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Original Research Article

Keratinophilic Fungi in Wetland Agroecosystem

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Abstract: Keratinophilic fungi are ecologically and epidemiologically significant group of fungi that cycle one of the most abundant and highly stable animal proteins on earth - keratin. Keratinophilic fungi exemplify a vast biodiversity of form, habitat and substrates. However, studies are rather meager in wetland agroecosystems and the present work has been undertaken to record the presence of keratinophilic fungi in a wetland agroecosystem in Kerala, India. Altogether, a total of 38 species of fungi comprising 22 genera were identified from the 120 soil samples collected from different areas of oil palm agricultural fields in the wetland agroecosystem from July 2015 to July 2016. A total of 11 fungi were recorded throughout the study irrespective of seasons. North East monsoon season fetched more fungi (30 species) followed by early summer (29 species), late summer (19 species) and south west monsoon season (18 species). It is clear from the results that soils of Vembanadu wetland agroecosystem provides ideal environment for the growth of keratinophilic fungi and dermatophytes which is be attributed to the high organic debris and keratinous substrates like rodent hair and feathers from birds and other animals and plant litter present in these soils. Thus the presence of these fungi which are human and animal pathogens are considered as bioindicators of environmental pollution with keratinous substrate which can pose risk of human and animal mycoses in the region.
Keywords: Vembanadu Wetland Agroecosystem, Fungi, Keratinophilic Fungi, Hair Baiting.

INTRODUCTION

Keratinophilic fungi are significant ecologically and epidemiologically with striking role in natural degradation of keratinous substrates [1, 2]. The distribution of keratinophilic fungi is varying with the environment and depends on several factors like human or animal presence and presence or absence of keratinous substances. The soil inhabiting keratinophilic fungi has a special affinity for keratinous substrates, as these are able to invade and live on cornified tissues by enzymatic digestion for their growth and nutrition [3]. Various keratinophilic fungi along with some dermatophytes are responsible for a number of skin infections, but little epidemiological data are available in this regard [4]. Knowledge of the frequency and extension of etiological agents of humans and animal mycosis and other potentially pathogenic fungi in soil is of paramount importance for understanding of epidemiological cycle of these fungi. Keratinophilic fungi exemplify a vast biodiversity of form, habitat and substrates. Moreover, fungal studies are rather meager in wetland agroecosystems and the present work has been undertaken to record the presence of keratinophilic fungi in a wetland agroecosystem in Kerala, India. The study will help to recognize the frequency and occurrence of keratinophilic fungi and risk of mycoses in the region. The presence of fungi in soil also indicates the infection risk connected with contamination of the environment by possible fungal pathogen and public health risks associated with it.

MATERIALS AND METHODS

Vembanadu-Kol Wetland is the largest lake in Asia (Ramsar site) and fringe area occupies the most extensive agricultural fields of the state. A total of 120 soils samples were collected from different areas of oil palm agricultural fields in the ecosystem (July 2015 to July 2016). Soil samples were collected in sterile polyethylene bags and brought to the laboratory for further mycological analysis. In order to find the fungal load of the soil samples, serial dilution followed by spread plating in Sabouraud Dextrose Agar (SDA) (with antibiotics) was adopted. The developed colonies were counted and identified up to species level. 10 gm of the soil sample were baited with sterilized human hair as described in Vanbreuseghem hair baiting technique [5] and incubated at room temperature for 10 to 30 days. Fungi colonized on hair fragments were subcultured on SDA and incubated at room temperature for 5 to 10 days. The sufficiently grown colonies were counted and identified by performing Scotch tape

method with Lactophenol Cotton Blue [6]. Macroscopic features like pigmentation, colony topography, colony texture, exudates production, rugal fold, reverse pigmentation and microscopic features like hyphal characteristics and conidia ornamentation were observed.

Fig-1: A view of the study area

RESULTS AND DISCUSSION

A total of 38 species of fungi comprising 22 genera were identified from the 120 soil samples collected from different areas of oil palm agricultural fields in the wetland agroecosystem from July 2015 to July 2016. Genus *Aspergillus*, *Microsporum*, *Penicillium* and *Trichophyton* are more common represented by four species each. A preponderance of *Chrysosporium* sp. (90.83%) followed by *A. niger* (77.50%), *A. flavus* (74.16%) and *F. chlamydosporum* (39.16%) was noted. The presence of dermatophytes like *Microsporum*, *Trichophyton* and *Epidermophyton* in a wetland agroecosystem is quite alarming, as human-soil contacts are inevitable in such environment. Several reports are available from various parts of India showing the rich variety of keratinophilic fungal flora in the country's soils [7-10]. However, there is no evidence of any study regarding fungi in wetland agroecosystem soils in Kerala. It can be concluded that the typical agro-climatic conditions in the agroecosystem are conducive for the keratinophilic fungi.

Table 1: List of fungi isolated (n=120)

Sl. No.	Fungi isolated	Frequency of occurrence (%)
1.	*Absidia corymbifera*	21.66
2.	*Alternaria alternata*	12.50
3.	*Aspergillus flavus*	74.16
4.	*A. fumigatus*	40
5.	*A. nidulans*	10
6.	*A. niger*	77.50
7.	*Chaetomium atrobreunneum*	19.16
8.	*Chrysosporium* sp.	90.83
9.	*Cunninhamella bertholletiae*	10
10.	*Curvularia geniculata*	32.50
11.	*Epidermophyton flococcosum*	2.50
12.	*Fusarium chlamydosporum*	39.16
13.	*F. dimerum*	15.83
14.	*F. semitectum*	25
15.	*Gliocladium* sp.	7.50
16.	*Humicola sp.*	3.33
17.	*Microsporum audouinii*	14.16
18.	*M. gypseum*	31.66
19.	*M. nanum*	11.66
20.	*M. cookei*	15.83
21.	*Paecilomyces lilacinus*	25.83
22.	*P. variotii*	17.50
23.	*P. chrysogenum*	9.16
24.	*Penicillium citrinum*	5.83
25.	*P. janthinellum*	3.33
26.	*P. verrucosum*	25
27.	*Phialimonium obovatum*	24.16
28.	*Pseudallescheria boydii*	2.50
29.	*Rhizopus sp.*	34.16
30.	*Rhizopus stolonifer*	32.50
31.	*Scopulariopsis brevicaulis*	9.16
32.	*Syncephalastrum racemosum*	21.66
33.	*Trichoderma* sp.	11.66
34.	*Trichophyton mentagrophytes*	8.33
35.	*T. rubrum*	5.50
36.	*T. tonsurans*	4.16
37.	*T. verrucosum*	4.16
38.	*Verticillium sp*	9.16

A total of 11 fungi were recorded throughout the study irrespective of seasons like Early summer (December- February); Late summer (March- May); South West monsoon (June –August); North East monsoon (September-November) (*A. falvus, A. niger, Chrysosporium* sp., *M. gypseum, P. chrysogenum, P. verrucosum, P. obovatum, Rhizopus* sp., *R. stolonifer, S. recemosum* and *Verticillium* sp.) (Table 2). North East monsoon season fetched more fungi (30 species) followed by early summer (29 species), late summer (19 species) and south west monsoon season (18 species).

Table 2: Seasonal distribution of fungi isolated (n=120)

Fungi isolated	Early summer (December-February)	Late summer (March-May)	South West monsoon (June – August)	North East monsoon (September-November)
Absidia corymbifera	+	+	-	+
Alternaria alternata	-	-	+	+
Aspergillus flavus	+	+	+	+
A. fumigates	+	+	-	+
A. nidulans	+	-	-	-
A. niger	+	+	+	+
Chaetomium atrobreunneum	-	-	+	+
Chrysosporium sp.	+	+	+	+
Cunninhamella bertholletiae	-	-	-	+
Curvularia geniculata	-	-	+	+
Epidermophyton flococcosum	+	+	-	-
Fusarium chlamydosporum	+	-	-	+
F. dimerum	-	-	-	+
F. semitectum	+	+	-	+
Gliocladium sp.	+	-	-	+
Humicola sp.	-	-	-	+
Microsporum audouinii	-	-	+	+
M. gypseum	+	+	+	+
M. nanum	+	+	-	-
M. cookie	+	-	-	+
Paecilomyces lilacinus	-	-	+	+
P. variotii	-	-	+	+
P. chrysogenum	+	+	+	+
Penicillium citrinum	+	+	-	-
P. janthinellum	+	-	-	-
P. verrucosum	+	+	+	+
Phialimonium obovatum	+	+	+	+
Pseudallescheria boydii	+	+	-	-
Rhizopus sp.	+	+	+	+
Rhizopus stolonifer	+	+	+	+
Scopulariopsis brevicaulis	+	-	-	+
Syncephalastrum racemosum	+	+	+	+
Trichoderma sp.	+	-	-	+
Trichophyton mentagrophytes	+	+	-	-
T. rubrum	+	-	-	+
T. tonsurans	+	-	+	+
T. verrucosum	+	-	-	-
Verticillium sp	+	+	+	+

Fig-2: Keratinophilic fungal colonization on hair baits

Deshmukh [21] reported the presence of keratinophilic fungi among the collected 158 soil samples from various areas of four districts in Kerala and noted the presence of eight genera with 15 species *viz*., *Arthroderma simii, Chrysosporium indicum, C. keratinophilum, C. lobatum, C. pannicola, C. tropicum,* Chrysosporium state of *Arthroderma cuniculi*, Chrysosporium state of *Ctenomyces serratus, Gymnascella hyalinospora, Malbranchea aurantiaca, M. fulva, Microsporum gypseum* complex, *Pseudogymnoascus roseus, Trichophyton mentragrophytes* and *T. terrestre.* Mini *et al.* [11] also reported the presence of keratinophilic fungi among soil from Ernakulam and Thrissur districts in Kerala.

It should be noted that majority of fungi producing diseases exist freely in nature as soil saprophytes and gain entrance into body through abrasion, implantation or inhalation. Vembanadu is well known as rice the bowl of Kerala, and it´s cultivation required comparatively high relative humidity in soils which favor the growth of keratinophilic fungi. The presence of different species of water birds, especially migratory in nature may also contribute to the rich flora of keratinophilics, as the soil is rich in keratin substrates. The diversity and abundance of water birds in the region are well established [12]. Thomas *et al*. [13, 14] also reported the high prevalence of fungi including keratinophilic and dermatophytes among rats inhabiting Vembanadu wetland agroecosystem. The dermatophytes attack epidermal tissue, which came into the contact of soil during various agricultural and recreational activities, hence, can be infected.

A plethora of investigations are done in various part of India during last few years and showed that rich variety of keratinophilic fungal flora in soil [15-18]. The obtained results are also in agreement with these reports. It should be noted that fungal infection of human beings is more common in India and other tropical countries due to environmental factors like heat and humidity. All these factors and personal hygiene are involved in flourishing of fungal infections and causing fungal diseases. Recently, human exposure to fungi is a matter of health risk, as the population of immunocompromised people is increasing day by day. Several nonpathogenic fungi are now being reported as opportunistic pathogen with much complex pathognomonic features. Identifying both environments and fungi where people are exposed to them is of paramount importance in public health purview.

Shrivastava *et al*. [19] studied the prevalence of keratinophilic fungi in paddy field soil during different stages of cultivation *viz*., transplanting, tillering, milking and maturation. Fourteen species belonging to a single genus *Chrysosporium* were isolated throughout the cropping season. *C. keratinophilum* (17. 1 %) followed by *C. tropicum* (13.15 %) were found to be the most dominating geophilic species. The highest percent distribution of keratinophilic fungi appeared during the milking stage (100 %) of paddy cultivation, followed by the maturation stage (89.47 %).

It is clear from the results that soils of Vembanadu wetland agroecosystem provides ideal environment for the growth of keratinophilic fungi and dermatophytes which is be attributed to the high organic debris and keratinous substrates like rodent hair and feathers from birds and other animals and plant litter present in these soils. The isolation of fungi was not uniform in the present study, as it depends on organic matter and animal presence. Organic matter content of soils is one of the major factors affecting the presence of keratinophilic fungi in soils [20]. Moreover, the distribution of keratinophilic fungi is influenced mostly by the amount of keratinized material available in the soil, factors like waterlogged condition, nitrogen rich environment, pH, other physicochemical factors etc. The study clearly indicates the varied distribution of keratinophilic fungi and dermatophytes in soils of Vembanadu wetland agroecosystem.

CONCLUSION

It is clear from present investigation that soils of Vembanadu wetland agroecosystem are ideal environment for the keratinophilic fungi which is attributed to high organic debris and keratinous substrates present in these soils. The organic matter content of soils and presence of keratinous substrates are the major factors affecting the presence of keratinophilic fungi in soils. Thus the presence of these fungi which are human and animal pathogens are considered as bioindicators of environmental pollution with keratinous substrate which can pose risk of human and animal mycoses in the region.

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