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Keratinophilic and Thermophilic Fungi from Animal Manures and Floor Dust in the Vicinity of Ayatt, Giza, Egypt.

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FORTY SEVEN samples of animal manures and thirty eight samples of floor dust were randomly collected from the vicinity of Ayatt City during September – November 2013. Keratinophilic and thermophilic fungi were isolated. A total of 26 sp belonging to 15 genera and 23 sp belonging to 17 genera of keratinophilic and thermophilic fungi were identified, respectively. Out of 76 encounters of animal manures *Thamnostylum piriforme* was the most frequent (10.52 %) followed by *Aspergillus brasiliensis* = *Chrysosporium keratinophilum* (7.89%) each. 78 encounters were isolated from floor dust. The most frequent was *Arachniotus dankaliensis* (10.25%) followed by *Candida albicans* (7.69%). 76 isolates of thermophilic fungi were isolated from animal manure. *Aspergillus fumigatus* is the most frequent (9.21%) followed by *Aspergillus flavus* var. *columnaris* (7.89%). 75 isolates were identified from floor dust. Again, *Aspergillus fumigatus* is the most frequent (9.33%). *Aspergillus brasiliensis* = *A. carneus* = *A. flavus* var. *columnaris* = *Rasamsonia byssochlamydoides* = *Chrysosporium zonatum* (6.66%) each comes second. The clinical importance of certain fungi was discussed.

Keywords: Fungi, Animal manure, Floor dust, Egypt.

Keratinofers are opportunisitic and/ or pathogenic fungi infecting man and animals. Keratinolytic fungi are able to attack and decompose keratin, whereas keratinophilic ones accompany them utilizing the products of keratin degradation. Keratinofers have been divided into three categories according to their natural habitats: anthropohilic that infects humans, zoophilic which infects animals and geophilic when soil is the natural habitat. Presence of humans and animals in the environment increases the incidence of keratinofers. Biology, physiology and ecology of these fungi have been invistigated throughout the world (Abdel-Fatlah *et al.,* 1982; Garg *et al.,* 1985; Abdel-Hafez *et al.,* 1990; Kunert, 2000; Katiyar & Kushwaha, 2002; Bohacz and Kornittowicz-Kowalska, 2012; Sharma & Ghoudhary, 2014; Bisen & Tiwari, 2015 and Khan & Bhadauria, 2015).

Heat- tolerant fungi can be classified as thermophiles or thermotolerant, depending on their cardinal growth temperatures. The definition of Cooney &

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Emerson (1964) consider thermophilic fungi to be those with maximum growth temperature of 50 ^{o}C or above and minimum growth temperature of 20 ^{o}C or above. Thermotolerant fungi are those that grow at maximum temperature up to 50 ^{o}C with a minimum temperature below 20 ^{o}C. Although this classification is quite practical, it is not applicable to all fungi. For example *Aspergillus fumigatus* is able to grow at temperature above 50 ^{o}C and below 20 ^{o}C. Alternatively, Maheshwari *et al.,* (2000) proposed that thermophilic fungi are those with an optimum growth temperature of 45 ^{o}C or above. Thermophilic fungi play an important role in composting in soil, some are opportunistic pathogens and others are allergenic (Sirinivasan *et al.,* 2005). Comprehensive reviews are available: (Cooney and Emerson, 1964; Maheshwari *et al.,* 1987 & 2000; Mouchaea, 1997 & 2007, Salar and Aneja, 2007 and Singh and Satyanarayana, 2014).

The objective of this study is the isolation and identification of both keratinophilic and thermophilic fungi from animal manures and floor dust in the vicinity of Ayatt, Giza, Egypt.

Material and Methods

Collection of Soil Samples

A total of 47 and 38 samples of animal manure and floor dust was randomly collected during September – December 2013 in sterile plastic bags and brought to laboratory and stored at 8 ^{o}C.

Isolation of Keratinophilic Fungi

Keratinophilic fungi were isolated by the hair – baiting technique (Vanbreseghem, 1952). Twenty g. from each soil samples were placed in 90 mm sterile Petri plate in five replicates. A sterile defatted human hair fragments (1-2 cm) were scattered on the surface of soil. The plates were moistened with antibiotic solution containing 50 mg L^{-1} chloramphenicol and 500 mg L^{-1} cyclohoximide. Petri plates were sealed with cellophane tapes and incubated at 28 ^{o}C for 4-6 weeks and moistened periodically with sterile water. If fungal growth was observed, the baits were transferred to plates containing Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol and cycloheximides as previously mentioned and inoculated at 28 ^{o}C. Fresh developed colonies were isolated, purified, examined and identified.

Isolation of Thermophilic Fungi

Ten mg of animal manure or floor dust were transferred asceptically to 90 mm Petri plates using sterile spatula. Twenty ml of semi- liquid yeast-glucose agar medium (Cooney and Eerson, 1964) were poured in each plate and hand swirled and left to solidify. Plates were then incubated at 45 ^{o}C for 4-6 days. The developed colonies were isolated, purified, examined and identified.

Identification of Fungi

Pure culture was maintained on the appropriate media and identified according to the following discriptive manuals: (Cooney and Emerson, 1964; Frey *et al.,* 1979; Domsch *et al.,* 1980; van Oorschot, 1980; Moubasher, 1993; de

Hoog and Guarro, 1995; Mouchaea, 1997; Latge, 2003; Ellis *et al.*, 2007 and Campell *et al.*, 2013). Identification of certain isolated was confirmed by the Authorities of AUMC (Assuit, Egypt, 71516) to whom the authors are greatly indebted. Number of fungus encounters and frequency for each species were calculated and represented in tables. Current names of fungi were used.

Results and Discussion

A total of 18 species belonging to 11 genera and 22 species belonging to 14 genera of keratinophilic fungi was isolated from animal manures and floor dust samples, respectively (Table 1). Out of 76 encounters of animal manure, *Thamnostylum piriforme* comes first (8 encounters and 10.52% frequency). In the second order comes *Aspergillus brasiliensis* = *Chrysosporium keratinophilum* (6 and 7.89%). The order of rest of fungi in descending manner is found to be: *Mucor circinilloides* = *M. hiemalis* = *Aspergillus flavus* = *A. fumigatus* = *Exserohilum rostratum* = *Fusarium solani* (5), 6.57% each >*Aspergillus ustus* = *Phoma glomerata* (4), 5.26 each> *Rhizopus stolonifer* = *Aspergillus sydowii* = *Penicillium duclauxii* = *Trichophyton terrestre* (3), 3.94%> *Cunninghamella echinulata* = *Mucor racemosus* = *Penicillium purpurogenum* (2), 2.63% each, respectively.

Concerning floor dust *Arachniotus dankaliensis* comes first (8), 10.25% followed by *Candida albicans* (6), 7.69%. In descending order the rest of fungi is found to be *Aspergillus brasiliensis* = *A. fumigatus* = *A. parasiticus* = *Phoma glomerata* = *Scopulariopsis brevicaulis* (5), 6.41> *Circinella muscae* = *Aspergillus flavus* (4), 5.13%> *Absidia corymbifera* = *Chrysosporium indicum* = *C. tropicum* = *Fusarium solani* = *Penicillium duclauxii* = *P. purpurogenum* (3), 3.85% > *Cunninghamella echinulata* = *Mucor hiemalis* = *M. racemocus* = *Rhizopus stolonifer* = *Aspergillus ustus* = *Chrysosporium keratinophillum* (2), 2.56% > *Trichophyton terrestre* (1), 1.28% each respectively.

Keratinophilic fungi were isolated from animal sheds and floor dust throughout the world (Maghraby *et al.*, 2008; Singh *et al.*, 2009; Al-Humiany, 2010; Jain and Sharma, 2012; Deshmukh and Verekar, 2012; Kacinova *et al.*, 2013; Sarkar *et al.*, 2014 and Deshmukh and Verekar, 2014). Keratinophilic fungi are associated with human and/ or animal activities (Table 2)

Concerning thermophilic fungi 76 encounters belonging to 20 spp from 15 genera and 75 encounters belonging to 20 spp from 14 genera were isolated from animal manure and floor dust, respectively. *Aspergillus fumigatus* was the most frequent in animal manure (7 encounters and 9.21% frequency, followed by *Aspergillus flavus* var *columnaris* (6) 7.89%> *Absidia corymbifera* = *Rhizomucor pusillus* = *Aspergillus brasiliensis* = *Chrysosporium zonatum* (5) 6.57% each>*Chaetomium thermophilum* = *Myceliophthora thermophila* = *Papulospora thermophila*= *Scytalidium thermophilum* (4) 5.56% each> *Rhizopus rhizopodoformis* = *Aspergillus terreus* = *Emericella nidulans* = *Malbranchea cinnamomea* = *Thermoascus aurantiacus* = *Thermomyces lanuginosus* (3) 3.94% each> *Rhizomucor miehei* =*Rasamsonia emersoni* = *Remersonia thermophila* (2) 2.63% each respectively.

TABLE 1. Fungal species encounters (ENC) and percentage frequency(%) of keratinophilic fungi isolated from animal manures (AM) and floor dust (FD).

Fungal species	A M		F D	
	ENC	%	ENC	%
Absidia corymbifera (Cohn) Saccardo & Totter	0	0	3	3.85
Circinela muscae (Sorokin) Berlse & Toni	0	0	4	5.13
Cunninghamella echinulata (Thaxter) Thaxter	2	2.63	2	2.56
Mucor circinilloides van Teighem	5	6.57	0	0
Mucor hiemalis Wehmer	5	6.57	2	2.56
Mucor racemosus Fresenius	2	2.63	2	2.56
Rhizopus stolonifer (Erhenberg) Vuillemin	3	3.94	2	2.56
Thamnostylum piriforme (Bainier) von Arx & Upahyay	8	10.52	0	0
Aspergillus brasiliensis Varga, Frisvad & Samson	6	7.89	5	6.41
Aspergillus flavus (Link)	5	6.57	4	5.13
Aspergillus fumigatus Fresenius	5	6.57	5	6.41
Aspergillus parasiticus Speare	0	0	5	6.41
Aspergillus sydowii (Bainer & Sartory)Thome & Church	3	3.94	0	0
Aspergillus ustus (Bainier) Thom & Church	4	5.26	2	2.56
Arachniotus dankalensis (Castellani) va Beyma	0	0	8	10.25
Candida albicans (Robin) Berkhout	0	0	6	7.69
Chrysosporium indicum (Randhawa & Sandha) Garg	0	0	3	3.85
Chrysosporium kertinophilum Frey (Carmichael)	6	7.89	2	2.56
Chrysosporium tropicum (Carmichael)	0	0	2	3.85
Exserohilum rostratum (Drechsler) Leonard & Sugg	5	6.57	0	0
Fusarium solani (Martin) Saccardo	5	6.57	3	3.85
Penicillium duclauxii Delacroxi	3	3.94	3	3.85
Penicillium purpurogenum Stoll	2	2.63	3	3.85
Phoma glomerata (Corda) Wollenweber & Hochapfel	4	5.26	5	6.41
Scopulariopsis brevecaulis (Saccardo) Bainier	0	0	5	6.41
Trichophyton terrestre Durie & Frey	3	3.94	1	1.28
No. of Total Encounters	76		78	
No. of Species	18		22	
No. of Genera	11		14	

TABLE 2. Fungal species encounters(ENC) and percentage frequency(%) of thermophilic fungi isolated from animal manures (AM) and floor dust (FD).

Fungal species	A M		F D	
	ENC	%	ENC	%
Absidia corymbifera (Cohn) Saccardo & Trotte	5	6.57	3	4.00
Rhizomucor miehei (Cooney & Emerson) Schipper	2	2.63	2	2.66
Rhizomucor pusillus (Lindt) Shipper	5	6.57	3	4.00
Rhizopus rhizopodoformis (Cohn) Zopf	3	3.94	2	2.66
Aspergillus brasiliensis Varga, Frisvad & Samson	5	6.57	5	6.66
Aspergillus carneus Blockwitz	3	3.94	5	6.66
Aspergillus flavus var *Columnaris* Raper & Fennel	6	7.89	5	6.66
Aspergillus fumigatus Fresenius	7	9.21	7	9.33
Aspergillus terreus Thom	3	3.94	4	5.33
Chaetomium thermophilum La Touche	4	5.56	2	2.66
Chrysosporium zonatum Al-Musallam & Tan	5	6.57	5	6.66
Emericella nidulans (Eidam) Vuillemin	3	3.94	3	4.00
Fennellia nivea (Wiley & Simmons) Samson	0	0	3	4.00
Malbranchea cinamomea (Libert) van Oovshot & de Hoog	3	3.94	4	5.33
Myceliophthora thermophila (Apinis) van Oorshot	4	5.56	3	4.00
Papulospora thermophila Fergus	4	5.56	0	0
Paceliomyces variotii (Thom) Samson	0	0	4	5.33
Rasamsonia byssochlamydoides (Stolk&Samson)Houbraken&Frisvad	0	0	5	6.66
Rasamsonia emersonii (Stolk) Houbraken & Frisvad	2	2.63	0	0
Remersonia thermophila (Fergus) Seifert & Samson	2	2.63	0	0
Scytalidium thermophilium (Cooney & Emerson) Austwick	4	5.56	4	5.33
Thermoascus aurantiacus Miehe	3	3.94	4	5.33
Thermomyces lanuginosus Tsiklinsky	3	3.99	2	2.66
No. of Total Encounters	76		75	
No. of Species	20		20	
No. of Genera	11		15	

In floor dust *Aspergillus fumigatus* was the most frequent (7) 9.33% followed by *Aspergillus brasiliensis* = *A. carneus* = *A. flavus* var *columnaris* = *Rasamsonia byssochlamydoides* = *Chrysosporium zonatum* (5) 6.66% each > *Aspergillus terreus* = *Malbranchea cinamomea* = *Paceliomyces variotii* = *Scytalidium thermophilum* = *Thermoascus auratiacus* (4) 5.33% each > *Absidia corymbifera*= *Rhizomucor pusillus* = *Emericella nidulans* = *Fennillia nivea* = *Myceliophthora thermophila* (3) 4.0% each >*Rhizomucor miehei* = *Rhizopus rhizopodoformis* = *Chaetomium thermophilum* = *Thermomyces lanuginosus* (2) 2.66% each respectively. Thermophilic and thermotolerant fungi play an important role in animal manure compositing. Mature (cured) animal manure is a good substrate as organic fertilizer. Application of immature animal manure (in mesophilic or thermogenic phases) is not saved from the hygenic point of view. Thermophilic fungi with clinical importance have been reported (El-Gindy *et al.,* 2002; Al-humiany, 2010; Rajavanaram *et al.,* 2010; Sreelatha *et al.,* 2013 and Singh and Satyanarayana, 2014).

Yang *et al.,* (2016) reviewed soil fungi implicated in animal and human pathogenesis.

Frequent occurrence of *Thamnostylum piriforme* has been reported earlier (Benny and Benjamin, 1975). In Egypt, *T. piriforme* was reported from buffalo-cows that failed to conceive (Morsy, 2007).

Aspergillus brasiliensis is newly described species (Varga *et al.,* 2007), which was in part created by the transfer of several existing *A. niger* strains to the new sp. *A. brasiliensis* is upiquitous soil fungus. Up to now *A. brasiliensis* has not been implicated in human diseases.

Chrysosporium spp is frequent keratinophilic soil fungi. Localised and dissiminated infections in healthy and immunocsmpromised individuals due to *Chrysosporium keratinophilum*, *C. zonatum* (both are thermotolerant) and *C. tropicum* may occur. Primary cutaneous infections by *Chrysosporium* spp. are relatively rare (Suchonwanit *et al.,* 2015).

Arachniotus dankaliensis is rare keratinophilic fungus. It was isolated from the house dust of Aden- Yemen (El-Gindy *et al.,* 2002).

Candida albicans is the most prevalent fungal species of human microbiota. It colonizes asyptomatically the gastrointestinal and genitourinary tracts of healthy individuals. Immunosupressed individuals can be infected with superficial mucosal to hematogenously dissiminated candidiasis (Noble & Johnson, 2015).

Aspergillus fumigatus followed by *A. flavus* are the most encountered human pathogens, allergens and mycotoxigenic fungi (Shahhossein *et al.,* 2011 and Hedayati *et al.,* 2007, respectively).

Rasamsonia byssochlamydoides a thermophilic fungus, newly reported of piles of tea fermentation (Zhang *et al.*, 2016). The fungus has human pathogenesis potentialities.

Exserohilum rostratum was isolated only from animal manure samples (5 isolates and 6.57% frequency). This fungus is a cross- kingdom jump pathogen of plants and humans. The fungus was newly reported from humans: keratomycosis (Joseph *et al.*, 2012), fungal meningitis (Casadevall and Pirofski, 2013) and invasive rhinusinsitis (Gupta *et al.*, 2014).

Trichophyton terrestre was the only true dermatophyte encountered from our samples. It was reported from mycotic granuloma (Frey *et al.*, 1974). *Phoma glomerata* was also isolated from our samples. Several cases of human diseases have been observed: mycotic granuloma, otomycosis, rhinitis and allergenic effects (Boerema *et al.*, 1965). A rare case of fungal keratitis was due to *P. glomerata* was also reported (Mc Elnea *et al.*, 2015).

Conclusion

The presence of keratinophilic and thermophilic fungi in the vicinity of Ayatt draws immediate attention. The pathogenic potentials of these fungi have a high risk for humans and animals. Certain isolates could become useful in managing the environment. Our study will continue for exploitation of some fungi encountered in our soil samples to produce a thermostable keratinase enzyme.

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الفطريات المحللة للكراتين و المحبة للحرارة المعزولة من عينات المخلفات الحيوانية و غبار

أحمد عوض الجندى ، أميمة محمد المهدى ، زينب محمد ابراهيم ، هدى عزيز.
المنزل من انحاء مدينة العياط ، الجيزة ، مصر.
قسم العلوم البيولوجية و الجيولوجية – كلية التربية جامعة عين شمس –القاهرة ، مصر.

تم تجميع سبعة و اربعون عينة من المخلفات الحيوانية و ثمانية و ثلاثون عينة من غبار المنزل عشوائيا من انحاء مدينة العياط خلال شهرى سبتمبر و نوفمبر ٢٠١٣ . و تم عزل الفطريات المحللة للكراتين و المحبة للحرارة .وتم تعريف ستة و عشرون نوع ينتمى الى سبعة عشر جنس من الفطريات المحللة للكراتين و المحبة للحرارة على التوالى . و من الست و سبعون عينة من المخلفات الحيوانية كان *Thamnostylum piriforme* هو الاكثر تكرارا (10.52%) و يليه *Aspergillus brasiliensis, Chrysosporium keratinophilum*(7.89%) و تم عزل ثمانية و سبعون عينة من غبار المنزل . و كان الاكثر تكراراهو *Arachniotus dankaliensis*(10.25%) ويليها *Candida albicans*(7.69%) . و تم عزل ستة و سبعون عزلة من الفطريات المحبة للحرارة من المخلفات الحيوانية و كان *Aspergillus fumigatus* هو الاكثر تكرارا بنسبة (29.21%) و يلية *Aspergillus flavus* var. *columnaris*(7.89%) . و تم تعريف خمسة و سبعون عزلة من غبار المنزل . مرة اخرى كان *Aspergillus fumigatus*بنسبة .(9.33%) ويليهم تكرارالاكثر هو *Aspergillus brasiliensis , A. carneus, A.flavus* var. *columnaris, Rasamsonia byssochlamydoides, Chrysosporium zonatum*(6.66%) و تم مناقشة الاهمية الطبية لبعض هذة الفطريات.