source. The keratin present in feather is comparatively less hard and can be easily broken down by the extracellular enzymes of the fungi. The isolated fungal colonies were cultured and purified on SDA media supplemented with chloramphenicol. All the soil samples collected from different sites of Sehore district of Madhya Pradesh were found to be positive for the growth of keratinophilic fungi. The keratinophilic fungi isolated by hair bait technique in our study have been grouped in to Four catagories i.e., (i) **human hair** (ii) **animal hair (iii) human nail clipping and (iv) chicken feather** . A total of 303 isolates belonging to 14 genera were isolated from different soil samples. Based on the frequency distribution patterns fifty isolates of keratinophilic fungi showing maximum frequency were selected for further studies[19]. Morphologically some of the isolated species had almost same phenotypic characteristics which made their differentiation a difficult task. But at the molecular level the differentiation was revealed using different genetic

markers.

Isolated fungi were identified on the basis of the monographs of Sigler and Carmichael (1976), Oorchschot (1980), Currah (1985), Von Arx (1986), Guarro (1994) and Sigler *et al*., (2002), Cano and Guarro (1990), Vidal (2002) by studying macro and micro-morphological characters of these cultures[20]. *Trichophyton tonsurans* was the most frequently isolated keratinophilic fungus present in all the soil samples examined followed by Acremonium spp. Penicillium spp., and Microspourum spp. The other genera isolated were Aspergillus spp., Arthroderma fulvum, Cephalotheca foveolata, Chrysosporium spp. *Cladosporium, Trichoderm harzianum, Fusarium spp., Aspergillus, Cladosporium cladosporoides Debaryomyces hansenii, Fusarium spp. Gymnoascus reessii, Microsporum spp. Paecilomyces javanicus, Porothrix schenickii*. Most of the keratinophilic fungi, viz. species of Trichophyton, Fusarium, Aspergillus, Microsporum, *Penicillium,* etc. are common saprophytes in soil. The frequency of different keratinophilic fungal genera is shown in (Fig. 4.3). Many investigations have been carried out in the recent years on the distribution and occurrence of keratinophilic fungi in soil in many parts of the world (Jain and Sharma 2012). Reports on the presence of these fungi in different soil habitats from different countries, Egypt, Australia, Palestine, Spain, India, Kuwait, Ukraine and Malaysia, have indicated that they are distributed worldwide (Anbu *et al.,* 2004). Various workers Rathore and Kumar (2014), Anane *et al*., (2015) Deshmukh and Verekar (2014) have reported these fungi from Indian soils. Deshmukh (2002a) reported the prevalence of keratinophilic fungi and related dermatophytes in sites located along the banks of glaciers, particularly Gulmarg[21], Khilanmarg, Sonamarg and Tangmarg. Studies on Isolation and Identification of dermatophytes from soil samples of Jabalpur city of Madhya Pradesh J Iqbal, et al., 2016.

Different species belonging to different genera which includes *Fusarium merismoides, Trichophyton equinum, Penicillium chrysogenum, Acremonium persicinum, Trichophyton mentagrophytes, Chrysosporium indicum, Chrysosporium keratinophilum, Microsporum fulvum, Paecilomyces javanicus, Chrysosporium queenslandicum, Fusarium oxysporum, Debaryomyces hansenii, Chrysosporium articulatum, Fusarium Chlamydosporum, Arthroderma fulvum, Cladosporium cladosporioides, Acremonium implicatum, Cephalotheca foveolata, Trichothecium roseum, Porothrix schenickii, Penicillium griseofulvum, Aspergillus allahabadii, Gymnoascus reessii, Microsporum gypseum, Fusarium redolens , yeasts and moulds*

Table. 4.3: Isolation of keratinophilic fungi from soils samples of various habitats of Industrial fields in sehore district by using different keratin substrates

S.No.	Site of soil collection	Human Hair	Animal Hair	Human nail Clipping	Chicken Feather	Probable Genera
1	Krishi Upaj Mandi	+	+	+	-	Fusarium merismoides
2	Ayodhya Seeds Private Limited	+	+	-	-	Trichophyton equinum, Microsporum gypseum
3	Nikky Bawa Ladies Salon, Sehore	+	-	+	-	Penicillium chrysogenum
4	Observe Healthcare (OPC) Private Limited	-	-	+	-	Acremonium persicinum, Microsporum gypseum
5	Crescent Spa And Resorts (Indore) Private Limited	-	-	-	+	Trichophyton mentagrophytes
6	Sisodia Poultry Farm Amlaha Sehore	-	+	+	-	Chrysosporium indicum, Microsporum gypseum
7	Vardhman Fabrics (A unit of vardhman Textile	+	+	-	-	Chrysosporium keratinophilum
8	Alfa Proteins (Pvt. Ltd) Krishi Upaj Mandi,	-	+	-	-	Microsporum fulvum
9	ABS Poultry farm, Astha	-	-	-	+	Paecilomyces javanicus, Gymnoascus reessii
10	Alfa Proteins Pvt. Ltd.	+	+	-	-	Chrysosporium queenslandicum
11	New kesh kalagents parlour, Sehore	+	+	+	-	Fusarium oxysporum
12	Sisodia Poultry Farm, Sehore	+	+	+	+	Debaryomyces hansenii
13	Municipal landfill site, Sehore	+	+	+	+	Chrysosporium articulatum
14	New look hai saloon sehore	+	-	+	-	Fusarium Chlamydosporum
15	Rural Agro Resource Development Private Limited	-	-	-	-	Arthroderma fulvum
16	Shudha Oils Private Limited	-	-	+	-	Cladosporium cladosporioides, Aspergillus allahabadii

17	Sathvik Agro-Food Industries Private Limited	+	-	-	-	Acremonium implicatum, Penicillium griseofulvum
18	Dodi Foods Private Limited	+	-	-	-	Cephalotheca foveolata
19	Nivedita Handlooms Private Limited	+	+	-	-	Trichothecium roseum
20	Chopal Sagar Poetry Farm	-	-	-	+	Porothrix schenickii

IDENTIFICATION OF KERATINOPHILIC MICROFLORA

4.2.1. Identification of Keratinophilic Fungi

From soil sample S. No. 01 to S.No. 100, a total of 303 colonies of keratinophilic fungi were isolated[22]. The fungal isolates belonged to 14 genera and 77 species. *Trichophyton* (10.89%) genera were the most predominant in this study. The 17 genera are as follows *Cephalotheca foveolata* (6.61%), *Trichophyton spp* (10.89%), *Penicillium* (10.2%), *Microsporum gypseum* (5.28%), *Trichothecium roseum* (7.26%), *Paecilomyces javanicus* (6.27%), *Fusarium spp.* (4.29%), *Aspergillus spp.* (2.3%), *Trichophyton equinum* (5.28%), *Acremonium* (7.59%), *Cladosporium cladosporoides* (4.62%), *Microsporum fulvum* (6.55%), *Arthroderma fulvum* (1.32%), *Chrysosporium spp* (4.62%), *porothrix schenickii* (6.23%), *Gymnoascus reessii* (3.96%), *Debaryomyces hansenii* (4.62%), and *Unidentified species* (2.31%), The frequency of different keratinophilic fungal genera is shown in (Table: 4.3; Figure. 4.3).

Table 4.4: Different site of soil collection Kerationophilic fungal flora

S.No.	Site of soil collection	*Cephalotheca foveolata*	*Trichophyton spp.*	*Penicillium*	*Microsporum gypseum*	*Trichothecium roseum*	*Paecilomyces javanicus*	*Fusarium spp.*	*Aspergillus allahabadii*	*Trichophyton equinum*	*Acremonium*	*Cladosporium cladosporoides*	*Microsporum fulvum*	*Arthroderma fulvum*	*Chrysosporium spp.*	*porothrix schenickii*	*Gymnoascus reessii*	*Debaryomyces hansenii*	*Unidentified species*	Total	Total Soil Sample	+ Soil samples
1	Krishi Upaj Mandi,	-	1	2	1	1	2	1	1	-	-	-	-	2	-	-	1	2	1	16	5	5
2	Ayodhya Seeds Private Limited	1	2	1	-	2	-	1	-	-	-	2	1	-	-	1	-	-	-	11	5	4
3	Nikky Bawa Ladies Salon, Sehore	1	3	2	-	1	-	3	-	1	-	3	-	-	2	1	1	-	-	18	5	5
4	Observe Healthcare (OPC) Private Limited	-	2	1	2	1	2	-	-	1	-	1	1	-	-	1	-	-	2	12	5	4
5	Crescent Spa And Resorts (Indore) Private Limited	1	3	-	3	1	1	-	-	1	2	-	2	-	2	-	-	1	-	17	5	5
6	Sisodia Poultry Farm Amlaha Sehore	-	4	1	2	4	-	1	-	3	4	-	1	-	-	3	-	1	-	24	5	5
7	Vardhman Fabrics (A unit of vardhman Textile	1	-	1	1	-	1	1	-	1	1	-	-	-	1	1	-	-	-	9	5	5
8	Alfa Proteins (Pvt. Ltd) Krishi Upaj Mandi	1	-	1	2	-	1	-	-	-	-	1	-	-	-	1	-	1	1	9	5	5

9	ABS Poultry farm, Astha	-	3	4	2	-	2	2	-	-	4	-	1	-	2	4	2	2	-	28	5	5
10	Mansuri Poultry Farm Satorniya	1	3	2	-	4	-	1	1	-	1	1	1	-	-	1	-	-	-	16	5	5
11	New kesh kalagents parlour, Sehore	1	3	2	1	2	3	-	-	3	4	-	3	1	-	-	-	1	-	24	5	5
12	Sisodia Poultry Farm, Sehore	4	-	3	1	-	3	-	-	1	-	2	3	-	-	1	-	-	-	18	5	5
13	Municipal landfill site, Sehore	1	-	3	-	1	1	-	-	2	-	2	-	-	2	-	1	4	1	19	5	5
14	New look hai saloon sehore	1	2	4	-	-	1	-	2	-	1	-	4	-	1	-	2	-	-	18	5	5
15	Rural Agro Resource Developme nt Private Limited	1	1	1	-	-	1	-	2	1	-	-	-	1	-	-	1	1	-	10	5	5
16	Shudha Oils Private Limited	-	2	-	1	2	-	-	-	-	2	1	-	-	2	1	-	1	-	12	5	5
17	Sathvik Agro-Food Industries Private Limited	1	-	1	-	-	-	1	-	-	-	1	1	-	-	1	-	-	1	7	5	5
18	Dodi Foods Private Limited	-	-	1	-	-	-	2	-	-	1	-	-	-	1	-	1	-	-	6	5	4
19	Nivedita Handlooms Private Limited	2	2	-	-	1	1	-	-	-	2	-	2	-	-	1	1	-	1	13	5	5
20	Chopal Sagar Poetry Farm	-	2	1	-	2	-	-	1	2	1	-	-	-	1	4	2	-	-	16	5	5
	Total	17	33	31	16	22	19	13	7	16	23	14	20	4	14	21	12	14	7	303	100	97
	%	6.61	10.89	10.2	5.28	7.26	6.27	4.29	2.3	5.28	7.59	4.62	6.55	1.32	4.62	6.23	3.96	4.62	2.31		100.02	

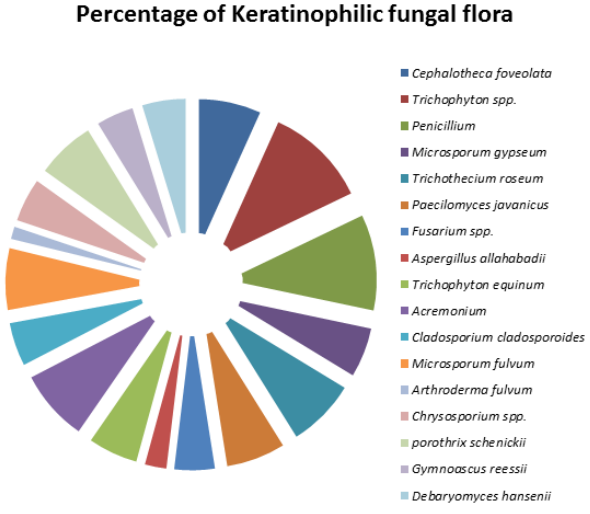

Figure 4.4: Percentage of different Kerationophilic fungal flora obtain from different sites

Although few investigations have been made on keratinophilic fungi from various parts of Madhya Pradesh which shows high prevalence of keratinophilic fungi in these soils (Bisen P 2015). However, there was no evidence of any study on the soil samples selected in the present course of study also; no reports on the molecular characterization of these fungi from Madhya Pradesh have been reported[23-24]. Usually two general methods are used for the laboratory identification of keratinophilic fungi by the researchers: a) identification on the basis of phenotype differences (conventional methods) and b) identification on the basis of molecular differences. Shrivastav *et al.* (2013) mentioned that identification of keratinophilic fungi and other dermatophyte species by conventional methods requires the examination of colony, particularly with the method of slide culture and microscopic morphological structures. Most of the morphological and physiological features are dynamic and outside factors such as temperature variation, medium and chemotherapy greatly influence the phenotypic characteristics and consequently make the identification more difficult (Aala *et al.*, 2012). According to Sehgal *et al.* (2011) many fungi do not produce characteristic spores which are the key to fungal species identification, in such cases molecular based approaches has proved to be potent and useful in the taxonomical classification of the fungi which are morphologically indistinguishable. Species identification

based on conventional phenotypic methods is often time-consuming, laborious and is hindered by the unstable and subjective nature of phenotypic characteristics, which are readily influenced by culture conditions (Pryce *et al.,* 2003). Conversely, molecular methods yield results that are uninfluenced by growth conditions and are frequently more rapid than phenotypic approaches, they are promising because of their simplicity, specificity and sensitivity and are capable of discriminating between fungi that fail to produce distinctive morphological features (Gugnani *et al*., 2014).

CONCLUSION:

The isolation of kerotinophilic from different sites (Table 1) is not uniform this could be due to difference in organicmatter of the soil. Organic matter content of soil is one of the major factors affecting the presence of kerotinophilic fungi in soil (chmel et al., 1972). The present study reveles that soils of garden, schools, poultry and garbage dumping sites are rich in keratineophilic fungi.

The data adds the information on the flora of kerttinophilic fungi of india. Although the study of keratinophilicfungi is a challenging task we have put our efforts to isolate keratinophilc fungi from few placea of sehore. Immense need of work is yet to be done on this particular aspect in this specific area.

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