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Characterization and Isolation of Fungi from Domestic Pigeon Droppings in the Governorate of Erbil and its Suburban Area

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Abstract

A total of 200 samples of domestic bird droppings were collected from 54 regions in Erbil Governorate and its suburbs, and 23 of the isolates were fungi obtained by using the Niger bird seed medium and Sabouraud dextrose agar medium. The fungal genera were identified through macroscopic, microscopic, and phenotypic properties on agar plates and slides by staining with Lactophenol cotton blue stain. Findings belong to nine genera (*Aspergillus*, *Alternaria*, *Chrysosporium*, *Cunninghamella*, *Helminthosporium*, *Mucor*, *Penicillium*, *Rhizomucor*, and *Rhizopus*). Among the isolates, six species belonging to *Aspergillus* were identified which also had the highest frequency (44.4 %) in total genera, while the lowest frequency was (0.1 %) for *Alternaria* sp., *Chrysosporium* sp., *Cunninghamella* sp. Results reveal that 17 sites' samples of bird droppings produced negative results when tested on Niger bird seed agar, while only two locations produced no results when tested on Sabouraud dextrose agar. Thus, Sabouraud dextrose agar (SDA) is thought to be better than Niger bird seed agar (NBSA) for isolating fungi since it is considered a generally rich media for them.

Keywords: Fungi, Pigeon droppings, *Aspergillus* sp.

INTRODUCTION

Mycotic infections are the most common in all types of birds. However, they are less dominating when compared to bacterial and viral illnesses. Aspergillosis, Candidiasis, Dactylariosis, Cryptococcosis, Favus, Rhodotorulosis, Torulopsis, Mucormycosis, Histoplasmosis, and Cryptococcosis are among the fungal pathogens. (Dhama et.al. 2013). As environmental indicators, pigeons and free-flying wild birds (Doves) (Streptopelia) play a critical role in public health. Several fungal species are commonly detected in bird feces, especially yeasts of the genera Cryptococcus Vuill, Candida Berkh, Trichosporon Behrend, and Rhodotorula, in addition to filamentous fungi belonging to the genera Aspergillus Michelli, and Penicillium spp (Elhariri, Hamza et al. 2015 ; Mendes et al., 2014; Santos et al., 2009; Fraga et al., 2011).

Approximately 50% of all birds are regarded to be reservoirs and carriers of fungi that could be dangerous to both birds and humans (Dynowska et al. 2015). Grisin et al. (2017) concluded that birds and their droppings can carry over 60 diseases, many of which are airborne and can be transmitted to humans merely by being close to them.

This is because dried bird droppings are generally very fertile ground for fungal species growth, due to high concentrations of nitrogenous bases and because as the droppings age, they contain higher concentrations of fungi than when recently eliminated (Silva & Paula,1963; Mendes et al. 2014; Elhariri et al. 2015).

Fungal species connected with bird nests have been studied in both terrestrial and wetland bird nests; nevertheless, data on this topic is still limited. The body temperature of birds (about 39-43^{o}C) and the unique conditions that exist in nests make them an ideal setting for fungus development. Many suitable substrates encourage microbial growth such as plant materials used to build nests, bird feces, animal remains, and keratin-rich substrates (feathers or hair). The nest position also affects the diversity of fungal species inside the pigeon nest, which affects the source of the nutrients (Hubálek et.al. 1976; Kornilłowicz-Kowalska et.al. 2011). Recent research has focused on teratophiliac fungi (fungi that obstruct wetland bird nests due to high amounts of keratin and favorable climatic conditions, such as greater humidity). (Kornilłowicz-Kowalska et.al.2011& 2013).

Our research aims to find out what types of molds inhabit pigeon droppings in Erbil city and its suburbs.

MATERIALS AND METHODS

Sample Collection

Two hundred samples of pigeon droppings were collected from 54 different regions in Erbil city and its suburbs from November 2021 to October 2022. Samples have been collected and transferred in sterilized and sealed containers into the advanced mycology laboratory at Science College/ Salahaddin University.

Isolation of Fungi

A biosafety cabinet was used for homogenizing and processing all samples. 20 grams of Pigeon (*Columbia Livia)* dropping samples were suspended in sterilized phosphate-buffered saline (PBS) at a 1:5 ratio by vortexing for 5 minutes and centrifuged at 500^{X} g for 5 min. 100 µL from the supernatant from each tube was inoculated onto Niger Bird Seed Agar **(**NBSA**)** in a Petri dish and onto Sabouraud dextrose agar (SDA) plates containing 1.0 g creatinine and 40 mL chloramphenicol (50 µg/mL). The Petri dishes were incubated in the dark at 25 °C in humid conditions for 3-10 days (Chae et. al. 2012)

Identification methods

In general, the identification of filamentous fungi was based on classic taxonomy (macro and microscopic characteristics). The surface and the reverse of the colonies were observed, as well as diameter, conidial color, texture, and presence of soluble pigments (Tell, 2005; Dugan, 2006; Balajee, et.al. 2007).

RESULTS

In Erbil city and its surroundings, 200 samples of regional pigeon excretion were gathered from 54 different areas, and 23 of the isolates were fungi obtained by using the Niger bird seed medium. The fungal genera were identified through macroscopic and microscopic properties on agar plates and slides, which belongs to 8 genera (*Aspergillus, Alternaria, Chrysosporium, Helminthosporium, Mucor, Penicillium, Rhizomucor and Rhizopus*). Among the isolates, four species belong to the genus *Aspergillus,* while for *Alternaria, Chryspsprium, Helminthospori-*

um, and *Rhizomucor* only the genus has been identified with one isolate for each, as shown in table-1. *Aspergillus niger* and *Mucor* had the highest prevalence of the isolates (8.7%) in Bnaslawa. Other species (*Alternaria, Chrysosporium,* frequency (4.3 %) in most of the areas, as shown in the Table 1.

Table (1). Mold isolated from pigeon droppings on Niger bird seed agar medium.

No.	Sample source	Mold	No. of colonies	%Frequency
1.	Bahare nwey	0	0	0
2.	Bahare kon	0	0	0
-+3.	Barzan	0	0	0
4.	Berkote nwey	0	0	0
5.	Bnaslawa	*Aspergillus niger*	2	8.7
		A.ochraceous	1	4.3
		Mucor sp.	2	8.7
6.	Chnar	*A.fumigatus*	1	4.3
		A.ochraceous	1	4.3
		Rhizopus sp.	1	4.3
7.	Chwarchra	*A.candidus*	1	4.3
		Penicillium sp.	1	4.3
8.	Daratu	0	0	0
9.	Darwazae shar	0	0	0
10.	Framanbaran	0	0	0
11.	Galawezh	0	0	0
12.	Havalan	*A.niger*	1	4.3
		Mucor sp.	1	4.3
13.	Kany gany	0	0	0
14.	Kasnazan	*A.niger*	1	4.3
		Rhizomucor sp.	1	4.3
15.	Mahala arab	*Helminthosporium sp.*	1	4.3
16.	Makhmur	*Penicillium sp.*	1	4.3
17.	Mala omer	0	0	0
18.	Mamostayan	A.ochraceous	1	4.3
		Penicillium sp.	1	4.3
		Rhizopus sp.	1	4.3
19.	Masif salahaddin	0	0	0
20.	Mufti	0	0	0
21.	Nawroz	0	0	0
22.	Science college	0	0	0
23.	Setaqan	0	0	0
24.	Shawes	0	0	0
25.	Shurtawa	*A.niger*	1	4.3
26.	Tayrawa	*A.niger*	1	4.3
27.	Xanaqa	*Alternaria sp.*	1	4.3
28.	Zanko	*Chrysosporium sp.*	1	4.3
29.	Zanko village	0	0	0
Total		23		

The findings in Table-1 and Table-2 reveal that 17 sites' samples of bird droppings produced negative results when tested on Niger bird seed agar. While only 2 locations produced no results when tested on Sabouraud dextrose agar. Thus, Sabouraud dextrose agar is thought to be better than NBSA for isolating fungi.

It is worth mentioning that no results were obtained in 17 regions from NBSA media. In contrast a total of 76 isolates were gained from Sabouraud dextrose agar, as mentioned in Table-2.

They belong to the genera (*Aspergillus, Cunninghamella sp., Fusarium, Mucor, Penicillium, Rhizomucor,* and *Trichoderma*) among them, the highest frequency was for unidentified *Penicillium* sp. (9.2%) in Nawroz and (5.2%) in Bahare kon respectively. While (3.9%) was the frequency obtained for the genera (*Aspergillus niger, Aspergillus fumigatus, A.ochraeous, A.terrus, A.flavus, Cunninghamella* sp., *Mucor* sp., *Rhizomucor* sp., and *Penicillium* sp.) in most of the regions. It merits mentioning that only two areas gave negative results, and no fungi have been identified, which were Chwarchra and Mamostayan.

In the isolating fungi on NBSA and SDA, eleven genera have been detected. *Aspergillus* had a superior number of (6 species) and the highest frequency (44.4%), followed by *Penicillium* (2.6%) and *Mucor* sp. (1.6%). While the *Fusarium, Rhizomucor Rhizopus,* and *Trichoderma* were recorded on (0.2 %) of the isolates. However, among isolates, *Cunninghamella, Chrysosporium, Alternaria,* and *Helminthosporium* had the lowest prevalence (0.1%), as shown in Table-3 and Fig. 1.

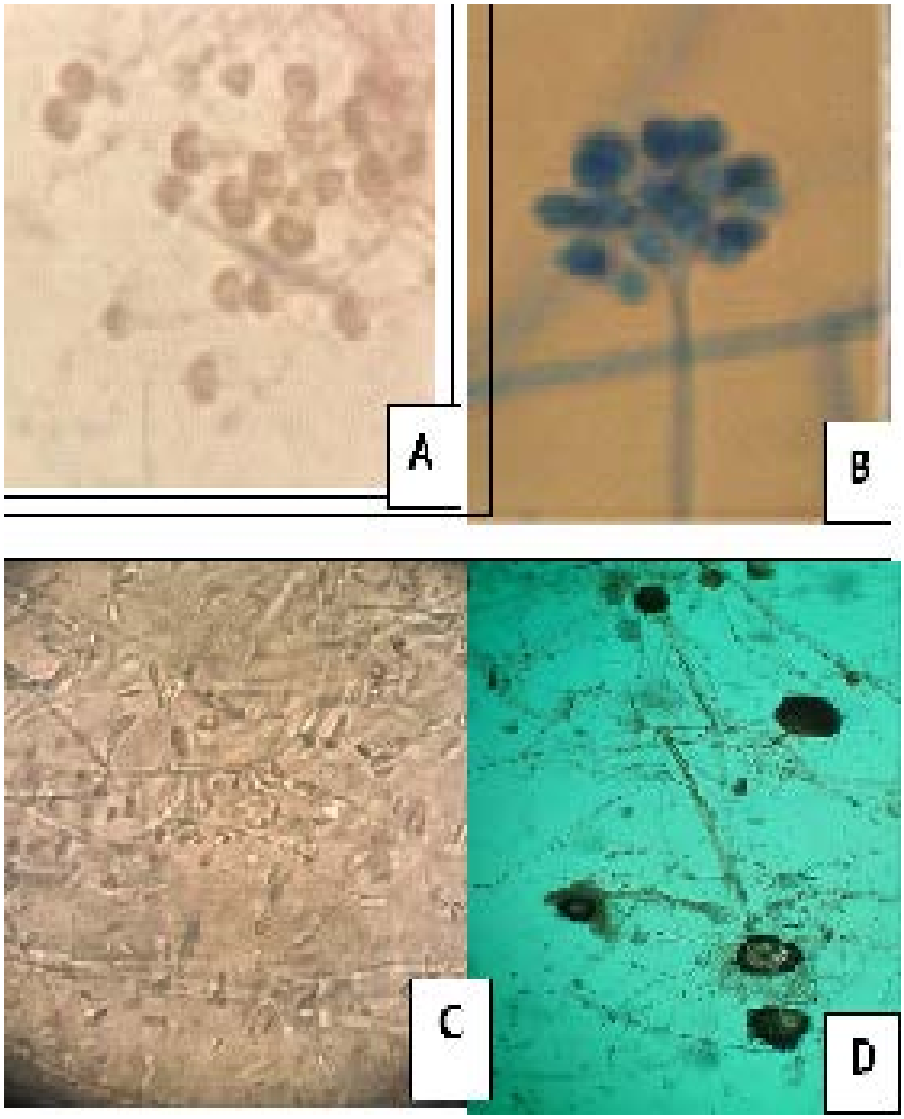

Figure (1). Shows the most composition of fungi isolated from droppings of domestic birds. a- *Aspergillus* b- *Cunninghamella,* c- *Fusarium,* d- *Rhizopus.*

Table (2): Molds isolated from pigeon droppings on Sabouraud Dextrose agar medium.

No.	Sample source	Mold	No. of colonies	% Frequency
1.	Bahare nwey	*Aniger*	1	1.3
		A.fumigatus	1	1.3
		Penicillium sp.	2	2.6
2.	Bahare kon	*A.niger*	1	1.3
		Penicillium sp.	4	5.2
3.	Barzan	*A.niger*	1	1.3
		Mucor sp.	1	1.3
		Penicillium sp.	1	1.3
4.	Berkote nwey	*A.niger*	3	3.9
5.	Bnaslawa	*A.niger*	2	2.6
		A.ochraceous	1	1.3
		Mucor sp.	3	3.9
		Penicillium sp.	1	1.3
6.	Chnar	*A.flavus*	1	1.3
7.	Chwar chra	*0*	0	0
8.	Daratu	*A.niger*	1	1.3
9.	Darwazae shar	*Penicillium sp.*	1	1.3
10.	Framanbaran	*Mucor sp.*	3	3.9
		Trichoderma sp.	2	2.6
11.	Galawezh	*A.niger*	2	2.6
		A.terrus	1	1.3
		Cannicamella sp.	1	1.3
		Fusarium sp.	2	2.6
		Rhizopus sp.	1	1.3
12.	Havalan	*A.flavus*	1	1.3
		Mucor sp.	1	1.3
13.	Kany gany	*A.niger*	1	1.3
14.	Kasnazan	*Rhizomucor sp.*	1	1.3
15.	Mahala arab	*Mucor sp.*	1	1.3
		Penicillium sp.	1	1.3
16.	Makhmur	*Penicillium sp.*	1	1.3
17.	Mala omer	*A.niger*	1	1.3
		Mucor sp.	1	1.3
		Penicillium sp.	3	3.9
18.	Mamostayan	*0*	0	0
19.	Masif salahaddin	*Aniger*	1	1.3
		Penicillium sp.	1	1.3
20.	Mufti	*A.niger*	1	1.3
		Mucor sp.	1	1.3
21.	Nawroz	*A.niger*	2	2.6
		Penicillium sp.	7	9.2
22.	Science college	*A.niger*	1	1.3
23.	Setaqan	*A.niger*	1	1.3
24.	Shawes	*A.niger*	3	3.9
		Mucor sp.	2	2.6
		Penicillium sp.	1	1.3
25.	Shurtawa	*Aniger*	1	1.3
		A.flavus	1	1.3
26.	Tayrawa	*A.niger*	1	1.3
27.	Xanaqa	*A.niger*	1	1.3
28.	Zanko	*A.niger*	1	1.3
29.	Zanko village	*A.ochraceous*	1	1.3
Total		76		

Table:(3). Total mold colonies isolated from the pigeon droppings in Erbil and its suburbs.

Fungi	Media		Total	% Frequency	Total	% Frequency
	NBSA	SDA				
	Total colony number					
Aspergillus candidus	1	0	1	1.0		
A.flavus	0	3	3	3.0		
A.fumigatus	1	1	2	2.0	44	44.4
A.niger	6	26	32	32.3		
A.ochraceous	3	2	5	5.0		
A.terrus	0	1	1	1.0		
Alternaria sp.	1	0	1	1.0	1	0.1
Cunninghamella, sp.	0	1	1	1.0	1	0.1
Chyrysosporium sp.	1	0	1	1.0	1	0.1
Fusarium sp.	0	2	2	2.0	2	0.2
Helminthosporium sp.	1	0	1	1.0	1	0.1
Mucor sp.	3	13	16	16.0	16	1.6
Pencillium sp.	3	23	26	26.0	26	2.6
Rhizomucor sp.	1	1	2	2.0	2	0.2
Rhizopus sp.	2	0	2	2.0	2	0.2
Trichderma sp.	0	2	2	2.0	2	0.2
Total	23	76	99	-	-	-

DISCUSSION

Table:(3). Total mold colonies isolated from the pigeons' dropping in Erbil and its suburbs.

This study isolated and characterized several fungi groups known to pose significant opportunistic risks in their presence within pigeon feces and contamination of the human environment and should, therefore, raise public health concerns, especially for older adults and the immunosuppressed. The growth of bacteria and fungi is highly grown in a nitrogen-rich environment that has been contaminated with birds' droppings (John et.al. 2001). The chemical characteristics and composition of pigeon feces (pH, uric acid, and nitrogen) provide a good substrate for fungal spore propagation. Pathogenic fungus abundance has been connected to weather (humidity, temperature, and radiation), vegetation, and bacteria associated with guano (Lee et.al.2017).

Humans have been known to suffer considerable morbidity and mortality from *Aspergillus* species. They are linked to several clinical manifestations, including disseminated infections, respiratory infections, subcutaneous infections, rhino-cerebral infections, skin and nail infections, ear infections, and keratitis (Rodrigues et.al. 2014).

In the current study, the genus *Aspergillus* was the most prevalent among the isolates, with a frequency of (44%) followed by *Penicillium* sp. with a frequency of (26%) These findings are in accordance with (Maryam, *et.al*.2013), who proved that pigeon droppings are associated with different pathogenic fungal species, including *Penicillium* spp. (n=30), *Apergillus* spp. (n=25), *Mucor* spp. (n=18), *Rhizopus* spp. (n=14), *Paecilomyces* spp. (n=11), *Fusarium* spp. (n=4), and *Cladosporium* spp. (n=2). Similarly, (Hashemi et. al. 2014) reported the presence of these opportunistic fungi such as *Aspergillus, Alternaria, Rhizopus, Mucor,* and yeast-like fungi in various domestic birds presented to veterinary clinics in Tehran. However, our findings partially agree with Abbas et al. (2017), who indicated that *Penicillium* (19%) achieved the highest frequency in the droppings of pigeons. It was followed by *Mucor* (9%), *Rhizopus* (7%), *A. niger* (6%), *A. fumigatus* (5%), *A. flavus* (4%), *Cladosporium* (3 %), and *Alternaria* (2%).

Abulreesh et al. 2015, reported that Mucorales were represented by 8 species related to 6 genera, from which R. stolonifer, *S. racemosum,* and *Mucor hiemalis* were the most common and appeared in 16.1%, 11.6%, and 10.7% of the examined samples, respectively. According to the diversity of genera, the genus Aspergillus ranked second to Mucorales isolated from pigeon fecal samples and was represented by five species, namely, *A. flavus*, *Aspergillus niger* (*A. niger*), *Aspergillus parasiticus*, *Aspergillus tamarii,* and *Aspergillus terreus*. So our findings are disagreeing with it. Some of the unfavorable outcomes in our study could be attributed to hatchlings defecating in the nest, which raises the salt and alkalinity of the nest lining, promoting alkalitolerant fungus species. (Korniłłowicz-Kowalska et. al. 2018) The adoption of culture-based methods for isolating fungi may have also resulted in a restricted spectrum of fungal species. Traditional methods are also more widespread and less expensive than high-throughput sequencing procedures. However, this approach has several disadvantages, including the impossibility of detecting unculturable elements (Pasanen,2001; Rastogi 2011). Also, the effectiveness of culture-based analysis of fungal species is affected by factors such as incubation temperature and culture medium type. (Meletiadis et. al. 2001; Marshall & Poulson-Cook, 1998).

On the other hand, the isolation of both yeasts and molds (i.e., fungi) from clinical and environmental samples can be complicated by various factors. Fungal growth in clinical or environmental samples may often be inhibited due to increased growth rates of bacteria as well as bacterial production of deleterious metabolites. These factors can fail to detect fungi in mixed cultures (Hockey et al., 1982). Additionally, fungi frequently have various and particular dietary requirements, which might limit the range and intensity of an organism's growth in the absence of a certain nutrient (Gao & Liu, 2010).

It should be noted that the addition of antibiotics such as chloramphenicol to growth media may also have a negative impact on the growth of certain fungal species (Touimi-Benjelloun et al., 1976). Thus, it can be concluded that there is a potential role of pigeons, as well as other birds, in the propagation of zoonotic yeasts in the environment affecting humans and other animals, which needs to be further investigated.

CONCLUSION

We concluded that pigeon droppings may be a source of many health problems since they contain different types of molds, which may cause clinical manifestations in humans and other animals.

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ETHICS

Animal Research Ethics Committee (AREC): Reference No.: 4S/482 on 20/9/2021.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: Neveen N. Rajab has collected the samples, performed the data analysis, and written up the paper. Nadeem A. Ramadan supervised the work, reviewed the paper,

and participated in writing the paper.

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