RESEARCH ARTICLE
Eurasian J Bio Chem Sci, 6(1):26-30, 2023
https://doi.org/10.46239/ejbcs.1276554

EJBCS

Eurasian Journal of Biological and Chemical Sciences

Journal homepage: www.dergipark.org.tr/ejbcs

Morphological and molecular identification of fungi isolated from various habitat in Kirkuk city – Iraq

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Received : 04/04/2023
Accepted : 11/05/2023

Abstract: The aim of this study is isolate and identify fungi from different habitat in Kirkuk City - Iraq. The fungal species were isolated from soil and water in four season 2021-2022, collected the samples from various geographical habitat in Kirkuk City. The fungi isolation from soil and water done by inoculating (1ml) from serial dilutions on Potato Dextrose Agar (PDA) plates. The molecular identification of the isolated fungi at the species level, by PCR using specific internal transcribed spacer primer (ITS1/ITS4). The PCR products were sequenced and compared with the other related sequences in GenBank (NCBI). Seven fungal species were identified. The results showed that the (*Aspergillus flavus* 20.83%) was the most abundant fungus, while the (*Penicillium citrinum* 8.30%) was the less prevalent one in all resources and locations. The seven local fungal isolates were registered within NCBI, and this is the first record of these isolates in Iraq

Keywords: *Aspergillus flavus, Penicillium citrinum,* Kirkuk

1. Introduction

Fungi are eukaryotic organisms is surrounded by a bilayer nuclear membrane and cytoplasm containing the endoplasmic reticulum, mitochondria, the Golgi apparatus, and other cytoplasmic organelles. Fungi lack plastids, and this is what distinguishes them from plant cells, so they are not self-feeding, but depend for their food on external sources, either decomposing organic materials or other organisms such as plants and animals, and on this basis, they are parasitic, Saprophytic or symbiotic (Gravesen *et al*., 1994).

Fungi are one of the most widespread living organisms in nature, as the number of diagnosed species reached (100,000) species. It exists in humans, animals and plants Its can spread in the soil, air and waters, some factors affect the growth and distribution of fungi as temperature, pH, moisture, amount and type of nutrients (Pellon *et al*,. 2020). Fungi are importance in nature through their relationship to human life and work on recycle elements and break down organic matter (Webster & Weber, 2007). Pathogenic fungi can cause harm to humans, animals and plants (Wang *et al*., 2014).

Fungi are used to treatment different types of wastewater becauseit its ability to analyzes many organic pollutants by its enzymes that secrete it, such as estrase and cellulase an enzyme and return it to its original components therefore it has the ability to maintain ecological balance, especially in aquatic environments, freshwater (Bermingham *et al*., 1996). The aimed of study to find out the geographical distribution of the fungi isolated from the soil and water from Different habitat in Kirkuk City - Iraq.

2. Materials and Method

2.1. Describing The Study Area

The study area is located within the borders of Kirkuk City and is 255 km away from the capital, Baghdad (Kamel, 2013). As shown in the figure (1) the distance between the first site and the second site was 5.2 km, while the distance between the second and third site was 8.1 km, meaning that the distance between the first and third site is 13.3 km. As for the distance between the fourth site and the fifth site, it

was 1.6 km, and between the fifth and sixth sites was 0.8 km, meaning that the distance between the fourth and sixth sites is 2.4 km. The first, second and third sites are located on Khasa River channels while the sites fourth, fifth and sixth sites are located on North Oil Company channel.

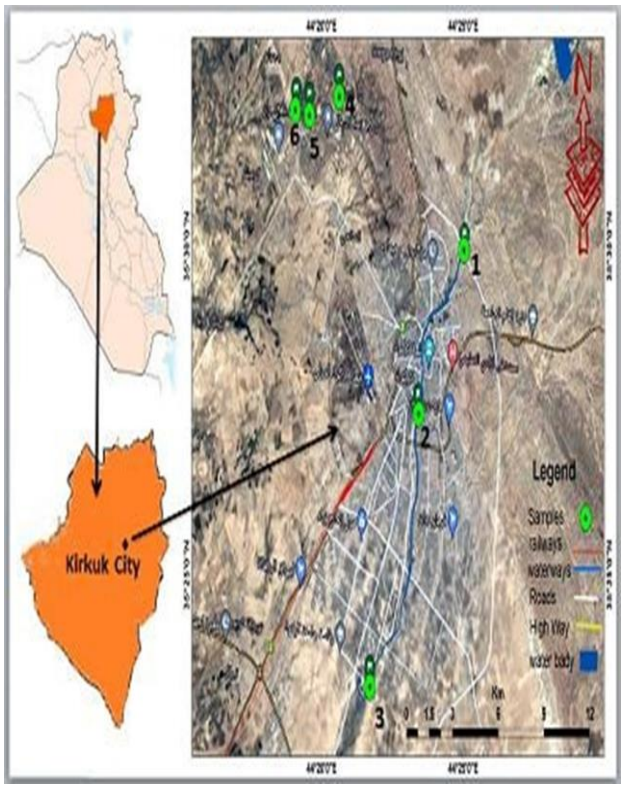


Fig. 1 Shows the locations of sampling collection

2.2. Samples Collected

The soil and water samples were collected from the North Oil Company and Khasa River channels in Kirkuk City - Iraq (September 2021 to September 2022). The samples of soil collected about 100g with sterilized bags from top soil layer (0-30 cm in depteh) after that the samples of soil separated and labeled according to their location (Gaddeyya *et al*., 2012). The samples of water collected by 5 liter polyethylene bottles and washed with 10% dilute hydrochloric acid and then rinsed with distilled water (Nollet, 2007).

2.3. Isolation and Examination of Fungi

The method of dilution was used to isolate the fungi from the soil and water samples by taken 1 gm of samples (soil or water) added to 100 ml sterile water, then the mixture was shaken for one minute and left to settle for a period of ten minutes. After preliminary experiments, the third dilution was chosen, 1ml of each dilution was with on Potato Dextrose Agar (PDA) supplemented with chloramphenicol move the plate to increase the spread of the sample. The plates were incubated at 28˚C in the dark for 5-7 days (Reddy *et al*., 2014). After that pure colonies of fungal observed and maintained for examination (Jasuja *et al.*, 2013).

Fungal morphology was studied microscopically by observing colony traits (colour, shape, size, and hyphae), and microscopically by compound microscope using a lactophenol blue-stained slide fixed with a small part of the mycelium (Cappccino & Sherman, 1996).

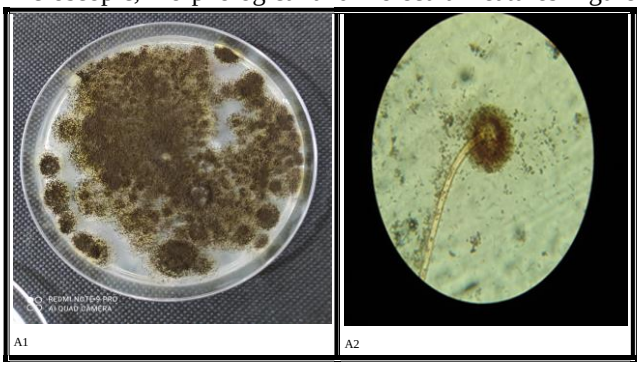
2.4. DNA Extraction and PCR Amplification

Genomic DNA was isolated from Fungal growth according to the protocol of ABIOpure Extraction (ABIOpureTM Total DNA). The DNA purity was measured using a nano-spectrometer. For molecular identification of fungi species used the universal primers (ITS1 and ITS4). The sequences of primers were:

Primer Name	Seq.	Annealing temp. (ºC)	Product size (bp)
ITS1	5`-TCCGTAGGTGAACCTGCGG-3`	55	≈600
ITS4	5`-TCCTCCGCTTATTGATATGC-3`		

The PCR products were resolved by horizontal electrophoresis in a 1% agarose gel using UV light after treatment with dye (safe red) and with a digital camera and the PCR products were sequenced and analyzed by comparison with all available sequences in the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) using the Basic Alignment Sequence Tool (BLAST): (http://blast.ncbi.nlm.nih.gov/Blast.cgi), (Javadi *et al*., 2012).

3. Results: The isolated and identified seven fungal species in this study were checked on the basis of cultural, microscopic, morphological and molecular features Figure (2 – 8).



The *Aspergillus niger* shows as ablack color in colony (Figure 2- A1) and the microscopic arrangement of conidia (Figure 2- A2).

Fig. 2 A1 *Aspergillums niger* colony and A2 conidia

The *Aspergillus terreus* shows as abrown color in colony (Figure 3- B1) and the microscopic arrangement of conidia (Figure 3- B2).

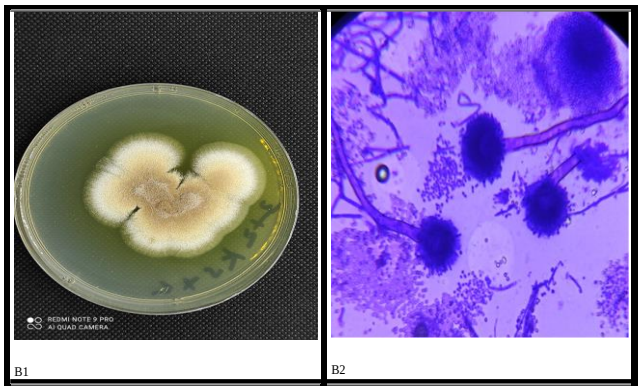


Fig. 3 B1 *Aspergillus terreus* colony and B2 conidia

The *Aspergillus flvus* shows as green color colony (Figure 4- C1) and the microscopic arrangement of conidia (Figure 4- C2).

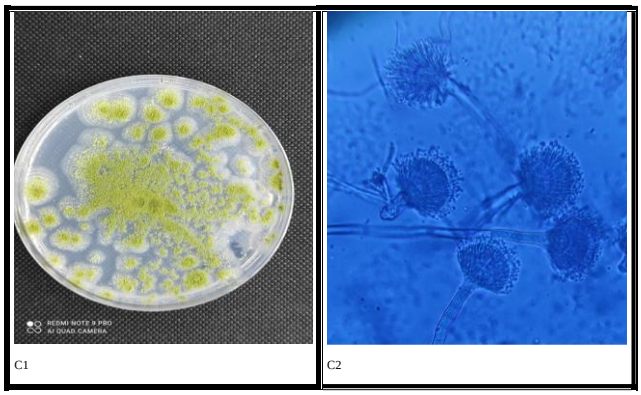


Fig. 4 C1 *Aspergillus flvus* colony and C2 conidia

The *Alternaria alternate* shows greenish-black surface colony (Figure 5- D1) and the microscopic observed macroconidia (Figure 5- D2).

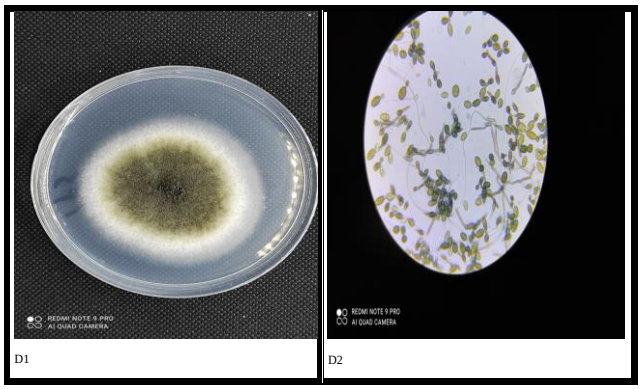


Fig. 5 D1 *Alternaria alternate* colony and D2 macroconidia

The *Penicillium citrinum* shows as a bluish-green colony (Figure 6-E1), and the brush arrangement of phialospores (Figure 6-E2).

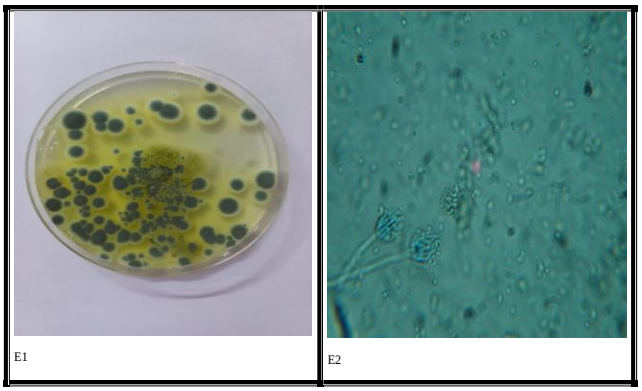


Fig. 6 E1 *Penicillium citrinum* colony and E2 phialospores

The revealed morphological features of *Trichoderma asperellum* are shown in (Figure 7- F1) include the mycelia were white and dark green, and arranged in concentric rings

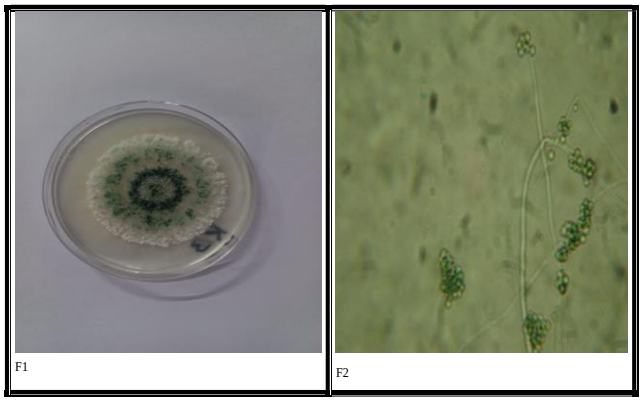


Fig. 7 F1*Trichoderma asperellum* and F2 conidi-ogenous

and small green or white conidiophores of cells called conidi-ogenous located at the ends of the many branches of conidiophores (Figure 7- F2).

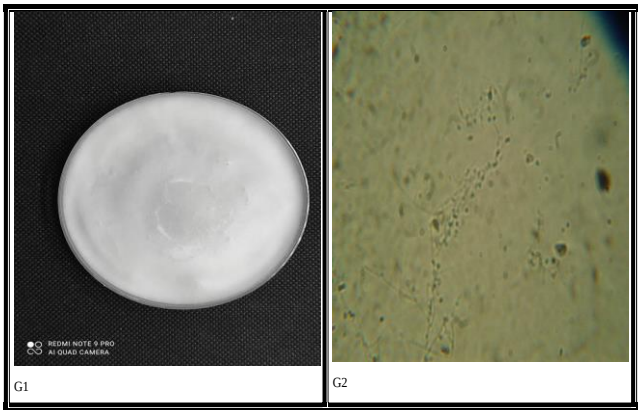


Fig. 8 G1 *Lecanicillium aphanocladii* and G2 aphanophilides

The *Lecanicillium aphanocladii* are distinguished by their white color and high on the surface of the mide PDA (Figure 8- G1) and Distinctive microscopic features such as aphanophilides that are arranged singly, in pairs, or in a group are shown in (Figure 8- G2) .

4. Discussion: In this study, used morphological and molecular (rDNA ITS sequences comparison and analysis) examination methods to isolated and identified the 7 species of fungi

from soil and water in Kirkuk City, Iraq. It is (*Aspergillums niger, Aspergillus terreus, Aspergillus flvus, Alternaria alternate, Penicillium citrinum, Trichoderma asperellum and Lecanicillium aphanocladii*). All of them identified species were isolated from the soil and water except (*A. terreus*) were isolated more from the soil and this match with soil is the basic source of the fungi (Chandrashekar *et al*., 2014).

The distribution and growth of fungi affect with differenet factors as soil pH, moisture, salinity, temperature and organic matter and that lead to variation in ratio of growth fungi (Sharma & Raju, 2013).

For the identification of fungi to the genus level, can be dependence on morphological examination (Wang *et al*., 2016). But for a more accurate identification we need to a molecular examination (Lutzoni *et al*., 2004), that carried out by DNA barcoding using the ITS region sequencing (ITS1 – ITS4). The DNA sequences were compared to those in the databases using NCBI-BLAST. The seven local fungal isolates were registered at the National Center for Information Technology (NCBI) under No. OP268345.1 for *Aspergillues niger*, No. OP268344.1 for *Aspergillus flavus*, No. OP268334.1 for *Aspergillus terreus*, No. OP268285.1 for *Alternaria alternata*, No. OP268332.1 for *Trichoderma asperellum* No. ON908684.1 for *Penicillium citrinum* and the No. OP020444.1 for *Lecanicillium aphanocladii*, all the fungal first recorded of these isolates in Iraq.

Table 1 The Percentage of Fungal Species Isolates at Study Sites.

Scientific name of fungal isolate	Collection sites - Kirkuk City				The sum of fungal isolates	fungal isolate %
	The North Oil Company Channel		Khasa River Channel			
	soil	water	soil	water		
Aspergillus flavus	8	7	8	7	30	20.83
Aspergillus niger	8	5	7	6	26	18.06
Alternaria alternate	7	4	7	4	22	15.30
Trichoderma asperellum	5	4	6	5	20	13.90
Lecanicillium aphanocladii	5	3	6	4	18	12.50
Aspergillus terreus	7	1	6	2	16	11.11
Penicillium citrinum	5	1	4	2	12	8.30
The total sum of the isolates	43	27	43	31	144	100

According to the results of this study, (Table 1) that all species of fungi were present throughout the study period, and this indicates the high ability of these species to adapt to difficult environmental conditions. The results shown that)*Aspergillus flavus* 20.83 (%was the most abundant fungus, while (*Penicillium citrinum* 8.30%) was the less prevalent one in all resources and locations.

5. Conclusion: Fungi were isolated and identified from the Khasa River channel and North Oil Company channel in Kirkuk City showed the presence of different species of fungi in these environments and the presence of Aspergillus spp more than others. The study indicated that the molecular identification of fungi added the ability to accurately identify the species of fungi, and the result of this study was seven local fungal isolates were registered at the National Center for Information Technology (NCBI). So, this study recommends more work in the future to be done in this habitat to isolate and identify fungi.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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