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New Records in Iraq and Arab Nations for some Fungi Isolated from Al-Barakia wastewater treatment plant in Al-Najaf Province

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ABSTRACT

This study was conducted in 2020 in the wastewater treatment plant in Al-Barakia Najaf, where samples were taken in November from the Bioshft unit and the compact unit from the plant to know the efficiency of the plant in treatment. The process of isolation and purification was Microbiology Laboratory_ Ecology and Pollution Department - College of Science - University of Kufa. Its diagnosis was carried out at the Plant Virus Laboratory - College of Agriculture - the University of Karbala, and (19) fungal isolates isolated from wastewater treatment plants were diagnosed. These isolates were analyzed using the PCR technique and determination of the nucleotide sequences of the polymerase chain reaction products that were amplified from those isolates using ITS4 and ITS1 primers. It belonged to the fungus *A. caespitosus*. and isolated *A flavus* (7,8), *T. asperellum* (9-11) *A.tubingensis* (12), *A.terrus(13), A.niger (14,15), A. alternata (16), C.sphaerospermum (17), A.oryzae (18), Acremonium sp* (19), similarity rate of 100% with isolates registered with the NCBI. The results show that all the isolated fungi diagnosed in this study are recorded globally. However, they are not recorded in Iraq, and (5) isolates are not recorded in the Arab nation and Iraq, including A. tubingensis, C.sphaerospermum, A. alternate, and A.oryzae, while the isolate *Acremonium sp*. According to the National Center for Biotechnology Information NCBI, it is registered only in Germany under the number (AJ557731), and the similarity rate is 100% with the isolate diagnosed in this study.

Keywords: wastewater; Molecular Identification; fungi; Polymerase chain reaction (PCR); DNA sequence analysis**.**

INTRODUCTION

Water covers about 71% of the Earth's surface and makes up 65% of our body. We all want cleaning water - to relax, drink, and enjoy. If polluted, this water loses economic and aesthetic value, and it can pose a significant hazard to health and aquatic life. Such as the fish that live in it and the wildlife that depends on it[1]. One of the more serious environmental issues is the chemical pollution of rivers and lakes. Because chemical pollution is carried by water and enters rivers and streams, it causes massive destruction[2,3]. Although some types of water pollution may occur through natural processes, they are primarily the result of human activity. Humans use water every single day in industry and homes. Used water is taken from lakes, rivers, and underground; after use pollution, most of the water returns to the same sites. This use of water is called "wastewater." Water not treated before is released into rivers, leading to severe pollution [1]. Wastewater is a mixture of water-borne effluents disposed of through establishments, dwellings and industrial facilities, as well as groundwater and surface water[4]. This water generally contains a large amount of waste that requires an amount of oxygen and pathogens (microbes), Organic matter, plant growth-stimulating nutrients, minerals, sediments and inorganic chemicals. The water may also contain toxic compounds [5]. Wastewater can be defined as a mixture of water-borne effluents discharged from dwellings, commercial and industrial establishments, and institutions, along with groundwater, rainwater, and surface water. Generally, it contains a large amount of waste that requires oxygen, pathogens or pathogens, organic matter, nutrients for plant growth, chemicals, sediments, inorganic materials, and minerals. It may also include toxic substances. [1]

Wastewater is divided into four types:

1. Household sewage: wastewater emitted from business establishments, households, and similar facilities; Industrial sewage: containing a variety of wastes

2. Leakage or/Outflow: Water leaks into the sewage system via indirect or direct means like leakage connections

3. Porous or cracked walls Storm water that enters the sewage system through rainfall drainage connections

4. Rainwater: runoff resulting from floods as a result of rainfall. For several years, the main objective of wastewater treatment in cities has been to lower the concentration of suspended particles, oxygen-requiring chemicals, dissolved substances, and potentially dangerous bacteria and fungi. However, in recent times, there has been increased emphasis on improving the ways of solid waste disposal from municipal treatment operations. Wastewater is the primary storage location for all microorganisms, including fungi and heavy metals. [6,7]. Wastewater is the primary reservoir for every microorganism, including fungal ones [8,9]. Fungi are eukaryotic microorganisms in a separate kingdom; about 95% of these species are currently unknown and must be discovered—discovery [10,11]. The classification of fungi based on their morphological characteristics may often give accurate results. However, many researchers do not rely on morphological characteristics because it may require sufficient experience in the field of classification, especially since there are groups of fungi that are very similar and require significant effort and time. It is also often an inaccurate method. After all, it is affected by environmental factors that may influence the shape and colors of spores, size and fungal colonies [12,13]. PCR technology is the molecular technique that relies on the selection and amplification of a specific area of the organism's genome, and thus knowledge of the genetic relationships in terms of similarity and the difference between the species of fungi that will help in the phenotypic diagnosis of the study fungi [14,15]. This study aimed to isolate and diagnose fungi isolates using (PCR), determine the sequence of nitrogenous bases, and identify similarities and differences in genetics between the isolates—compared with the same fungi internationally diagnosed recorded in (NCBI).

MATERIALS AND METHODS

Al-Barakia wastewater treatment plant is located in Najaf Governorate to the southeast of the city of Kufa on the Euphrates River (Shatt al-Kufa) at the coordinates (N 32 00 40, E 44 25 20) and as shown in Figure (1): The wastewater treatment plant consists of two projects, where samples were collected from the modern project, which contains two treatment units, Bio_Sheft unit, and compact unit. Water samples were collected from the Al-Barakah sewage treatment plant in the morning of November 2020, and (3 replicates) were taken for the Biosheft unit and the Compact unit. A sample was taken from the al-Kufa River (1 km) before entering the site to carry out laboratory tests, isolate and diagnose fungi from the sewage station, where they are collected using polyethylene containers with a capacity of (5) liters that are washed with distilled water before use. According to (Himedia), PDA was prepared for the isolate of fungi, as (39 g) PDA powder was dissolved in 1 liter of "distilled water" and autoclaved at 121 °C, then 15 ib.ng-1 pressure. The media is then cooled, and an antibacterial, chloramphenicol (250) mg/liter, is added to stop bacterial growth before pouring.

Furthermore, the fungi are isolated and purified. A separate method (1 ml) is taken from water samples for the two sites and placed in Petri dishes, 15 ml of PDA medium is added, and three replicates are taken for each site. The media is left until the PDA hardens and then incubated at 25 -$^{+}$ 28 °C for five days until the microbial colonies grow correctly. The process of purification and diagnosis was carried out, including morphology and microscopic features of the fungi.

Molecular diagnosis of fungi isolates

The DNA extraction technique of fungi isolates, by the method described by the American company Zymo0 Research, was used, with the kit (Cat. No. D6005) provided by the same company.

Polymerase chain reaction (PCR)

To diagnose isolates fungus isolated in this study, a test polymerase chain reaction(PCR) through the use of the Kit (Maxime PCR PreMix (i-Taq), Cat. No.25026), made by the Korean company (iNtRoN). They performed a (PCR) with total volumes of the (20µl), which contained (1µl) of each initiator's front (ITS1:TCCGTAGGTGAACCTGCGGG) and posterior (TCCTCCGCTTATTGATATGC: TS4) (White et al.,1990) as well as (1 µl) of DNA extracted. All of the above components were placed inside a tube provided by the manufacturer, and the volume was 20 µl of nuclease-free water. The subsequent steps have doubled the DNA of the fungal isolates. PCR conditions are: DNA denaturation first for five minutes at 98$^{\circ C}$, followed by the final denaturation process. that consists of 35 cycles during 40 seconds at 94$^{\circ C}$, and the primer annealing process continues during period40$_s$ at 55 $^{\circ C}$, then the initial elongation by the PCR, amplified products during the period (1 minute)at 72$^{\circ C}$. Elongation step at 72$^{\circ C}$. Finally, the PCR ended by one step with the final elongation at 72$^{\circ C}$ [16].

DNA sequence analysis for fungi

This study aims to diagnose the fungal isolates (1-19) and those isolated from the Al-Barakia wastewater treatment plant by PCR. Therefore, the primer (ITS1 and ITS4) was delivered to the Macrogen Company of South Korea to determine The sequence of the nitrogenous bases. All nitrogenous base sequences are analyzed by BLAST to compare the results with the facts provided in NCBI) belonging to the like fungus at (NCBI) Phylogenetic trees analysis is using drawn MEGA X[17].

RESULTS

solation and Identification of Fungi

Table (1) identifies 19 fungal isolates from the Al-Baraka wastewater treatment plant in Najaf. The fungi were identified by molecular diagnosis by PCR technique, and the most fungi were *Aspergillus (13) isolates, Trichoderma (3) isolates, A. alternate (1), Acremonium sp (1), and C. sphaerospermum(*(1) isolate and the similarity and difference ratios were compared with fungi isolates previously registered in NCBI (BLAST) as shown in Table 1

The isolate identified in this study	Nearest relative isolate (Accession No.)	Nucleotide sequence similarity (%)	Origin
Aspergillus caespitosus (1,2,3)	MF319914	100%	India
Aspergillus caespitosus (4,5,6)	MT573201	100%	Saudi Arabia
Aspergillus flavus (7,8)	MT292809	100%	India
Trichoderma asperellum (9,10,11)	LC514692	100%	Indonesia
Aspergillus niger (12,13)	GU951769	100%	China
Aspergillus oryzae (14)	KJ941129	100%	India
A. alternate (15)	MK108918	100%	South Korea
Acremonium sp.(16)	AJ557731	100%	Germany
Aspergillus terrus (17)	KT310990	100%	China
C.sphaerospermum(18)	MN947604	100%	China
Aspergillus tubingensis(19)	MT446135	100%	China

Table 1. Fungi isolated from wastewater treatment plant.

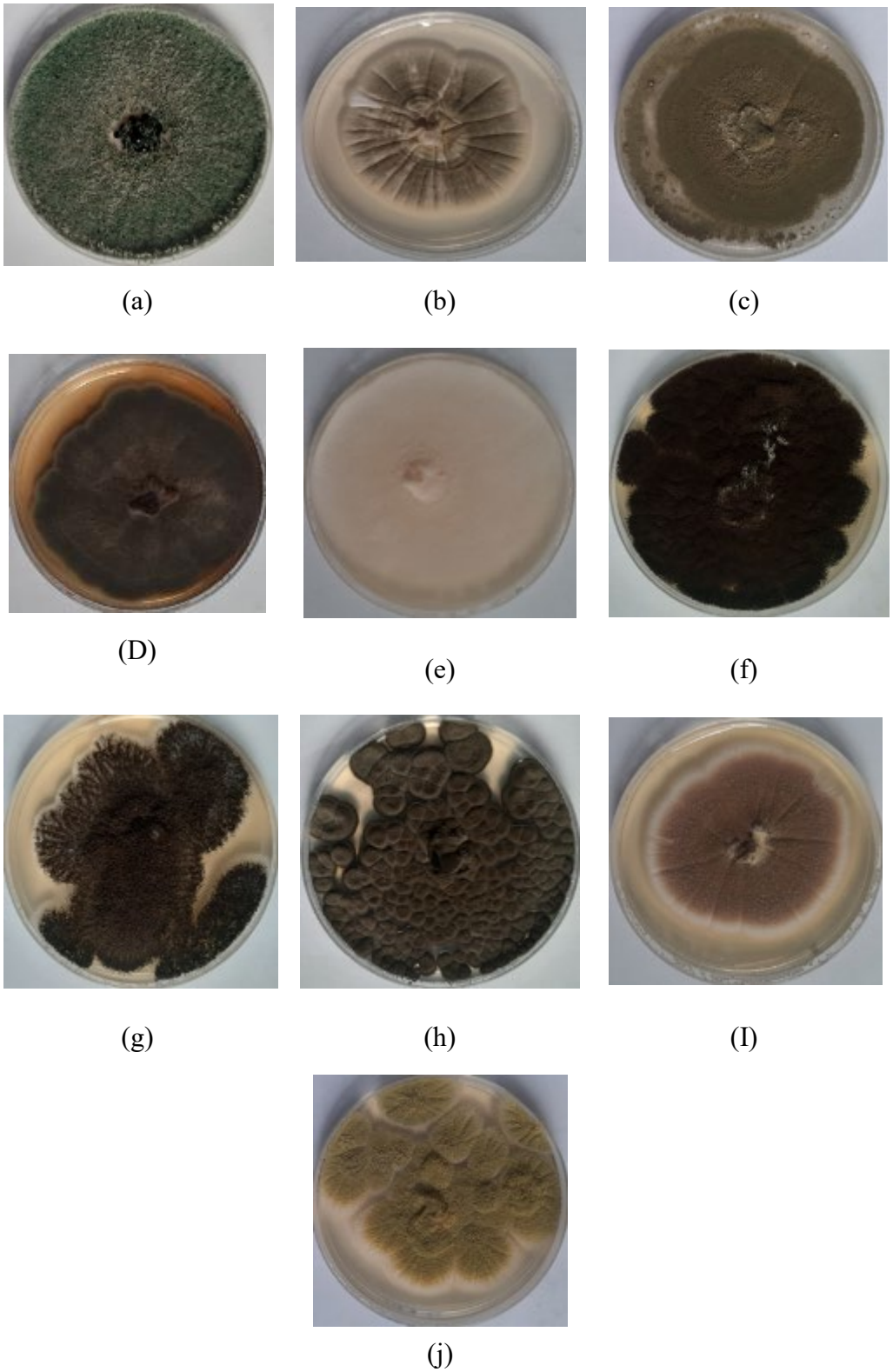

Figure 1. ***(a) Trichoderma asperellum. (b) Aspergillus caespitosus.*****(c)** ***Aspergillus flavus (d) Alternaria alternate.*** **(e)** ***Acremonium sp.*** **(f)** ***Aspergillus niger (*****g)** ***Aspergillus tubingensis*** **(h)** ***Cladosporium sphaerospermum*** **(I)** ***Aspergillus terrus*** **(J)** ***Aspergillus oryzae.***

Molecular Diagnostics of Fungi by Using PCR Technology

The results of DNA extraction from fungal isolates (1-19) and PCR exposure revealed (Figure 3.2) the capability of multiplying PCR-amplified product with sizes ranging from (620 bp) and using the pair of primers (ITS1) and (ITS2) (ITS4). M = DNA ladder marker (100bp DNA ladder marker with volume each fastened to the number of nitrogen base pairs (bp) on the Figure's left side). N.C. refers to unfavorable Comparison (a mixture containing PCR components without DNA addition). as shown in Figure 2.

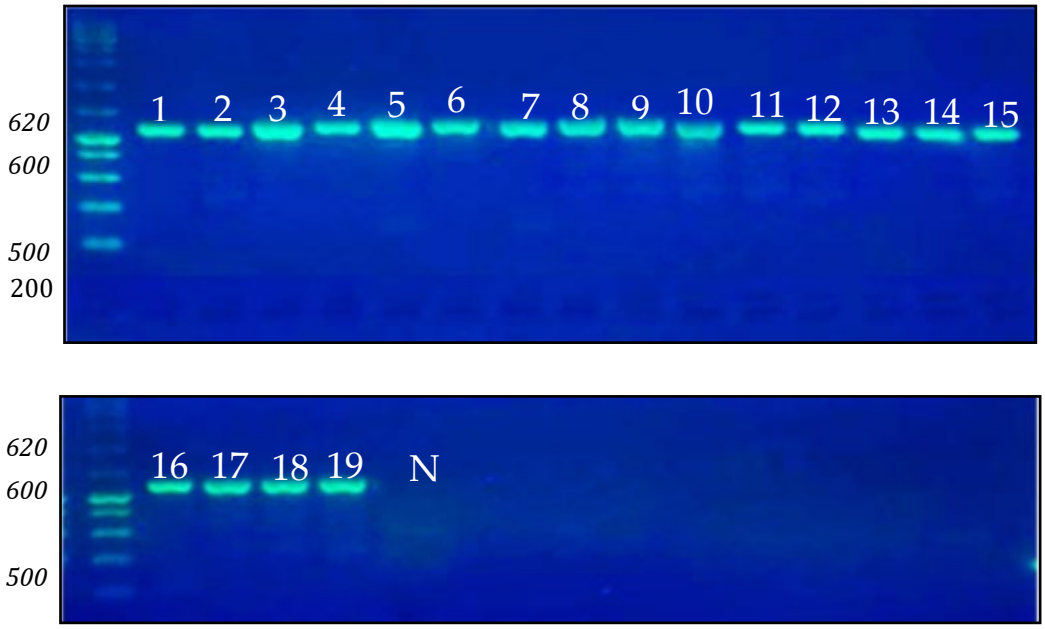

Figure 2. DNA products amplified by polymerase chain reaction (PCR) from fungi isolated (1-19) from the wastewater treatment plant.

The nucleotide sequence analyses of the multiplexed DNA produced by the fungal isolate were shown in the results. Making utilization of (the BLAST) program proves that all the fungal isolates belong to the fungus *A caespitosus*. Analysis results showed the nucleotide sequences generated from the identified. *A. caespitosus* isolates. The similarity rate was 100%. Among isolates (1, 2, 3), the similarity percentage was 100% between isolates (4, 5, 6), while the similarity percentage was 99% between isolates (1, 2, 3) and (4,5 and 6) diagnosed in this study. As shown in Figure (3)

A. caespitosus

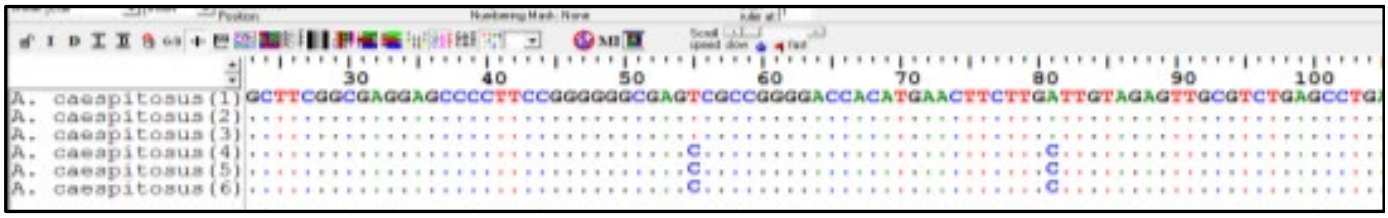

Figure 3. Nucleotide sequence alignment of the PCR products amplified by (PCR) from *A. caespitosus* isolates (1, 2, 3, 4, 5, and 6) isolated in this study.

The numbers on the right side of the fungi name refer to the sequences of nitrogen bases in the DNA products of the fungi in this experiment.

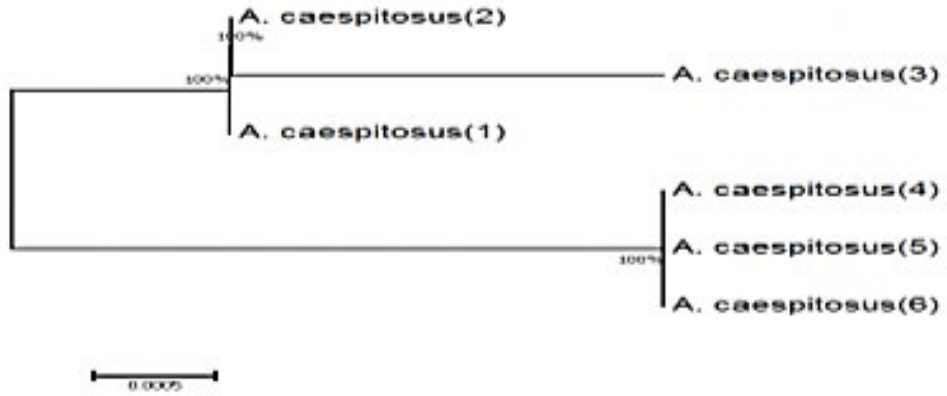

Figure 4. A phylogenetic tree shows the genetics between the *A. caespitosus* isolates (1, 2, 3, 4, 5, and 6) isolated from the wastewater treatment plant.

Fungus	Isolate or strain name.	Origin	The most similar sequences in the GenBank database	
			GenBank Accession Number	Sequence similarity (%)
A.caespitosus	BAB-6587	India	MF319914	**100**
A.caespitosus	5H0205	Thailand	KT385755	100
A.caespitosus	K Air-29	Slovakia	KM063244	99
A.caespitosus	TSF04	India	HE653994	99
A.caespitosus	AS20	Saudi Arabia	MT328529	99
A.caespitosus	AS21	Saudi Arabia	MT328531	99
A.caespitosus	NRRL 1929	USA	NR_131288	99
A.caespitosus	CBS 103.45	Japan	AB267813	99
A.caespitosus	DTO 325-C1	Netherlands	KU866669	98

A.caespitosus	UOA/HCPF11 625	Greece	KC253948	98
A.caespitosus	CBS 654.74	Egypt	KU866578	98
A.caespitosus	SRRC 308	USA	AY373841	98
A.caespitosus	SQU-MA12	Oman	KU945912	98
A.caespitosus	CSVNU_JP3	India	MT107145	98
A.caespitosus	CCTU1164	Iran	KY046248	98
A.caespitosus	EGJMP17	India	KF234003	98
A.caespitosus	EF652428	Saudi Arabia	LN812957	98
A.caespitosus	1071	USA	KT826651	98
A.caespitosus	KAUH10	Saudi Arabia	LN827684	97

Table 2. Comparison between the similarity ratios of *Aspergillus caespitosus* isolates from a wastewater treatment plant with the other isolate of the same fungi previously recorded in NCBI.

Isolation of *A.caespitosus* * fungus isolates in this study.

Fungus	**Isolate or strain name.**	**Origin**	**The most similar sequences in the GenBank database**	
			GenBank Accession Number	**Sequence similarity (%)**
A. flavus	A1	USA	CP051065	100
A. flavus	K54A	USA	CP051089	100
A. flavus	VCG1	USA	CP051097	100
A. flavus	NRRL 2999	Uganda	CP051033	100
A. flavus	ST3	India	MT292809	100
A. flavus	NRRL 3357	USA	CP044617	100
A. flavus	ADI B9	Pakistan	MK139781	100
A. flavus	DTO 389-C8	Netherlands	MH279451	100
A. flavus	AFc35	Benin	KC964101	100
A. flavus	DMGS7	India	MW020891	100
A. flavus	SF6	Nigeria	MF668181	100
A. flavus	176 1 B2	Brazil	KP784374	99
A. flavus	GFRS16	China	MT447484	99
A. flavus	DTO 213-I2	Netherlands	MH279408	99
A. flavus	MUM:10.200	Portugal	HQ340101	99
A. flavus	NA-DY34	Viet Nam	MF599709	99
A. flavus	**SG_4**	Italy	MN845198	99
A. flavus	KSRCT-BTMS5	India	MT509808	99
A. flavus	Maci262	France	MG745384	99
A. flavus	SG_10	Italy	MN845204	99
A. flavus	ZGCL17	Pakistan	KT970478	99

Table 3. Comparison between the similarity ratios of *Aspergillus flavus* isolates from a wastewater treatment plant with the other isolate of the same fungi previously recorded in NCBI.

Isolation of *A.flavus* * fungus isolates in this study.

The result showed that using the BLAST program, the nucleotide sequence analyses of the PCR-amplify-produced fungal show that all isolates belong to *Trichoderma asperellum*—analysis results of the nucleotide sequences generated from the identified. *Trichoderma asperellum* isolates show. The similarity isolates between (9, 10, and 11) are 100%. They were diagnosed in this study (Figure 5).

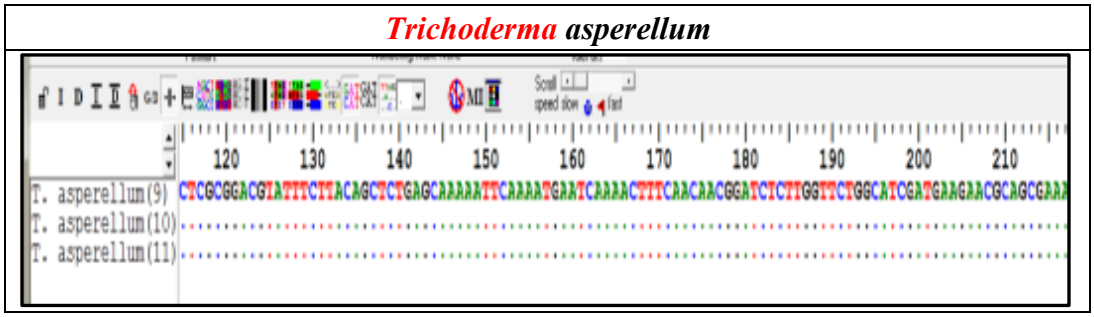

Figure 5. Nucleotide sequence alignment of the PCR products amplified by (PCR) from *T. asperellum* isolates (9,10 and 11) isolated in this study.

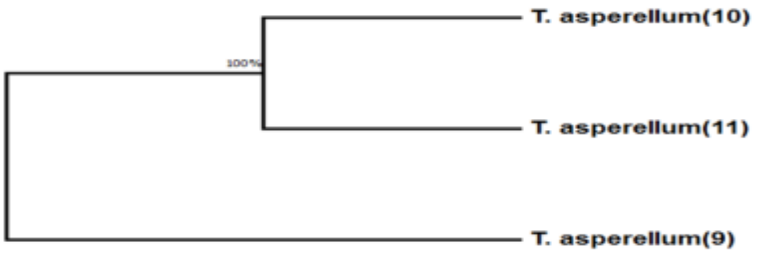

Figure 6. The phylogenetic tree shows the genetic relationships of *T.asperellum* (9, 10, and 11) isolates. This experiment isolates it from the wastewater treatment plant.

Fungus	Isolate or strain name.	Origin	The most similar sequences in the GenBank database	
			GenBank Accession Number	Sequence similarity (%)
T.asperellum	19MT-04-3	Indonesia	LC514692	**100**
T.asperellum	IIPRCPT-94	India	MK841023	100
T.asperellum	ECK8	India	MK253266	100
T.asperellum	US 18S	USA	MH130213	100
T.asperellum	CBS 125572	Peru	MH863552	100
T.asperellum	TR24	India	MH400817	100

T.asperellum	TL1	Brazil	KU341017	100
T.asperellum	T337	Mexico	KP059114	100
T.asperellum	TUB F-755	Austria	AY857217	100
T.asperellum	CPTrZC-12	Colombia	MG687498	100
T.asperellum	TV255	Venezuela	KP263716	100
T.asperellum	TF1	Brazil	KU341007	100
T.asperellum	ASP5	Egypt	KC898194	100
T.asperellum	TS-RP	Morocco	MW074111	100
T.asperellum	ITS1F_837	Poland	MW713493	99
T.asperellum	RIZ11-3S	Nigeria	MW250198	99
T.asperellum	G93	India	MW812028	99
T.asperellum	TAS107	Pakistan	MW785568	99
T.asperellum	Ta31KR	Malaysia	MW543025	99
T.asperellum	JP2	Malaysia	MW082791	99

Isolation of *T.asperellum* * fungus isolates in this study.

Table 4: Comparison between the similarity ratios of *Trichoderma asperellum* isolates from a wastewater treatment plant with the other isolate of the same fungi previously recorded in NCBI.

Fungus	Isolate or strain name.	Origin	The most similar sequences in the GenBank database	
			GenBank Accession Number	Sequence similarity (%)
A.tubingensis	ZMXR30	China	MT446135	**100**
A.tubingensis	ZMQR16	China	MT446097	100
A.tubingensis	M1	China	MH055392	100
A.tubingensis	NEF10	India	MT322427	99
A.tubingensis	BJR6	China	MT856267	99
A.tubingensis	DGY03	China	MH055394	99
A.tubingensis	37	Nigeria	MH345877	99
A.tubingensis	RT95IT	Mexico	MW193215	99
A.tubingensis	KIF 1	Indonesia	MW466769	99
A.tubingensis	ND5	Zimbabwe	MG659599	99
A.tubingensis	EX2019-M10	China	MT495451	99
A.tubingensis	AS181	South Korea	LC467944	99
A.tubingensis	CMXY25850	Netherlands	MG991653	99
A.tubingensis	HKAS 93727	Thailand	KX165340	99
A.tubingensis	**1210**	China	KF435032	99
A.tubingensis	IFO 4308	Japan	AP024428	98
A.tubingensis	RCZ2B-2	Nigeria	MW260084	98

A.tubingensis	NBRC 31125	Japan	LC573619	98
A.tubingensis	NBRC:4407	Japan	LC573617	98
A.tubingensis	NBRC:4050	Japan	LC573615	98

Isolation of *A.tubingensis** fungus isolates in this study

Table 5. Comparison between the similarity ratios of *Aspergillus tubingensis* isolates from a wastewater treatment plant with the other isolate of the same fungi previously recorded in NCBI.

Fungus	Isolate or strain name.	Origin	The most similar sequences in the GenBank database	
			GenBank Accession Number	Sequence similarity (%)
A.terrus	MSEF93	China	KT310990	**100**
A.terrus	R7	Iran	MN944451	99
A.terrus	4100062L51-1	South Korea	MN559622	99
A.terrus	MSEF75	China	KT310979	99
A.terrus	DTO 403-C9	Italy	MT316343	99
A.terrus	QCX-28	China	MK418744	99
A.terrus	ATCC 1012	USA	NR_131276	99
A.terrus	A1S4_D36	Malaysia	JX501361	99
A.terrus	UOA/HCPF 10536	Greece	FJ878634	99
A.terrus	wb464	Austria	AF455426	99
A.terrus	2aWF	India	MH196570	99
A.terrus	FJAT-31011	China	KU687809	99
A.terrus	LPSC 1180	Argentina	KF753943	99
A.terrus	NRRL 680	USA	EF669618	99
A.terrus	H15	Germany	AY939788	99
A.terrus	AY839	Namibia	MG250398	99
A.terrus	A8	Australia	JN129182	99
A.terrus	RGS	Egypt	MW282328	99

Isolation of A.*terrus* * fungus isolates in this study.

Table 6. Comparison between the similarity ratios of *A.terrus* isolates from a wastewater treatment plant with the other isolate of the same fungi previously recorded in NCBI.

Fungus	Isolate or strain name.	Origin	The most similar sequences in the GenBank database	
			GenBank Accession Number	Sequence similarity (%)
A.alternate	KNUZS2	South Korea	MK108918	**100**
A.alternate	E20	India	MT524319	99

A.alternate	1	Poland	MW720803	99
A.alternate	JN10	China	MN589744	99
A.alternate	HETIAO-04	China	KJ002058	99
A.alternate	TJZYM-341	China	MW008923	99
A.alternate	19-4d-1	China	KX073994	99
A.alternate	R	Iran	MG198619	99
A.alternate	ET 90	Turkey	MK752741	99
A.alternate	OISB-1	Pakistan	MH553296	99
A.alternate	fung2	Poland	MT635274	99
A.alternate	XBC1-3	China	MH588263	99
A.alternate	WZ-267	China	MN856385	99
A.alternate	Aa-23	India	MK979375	99
A.alternate	PAK37	Pakistan	KT280010	99
A.alternate	GG3F23	India	KY949584	99
A.alternate	RdKyA-4b	India	MF116306	99
A.alternate	5-F36	China	MW081364	99
A.alternate	GG2F35	India	KY419541	99
A.alternate	ZLVG 331	Slovenia	HE774489	99

Isolation of *A. alternate* * fungus isolated in this study.

Table 7. Comparison between the similarity ratios of *Alternaria alternata* isolates from a wastewater treatment plant with the other isolate of the same fungi previously recorded in NCBI.

Fungus	**Isolate or strain name.**	**Origin**	**The most similar sequences in the GenBank database**	
			GenBank Accession Number	**Sequence similarity (%)**
A.niger	COF01	China	KP764197	**100**
A.niger	ZMQR6	China	MT446087	99
A.niger	F8143	China	MN429196	99
A.niger	T8	India	MN180811	99
A.niger	2-1	China	MK898825	99
A.niger	mkh-40	Iran	MG775228	99
A.niger	GF6	India	MG759551	99
A.niger	S13	Brazil	MG654699	99
A.niger	BPb7	China	KP940594	99
A.niger	BPb8	China	KP940595	99
A.niger	BPb6	China	KP940593	99
A.niger	BPb4	China	KP940591	99
A.niger	MO-25	Turkey	KF939141	99
A.niger	AL-25 18S	Mexico	KC341970	99

A.niger	YLA3	Thailand	LC496501	99
A.niger	ORTO514	Turkey	MW418321	99
A.niger	BNYG	Egypt	LC582533	99
A.niger	45	Nigeria	MH345885	99
A.niger	J8M-31	China	JN226924	99
A.niger	ND4	Zimbabwe	MG659598	99

Isolation of *A. niger* * fungus isolated in this study.

Table 8. Comparison between the similarity ratios of *Aspergillus niger* isolates from a wastewater treatment plant with the other isolate of the same fungi previously recorded in NCBI.

	Isolate or strain name.	Origin	The most similar sequences in the Gen-Bank database	
			GenBank Accession Number	Sequence similarity (%)
Acremonium sp.	20	Germany	AJ557731	**100**

Isolation of Acremonium *sp.* * fungus isolates in this study.

Table 9. Comparison between the similarity ratios of *Acremonium sp* isolates from a wastewater treatment plant with the other isolate of the same fungus previously recorded in NCBI.

Fungus	Isolate or strain name.	Origin	The most similar sequences in the GenBank database	
			GenBank Accession Number	Sequence similarity (%)
C.sphaerospermum	H89	Oman	MN947604	**100**
C.sphaerospermum	C5-03	Japan	AB572902	100
C.sphaerospermum	H91	Oman	MN947606	99
C.sphaerospermum	CCTU1121	Iran	KY046241	99
C.sphaerospermum	JnUBD27	Bangladesh	MH393182	99
C.sphaerospermum	6018	Brazil	KX363452	99
C.sphaerospermum	CCTU1124	Iran	KY046243	99
C.sphaerospermum	CCTU1125	Iran	KY046242	99
C.sphaerospermum	WM 05.11	Australia	EF568045	99
C.sphaerospermum	1254	East Pacific O	AM176719	99
C.sphaerospermum	SCSIO z030	China	KX258804	99
C.sphaerospermum	SD-26	India	MF467882	99
C.sphaerospermum	TSY0853	Japan	AB572898	99
C.sphaerospermum	Cla02	Japan	AB572904	99

C.sphaerospermum	NS0138	Japan	AB572894	99
C.sphaerospermum	H6	Oman	MN947602	99
C.sphaerospermum	26R-5-F04	Japan	KX958081	99
C.sphaerospermum	Cla15	Japan	AB572906	99
C.sphaerospermum	C4-11	Japan	AB572901	99
C.sphaerospermum	TSY0379	Japan	AB572896	99
C.sphaerospermum	NH1426	Japan	LC375369	99
C.sphaerospermum	SW67	Germany	MH482916	99
C.sphaerospermum	F23T3IIIA	Brazil	MW533017	99
C.sphaerospermum	AUMC 10865	Egypt	MN826828	98

Isolation of *C.sphaerospermum* * fungus isolated in this study

Table 10. Comparison between the similarity ratios of *Cladosporium sphaerospermum* isolates from a wastewater treatment plant with the other isolate of the same fungi previously recorded in NCBI.

Fungus	**Isolate or strain name.**	**Origin**	**The most similar sequences in the GenBank database**	
			GenBank Accession Number	**Sequence similarity (%)**
A.oryzae	KVCET7	India	KJ941129	**100**
A.oryzae	DTO 213-C8	Netherlands	MH279400	99
A.oryzae	M45	Viet Nam	MH746006	99
A.oryzae	NRRL 35226	USA	EF634406	99
A.oryzae	RIB 40	Japan	AP007173	99
A.oryzae	RIB 40	Japan	AP007172	99
A.oryzae	NRRL 506	USA	AF459735	99
A.oryzae	CBS 100925	France	MF668185	99
A.oryzae	GCU-DAB-S19.2	Pakistan	KU312066	99
A.oryzae	Y1	China	MF374341	99
A.oryzae	ZGCL12	Pakistan	KT964480	99
A.oryzae	ASOP GRD113	India	JX110980	99
A.oryzae	NRRL 35191	USA	EF591304	99
A.oryzae	NRRL 447	USA	EF661560	99
A.oryzae	ZGCL43	Pakistan	KT970479	99

Isolation of ***A.oryzae*** * fungus isolated in this study

Table 11. Comparison between the similarity ratios of *A.oryzae* isolates from a wastewater treatment plant with the other isolate of the same fungi previously recorded in NCBI.

DISCUSSION

It shows that the highest percentage of 100% genetic similarity for *A. caespitosus* isolates (1,2,3,4,5,6) was with *A.caespitosus* isolates previously isolated from India and Thailand (MF319914 and KT385755, respectively), followed by the diagnosed fungal isolate in Slovakia (KM063244). India (HE653994), Kingdom of Saudi Arabia (MT328529), with a similarity rate of 99%, although genetically distant from the isolate of *A. caespitosus* isolated from Saudi Arabia (LN827684, with a difference of 97%. Also, It showed the proportion of similarity between this isolate and isolates from the world that has been recorded (NCBI). Furthermore, these isolates were not recorded in Iraq. Table 3. shows by comparing the DNA base the sequence of the dual-stranded DNA from *A. flavus* isolate (7,8) with the data at the (NCBI) that the highest similarity percentage (100%) was with the fungus isolates isolated from USA (CP051065, CP051089, CP051097) Uganda, India and China (CP051033, MT292809 and CP047255), respectively. It was also found that the lowest genetic similarity percentage (99%) was for the fungal isolates isolated from India (MT509808, France, Italy, and Pakistan(MG745384, MN845204, KT970478). Respectively. These isolates from wastewater were not recorded in the Arab nations and Iraq.

The results presented in Table 4. show that the highest percentage of 100% genetic similarity for *T.asperellum* (9,10,11) isolates were with *T.asperellum* isolates previously isolated from Indonesia (LC514692) India (MK841023, MK253266), and the USA (MH130213) Isolates were recorded in the Arab world 100% similarity from Egypt (KC898194) and Morocco (MW074111). Although genetically distant from the isolate of *T.asperellum* isolated from India (MW493185) and Malaysia (MW082791) with a difference of 99%, The above Table Shows the proportion of similarity between this isolate and isolates from across the world that has been recorded (NCBI). It was not recorded in the Arab nations and Iraq. The results presented in Table 5. were also presented by comparing the sequence of DNA's nitrogenous bases double for the *A.tubingensis* isolate (12) with data available in the (NCBI) that the highest genetic similarity rate of 100% with Previously isolated fungus isolates from China (MT446135, MT446097, MH055392) followed by the diagnosed fungus isolates from India (MT322427, MT443912) China (MT856267, MH055394), Nigeria (MH345877) and Mexico (MW193215) with a similarity rate of 99% The above Table It showed the proportion of similarity between this isolate and isolates from across the world that has been recorded (NCBI). It was not recorded in the Arab nations and Iraq.

The results presented in Table *6.* were also presented by comparing the sequence of the DNA's nitrogenous bases double for the *A.terrus* isolate (*13*) with data available in the (NCBI) that the highest genetic similarity rate of 100% with Previously isolated fungus isolates from China (KT310990) followed by the diagnosed fungus isolates from Iran (MN944451), South Korea (MN559622), China (KT310979, MK418744), Italy (MT316343), USA (NR_131276) and Egypt (MW282328) with a similarity rate of 99% The above Table It showed the proportion of similarity between this isolate and isolates from across the world that has been recorded (NCBI), where it was not recorded in Iraq.

According to Table 7, comparing the series of the DNA's nitrogenous bases double for the *A.alternate* isolate (16) with the data available in the (NCBI) the highest genetic similarity rate of 100% with Previously isolated fungus isolates from South Korea (MK108918) followed by the diagnosed fungus isolates from India, Poland (MT524319, MW720803) China (MN589744, KJ002058, MW008923, KX0739949) and Iran (MG198619) with a similarity rate of 99% The above Table It showed the proportion of similarity between this isolate and isolates from across the world that has been recorded (NCBI), where it was not recorded in the Arab nation and Iraq.

The results shown in Table 8 comparing the series nitrogenous bases of the DNA double *A.niger* isolates (14, 15) with the available Information in (NCBI) show that the highest genetic similarity rate of 100% with Previously isolated fungus isolates from China (KP764197), followed by the diagnosed fungus isolates in China (MT446087, MN429196) India (MN180811) with a similarity of 99% and also recorded in Egypt (LC582533)) with a similarity rate of 99%, The above Table It showed the proportion of similarity between this isolate and isolates from across the world that has been recorded (NCBI), where it was not recorded in Iraq.

Table 9 shows that by comparing the DNA base and the sequence of the dual-stranded DNA from *Acremonium sp*. isolate (19) with the data at the (NCBI) only one isolate was recorded in the world, with a genetic similarity rate of 100%, with a fungus isolate previously isolated from Germany (AJ557731), where no recorded in the Arab world and Iraq.

The results presented in Table 10 were also presented by comparing the sequence of the DNA's nitrogenous bases double for the *C.sphaerospermum* isolate (17) with data available in the NCBI that a highest genetic similarity rate of 100% with Previously isolated fungus isolates from Oman (MN947604), Japan (AB572902) followed by the diagnosed fungus isolates from Oman (MN94760) Iran (KY046241, KY046243), Bangladesh (MH393182), Brazil (KX363452) and Egypt (MN826828) with a similarity rate of 99% The above Table It showed the proportion of similarity between this isolate and isolates from across the world that has been recorded (NCBI).. Where it was not recorded in Iraq.
Table 11 are, comparing by the series of the DNA's nitrogenous bases double for the *A.oryzae* isolate (18) rate of 100% with Previously isolated fungus isolates from India (KJ941129) followed by the diagnosed fungus isolates from Netherlands (MH279400), Viet Nam (MH746006), USA (EF634406) Japan (AP007173) with a similarity rate of 99% The above Table It showed the proportion of similarity between this isolate and isolates from across the world that has been recorded (NCBI), where it was not recorded in the Arab nation and Iraq.

CONCLUSIONS

It is concluded from this study that there is an unlimited diversity of types of fungi in the wastewater treatment plant in Al-Barakia, Najaf. It is also concluded from this learning that all fungi isolates were genetically different from each other. The results proved that these isolates were previously registered in the center, as mentioned above. However, not all isolates were registered in Iraq. In this study, the technique of PCR was used in many previous studies to diagnose many organisms, including fungi, to get free of diagnostic harm depending on the morphological features of fungi [12].
Although morphological diagnosis helps to confine the fungi understudy into smaller sets before using other diagnostic ways, there are some problems associated with a morphological diagnosis of fungi as they require excellent experience in this area and a lot of time and effort. Since some fungal species are found very close to each other in some morphological features, the diagnostic process is more complicated [15]. Moreover, fungi are affected by many environmental conditions that may alter the shapes, sizes, and colors of the spore and fungal colony[19].
Differences in DNA sequences of the ITS region (internal transcription spacer) [20]. They were highly effective and have been exploited to diagnose several fungi types. PCR-based identification has been used extensively in previous studies in diagnosing fungi such as *Acremonium sp, Aspergillus flavus, Alternaria alternata, Cladosporium sphaerospermum, Aspergillus tubingensis, Aspergillus niger* [21,22,23]

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