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DIVERSITY AND KERATINASE ACTIVITY OF DERMATOPHYTES AND OTHER MYCOKERATINOPHILES INHABITING THE FEATHERS OF SOME MIGRATORY BIRDS VISITING GHARANA WETLAND (INDIA)

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ABSTRACT

Investigations were undertaken to study the diversity of dermatophytes and other keratinophilic fungi inhabiting the feathers of two migratory birds viz., bar-headed geese and common teal, which visit Gharana wetland situated in Jammu province of J&K state (India). This group of fungal organisms is responsible for causing human and animal mycoses and may get dispersed to distant places through these birds while taking long flights. In view of this, an attempt was made to isolate and identify this unique group of mycokeratinophiles.

A total of 33 keratinophilic fungal species belonging to 17 genera were recovered from the feathers of bar headed geese and common teal. These included 2 species of dermatophytes and 31 species of non-dermatophytes. The dermatophytes consisted of two species of *Microsporum* (*M. gypseum* and *M. canis*), whereas the non- dermatophytes included 5 species each of *Chrysosporium* and *Aspergillus*, 4 species of *Penicillium*, 2 species each of *Fusarium, Curvularia, Mucor, Sarocladium* and 1 species each of *Acremonium, Purpureocillium, Alternaria, Cladosporium, Histoplasma, Sagenomella, Rhizopus, Syncephalastrum* and *Didymella.* During the investigation period, maximum number of keratinophilic fungal species (33) were recovered from the feathers of bar-headed geese, whereas only 21 species were recovered from that of common teal. Keratinophilic fungal species commonly found on the feathers of both the birds species included *Microsporum gypseum, M. canis, Chrysosporium indicum, C. keratinophilum, C. queenslandicum, Aspergillus flavus, A. fumigatus, A. versicolor, A. candidus, Acremonium fusidioides, Fusarium verticillioides, Purpureocillium lilacinum, Penicillium purpurogenum, Alternaria alternata, Curvularia lunata, C. pallescens, Histoplasma capsulatum, Mucor luteus, Sarocladium strictum, S. kiliense* and *Syncephalastrum racemosum.* All the recovered mycokeratinophiles showed keratinase activity. However, the dermatophytes possessed highest keratinase activity, whereas among the non-dermatophytes, *Chrysosporium* species showed maximum activity. In view of these observations, it can be concluded that most of the keratinophiles recovered from the feathers of migratory birds have the potential of causing mycosis.

INTRODUCTION

Keratinophilic fungi are highly specialized group of microorganisms, which continuously degrade the keratinaceous matter, added to the soil in the form of feathers, hair, horns, claws, nails, etc. Feathers of birds being rich in keratin matter are most suitable for the growth and multiplication of keratinophilic fungi. In addition, birds while taking long flights may carry the spores of these fungi on their keratin rich feathers to distant places. Since most of the birds find soil as the best place for feeding and breeding, they keep on adding feathers along with the keratinophilic flora to the soil, thus providing an important means of not only association but also long distance dispersal and survival in the soil.

From India, some researchers have offered comprehensive account on the distribution of keratinophilic fungi on the feathers of free living birds from different states like Tamil Nadu (Pugh, 1966), Orissa (Sur and Ghosh, 1980; Sarangi and Ghosh, 1991), Uttar Pradesh (Dixit and Kushwaha, 1991) and Maharashtra (Deshmukh, 2002). A close relationship is also known to exist between the keratinophilic fungi and some specific birds e.g., *Arthroderma curreyi* and *Turdus* (Pugh, 1964), *Ctenomyces serratus* and members of galliforme, especially patridges(Pugh,1966) and chickens (Rees,1967).

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The association , survival and dispersal of keratinophilic fungi with the feathers of different birds have been investigated by various researchers from many countries across the globe viz., United Kingdom (Pugh, 1964, 1965 and 1966), Australia (Rees, 1967), Yugoslavia and Czechoslovakia (Hubalek *et al*., 1973 ; Hubalek 1974) and Italy (Marsella *et al*.,1985).

Some migratory birds viz., bar-headed geese (*Anser indicus* Latham), common teal (*Anas crecca* Linn.), grey heron (*Ardea cinerea* Linn.), little grebe (*Tachybaptus ruficollis* Pallas) and purple swamphen (*Porphyrio porphyrio* Linn.) are known to visit Gharana wetland, R.S. Pura, Jammu every year during the winter months. Of these, the bar- headed geese and the common teal are the most important as they visit the wetland in large flocks and remain there for the entire winter. So far, no study has been done on the keratinophilic fungi associated with the feathers of migratory birds visiting Gharana wetland. Therefore, an attempt was made to investigate the occurrence of keratinophilic fungi on the feathers of bar-headed geese and common teal as this fungal group is responsible for causing human and animal mycoses and may get dispersed to distant places through the long flights of these migratory birds.

MATERIALS AND METHODS

Feathers of two commonly visiting migratory birds viz., bar headed geese (*Anser indicus* Latham) and common teal (*Anas crecca* Linn.) were collected from Gharana wetland by taking help from the local bird experts and members of the World Wide Fund for Nature (Chapter Jammu) who were working on the migratory birds. The feathers were brought to the laboratory in presterilised polythene bags and isolation of keratinophilic fungi from them was done by following Kaul (1995). Sterilized petriplates each containing 10-20 g of sterilised garden soil were moistened with sterilized water and feather samples of uniform length (4cm) were placed on them under aseptic conditions. These petriplates were incubated at 28±2° C for about 20 days and examined periodically for any sign of mycelial growth on the feathers. Direct transfer of fungal mycelium from the sampled feathers was made on petriplates plated with Sabouraud Dextrose Agar (SDA) medium supplemented with chloramphenicol (50mg/1000 ml).The keratinophilic fungal isolates were identified on the basis of their cultural and morphological details by following taxonomic keys of specific genera(Brown and Smith, 1957; Raper and Fennel, 1965; Tandon, 1968; Rifai, 1969; Booth, 1971; Ellis, 1971,1976; Barron, 1972; Pitt, 1979; Van Oorschot, 1980; Onions *et al*., 1981; Gams, 1997, Pounder *et al*., 2005).

Frequency occurrence was calculated as follows

$$\text{Frequency occurrence}(\%) = \frac{\text{Number. of samples from which an organism was isolated}}{\text{Total Number of samples tested}} \text{x} 100$$

Comparison of diversity indices of the recovered keratinophilic fungal species from feather samples

To compare the diversity of recovered fungal species, following indices were used:

Species richness (S) is the number of species recorded at the sampled area (Magurran, 1988).

Shannon-Wiener index *(H')* (Shannon and Wiener, 1949). This index was originally proposed by Claude Shannon to quantify the uncertainty associated with the prediction that any two organisms sampled from a site belong to same species. It is calculated as given below:

Shannon-Wiener index *(H')* $= -\sum_{i=1}^{s} p_i \ln p_i$

where *p*i is the relative importance value of species *i.*

Simpson's dominance index (Cd) (Simpson, 1949). The Simpson dominance index is used to measure the degree of concentration when individuals are classified into types. Its measure equals the probability that two entities taken at random from the site of interest represent the same type. Its value ranges from 0 to 1, with values near 0 corresponding to low concentrated and more homogeneous sites while values near 1 corresponding to highly concentrated and heterogeneous sites.

Simpson's dominance index *(*Cd*)* $= \sum_{i=1}^{s} (p_i)^2$

where, p_i, is the relative importance value of species ***i.***

Margalef's index (Margalef, 1958). Margalefs index was used as a simple measure of species richness.
Margalef's index (D_{Mg})= (S - 1)/ ln N
S = total number of species
N = total number of individuals in the sample
ln = natural logarithm

Menhinick's index (Menhinick, 1964) was also used as a simple measure of species richness.
Menhinick's index (D_{Mn}) = $s/\sqrt{N}$
where s = the number of different species
N = the total number of individual organisms.

Estimation of Keratinase Activity

Keratinase activity of the recovered keratinophilic fungi was estimated by following the method given by Vigneshwaran *et al.* (2010).

Preparation of Feather meal powder: It was prepared by following Agrahari and Wadhwa (2010). In this method, feathers of birds were washed, defatted and then dried in a hot air oven. Thereafter, dried feathers were pulverised and the powder so formed was used as a feather meal for determination of keratinase activity.

Preparation of crude enzyme: Each Erlenmeyer flask of 250ml capacity containing 50ml of sterilized Sabouraud's dextrose broth supplemented with 50 mg feather meal powder as keratin source was inoculated with fungal disc (5mm diameter) from the periphery of actively growing seven days old culture by using a sterile circular cutter. Flask containing medium with a disc of agar without the fungus served as control. Three replicates of the test flasks and one control set were maintained for each isolate. These were incubated at 28$\pm$2^{o}C for 4 days on shaker and then for 4 days in static condition. The broth was then centrifuged at 10,000 rpm for 10 minutes and the supernatant so formed was used as a crude enzyme.

Preparation of keratin solution: Keratinolytic activity was measured with soluble keratin (0.5%, w/v) as substrate. Soluble

keratin was prepared from white chicken feathers by the method of Wawrzkiewicz *et al.* (1987). Native chicken feathers (10 g) in 500 ml of dimethyl sulfoxide were heated in a hot air oven at 100 °C for 2 hours. Soluble keratin was then precipitated by addition of cold acetone (1 L) at -70 °C for 2 hours, followed by centrifugation at 10,000 rpm for 10 minutes. The resulting precipitate was washed twice with distilled water and dried at 60 °C in a hot air oven for 20 minutes. One gram of quantified precipitate was dissolved in 20 ml of 0.05M NaOH. The pH was adjusted to 7.0 with 0.1M hydrochloric acid and the solution was diluted to 200 ml with 0.05 mol/L phosphate buffer (pH 7.0).

Keratinase assay: For assessment of keratinolytic activity, 1.0 ml of crude enzyme was diluted with 2ml of phosphate buffer (0.05 M of pH 7.0) and was then incubated with 1 ml keratin solution at 50 °C in a water bath for 10 min. The reaction was stopped by adding 2.0 ml of 0.4M trichloroacetic acid (TCA). Then centrifugation was done at 1500 rpm for 30 minutes and the absorbance of supernatant was determined at 280 nm (Shimadzu,UV-1800 spectrophotometer). One unit of keratinase activity was defined as the amount of enzyme required to liberate 1µg of tyrosine/ ml in I minute under experimental conditions used.

Keratinase Unit (KU) = µ mol/ml/min
On the basis of keratinase activity, the fungal species were categorized into three classes:

1. Low activity (0 to 15KU)
2. Moderate activity(16 to 30KU)
3. Maximum activity(31 to 45KU)

Preparation of L-tyrosine standard curve: Tyrosine standard stock solution (1mM) was prepared in de-ionized water by gently heating in a water bath until tyrosine dissolved completely. Different aliquots in the range of 1.0 µmoles to 3.0 µmoles were prepared. The standard curve was generated by reading the absorbance in a spectrophotometer (Schimadzu UV-1800) at 280nm.The relationship between the absorbance and tyrosine (mM L) was then plotted.

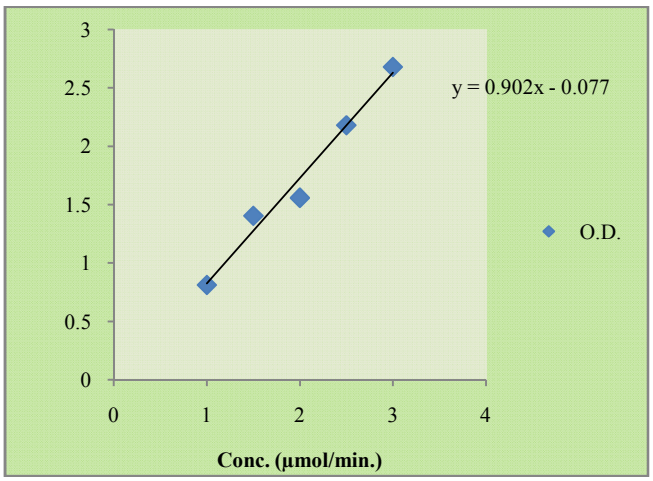

Figure 1 Tyrosine standard curve for keratinase estimation

RESULTS

During the period under investigation (November,2013-February,2015), feather samples of bar-headed geese (greyish white coloured) and common teal (brownish coloured) were collected from Gharana wetland during the winter months of December, January and February by taking help of local bird experts. The samples were placed in clean and sterilized plastic bags, appropriately labelled and brought to the laboratory for screening the diversity of associated keratinophilic fungal flora by following the method of Kaul (1995). As depicted in table 1, approximately 76% of the investigated feather samples of bar-headed geese and 62% feather samples of common teal were found to be positive for the presence of keratinophilic fungi. This indicates that most of the feathers of migratory birds are associated with keratinophiles, which may get dispersed to distant places as the migratory birds move from one place to another.

Persual of data given in table 2 shows that the positive feather samples of bar- headed geese and common teal yielded a total of 33 keratinophilic fungal species belonging to 17 genera. The recovered keratinophiles included 2 species of dermatophytes and 31 species of non-dermatophytes (Figure2). The dermatophytes consisted of two species of *Microsporum* (*M. gypseum* and *M. canis*), whereas the non- dermatophytes included 5 species each of *Chrysosporium* and *Aspergillus*, 4 species of *Penicillium*, 2 species each of *Fusarium, Curvularia, Mucor, Sarocladium* and 1 species each of *Acremonium, Purpureocillium, Alternaria, Cladosporium, Histoplasma, Sagenomella, Rhizopus, Syncephalastrum* and *Didymella.*

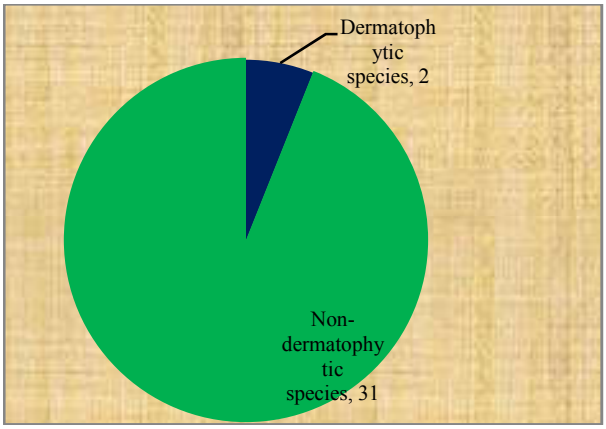

Figure 2 Keratinophilic fungal species recovered from the feathers of migratory birds

Data presented in figure 3 shows that maximum number(33) of keratinophilic fungal species were recovered from the feathers of bar- headed geese, whereas only 21 species were recovered from that of common teal. Keratinophilic fungal species common to both feather samples included *Microsporum gypseum, M. canis, Chrysosporium indicum, C. keratinophilum, C. queenslandicum, Aspergillus flavus, A. fumigatus, A. versicolor, A. candidus, Acremonium fusidioides, Fusarium verticillioides, Purpureocillium lilacinum, Penicillium purpurogenum, Alternaria alternata, Curvularia lunata, C. pallescens, Histoplasma capsulatum, Mucor luteus, Sarocladium strictum, S. kiliense* and *Syncephalastrum racemosum* (Table 2).

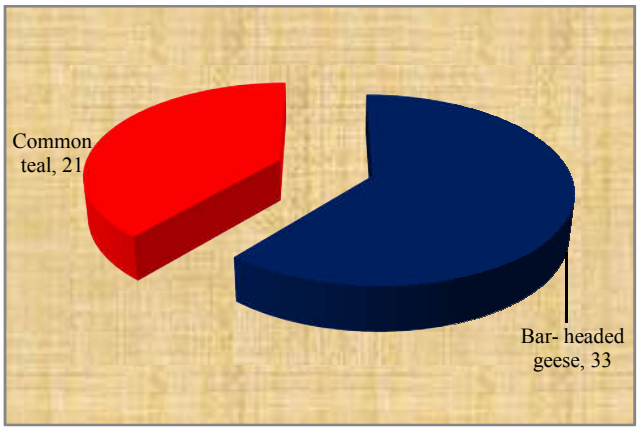

Figure 3 Number of keratinophilic fungal species recovered from the feathers of two different species of migratory birds

Bar- headed geese

As depicted in table 2, a total of 33 keratinophilic fungal species belonging to 17 genera were isolated from the feathers of bar-headed geese. Among the recovered keratinophiles, 2 dermatophytic species and 31 non- dermatophytic species were recovered. The recovered dermatophytes included two species of *Microsporum* (*M. gypseum* and *M. canis*) accounting for 6% of the total recovered diversity, whereas non- dermatophytic fungal species included 5 species of *Aspergillus* (*A. flavus, A. fumigatus, A. versicolor, A. candidus* and *A. niger*) and that of *Chrysosporium* (*C. indicum, C. tropicum, C. merdarium, C. keratinophilum* and *C. queenslandicum*), each representing 15% of the total fungal diversity (Figure 4). This was followed in decreasing order by 4 species of *Penicillium* (*P. olivicolor*, *P. purpurogenum*, *P. griseofulvum* and *P. puberulum*), which contributed 12% of the total fungal diversity. Next in decreasing order were two species each of *Fusarium* (*F. pallidoroseum* and *F. verticillioides*), *Mucor* (*M. luteus* and *M. hiemalis*), *Curvularia* (*C. lunata* and *C. pallescens*) and *Sarocladium (S. strictum and S. kiliense),* each of which contributed 6% of the fungal diversity(Figure 4). Least contribution of 1 species each was that of *Acremonium (A. fusidioides), Alternaria (A. alternata), Histoplasma (H. capsulatum), Rhizopus (R. arrhizus), Cladosporium (C. cladosporoides), Purpureocillium (P. lilacinum), Didymella (D. molleriana), Sagenomella (S. griseoviridis)* and *Syncephalastrum (S. racemosum*). Each of these genera represented 3% of the total recovered keratinophilic fungal diversity (Figure 4).

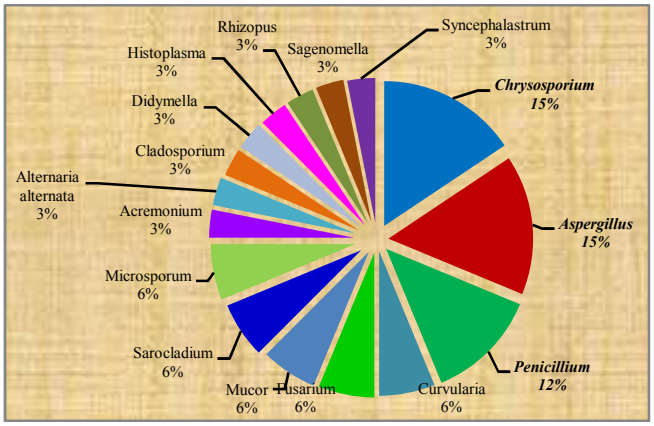

Figure 4 Percentage of species representing each fungal genus recovered from feathers of bar- headed geese

Common teal

As depicted in table 2, a total of 21 keratinophilic fungal species belonging to 14 genera were isolated from the feathers of common teal. These included 2 dermatophytic and 19 non-dermatophytic species. The recovered dermatophytes consisted of 2 species of *Microsporum* (*M. gypseum* and *M. canis*), which contributed 9% of the total species (Figure 5). The non-dermatophytic fungal species included 4 species of *Aspergillus (A. flavus, A. fumigatus, A. versicolor* and *A. candidus)* accounting for 18% of the diversity, followed in decreasing order by 3 species of *Chrysosporium (C. indicum, C. keratinophilum* and *C. queenslandicum*), which contributed 14% of the diversity. Next in decreasing order were *Curvularia* (*C. lunata* and *C. pallescens*) *and Sarocladium (S. strictum* and *S. kiliense)* each represented by 2 species and showing 9% of the recovered species diversity (Figure 5). The other keratinophilic species viz., *Acremonium fusidioides, Fusarium verticillioides, Purpureocillium lilacinum, Penicillium purpurogenum, Alternaria alternata, Mucor luteus, Histoplasma capsulatum, Rhizopus arrhizus* and *Syncephalastrum racemosum* each contributed 5% of the recovered diversity (Figure 5).

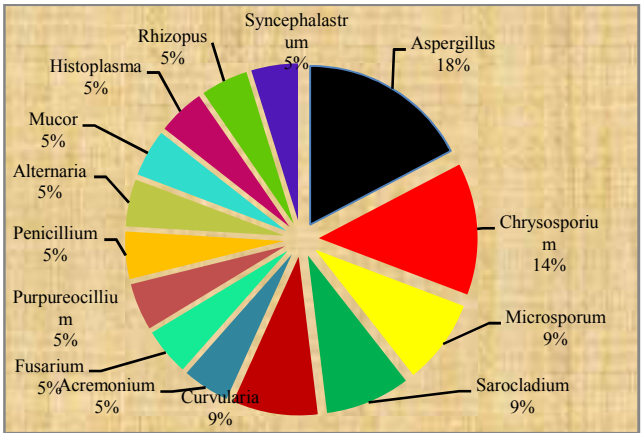

Figure 5 Percentage of species representing each fungal genus recovered from feathers of common teal

DISCUSSION

As given in table 2, the two dermatophytes viz., *Microsporum canis* and *M. gypseum* were recovered from the feathers of both the investigated migratory birds (bar- headed geese and common teal). However, frequency occurrence of *M. gypseum* was higher (24-26%) than that of *M. canis* (2- 4%) and it was recorded more from the feathers of bar-headed geese (Table 2). Earlier, Pugh (1966) reported *M. gypseum* from the nests of some birds, whereas Deshmukh (2004) isolated it from the feathers of pigeons. Later, Gugnani *et al.* (2012) isolated *M. gypseum* from feather samples of Caribbean dove, pigeon and duck and reported it to be responsible for cutaneous mycoses in humans and animals. During the present investigation, the keratinolytic activity of *M. gypseum* was detected to be 40.12 KU, which is quite high. Earlier, *M. gypseum* has been reported to cause ringworm of the scalp and glabrous skin of human beings and other animals (Ali-Shtayeh and Jamous, 2000). Another dermatophyte, *M. canis* was detected to show even more keratinase activity(41.0KU). It has also been reported earlier as causal agent of ringworm infection in pets and of tinea capitis and tinea corporis in humans(Bernardo *et al.*,

2005). However, isolation of *M. canis* from the feathers of migratory birds is being reported for the first time and is of concern due to its pathogenic nature and faster mode of dispersal.

Table 1 Frequency (%) of feathers showing association of keratinophilic fungi

Migratory birds	Feather samples investigated	Feather samples positive for keratinophilic fungi	Frequency (%) of positive samples
Bar-headed geese (*Anser indicus* Latham)	n= 50	n=38	76
Common teal (*Anas crecca* Linn.)	n=50	n=31	62

Among the non-dermatophytic keratinophiles, *Chrysosporium* emerged as the most important genus, which showed maximum keratinase activity that varied from 32.0-39.40KU and whose species were recovered from the feathers of both the investigated migratory birds. As depicted in table 2, five species of *Chrysosporium* (*C. indicum*, *C. tropicum*, *C. merdarium*, *C. keratinophilum* and *C. queenslandicum*) were recovered from the feathers of bar- headed geese, whereas only three species (*C. indicum*, *C. keratinophilum* and *C. queenslandicum*) were recovered from the feathers of common teal. Frequency occurrence of *Chrysosporium indicum* was detected to be maximum (upto 40%), followed in decreasing order by *C. tropicum* (upto 30%), *C. queenslandicum* and *C. keratinophilum* (upto 26%) and *C. merdarium* (upto 24%).

A large number of earlier workers have also isolated *Chrysosporium* species from dropped off feathers of birds viz., domestic fowls and wild birds (Sur and Ghosh, 1980; Olusola, 2002; Mandeel *et al*., 2009; Sharma *et al*., 2012), birds and their nests (Hubalek, 1974; Kornillowicz *et al.,* 2011) and from various soils enriched with bird feathers (Otcenasek 1978; Sur and Ghosh, 1980; Kaul and Sumbali, 1994).

Similarly, five keratinophilic species of *Aspergillus* viz., *A. niger, A. fumigatus, A. candidus*, *A. versicolor* and *A. flavus* were recovered from the feathers of bar- headed geese, whereas only four of them viz., *A. fumigatus, A. candidus*, *A. versicolor* and *A. flavus* were recovered from the feathers of common teal (Table 2). Among these, *A. fumigatus* occurred more frequently (upto 34%) on the feathers, whereas *A. flavus , A. niger and A. versicolor* showed frequency occurrence upto 28% . Most of these *Aspergillus* species have been reported earlier also to be dominant on the feathers of some Indian birds (Pugh, 1966; Hubalek, 1974; Abdel- Hafez, 1991; Gupta and Ramnami, 2006). Similarly, Kaul and Sumbali (2000) while investigating the feathers of poultry birds reported frequent occurrence of *A. flavus.* Recently, Singh *et al*. (2016) isolated *A. versicolor* while investigating keratinophilic fungal flora associated with the feathers of barnacle goose of Svalbard (Arctic).While investigating the keratinase activity of recovered aspergilli, all were detected to have moderate activity (20.02KU- 28.90KU).

Table 2 Frequency (%) and keratinase activity of keratinophilic species recovered from the feathers ofmigratory birds.

Keratinophilic fungi recovered	Bar- headed geese No. of feather samples examined(n)=50		Common teal No. of feather samples examined(n)=50		Keratinase
	No. of positive samples	Frequency %	No. of positive samples	Frequency %	Keratinase Units (KU)
Microsporum canis	02	4	01	02	41.0
Microsporum gypseum	13	26	12	24	40.12
Chrysosporium indicum	20	40	14	28	34.02
Chrysosporium tropicum	15	30	-	-	32.0
Chrysosporium merdarium	12	24	-	-	35.02
Chrysosporium keratinophilum	12	24	13	26	39.40
Chrysosporium queenslandicum	13	26	12	24	37.03
Aspergillus flavus	12	24	14	28	28.90
Aspergillus fumigatus	17	34	12	24	28.41
Aspergillus versicolor	14	28	14	28	26.02
Aspergillus candidus	11	22	11	22	22.43
Aspergillus niger	14	28	-	-	20.02
Acremonium fussidioides	15	30	11	22	30.89
Fusarium pallidoroseum	12	24	-	-	31.68
Fusarium verticilloides	13	26	12	24	31.40
Purpureocillium lilacinum	05	10	13	26	34.18
Penicillium olivicolor	12	24	-	-	24.70
Penicillium purpurogenum	11	22	11	22	24.39
Penicillium griseofulvum	10	20	-	-	24.04
Penicillium puberlum	12	24	-	-	24.40
Alternaria alternata	13	26	12	24	14.32
Didymella molleriana	04	8	-	-	14.87
Cladosporium cladosporoides	10	20	-	-	22.65
Curvularia lunata	13	26	12	24	25.10
Curvularia pallescens	15	30	11	22	24.02
Histoplasma capsulatum	20	40	18	36	33.14
Mucor luteus	10	20	11	22	30.08
Mucor hiemalis	12	24	-	-	31.06
Rhizopus arrhizus	10	20	-	-	16.49
Sarocladium strictum	12	24	12	24	25.41
Sarocladium kiliense	10	20	11	22	25.02
Sagenomella griseoviridis	12	24	-	-	32.35
Syncephalastrum racemosum	16	32	15	30	14.80

-, Absent

Keratinophilic isolates of *A. fumigatus, A. flavus* and *A. niger* have been reported earlier as pathogens of human and other animals either alone or in association with other potential pathogens (Velez and Diaz, 1985; Olusola, 2002; Singh *et al*., 2016).

Next to *Chrysosporium* and *Aspergillus* species were those of *Penicillium,* which were represented by *P. olivicolor, P. purpurogenum, P. griseofulvum* and *P. puberulum*. All these species showed moderate keratinase activity, which ranged from 24.04KU to 24.70KU (table 2). Pugh (1965) and Efuntoye (2002) have also isolated keratinophilic species of *Penicillium* from feathers of some birds and have reported their widespread occurrence. Kornillowicz *et al*. (2011) while studying the keratinophilic fungi, recovered *Penicillium* species even from the nests of birds. However, so far, *P. olivicolor* and *P. puberlum* have not been isolated from the feathers or any other keratinous substrate and therefore, are new additions to the list of keratinophilic *Penicillium* species.

Two species of *Fusarium* viz., *F. pallidoroseum* and *F. verticillioides* were isolated from the feathers of bar- headed geese, each with a frequency of 24% and 26% respectively. However, from the feathers of common teal, only a single species of *Fusarium* (*F. verticillioides)* with frequency of 24% could be recovered (Table 2). Earlier, both these fusarial species have been isolated by Abdel Hafez (1991) from the feathers of ducks and geese from Egypt, whereas Kaul and Sumbali (2000) reported these species from poultry birds of Jammu. Both these species were detected to possess keratinase activity of 31.68KU and 31.40KU respectively. Earlier, Velez and Diaz (1985) have reported keratinophilic isolates of *Fusarium* species to be responsible for causing onychomycosis amongst the people all over the globe. Recently, Bhou and Sumbali (2015) detected *Fusarium verticillioides* as an important mycotic agent of nail dystrophies among the farmers working in the rice fields around Gharana wetland.

Other keratinophilic fungi recovered from feathers of migratory birds included some members of dematiaceous hyphomycetes viz., *Curvularia* (*C. lunata and C. pallescens*), *Cladosporium* (*C. cladosporoides*) and *Alternaria (Alternaria alternata)*.Occurrence of *C. lunata* (26%) and *C. pallescens* (30%) was detected to be more on the feathers of bar- headed geese than on the feathers of common teal (Table 2). Earlier, Abdel- Hafez (1991) isolated *C. lunata* from the feathers of ducks and geese while investigating the poultry farms of Egypt. Later, Kaul and Sumbali (2000) isolated it from the feathers of poultry birds at Jammu. Both these species of *Curvularia* were detected to have moderate keratinase activity (Table 2) and are reported earlier to be responsible for causing cutaneous infections and nail dystrophies (Agrawal and Singh, 1995; Sharma and Sharma, 2010; Bhou, 2017). However, so far, *C. pallescens* has not been reported from the feathers of birds.

Similarly, *Cladosporium cladosporoides*, with moderate keratinase activity (22.65KU) and a frequency of 20% was detected to be more on the feathers of bar- headed geese than on the feathers of common teal (Table 2). Earlier, Hubalek (1976)) isolated *C. cladosporoides* from the feathers of house sparrows of Czechoslovakia and Abdel- Hafez (1991) recovered this species from the feathers of ducks and geese kept in the poultry farms of Egypt.

Another dematiaceous fungus, *Alternaria alternata* with low keratinase activity of 14.32KU was detected from the feathers of both the birds but its frequency was more on the bar-headed geese (26%) than on the common teal (24%). Earlier, Mbata (2009) recovered *Alternaria alternata* from the feathers of chickens and reported it to produce clinical skin superficial mycoses amongst chickens reared in warm regions. Recently, Bhou (2017) while surveying the toenails and fingernails of people residing around the Gharana wetland, observed *Alternaria alternata* as common causal agent of onychomycosis.

As depicted in table 2, *Histoplasma capsulatum*, the causal agent of histoplasmosis showed keratinase activity of 33.14KU and was found frequently associated with the feathers of both the bar- headed geese (40%) and common teal (36%). Emmons (1949) was the first to isolate *H. capsulatum* from the soil and bat guano in the United States of America. Other keratinophilic fungi recovered from the feathers of migratory birds included *Sarocladium strictum, S. kiliense, Acremonium fusidioides, Purpureocillium lilacinum, Didymella molleriana* and *Sagenomella griseoviridis* (Table 2). Of these, *Acremonium fusidioides* and *Purpureocillim lilacinum* showed maximum keratinase activity and frequency occurrence of 30% and 10% respectively on the feathers of bar- headed geese and of 22% and 26% respectively, on the feathers of common teal. *Didymella molleriana* was found exclusively from the feathers of bar-headed geese and it showed the least frequency of 8% as well as keratinase activity of 14.8KU, which is similar to the results of Kumar and Kushwaha(2014), who reported species of *Didymella* as poor producers of keratinases in submerged culture conditions. *Sarocladium strictum* showed frequency of 24% from the feathers of both the migratory birds, whereas *S. kiliense* showed slightly higher frequency (22%) on the feathers of common teal than on the feathers of bar-headed geese (20%). Recently, Awad (2017) isolated keratinophilic isolates of *Paecilomyces lilacinus* and *Didymella molleriana* from the fur of goat but there are no reports of *Sarocladium strictum*, *S. kiliense*, *Sagenomella griseoviridis and Didymella molleriana* from the feathers of birds.

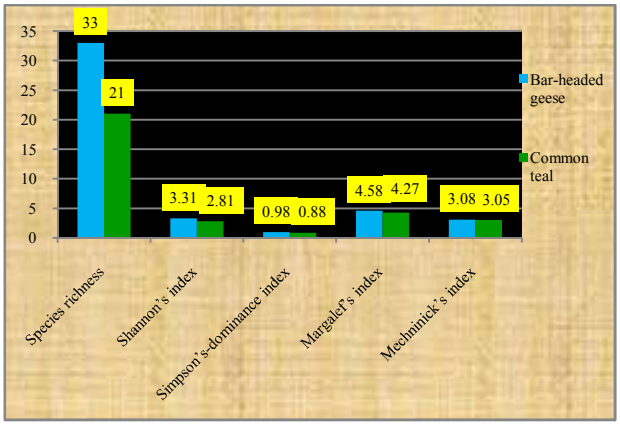

Figure 6 Diversity indices of keratinophilic fungi recovered from the feathers of bar- headed geese and common teal

Some members of the Class Zygomycetes were also detected to be keratinophilic and these included species of *Mucor*, *Rhizopus* and *Syncephalastrum* (Tables 2). They are mainly

responsible for causing zygomycosis and onychomycosis and depending on the site involved, they cause the formation of various clinical forms (Bala *et al*., 2015; Bhou, 2017). Among the recovered Zygomycetes, *Mucor hiemalis* showed frequency of 24% on the feathers of bar- headed geese, whereas *M. luteus* showed maximum frequency of 22% on the feathers of common teal. Both the species of Mucor were detected to show good keratinase activity and have been reported earlier by some workers to cause feather loss in many birds (Decostere *et al*.,2003; Quesada *et al*., 2007).Similarly, *Syncephalastrum racemosum* was recovered from the feather samples of both the migratory birds, but it possessed low keratinase activity (14.80KU). However, *Rhizopus arrhizus* with low keratinase activity of 16.49KU was recovered only from the feathers of bar- headed geese. Earlier, this keratinophilic species was reported from feathers of poultry birds of Egypt (Abdel - Hafez,1991).

Diversity indices were also calculated for the keratinophilic fungal species recovered from the feathers of bar-headed geese and common teal(Table 3). Since the feather samples were from two different species of migratory birds, therefore, significant differences were observed in their diversity indices. As depicted in figure 6, highest species richness(S) and Shannon diversity index (H') were recorded for the bar-headed geese(S= 33 species and H'=3.31), which shows that the feathers of bar- headed geese are more diverse and richer in keratinophilic species than the feathers of common teal. Simpson's diversity index, a measure of heterogeneity, shows that for the bar- headed geese (0.98) and common teal (0.88), the values are near to one, thereby showing more heterogenous nature of the keratinophilic fungal species present on the feathers of these birds. The highest Margalef's index value was recorded for the bar- headed geese (D_{Mg}=4.58) indicating more species diversity than that of common teal (D_{Mg} = 4.27). Similarly, Mehninick's index was calculated and the highest value was again obtained for the bar- headed geese (D_{Mn}=3.08), which supports the result obtained by Margalef's index (Table 3). From the calculated diversity indices, it is concluded that highest species richness and diversity of keratinophilic fungal species is present on the feathers of bar- headed geese.

Table 3 Diversity indices calculated for the keratinophilic fungi recovered from feathers of migratory birds

Diversity indices	Migratory birds	
	Bar-headed geese	Common teal
Species richness (S)	33	21
Shannon's index (H')	3.31	2.81
Simpson's dominance index (C_d)	0.98	0.88
Margalef's index (D_{Mg})	4.58	4.27
Mechninick's index (D_{Mn})	3.08	3.05

CONCLUSION

From the present investigation, it is concluded that the migratory birds may act both as reservoirs and carriers of keratinophilic fungal species during their long flights and thus disperse their spores to distant lands including wetlands. The wetland soil, which gets enriched with the keratinous feathers and claws of migratory birds along with the associated keratinophiles, provides most conducive environment for their growth, multiplication and further dispersal by even the local birds. Being opportunists, keratinophilic fungi may become parasitic by accident and cause various types of mycoses among the humans and animals living in the vicinity of the wetland as observed earlier by a researcher (Bhou, 2017).

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