

Isolation And Identification Of Fungi From Soil And Water In The Bahr Al-Najaf Depression

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Abstract

The purpose of the study is to ascertain the biological variety of the fungi living in the Bahr Al-Najaf depression's soil and water, where the study session was split into two seasons, with the first season beginning in mid-October and the second in mid-January. With three duplicates for each location, three water sample collection sites and three soil sample collection sites were chosen. In this study, the water and soil of Bahr Al-Najaf were used to identify 51 fungal isolates, which were distributed as follows: 34 fungal isolates for the first season and 17 fungal isolates for the second season. The fungus isolated during the first season by molecular diagnostics and polymerase chain reaction technology were two isolates of (*A. caespitosus*) (*C. cladosporioides*) 3 isolates, (*A. niger*) 4 isolates, (*A. tubingensis*) 2 isolates, (*A. oryzae*) Two isolates, (*A. flavus*) 4 isolates, (*A. terreus*), 4 isolates, (*Alternaria arborescens*) one isolate, (*C. allicinum*) one isolate, (*Talaromyces helices*) one isolate, (*C. iridis*), one isolate, (*Metarhizium anisopliae*) one isolate, (*Moesziomyces aphidis*) one isolate, (*F. solani*) one isolate, (*Talaromyces funiculosus*) one isolate, (*Neurospora tetrasperma*) one isolate, (*Trichoderma longibrachiatum*) one isolate, (*A. Irpex*) [1] isolate, (*A. niveus*) [1] isolate.

As for the fungal isolates isolated in the second season, they are *Fusarium sp* (2) isolates, *Mucor racemosus* (4) isolates, *Mucor sp* (2) isolates, *Paecilomyces sp* (3) isolates, *A. ibericus* (1) isolate, *Plectosporium tabacinum* (1) isolate, *A. fumigatus* (1) isolate, *Paecilomyces variotii* (1) isolate, *A. niger* (1) isolate, *F. culmorum* (1) isolate.

Five fungal isolates have been recorded globally:

(*C. allicinum*, *Moesziomyces aphidis*, *Talaromyces helices*, *Neurospora tetrasperma*, *A. turcosus*).

key words: Bahar AL-Najaf depression , fungi, soil fungi , aquatic fungi

1- INTRODUCTION

In the southwest of the Najaf Governorate, Bahr al-Najaf is situated between two circles at longitude 31.50-°32.50 and latitude 44.15-°44.21. A wide amount of agricultural land surrounds the permanent salt marsh that fills its core. (2008) Al-Janabi & Al-Khafaji The term "biodiversity" refers to the diversity of all kinds of life, including species, genealogies, and the ecosystems that make up the areas where neighborhoods are found. The extinction of a species or group of species in an ecosystem is a warning that something is wrong with the way this system is working since ecosystems depend on a stable system and subtle variety to sustain one another. (Sharma *et al.*, 2007). Fungi can be found in up to five times as many distinct types of ecosystems. 95% of the species of fungus are still undiscovered and waiting to be found. Huang and colleagues (2016); Yang *et al.*, 2007). Fungi may thrive in a variety of ecological zones, thus they are widely spread in nature. Asexual spores (conidia or sporangiospores), which are spread in the air over great distances and are the primary mode of reproduction for many fungus, allow them to live in habitats that are often dissimilar to their native ones. And as a result, it spreads throughout all media, whether in soil, air, or water, and has the capacity to swiftly adapt to any particular habitat in which it finds itself (Rocio *et al.*, 2010). Although fungi are among the most important creatures in the world, the majority of their species are currently unknown or poorly understood, and estimates of the number of fungal species vary significantly. This lack of fundamental understanding of taxonomic variation has a substantial influence on the many dimensions of biological evolution. The estimate of 1.5 million fungal species that is frequently referenced (Mueller & Schmit, 2007). Fungi are among the most significant creatures in the world due to their crucial involvement in ecological processes, their effect on people, and human-related activities. (Bills & Mueller, 2004). Soil is an important substrate for mineral and organic raw materials in addition to being the home to a variety of microorganisms, notably fungi that multiply prolifically in the soil (Behera & Mukerji, 1985; Moubasher, 1995; Azaz and Pekel, 2002). Some fungal species are found in soil, which may indicate that they are immune to plant harmful bacteria. These fungi are known to be used in biological control to inhibit the growth of some pathogenic species because they are strong competitors to other fungi and have the ability to eradicate a variety of microorganisms and animal organisms harmful to plants. They are more frequently found in soil devoid of pathogenic fungi than in *Trichoderma* soil. (2005) (Maghrabi). Researchers cannot rely on morphological characteristics to accurately classify fungi because they require extensive training and expertise, especially when dealing with closely related fungal isolates. Additionally, accuracy is frequently influenced by environmental factors that affect the size, shape, and color of spores and fungal col (Yang *et al.*, 2007, Huang & Wang, 2016; Zhang *et al.*, 2012). A key component of the molecular method known as PCR is the selection and

amplification of a particular area of the genome of an organism. Find the genetic links based on similarities, then compare the differences in the DNA sequence of that location.. The difference between the types of fungi supports the phenotypic diagnosis of the studied fungus (Erlacher et al.,2014 ; Hawksworth et al.,1995 ;Yang et al .,2007).

1-2- Aim of the study

The study aims to assess the biodiversity of fungi in the soil and water of the Bahr al-Najaf depression in the summer and winter season through the following topics:

1. Isolation of fungi from the water and the soils of Bahr al-Najaf depression.
2. Molecular identification of fungi of isolated fungi from the water and the soils of Bahr al-Najaf depression.
3. Morphological identification of fungi of isolated fungi from the water and the soils of Bahr al-Najaf depression.

2-MATERIALS AND METHODS :

2-1-Study area:

The study area was conducted in Bahr Al-Najaf depression is a water body with a length and width of about (75-45) kilometers and an area of about 435 km². Its water is seasonally stagnant depending on precipitation levels. The soil and water samples of the Bahr Al-Najaf depression were taken during two seasons, where the first season was mid-October of 2021, and the second season was mid-January of 2022. Three sites were selected from the study area. The geographical coordinates of the studied locations were taken using the GPS device (Picture 1) as follows: Location 1 (N 32° 00.670, E 44° 16.934), Location 2 (N 31° 56,603, East 44° 15.891), Location 3 (31° 58,572 E 44° 12.861) (Fig. 1). Three replicates were taken for each site and three samples were taken for each replicate.

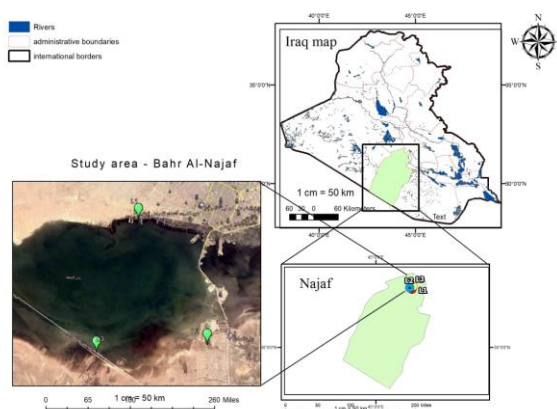


Fig (1): GIS image for study area the Bahr Al-Najaf Depression

2-3-Sample collection

Soil and water samples were collected from different locations in Bahr al-Najaf depression in two different seasons, the first season was in the middle of October, and the second season was in the half of January for the purpose of laboratory tests. Soil samples were collected at a depth of 5 cm. Water samples were collected from the mentioned sites using sterile 1000 ml water bottles.

2-4-Potato dextrose Agar (PDA)

The according to the (Himedia) method, potato dextrose agar which prepared for the isolation of fungi, where was (41)g of the PDA powder dissolved in a liter of the "distilled water" in a flask, and the media is sterilized by the autoclave at 121 °C and, at pressure 15 ib.ng-1. Then the medium was cooled and an antibiotic is added to inhibit the growth of bacteria, chloramphenicol (250) mg/l before pouring the Petri dishes and being cultured, used to isolation and purification of fungi.

2-5-Isolation and diagnosis of fungi

After preparing (PDA) according to (Himedia) company, the direct method for cultivation is used and it was used to isolate the fungi from the wastewater treatment plant by taking (1 ml) from a sample of two Petri dishes and pouring the medium on it. Three replicates were prepared for each site. The dishes were incubated at (25 ± 2) °C for 7 days to properly grow colonies of the fungi, then purified and the diagnosis was made morphologically (Rapper &Fennel, 1965).
Molecular identification of fungi using PCR

3.2.6.1 Extraction of DNA

Each fungal isolate is subjected to DNA extraction using the kit (Cat. No. FAPGK100) in accordance with the procedure outlined by Favorgen Corporation/ Taiwan-China.

- i.Take between 50 and 100 mg of the fungus to be identified and grow on potato dextrose agar PDA at the age of 3 to 6 days, then place in Eppendorf for crushing with a Micro Pestle.

- ii. Add 200 microliters of FATG buffer solution to the sample, shake for five minutes with a vortex, and incubate for ten minutes in a water bath at 70 oC while shaking the tube every three minutes.
- iii. Concuss the sample with 200 cc of 95 percent ethanol for ten seconds.
- iv. The mixture was then transferred to the FAPG tube, which was then spun in the centrifuge for five minutes at a speed of 14000 rpm/min to extract the DNA bound to the membrane inside the FAPG tube. moved to a fresh Collect tube.
- v. Then, add 400 ml of W1buffer solution and centrifuge for 30 seconds at 14000 rpm. Keeping the FABG tube in order to dispose of the filtrate.
- vi. After returning the FABG tube to the collection tube, 600 l of washing solution (Wash buffer) was added to it. Centrifugation was then performed for 30 seconds, and the filtered solution was discarded. Centrifugation was then repeated for 3 minutes to remove any remaining wash solution residues.
- vii. The FAPG tube is placed in a fresh (Eppendorf tube), to which (100) microliters of elution buffer are added, and the membrane containing the DNA is left exposed for three minutes at room temperature. The concentration and purity of the extracted nucleic acid (DNA) were determined and stored at -20 oC for subsequent use after being centrifuged at a speed of (13000) rpm/min.

3.2.6.2 Determining DNA's purity and concentration

To determine the acid nuclear (DNA) concentration, the following equation was applied using a spectrophotometer at a wavelength of (260 nm) or less.

DNA concentration (µg/ml)= light absorption at a wavelength of 260nm ×50 × Dilution factor

The following equation, which was described by (William *et al.*, 1997), was used to determine the purity of DNA:

$$\text{DNA purity} = \frac{\text{Amount of absorption along the wavelength 260nm}}{\text{amount of absorption along the wavelength 280nm}}$$

The polymerase chain reaction PCR was used to evaluate the DNA that had been isolated from the fungus isolates, which was at kept(-20C°).

3.2.6.3 Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) was used to diagnose the fungal isolates that were isolated for this investigation using a kit (Maxime PCR PreMix (i-Taq), Cat. No. 25026), created by a Korean firm (iNtRoN).

Polymerase chain reaction (PCR) was carried out with a total volume of (20 µl), which contains (1 µl) from the front of each initiator (ITS1: TCCGTAGGTGAACCTGCGGG) and the back (TCCTCCGCTTATTGATGC: TS4) (White *et al.*, 1990), and (1 µl) of extracted DNA All components were placed inside a tube provided by the manufacturer, and the volume was completed to 20 µl of nuclease-free water. Amplification was performed in thermal cycles of Agilent technologies table (3-3)

Gene	Temperature (°C)/Time				
	Initial Denaturation Cycle	Cycling condition 35 cycle			Final extension I cycle
		Denaturation	Annealing	Extension	
ITS	94°c/5min	94°c /30sec	55°c /30sec	72 °c /1min	72 °c/5min

Table(3-3): program were used in the PCR for ITS 1 gene

3.2.6.4 Electrophoresis using Agarose gel: When the temperature decreases, add 5 microliters of the stain (Ethidium bromide) to the layer of agarose gel that has been previously made by dissolving 1 gram of agarose powder in 100 milliliters of buffer solution (Tris boric acid EDTA buffer) until the mixture becomes clear. To drill through the gel layer, the agarose pouring mold, which has a comb at one end, was ready. Following the hardening of the agarose layer, the melted agarose was combined with the ethidium bromide dye and poured into the mold. It was then kept at room temperature until it solidified. The mold was put in position with the transfer device after the comb was gently pulled out of the way. The electrophoresis tank received the addition of 1 TBE solution, which roughly covered the agarose layer. (1 cm). Each pit from the pit layer of the Agarose gel receives 5 l of DNA that has been doubled by PCR. Additionally, 5 l of a (DNA ladder marker) were inserted into the hole on the opposite side of the samples. The power supply's electrodes are then connected to the mains and run for an hour at (150 mA). The agarose gel layer containing the multiplexed nucleic acid bundle (PCR products) is viewed under UV light once the electrophoresis migration process of the samples is complete, and photographs are taken.

3.2.6.5 DNA sequence analysis of fungal isolates: The PCR amplicons that were duplicated by PCR were transcribed, and the primers ITS1 and ITS4 were submitted to Macrogen Corporation in South Korea for the purpose of determining the nitrogenous base sequence in order to identify the fungi isolated (1-51) in this work. BLAST was used to examine each of the sequences' nitrogenous bases (Basic Local Alignment Search Tool). Utilizing MEGA X, the outcomes were

compared to information provided by the National Center for Biotechnology Information (NCBI), which pertains to each phylogenetic tree analysis (Kumar et al., 2018).

RESULTS AND DISCUSSION:

2.3 Morphological and Molecular identification of the fungi isolated from Bahr al-Najaf:

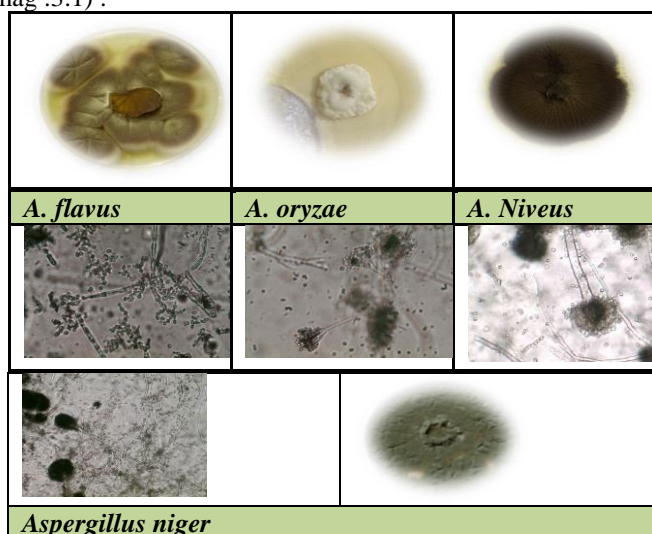
2.3.1 Morphological identification of the fungi isolated: The morphological traits identified by Leslie and Summerell were utilized to distinguish the fungus isolated for this investigation (2006). The morphological traits of the pure fungus after 5-7 days are displayed in table (4-2).

Species	Colony diameter	Colony color	Conidia		Conidio Phores
			Color/shape	Size	
<i>A.tubingensis</i>	65-72µm	Black	Subglobose	4-5 µm	-----
<i>A.niger</i>	67-70µm	Black to dark brown	Brown/rough	3-5 µm	Hyaline/ globose/ 200-400µm
<i>A . terrus</i>	500×30-50 µm	Brownish	Globose smooth-walled	2µm	Smooth/ 100-250×4-6
<i>A.oryzae</i>	60-75mm	pale yellow	Yellow green	2-3µm	-----
<i>A. flavus</i>	Grater than 400µm	green/rough	Yellowish-green Yellowish	3-6 µm	Rough globose to subglobose /400-800µm/ colorless
<i>T. longibrachiatum</i>	9≤ CM Typically colony radius 60 mm	Olivaceous / pale yellow	white /dark green/ broadly /oblong	< 250 µm	dark green, smooth (3.0–)5.7– 9.5(–12.7) µm
<i>C. iridis</i>	19–23 mm after 14 d at 25 °C	greenish olivaceous, smoke-grey to olivaceous – grey	Sub hyaline / pale olivaceous-brown/ cylindrical-oblong	(2–)2.5– 3.5(– 4) µm	very long/ up to 720 µm long, 6–11 µm wide
<i>Aspergillus ibericus</i>	38–43 mm	Black	Black / globose to subglobose	5–7 µm	smooth, thick walled 1200– 2000 µm
<i>Aspergillus fumigatus</i>	2.4-3.7 mm	white initially and then dark green, smoky gray	green / subglobose / globose	70.- 80. µm	hyaline, smooth / 208- 250 µm

Table(2-3): Macroscopic and microscopic features of fungi isolated from the soil and water of Bahr al-Najaf for both seasons.

2.3.1.fungi isolated from water: A group of fungi was isolated from the water of Bahar AL-Najaf depression for the first and second seasons and obtained according to the following sites:

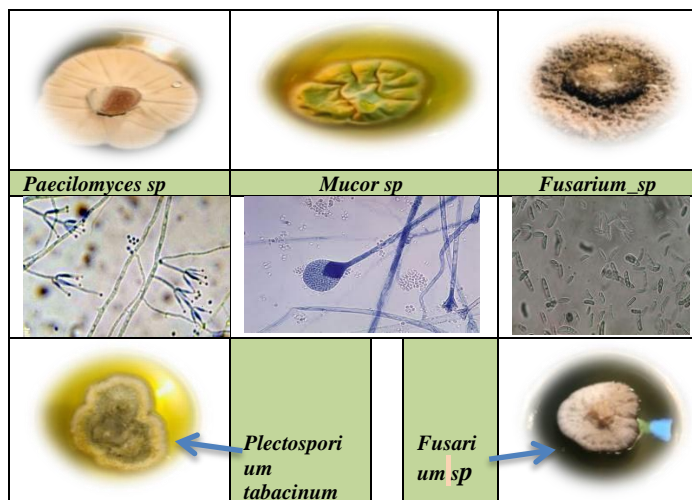
2.3.1.1.location.1.season.1:Four fungal isolates were isolated from the first site, the first season of water which are (*C. cladosporioides* [1] isolate , *Aspergillus oryzae* [1] isolate , *Aspergillus Niveus* [1] isolate , *Aspergillus flavus* [1] isolate, as shown in the following (Imag .3.1) :



Imag.(3.1) Fungi and Clamydospores that were isolated of first season (1 site) from water of Bahar Al- Najaf

2.3.1.2. location. 1, season. 2:

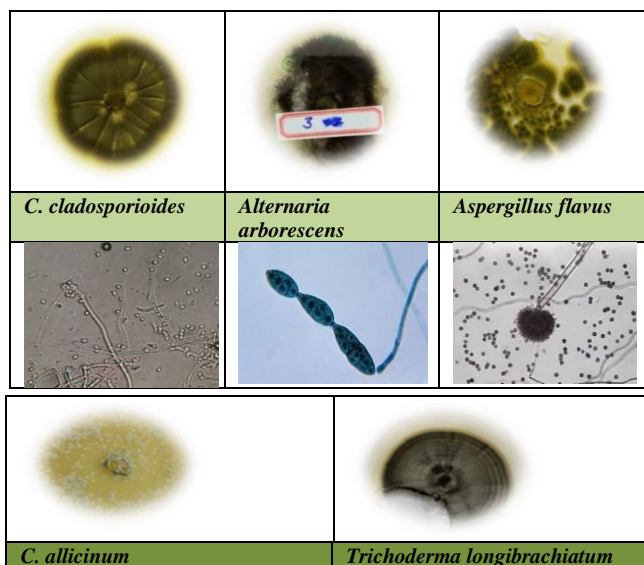
Six different fungal isolates were isolated from the first site of the second season of water as follows (*Paecilomyces* sp [1] isolate, *Paecilomyces variotii* [1] isolate, *Fusarium* sp [2] isolates , *Plectosporium tabacinum* [1] isolate, *Mucor* sp [1] isolate, and as shown in **Imag. (3.2)**:



Imag. (3-2): Fungi images and Clamydospores that were isolated of second season (1site from water of Bahar Al- Najaf

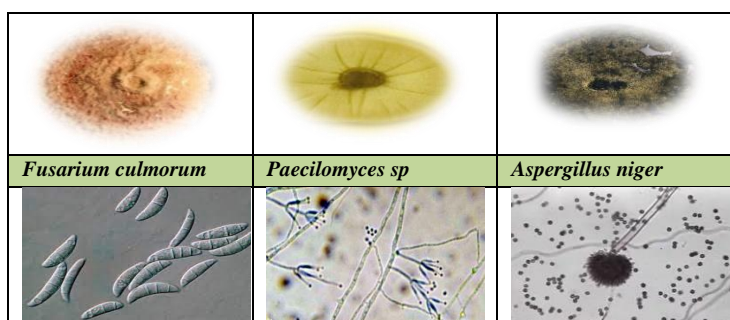
2.3.1.3.Loc.2, Season.1:

Eight fungal isolates were obtained from the second site, the first season of water, where 2 isolates of *A. oryzae* shown in the first site, 2 isolates of *A. terrus*, one isolate of *A. niger*, one isolate of *A. arborescens* ,one isolate of the fungus *C. cladosporioides* shown in first site , and one isolate of *C. allicinum* ,as shown in the following **Imag. (3.3)**



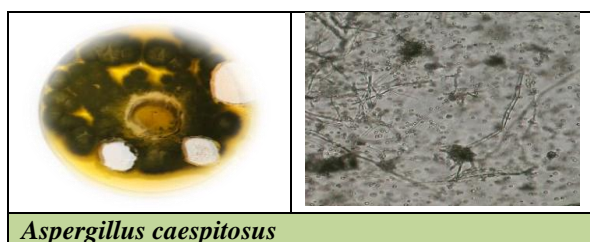
Imag. (4-3): Fungi and Clamydospores that were isolated of first season (2 site) from water of Bahar Al- Najaf

2.3.1.4.location.2,season2 :four fungal isolates were obtained from the second site, the second season of water, where 2 isolates of *Paecilomyces* sp ,one isolate of *F. culmorum*, and one isolate of *A. niger*, as shown in the following figure **Imag. (3.4)**:



Imag. (3.4) Fungi and Clamydospores that were isolated of second season (2 site) from water of Bahar Al- Najaf

4.2.1.1.5. Season .1. Location.3: Two fungal isolates were obtained from the third site, the first season of water, where one isolate of *A. terrus* shown in the second site ,and one isolate of *A. caespitosus* , as shown in following **Imag. (4.5)**

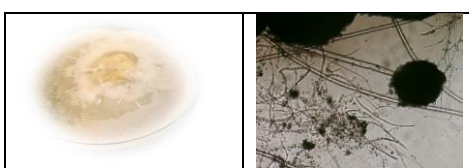


Aspergillus caespitosus

Imag (4.5) Fungi and Clamydospores that were isolated of first season (3 site) from water of Bahar Al- Najaf

2.3.1.6. Location.3,season2.

Only one isolate was obtained from the third site of the second season, which is (*M. racemosus*), as shown in the following **Imag. (3.6)**

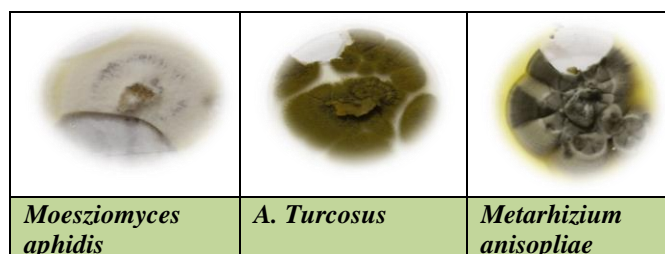


Mucor racemosus

Imag (3.6) Fungi and Clamydospores that were isolated of second season (3 site) from water of Bahar Al- Najaf

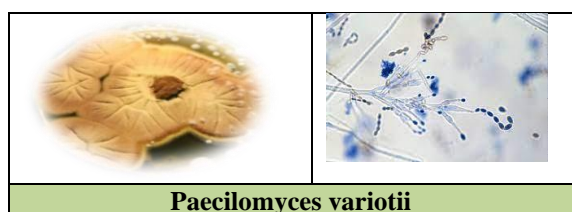
2.3.2. Fungi isolated from soil: A group of fungi was isolated from the soil for the first and second seasons and obtained according to the following sites:

2.3.2.1.first season .loc.1: six isolates of fungi were isolated from the soil for the first season from the first site, which are (*Cladosporium cladosporioides* [1] isolate, *A. Turcosus* [1] isolate, *M. aphidis* [1] isolate, *A. terrus* [1] isolate, *M. anisopliae* [1] isolate, *A. turcosus* [1] isolate) as in the following **imag. (3.7):**



Imag. (4.7) Fungi and Clamydospores that were isolated of second season (3 site) from soil of Bahar Al- Najaf

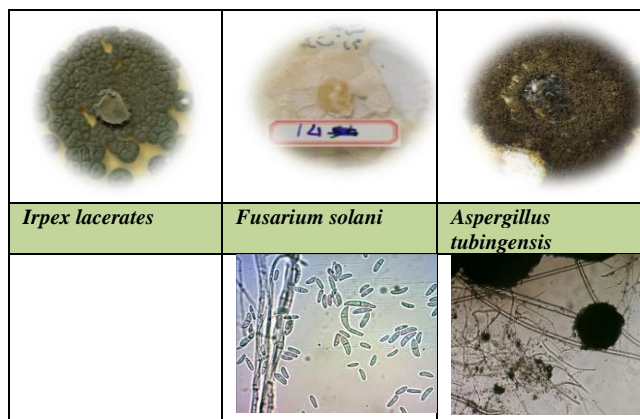
2.3.2.2.Second season . loc.1 One isolate obtained from the first site of the second season from the soil of Bahr Al-Najaf depression, as shown in the following **Imag. (3.8)**



Paecilomyces variotii

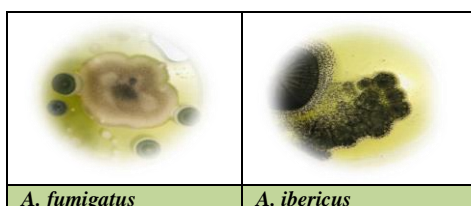
Imag. (3.8) Fungi and Clamydospores that were isolated of second season (1 site) from soil of Bahar Al- Najaf

2.3.2.3.first season .Loc.2: Saven isolates of fungi were isolated from the soil for the first season from the second site, which are (*A. terrus* [1] isolate, *A. niger* [1] isolate, *A. caespitosus* [1] isolate, *A. tubingensis* [1] isolate, *F. solani* [1] isolate, *A. flavus* [1] isolate , *I. lacerates* [1] isolate) as in the following (Imag. 3.9):



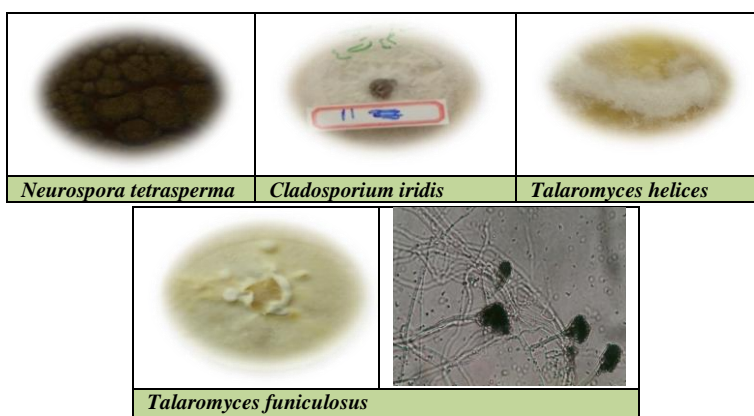
Imag. (3.9) Fungi and Clamydospores that were isolated of first season (2 site) from soil of Bahar Al- Najaf

2.3.2.4.Second season .loc.2: Only two fungal isolates were isolated from the soil of the depression in the second site of the second season, as follows (*A. ibericus* , *A. fumigatus*).as in **Imag.(3-10)**:



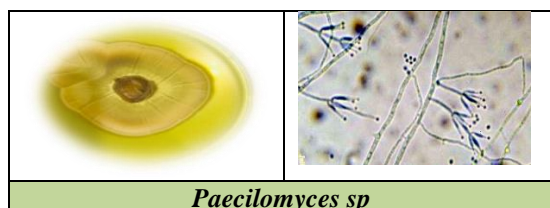
Imag. (3.10) Fungi that were isolated of second season (2 site) from soil of Bahar Al- Najaf

2.3.2.5.first season .loc.3: Five isolates of fungi were isolated from the soil for the first season from the second site, which are (*N. tetrasperma*, *A. tubingensis* as shown in site 2 first season, (*C. iridis*, *T. helices*, *T. funiculosus*), as in **Imag. (3.11)**



Imag. (3.11) Fungi and Clamydospores that were isolated of first season (3 site) from soil of Bahar Al- Najaf

2.3.2.6.second season .loc.3: two fungal isolates were isolated from the soil of the depression in the third site of the second season, as follows (*Mucor racemosus* as shown in second season loc.3 , *Paecilomyces sp*). as in **Imag. (3.12)**



Imag. (3.12) Fungi and Clamydospores that were isolated of second season (3 site) from soil of Bahar Al- Najaf

4.2.Molecular diagnostics of fungi using PCR technology

The results are shown in Figure (4-15) showed the possibility of doubling PCR-amplified products, each of, which has a size of approximately 600 base pairs (Base pairs, bp) by polymerase chain reaction (PCR) and in the presence of the B-base pair. (ITS1) and posterior (ITS4). M = DNA ladder ((100bp) Molecular-weight size marker with the number of base

pairs (bp) and sizes each fixed on the left side of the figure NC: comparison treatment [without adding DNA to the remaining components of the PCR mixture].

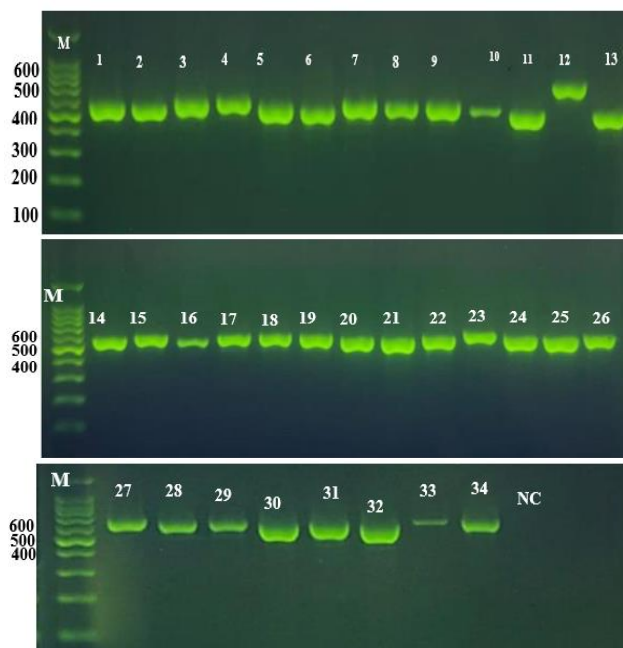


Fig.(4-15) DNA products (PCR products) multiplexed using polymerase chain reaction (PCR) and using primer pair ITS1 and ITS4 from(1- 34) isolates of Fungi isolated to this study from soil and water of Bahr Al-Najaf. M = DNA ladder ((100bp) Molecular-weight size marker with the number of base pairs (bp) and sizes each fixed on the left side of the figure NC: comparison treatment [without adding DNA to the remaining components of the PCR mixture].

The results of the nucleotide sequence analysis of the multiplexed DNA products from the isolates of the fungi that were made using BLAST program proved that the two isolates (1 and 2) belong to the fungus *Aspergillus caespitosus*. (1 and 2) the presence of an identical percentage of 100% ,it was also found that these two isolates have a similar percentage of 100% with most isolates belonging to the fungus and registered in (the National Center for Biotechnology Information) NCBI.

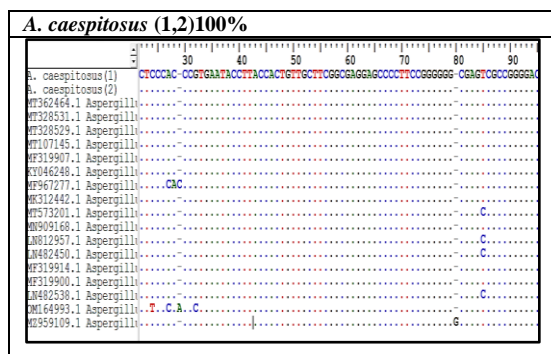


Fig (4-16) nitrogenous base sequences (sequence alignment) created from the multiplexed DNA products (PCR product) from A have similarities and differences in some areas. The nucleotide sequences of the two caespitosus isolates (1 and 2) that were isolated for this investigation may be found outside in NCBI (National Center for Biotechnology Information).

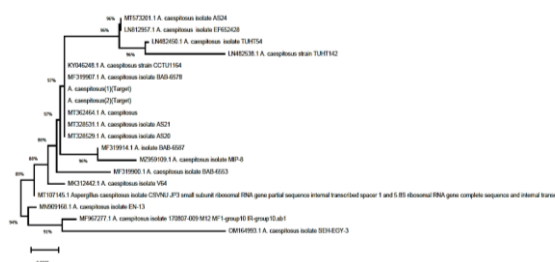
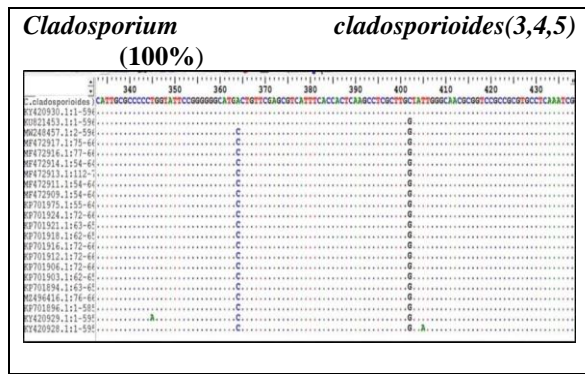


Fig (4-17): (Neighbor-Joining tree) that showing genetic relationship between *A. caespitosus* isolates (1 and 2) that were isolated in the study, as well as other isolates of the same fungus, are registered with (the National Center for Biotechnology Information) NCBI.



(Fig. 4-18) Similarities and differences in some regions of the sequence alignments formed from PCR product from *C. cladosporioides* (3,4and 5) isolated from this study, and the sequences of the other isolates of the same fungus recorded in the center National Biotechnology Information (NCBI).

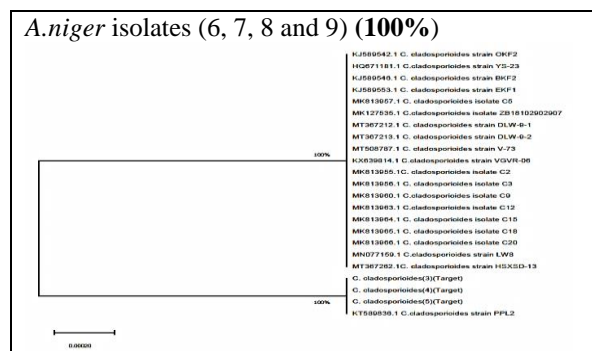
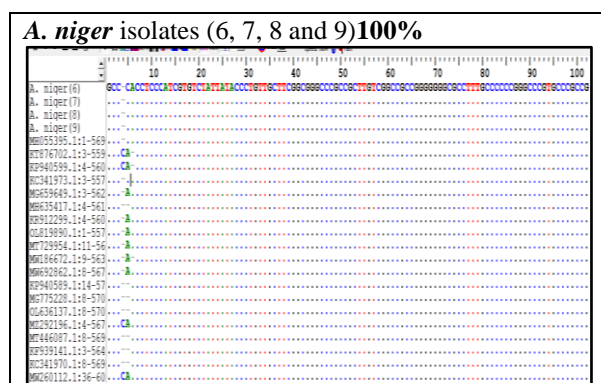


Figure (4-19) Neighbor_Joining tree that showing genetic relationship between isolates of *C. cladosporioides* (3, 4 and 5) that were isolated in this study, and other isolates of the same Fungus registered at the National Center for Technology Information Vital (NCBI)



Fig(4-20). One of the sequence alignments created from the PCR result from A has similarities and differences. nitrogenous base sequences of other isolates of the same fungus registered at the National Center for Biotechnology Information(NCBI), as well as the niger isolates (6, 7, 8, and 9) that were taken from the Bahar AL-Najaf depression for this investigation

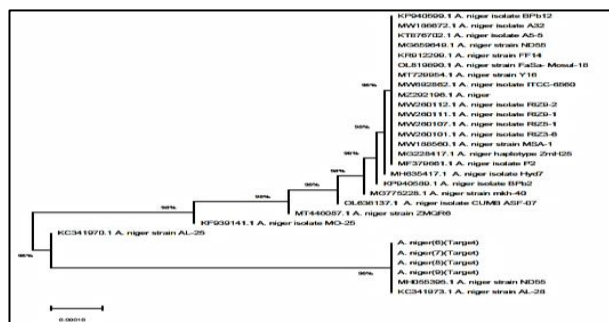


Fig (4-21) The neighbor-joining tree shows the genetic relationship between isolates of *A. niger* (6, 7, 8, 9) that were isolated from this study. and other isolates of the same fungus, registered at the National Center for Biotechnology Information (NCBI).

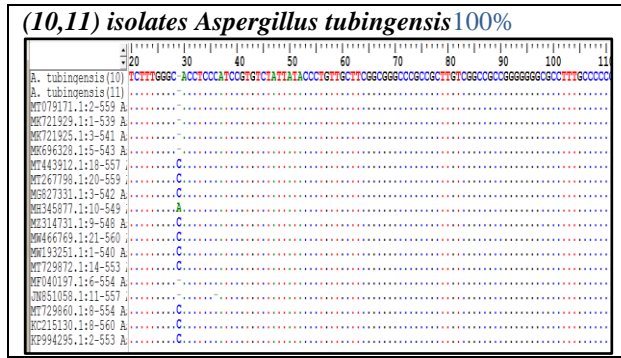


Fig.(4-22) Sequence alignments made from the PCR result of A isolates show similarities and differences. tubingensis (10 and 11) that were isolated for this research, as well as their isolate sequencing. The National Center for Biotechnology Information(NCBI). has several varieties of the same mushroom listed for registration.

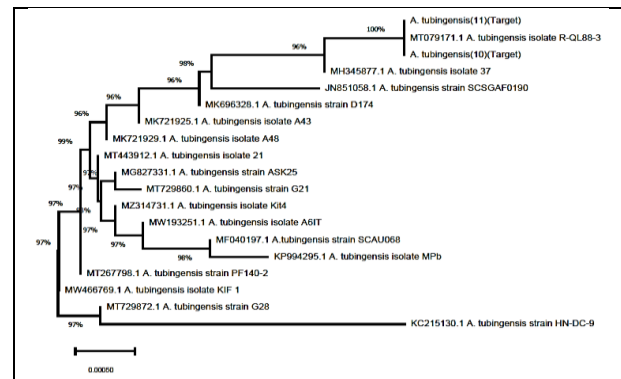


Fig. (4-23) Neighbor-Joining tree showing the genetic relationship between *A. tubingensis* isolates (10 and 11) that isolated from this study. as well as other isolates of the same fungus registered in (National Center for Biotechnology Information) NCBI.

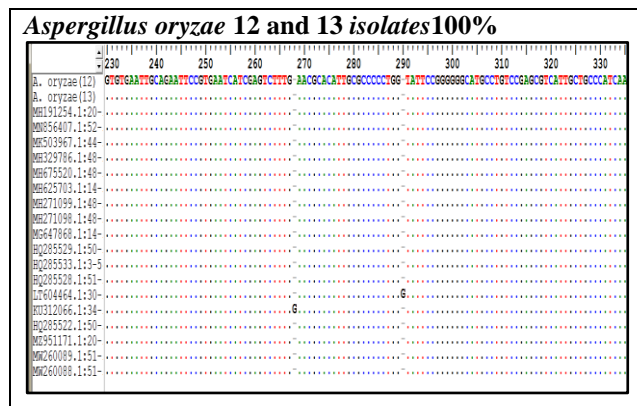


Fig.(4-24) Sequence alignments generated from the PCR result of the fungal isolate A show similarities and differences. the base sequences of the nitrogenous oryzae isolates (12 and 13) and other isolates of the same fungus recorded at the National Center for Biotechnology Information (NCBI).

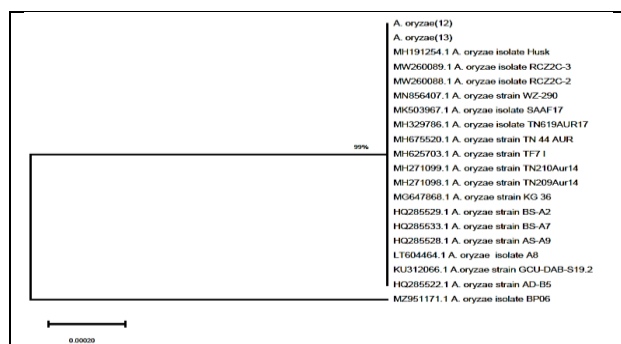


Fig.(4-25) Neighbor-Joining tree displaying A and B's shared genetic heritage. oryzae (12 and 13) that were discovered in the Bahar Al-Najaf depression, along with other isolates of the same fungus that were cataloged by the National Center for Biotechnology Information (NCBI).

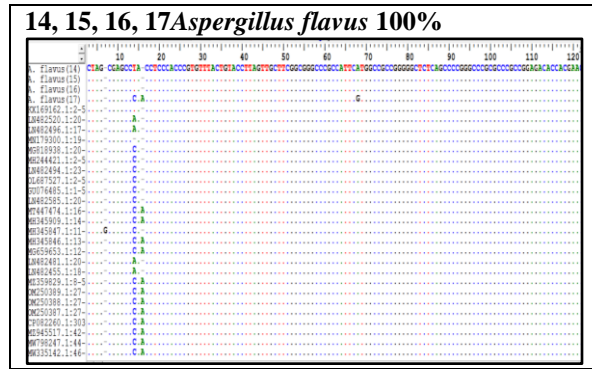


Fig.(4-26) One of the sequence alignments created from the PCR result from A has similarities and differences. Nitrogenous base sequences of further isolates of the same fungus, including flavus isolates (14, 15, 16 and 17) recovered from the Bahar Al-Najaf depression, were registered at the National Center for Biotechnology Information (NCBI).

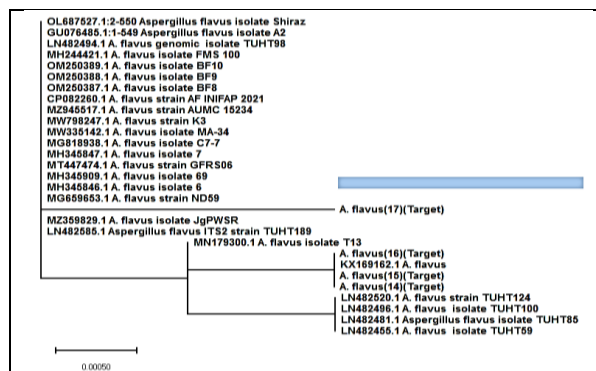


Fig. (4-27) The Neighbor-Joining tree showing the genetic relationship between *A. flavus* isolates (14, 15, 16 and 17) isolated from Bahar Al- Najaf depression , and other isolates of the same fungus registered at (National Center for Biotechnology Information) NCBI.

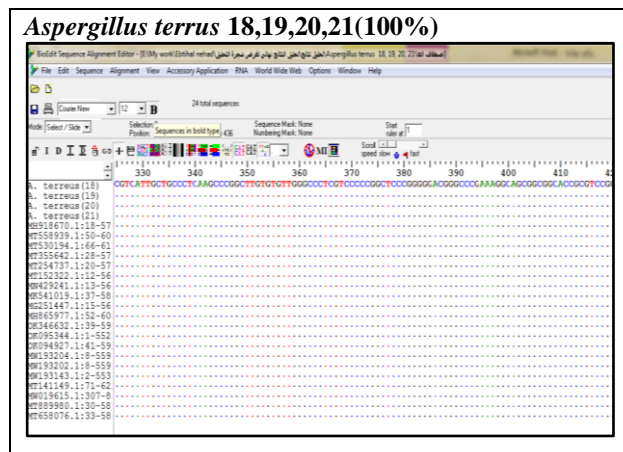


Fig.(4-28) Sequence alignments of *A. terreus* isolates (18, 19, 20, and 21) from the Bahar Al-Najaf depression and nitrogen base sequences of other isolates of the same fungus recorded at the National Center for Biotechnology Information (NCBI).were compared for similarities and differences



Figure (4-29) The Neighbor-Joining tree showing the genetic relationship between *A. terreus* isolates (18, 19, 20 and 21) isolated from Bahar Al- Najaf depression, and other isolates of the same fungus registered at (National Center for Biotechnology Information) (NCBI).

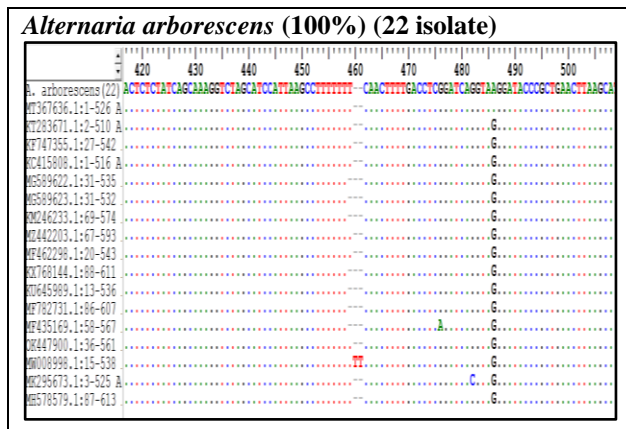


Fig.(4-30) Sequence alignments made from the isolate A's PCR result show similarities and differences. The same fungus is listed with the National Center for Biotechnology Information. (NCBI) arborescens (22) identified from the Bahar Al-Najaf depression, and the sequences of the other dependent isolates.

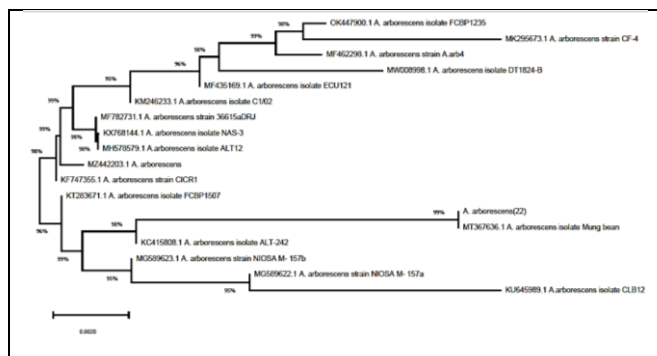


Fig. (4-31) Neighbor-Joining tree showing the genetic relationship between *A. arborescens* isolate (22) isolated from Bahar Al- Najaf depression, and other isolates of the same fungus registered at the National Center for Biotechnology Information (NCBI) .

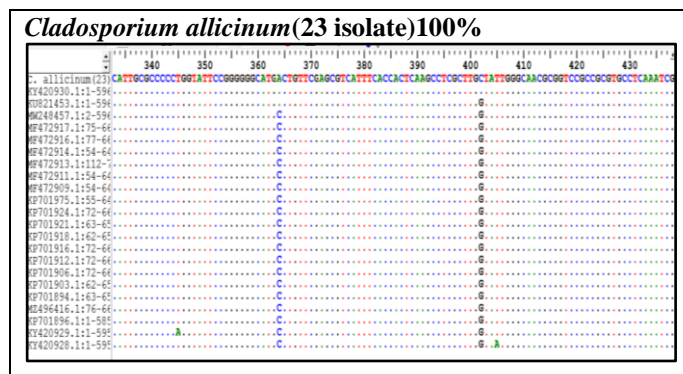


Fig.(4-32) Similarities and differences in one of the sequence alignments formed from the PCR product of the isolate *C. allicinum* (23) isolated from Bahar Al- Najaf depression, and the sequences of the other dependent isolates The same fungus is registered with the National Center for Biotechnology Information (NCBI).

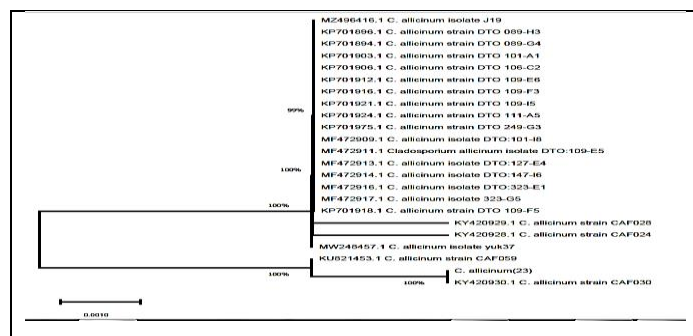


Fig. (4-33) Neighbor-Joining tree showing genetic relationship between the isolate of *C. allicinum* (23) isolated from Bahar Al- Najaf depression, and other isolates of same fungus registered in the National Center for Biotechnology Information (NCBI)

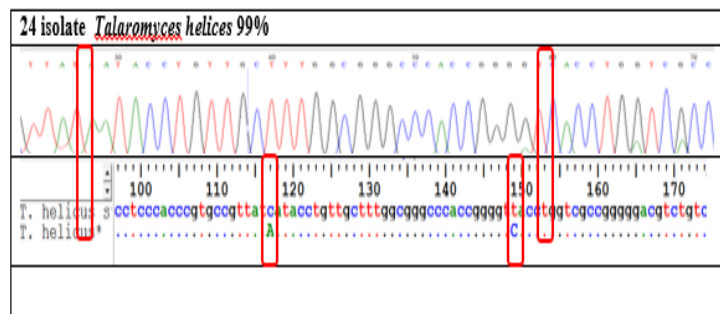


Fig.(4-34) The difference in one of the sites of the nitrogenous base sequence with the most closely related isolate of the fungus *T. helices* (24) previously recorded in the National Center for Biotechnology Information (NCBI).

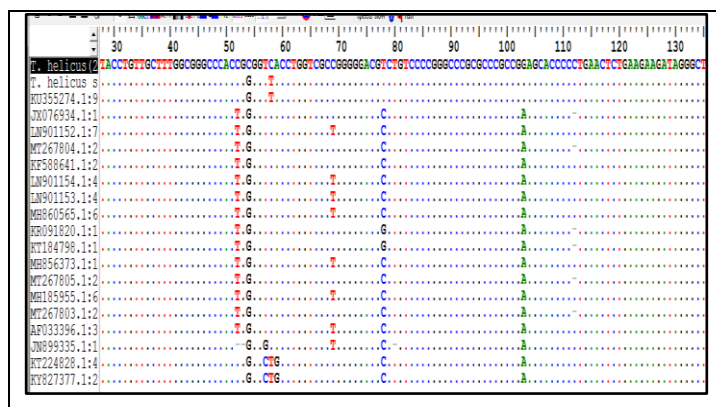


Fig.(4-35) Similarities and differences in one of the sequence alignments formed from the PCR product of the isolate *T. helices* (24) isolated from Bahar Al- Najaf depression, and the sequences of other dependent isolates The same fungus is registered with the National Center for Biotechnology Information (NCBI).

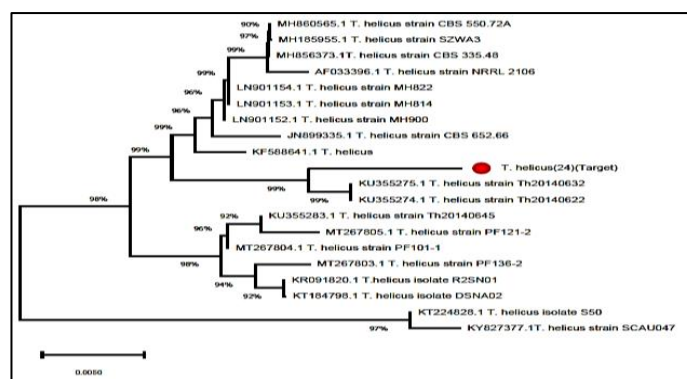


Fig. (4-36) Neighbor-Joining tree that showing genetic relationship between isolate of the fungus *T. helices* (24) isolated from Bahar Al- Najaf depression, and other isolates of same fungus registered in (National Center for Biotechnology Information) (NCBI) .

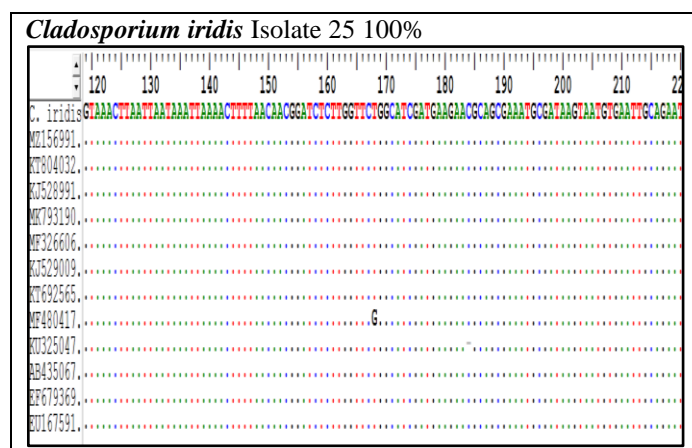


Fig.(4-37) Similarities and differences in one of the sequence alignments formed from the PCR product of the fungus *C. iridis* (25) isolated from Bahar Al- Najaf depression, and the sequences of the other isolates of the same fungus are registered with (the National Center for Biotechnology Information) (NCBI).

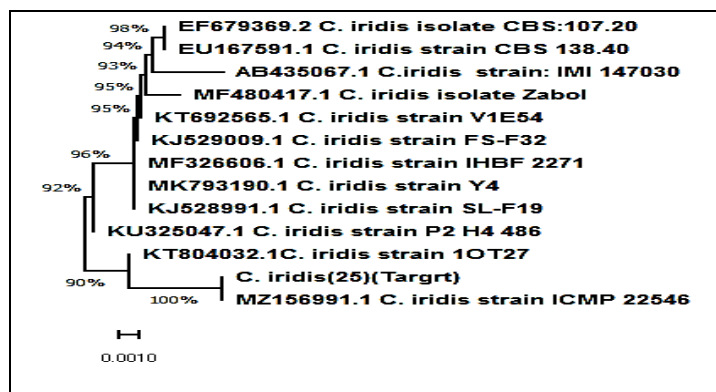


Fig. (4-38) Neighbor-Joining tree that showing genetic relationship between isolate of *C. iridis* (25) isolated from this study , and other isolates of same fungus registered in the National Center for Biotechnology Information (NCBI) .

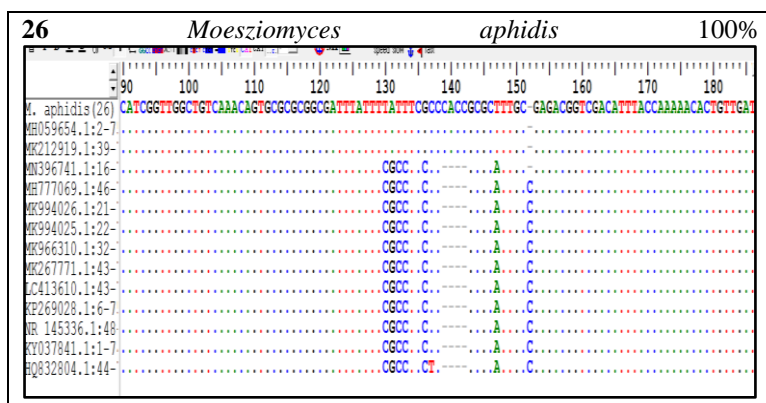


Fig. (4-39) Similarities and differences in one of the sequence alignments formed from the PCR product of the isolate *M. aphidis* (26) isolated from Bahar Al- Najaf depression, and the sequences of the other dependent isolates The same fungus is registered with (the National Center for Biotechnology Information) NCBI.

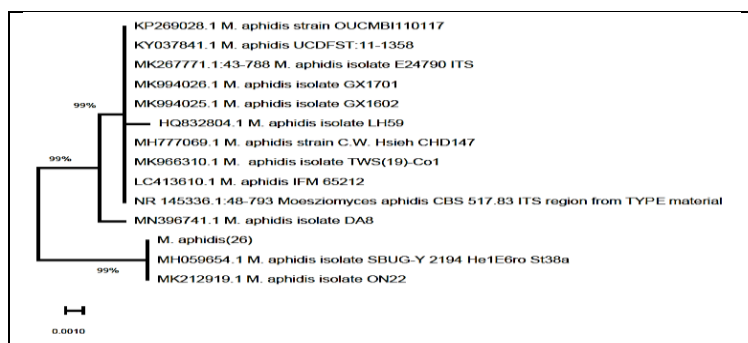


Fig.(4-40) Neighbor-Joining tree that showing genetic relationship between isolate of *M. aphidis* (26) isolated from this study, and other isolates of same fungus registered in the National Center for Biotechnology Information (NCBI) .

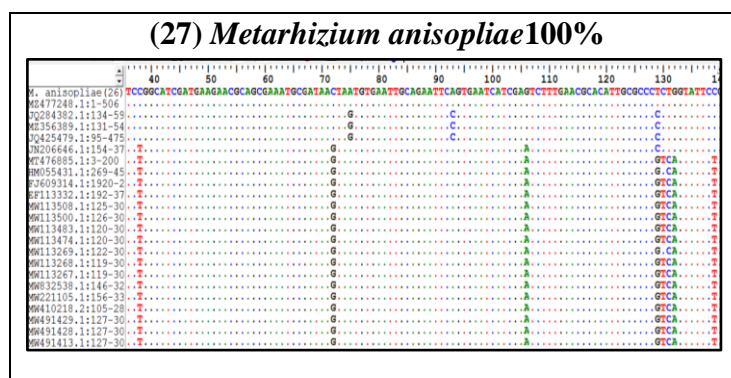


Fig.(4-41) Similarities and differences in one of the sequence alignments formed from the PCR product of the isolate *M. anisopliae* (27) isolated from Bahar Al- Najaf depression, and the sequences of other dependent isolates The same fungus is registered with (the National Center for Biotechnology Information) NCBI.

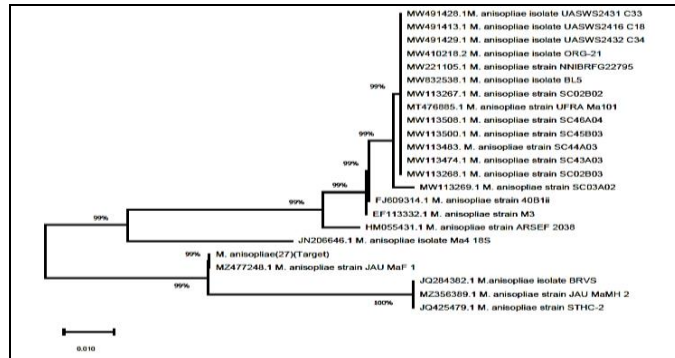


Fig. (4-42) Neighbor-Joining tree that showing genetic relationship between the isolate of *M. anisopliae* (27) isolated from Bahar Al- Najaf depression, , and other isolates of same fungus registered in (the National Center for Biotechnology Information) NCBI .

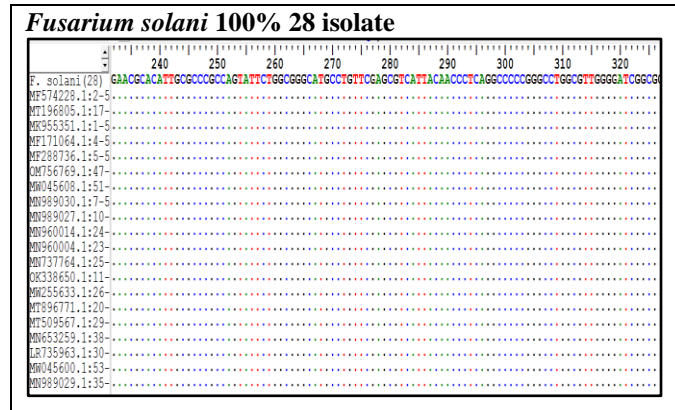


Fig.(4-43) Similarities and differences in one of the sequence alignments formed from the PCR product of the isolate *F. solani* (28) isolated from Bahar Al- Najaf depression, and the sequences of other dependent isolates The same fungus is registered with (the National Center for Biotechnology Information) NCBI.

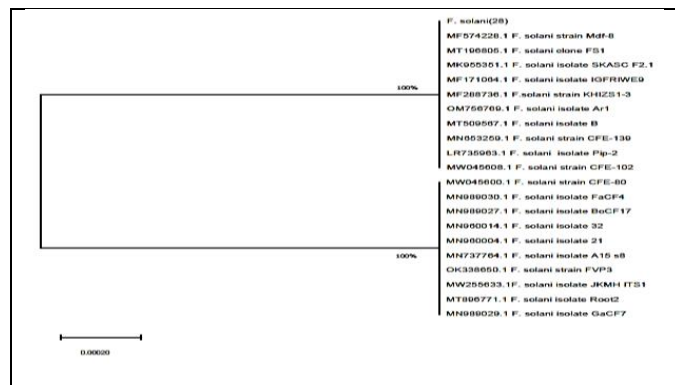


Fig. (4-44) Neighbor-Joining tree showing the genetic relationship between the isolate of *F. solani* (28) isolated in this study and other isolates of the same fungus registered in the National Center for Biotechnology Information (NCBI).

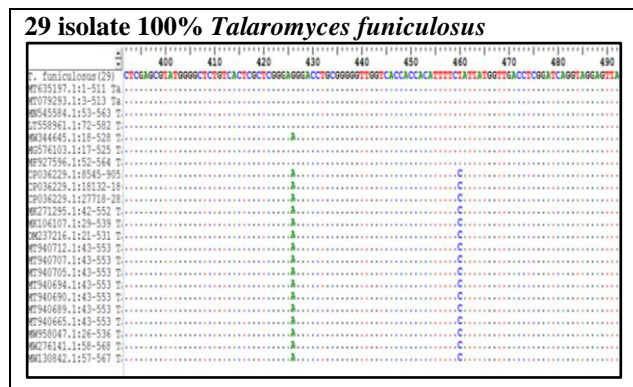


Fig. (4-45) Similarities and differences in one of the sequence alignments formed from the PCR product of the isolate of the fungus *T. funiculosus* (29) isolated from Bahar Al- Najaf depression, and the sequences of isolates. Others belonging to the same fungus are registered with (the National Center for Biotechnology Information) NCBI.

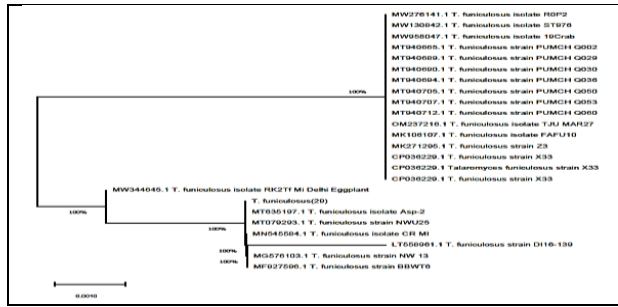


Fig. (4-46) Neighbor-Joining tree that showing the genetic relationship between the isolate of *T. funiculosus* (29) isolated from Bahar Al- Najaf depression, and other isolates of the same fungus registered at (the National Center for Biotechnology Information) (NCBI) .

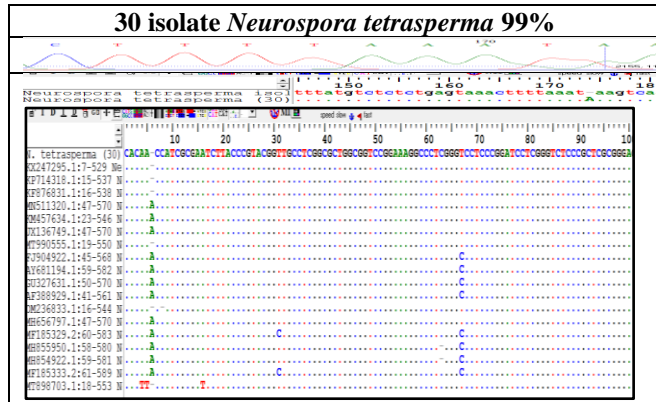


Fig. (4-47) Similarities and differences in one of the sequence alignments formed from the PCR product of the isolate of the fungus *N. tetrasperma* (30) isolated from Bahar Al- Najaf depression, and the sequences of isolates Others belonging to the same fungus are registered with the National Center for Biotechnology Information (NCBI).

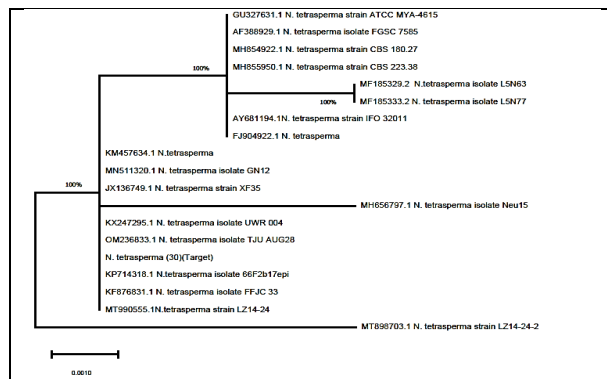


Fig. (4-48) Neighbor-Joining tree (showing the genetic relationship between *N. tetrasperma* isolate (30) isolated from Bahar Al- Najaf depression, and other isolates of the same fungus registered at (the National Center for Biotechnology Information) NCBI.

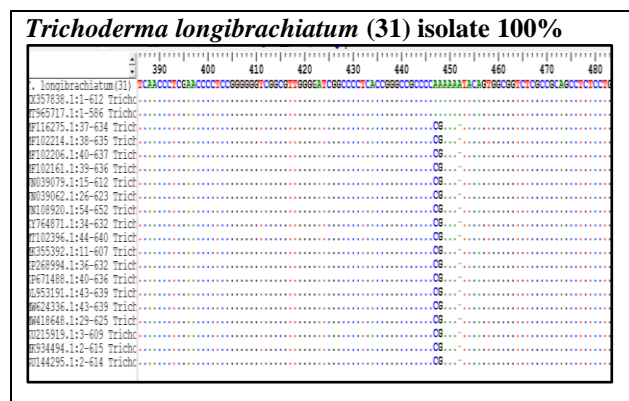


Fig (4-49) Similarities and differences in one of the sequence alignments formed from the PCR product of the isolate of the fungus *T. longibrachiatum* (31) isolated from Bahar Al- Najaf depression, and the sequences of isolates. Others belonging to the same fungus are registered with the National Center for Biotechnology Information (NCBI).

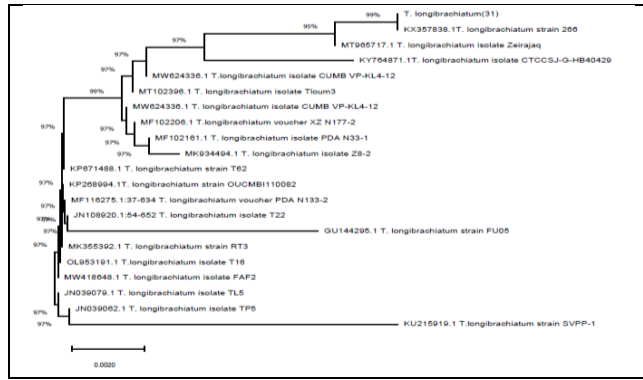


Fig. (4-50) Neighbor-Joining tree that showing the genetic relationship between the isolate of *T. longibrachiatum* (31) isolated from Bahar Al- Najaf depression, and other isolates of same fungus registered at the National Center for Biotechnology Information (NCBI).

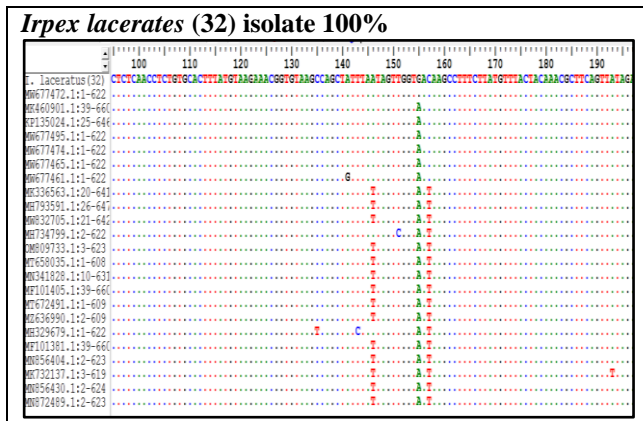


Fig.(4-51) Similarities and differences in one of the sequence alignments formed from the PCR product of the isolate of the fungus *I. lacerates* (32) isolated from Bahar Al- Najaf depression, and the sequences of isolates. Others belonging to the same mushroom are registered with (the National Center for Biotechnology Information) NCBI.

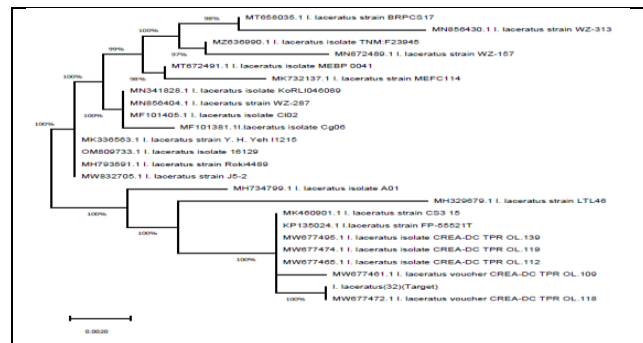


Fig. (4-52) Neighbor-Joining tree that showing the genetic relationship between *I. lacerates* isolate (32) isolated from Bahar Al- Najaf depression, and other isolates of the same fungus registered at (the National Center for Biotechnology Information) NCBI .

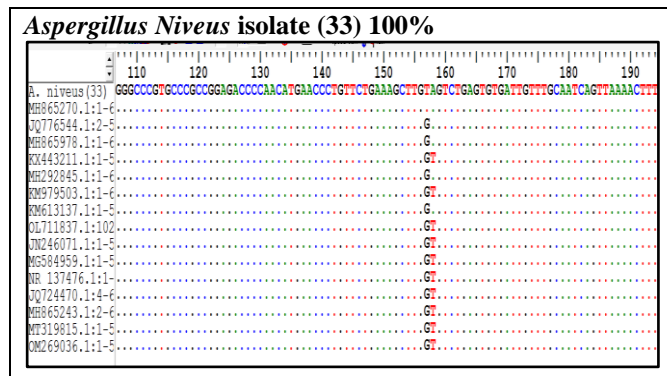


Fig. (4-53) Similarities and differences in one of the sequence alignments formed from the PCR product of the isolate of the fungus *A. niveus* (33) isolated from Bahar Al- Najaf depression, and the sequences of isolates. Others belonging to the same mushroom are registered with (the National Center for Biotechnology Information) NCBI.

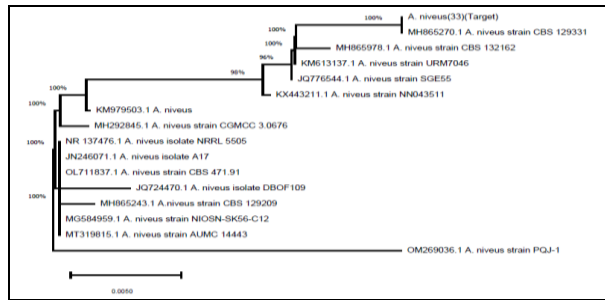


Fig.(4-54) Neighbor-Joining tree that showing the genetic relationship between the isolate of *A. Niveus* (33) isolated from Bahar Al- Najaf depression, and other isolates of the same fungus registered at (the National Center for Biotechnology Information) NCBI .

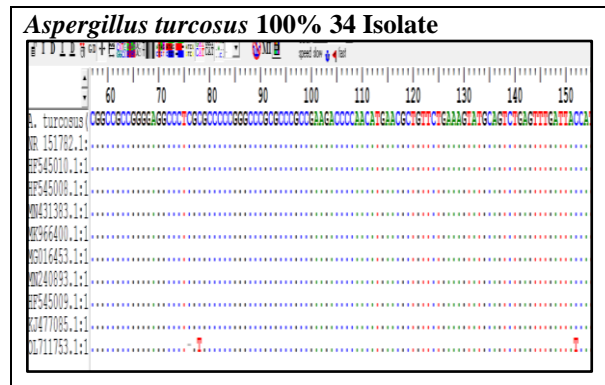


Fig.(4-55) : Similarities and differences in one of the sequence alignments formed from the PCR product of the fungus isolate *A. turcosus* (34) isolated from Bahar Al- Najaf depression, and the sequences of isolates Others belonging to the same fungus are registered with (the National Center for Biotechnology Information) NCBI.

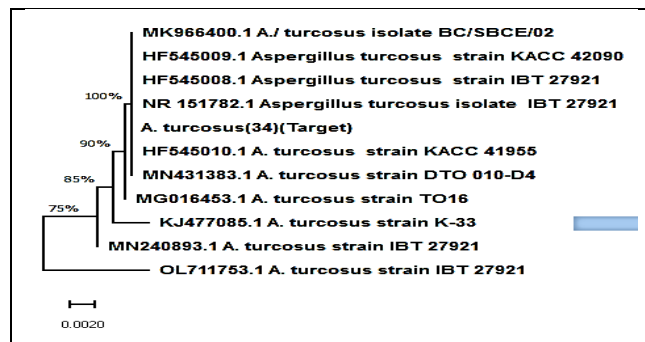


Fig. (4-56) The Neighbor-Joining tree shows the genetic relationship between the isolate of the fungus *A. turcosus* (34) The isolates from Bahar Al- Najaf depression, and other isolates of the same fungus registered in (the National Center for Biotechnology Information) NCBI.

Through the results and when isolating and diagnosing the fungi, it was found that the soil and water of the Najaf Sea contain large numbers of fungal organisms and that there is a high incidence of fungal diversity, which may be due to the fact that the water and soil in the study area are very suitable for the growth of microorganisms may be due to the physicochemical properties of the area and through Previous studies (Al-Khafaji & Al-Zurfi, 2018) and (Mutlag& hatimi ,2022) for the Bahr Al-Najaf region, which showed the presence of a percentage of nutrients, as well as the moderate pH of the soil, as well as the appropriate salinity, temperature and other characteristics that play a major role in the growth and inhibition of microorganisms.

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