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Isolation and Identification of Fungi from Some Aquatic Invertebrates in Baghdad Al- Jadryia

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ABSTRACT

The current study included the isolation and diagnosis of fungi from different places of the watercourse of Baghdad University in AL-Jadryia. The samples were collected twice a week for a period of three months, starting from October to December of the year 2022. The results showed average ranges of the air and water temperature with values of 20.6- 34 and 15.5- 24.5°C, respectively, while pH ranged from 7.4- 7.7; electrical conductivity ranges were 710- 714µS/ cm, and salinity ranged from 4.4- 4.5mg/ L. The highest percentage of genus identified from water samples was *Aspergillus niger*, comprising 18.9%. The isolation of adherent fungi on *Limnaea stagnalis* revealed a variety of fungal species, with the highest percentage being *Aspergillus* sp., accounting for 20.2%. The isolation of adherent fungi on the invertebrate *Spongilla lacustris* showed that *Aspergillus* sp. had the highest percentage, accounting for 27.1%. Furthermore, isolating fungi from inside the tissues of *Limnaea stagnalis*, after treating it with a chlorine solution diluted with water to remove all external microorganisms, revealed that *Achlya* sp. was the most prevalent genus, comprising 27.2%. The fungi obtained from inside *Spongilla lacustris* tissues showed that *Saprolegnia* sp. had the highest percentage, accounting for 17.5%. The current study aimed to identify the biological diversity of fungi that were isolated from the stream located in the Jadriyah area, which derives its water from the Tigris River from some types of invertebrates, as well as the possibility of using these types of invertebrates as traps and a natural alternative to cultural media.

I NTRODUCTION

A pond is a container for collecting and retaining water, typically an artificial water-filled depression that is low on the ground. It is created by digging a flat area or expanding a natural depression, which can be drained. One of the main characteristics of a pond is its ease of drainage **(Dudgeon, 2019)**. The primary purpose of ponds is to use the process of irrigation for crops, which depends on the management techniques employed. Additionally, ponds can be beneficial in providing aesthetic value to a location **(Osman *et al.*, 2013; Almond *et al.*, 2020)**. Ponds are home to a diverse range of living organisms, including invertebrate animals, aquatic plants, and microorganisms. This diverse community provides a suitable environment for many fungi to thrive, either freely or as parasites on invertebrate animals or plants **(Almond *et al*., 2020; Troppens *et al*., 2020)**.

The watercourse is supplied with water from the Tigris River, which brings in microscopic spores, larvae, and other organisms. With each filling, the pond is replenished with a variety of aquatic plants, small fish, amphibians, invertebrates, and microorganisms **(Mohammad & Habeb, 2016)**. Fungi are a significant group of organisms found in the watercourse, where water serves as a reservoir for these organisms **(Ekin & Şeşen, 2020; Al-Sheikhly, 2021)**.

Fungi can live freely, feeding on dead and decaying matter, as well as parasitizing organisms such as invertebrates that inhabit the aquatic environment. Some species, such as *Limnaea stagnalis* (Phylum: Mollusca, Class: Gastropoda, Family: Lymnaeidae) and *Spongilla lacustris* (Phylum: Porifera, Class: Demospongia, Order: Spongillida), provide a suitable habitat for fungi to grow and reproduce. Fungi can hide inside the snail's shell or live within its soft tissues, and they can also spread across sponges **(Amend *et al*., 2019; Safaei-Mahroo & Ghaffari, 2021)**. As heterotrophic organisms, fungi do not produce their own food and rely on organic matter in water. They can also form symbiotic relationships with other organisms, such as plants and animals, and use them as hosts for growth and reproduction. In some cases, fungi can even cause death **(Ann *et al*., 2019; Al-Hayanni *et al*., 2022)**. Environmental factors, such as temperature, oxygen levels, and minerals can encourage the growth and diversity of fungi in the region. The study aimed to review the fungal diversity in the region and compare the species emerging from water or from parasitizing certain types of invertebrates, viz. sponges and snails.

MATERIALS AND METHODS

1. Ecological measurement

a. Temperature: Air and water temperatures were measured by using mercuric thermometer.

b. pH: pH was measured by using a pH-meter HANNA -portable.

c. Electrical conductivity: Conductivity was measured by using conductivity- meter HANNA -portable.

d. Salinity: The salinity was calculated through the equation (Salinity % = E.C 14.78/ 1589.08) **(APHA, 2005)**.

e. Collection of samples: Samples were collected twice a week for a period of three months, starting from October to December 2022, from different locations of the watercourse of Baghdad University in Al-Jadriya, at a depth of 25- 100cm below the water surface **(Nashaat *et al.*, 2015; Mohammad & Habeb, 2016)**. The samples were collected using sterile, hermetically sealed plastic bottles of 100mL capacity, which were opened underwater and then sealed tightly after sampling. The samples were placed in a refrigerated box and transported to a laboratory for testing **(Ekin & Şeşen, 2020; Mohammad, 2023)**.

2. Snails and sponge collection

Samples were collected from the walls of the watercourse by manual excavation and hand shoveling to a depth of 100cm. The snails were placed on the culture medium in their entire shells, while small portions of sponge bodies were cut and prepared for isolation on petri dishes for adherent fungal checks. For internal fungal examination, small portions were taken from the internal tissues of both species **(Mohammad & Habeb, 2016; Noor *et al*., 2023)**.

3. Isolation of fungi

Two methods were used to isolate the fungus from the water: Initially, water samples were isolated from the insulation site. To isolate aquatic fungi that infect invertebrates (snails: *Limnaea stagnalis* and sponge: *Spongilla lacustris*) in the water, a method of baiting was employed using insect parts after sterilization with a closed device. The insects were used due to their high protein content. The samples were then distributed to sterile grafts at 1mL of sample water and 3 replicates for each sample on sterile petri dishes. To eliminate bacterial contamination, 1.5- 2mg/ L of chloramphenicol antibiotic was added to each dish, prepared by dissolving 250mg/ L of antibiotic in 250mL of water. The dishes were incubated for 7 days at a temperature of 25°C in an incubator. After incubation, the parts of insects infected with fungi were transferred to petri dishes containing potato dextrose agar and also incubated for 7 days at a temperature of 25°C in an incubator to diagnose the fungi resulting from the isolation and purification process. The properties and diagnosis of the isolated fungi were studied using a taxonomic key for fungi **(Ann *et al*., 2019; Yu *et al*., 2021)**.

The second method involved taking invertebrate animals (snails: *Limnaea stagnalis* and sponge: *Spongilla lacustris*) from the isolation site and subjecting them to two treatments. In the first treatment, the invertebrates were cut into small parts using a scalpel and sterile scissors and placed directly in petri dishes containing potato dextrose agar. The petri dishes were then incubated at a temperature of 25°C for 7 days to study the characteristics of the fungus **(Babič *et al*., 2016)**. The second treatment was used to diagnose fungi that adhere to the invertebrate animal. In this treatment, the invertebrates were sterilized using a chlorine solution at a ratio of 1:3 (chlorine:sterile water) for 2 minutes. After sterilization, the invertebrates were washed three times with gently distilled water to remove any residual chlorine. This method was used to obtain fungi that infect the tissues of an invertebrate animal. The sterilized invertebrates were then placed in petri dishes containing potato dextrose agar and incubated at a temperature of 25°C for 7 days to study the characteristics of the fungus **(Khalil, 2020; Troppens *et al*., 2020)**.

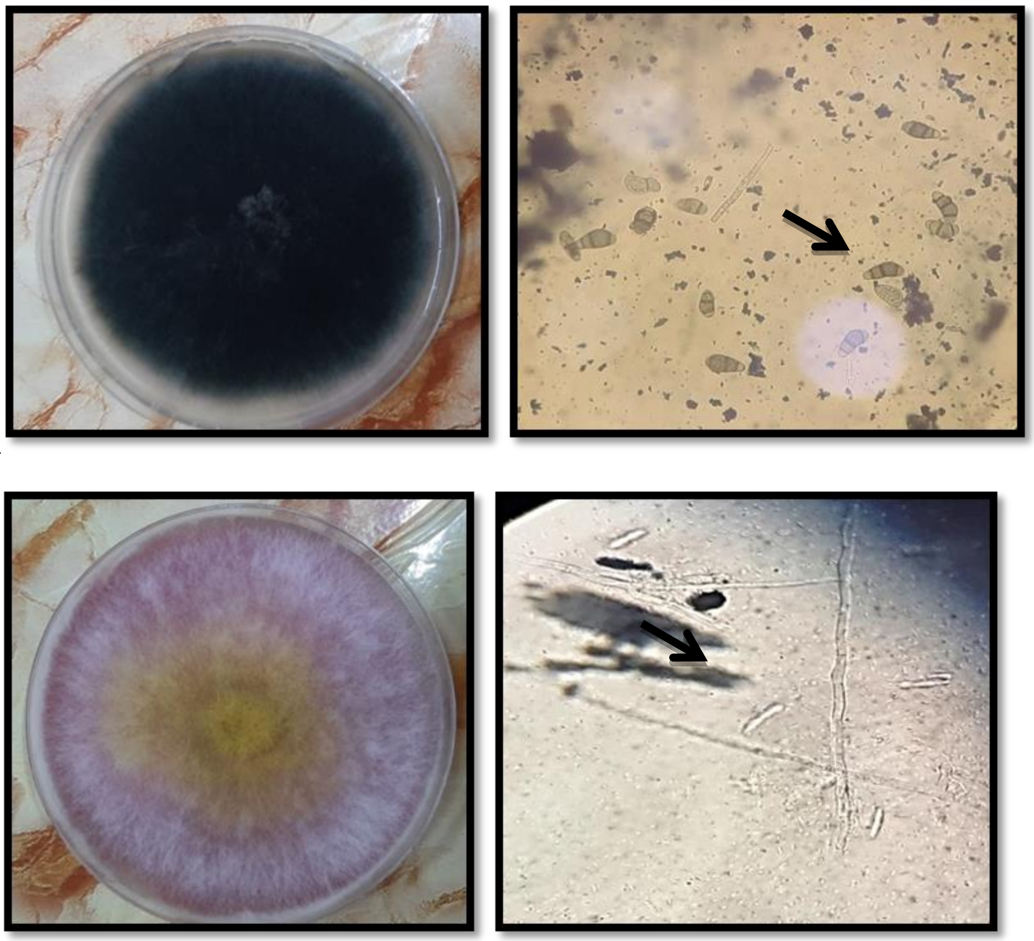
Calculation of the percentage frequency of fungi

The percentage of the appearance of the genera of fungi isolated from the collection site and for all coefficients was calculated using the following equation **(SAS, 2020)**:

$$\text{Frequency percentage} = \frac{\text{Number of fungi isolates}}{\text{Number of total isolates of all fungi}} * 100$$

RESULTS

The results of the ecological measurements for the current study revealed that the average temperature ranges were 20.6- 34°C for air and 15.5- 24.5°C for water, with pH values ranging from 7.7 to 7.4. The electrical conductivity ranged from 710 to 714µS/ cm, and the salinity was between 4.4 and 4.5mg/ L. The results of fungal isolation showed that many fungal species were found in the waters and diagnosed invertebrates, with a frequency percentage determined as folllows: *Aspergillus niger* 18.9%, *A. flavus* 15.9%, *Alternaria alternate* 12.4%, *Mycelia sterilia* 11.2%, *Mucor* sp. 10.6%, *Penicillium* sp. 9.4%, *Trichoderma* sp. 7.6%, *Fusarium solani* 6.5%, *Ulocladium* 4.1%, and *Phoma* 2.9%. Fig. (1) and Table (1) illustrate the various forms of fungi that were isolated from the water.



A

B

Fig. 1. Some types of fungi isolated from water on 40X and on potato dextrose agar **A.** *Alternaria Alternate* and **B.** *Fusarium Solani*

Table 1. Frequency of fungal genera isolated from waters of the Al-Jadriyah

No.	Fungal genera	No. of isolate	Frequency %
1.	*Aspergillus niger*	32	18.9%
2.	*A. flavus*	27	15.9%
3.	*Alternaria alternate*	21	12.4%
4.	*Mycelia sterilia*	19	11.2%
5.	*Mucor sp.*	18	10.6%
6.	*Penicillium sp.*	16	9.4%
7.	*Trichoderma sp.*	13	7.6%
8.	*Fusarium solani*	11	6.5%
9.	*Ulocladium*	7	4.1%
10.	*Phoma*	5	2.9%
	Total isolate: 169		

The results of the fungal isolation from snails *Limnaea stagnalis* also revealed a group of fungi with the following frequency percentage: *Aspergillus* sp. 20.2%, *Pythium* sp. 18%, *Rhizopus* 17%, *Calyptralegnia* 14.8%, *Cladosporium* 11.7%, *Saprolegnia* sp. 9.5%, and *Achlya* 8.5%. Fig. (2) and Table (2) illustrate these findings.

Table 2. Frequency of fungal genera isolated from adherent fungi on *Limnaea stagnalis* of the Al- Jadriyah

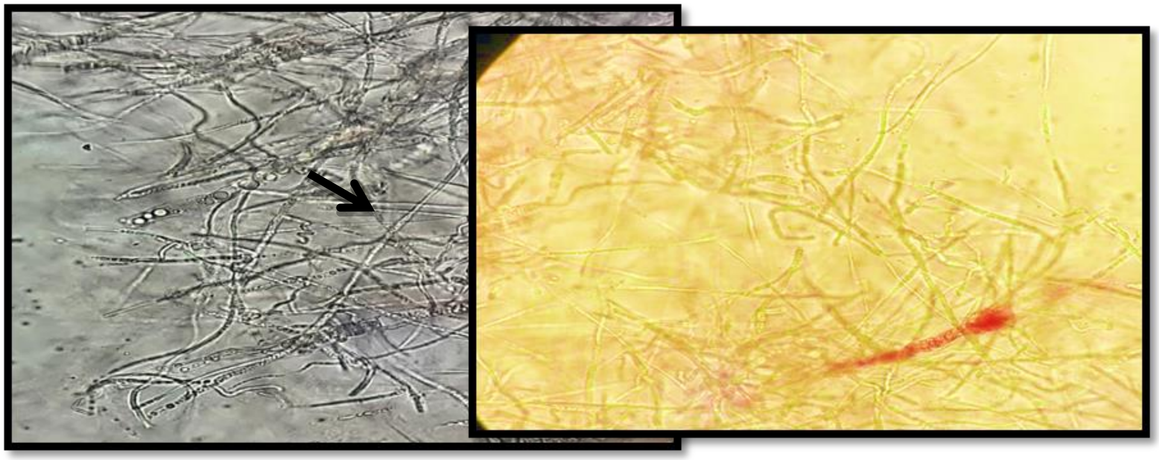
No.	Fungal genera	No. of isolate	Frequency %
1.	*Aspergillus* sp.	19	20.2%
2.	*Pythium* sp.	17	18%
3.	*Rhizopus*	16	17%
4.	*Calyptralegnia*	14	14.8%
5.	*Cladosporium*	11	11.7%
6.	*Saprolegnia sp.*	9	9.5%
7.	*Achlya*	8	8.5%
		Total isolate:94	

The results of the fungal isolation from the sponge *Spongilla lacustris* revealed a group of fungi with the following frequency percentage: *Aspergillus* sp. 27.1%, *Rhizopus* 22.2%, *Cladosporium* 20.9%, *Ulocladium* sp. 16.2%, and *Phoma* sp. 13.5%. Table (3) displays these findings.

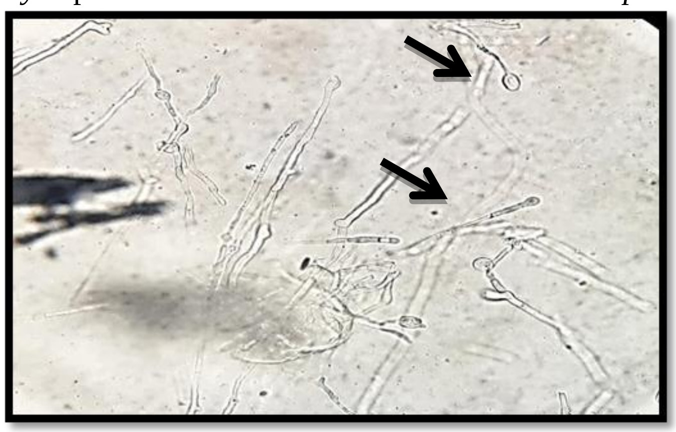
Table 3. Frequency of fungal genera isolated from adherent fungi on *Spongilla Lacustris* of the Al-Jadriyah

No.	Fungal genera	No. of isolate	Frequency %
1.	*Aspergillus* sp.	22	27.1%
2.	*Rhizopus* sp.	18	22.2%
3.	*Cladosporium* sp.	17	20.9%
4.	*Ulocladium* sp.	13	16.2%
5.	*Phoma* sp.	11	13.5%
		Total isolate:81	

The results of isolating fungi from the internal tissues of snails *Limnaea stagnalis* following treatment with a chlorine solution diluted with water and removal of external microorganisms to obtain fungi within the invertebrate tissues revealed the following percentages: *Saprolegnia* sp. 17.5%, *Achlya* sp. 16.2%, *Scopulariopsis* sp. 13.7%, *Calyptralegnia* sp. 12.5%, *Aspergillus niger* 11.2%, *A. flavus* 8.7%, *A. terreus* 7.5%, *Cladophora* sp. 5%, *Alternaria alternate* 3.7%, *Rhizopus stolonifer* 2.5%, and *Zygosaccharomyces bailii* 1.25%. Fig. (3) and Table (4) exhibit these findings.



A. *Achlya* sp. **B.** *Saperolegnia* sp.



C. *Pythium* sp.

Fig. 2. Some forms of adherent fungi on invertebrate animals on 40X

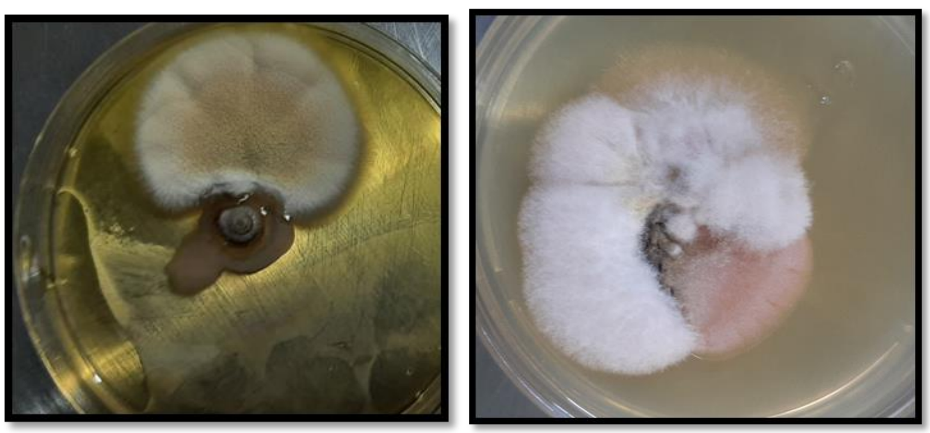
Table 4. Frequency of fungal genera isolated from fungi from the tissues of the *Limnaea Stagnalis* of the Al-Jadriyah

No.	Fungal genera	No. of isolate	Frequency %
1.	*Saprolegnia* sp.	14	17.5%
2.	*Achlya* sp.	13	16.2%
3.	*Scopulariopsis* sp.	11	13.7%
4.	*Calypeterlegnia* sp.	10	12.5%
5.	*Aspergillus niger*	9	11.2%
6.	*A. flavus*	7	8.7%
7.	*A. terreus*	6	7.5%
8.	*Cladophora* sp.	4	5%
9.	*Alternaria alternate*	3	3.7%
10.	*Rhizopus stolonifer*	2	2.5%
11.	*Zygosaccharomyces bailii*	1	1.25%
		Total isolate: 80	

The results of isolating fungi from the internal tissues of *Spongilla lacustris*, aiming at obtaining fungi within the invertebrate tissues, revealed the following percentages: *Achlya* sp. 27.2%, *Scopulariopsis* sp. 16.3%, *Yeast* sp. 16.3%, *Aspergillus* sp. 12.7%, *Trichoderma* sp. 10.9%, *Rhizopus* sp. 7.2%, *Penicillium* sp. 5.4%, and *Fusarium* sp. 3.6%. Fig. (3) and Table (5) illustrate these findings.

Table 5. Frequency of fungal genera isolated from fungi from the tissues of *Spongilla Lacustris* of the Al-Jadriyah

No.	Fungal genera	No. of isolate	Frequency %
1.	*Achlya* sp.	15	27.2%
2.	*Scopulariopsis* sp.	9	16.3%
3.	Yeast sp.	9	16.3%
4.	*Aspergillus* sp.	7	12.7%
5.	*Trichoderma* sp.	6	10.9%
6.	*Rhizopus sp.*	4	7.2%
7.	*Penicillium* sp.	3	5.4%
8.	*Fusarium* sp.	2	3.6%
		Total isolate: 55	



A B

Fig. 3. Some of fungi that infect the internal tissues of **A.** Snails: *Limnaea stagnalis*, and **B.** sponge: *Spongilla lacustri*

DISCUSSION

The study of fungal biodiversity in the watercourse of the Jadriya area, which is sourced from the Tigris River in Baghdad, is scarce, and research on the adhesion of fungi and their parasitism on the internal tissues of invertebrate animals is almost non-existent. The current study revealed the emergence of free-living fungal species and the occurrence of fungal colonies in the water environment, which contain a group of aquatic plants and invertebrate organisms. This finding is consistent with the results of **Barlocher *et al.* (2008)**, who elucidated a high percentage of fungal species appearing in water samples. These species are originated from temporary sources, resulting from air currents bringing fungal spores to the water surface, or from habitats within the water. Additionally, they could have been introduced or adhered to plants and animals in the water, and their spores were dispersed when they matured in water. This is also consistent with the findings of **Amend *et al.* (2019)**.

The current study coincides with that of **Babič *et al.* (2016)**, as the former reported the adhesion of fungi to certain types of invertebrates isolated from the same area. Specifically, the study found that the number of fungi adhering to *Limnaea stagnalis* increased by 94 isolates, while the results of isolating adherent fungi on *Spongilla lacustris* showed the presence of 81 isolates of adherent fungi, as shown in Fig. (4) **(Goldani & Wirth, 2017; Duarte, 2019)**. The increase in the number of fungus species adhering to the outer layer of the snail is attributed to fungal symbiosis on the snail and the greater susceptibility of the fungus to adhere to the surface of the snail compared to the sponge **(Kadhim, 2013; Rana *et al.*, 2022)**.

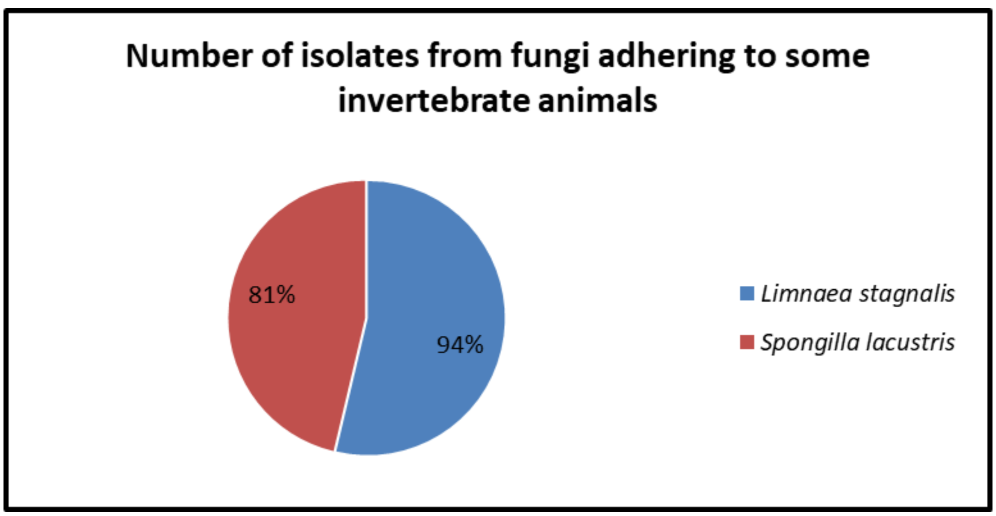


Fig. 4. The percentage isolates of adherent fungi

The results of the study on the presence of fungi inside the tissues of certain invertebrates isolated from water in the Jadriyah area revealed that 80 types of fungi were isolated from within the tissues of *Limnaea stagnalis*, while 55 types of fungi were isolated from within the tissues of *Spongilla lacustris* (Fig. 5).

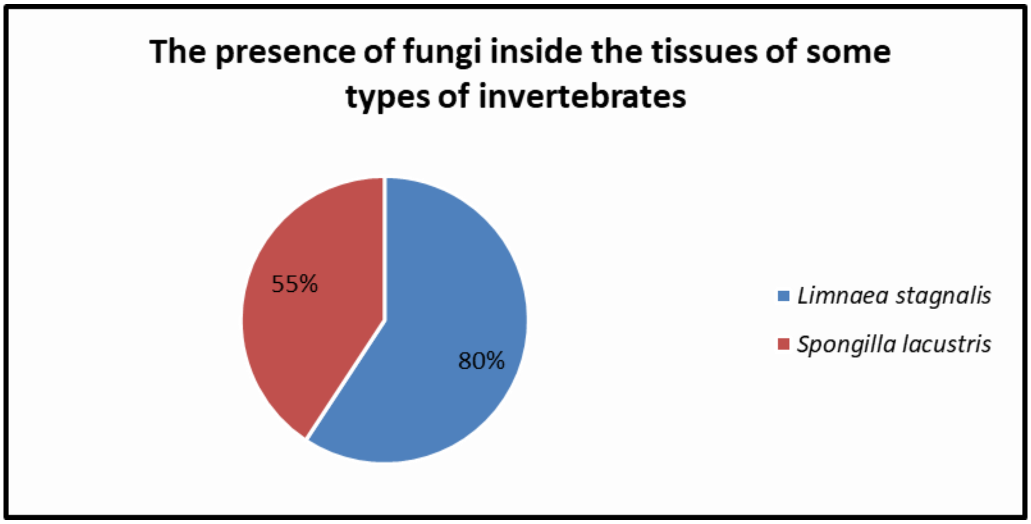


Fig. 5. The percentage isolates of fungi inside tissues

The presence of a greater number of fungal types in snails is attributed to the presence of nutrients within the tissues, which provide all the necessary growth requirements for the fungi. This is consistent with the findings of previous studies **(El-Khayat *et al*., 2018; Saad *et al*., 2019)**.

CONCLUSION

The study confirmed the feasibility of using snails as a trap bait to obtain a variety of fungal species and to assess the biological diversity of fungi in river water. Snails can also be used as a natural medium for fungal growth, either in their natural habitat or after sterilization in a controlled device, and can be employed as a substrate for fungi that parasitize tissues in the laboratory.

REFERENCES

Al-Hayanni, H.S.A.; Marwah, T.H.A.; Al-lami, R.A.H. and Zaboon, S.M. (2022). Antibacterial Effect of Silver Nanoparticles Prepared from *Sophora flavescens* Root Aqueous Extracts against Multidrug-resistance *Pseudomonas aeruginosa* and *Staphylococcus aureus*. J Pure Appl Microbiol. 16(4):2880-2890. doi: 10.22207/JPAM.16.4.61.

Almond, R.E.A.; Grooten, M. and Peterson, T. (2020). Living Planet Report Bending the Curve of Biodiversity Loss; World Wildlife Fund: Gland, Switzerland.

Al-Sheikhly, O. F. (2021). The Avifauna of Tigris and Euphrates River Basin. pp.913-935. In: Jawad, L. A. (ed), Tigris and Euphrates Rivers: Their Environment from Headwaters to Mouth. Springer, Cham.

Amend, A.; Burgaud, G.; and Cunliffe, M. (2019). Fungi in the marine environment: Open questions and unsolved problems. Ecol. Evol. Sci. 10: e01189-18.

Ann, V.; Freixa, A.; Butturini, A.; Romaní, A.M. (2019). Interplay between sediment properties and stream flow conditions influences surface sediment organic matter and microbial biomass in AMediterranean River. Hydrobiologia. 828(1):199-212.

APHA, (2005). American Public Health Association. Standard Methods for the Examination of Water and Wastewater. 21st ed. American Public Health Association, Washington DC, 1220p.

Babič, M.N.; Zalar, P. and Ženko, B. (2016). Yeasts and yeastlike fungi in tap water and groundwater, and their transmission to household appliances. Fungal Ecol. 20: 30-39.

Barlocher, F.; Seena, S.; Wilson, K. and Williams, D.D. (2008). Water temperature lowers diversity of hyporheic aquatic hyphomycetes. Freshwater Biol 53: 368–379.

Duarte, S.; Antunes, B. and Trabulo, J. (2019). Intraspecific diversity affects stress response and the ecological performance of a cosmopolitan aquatic fungus. Fungal Ecol. 41: 218-223.

Dudgeon, D. (2019). Multiple Threats Imperil Freshwater Biodiversity in the Anthropocene. Curr. Biol. 29, R960–R967.

Ekin, I. and Şeşen, R. (2020). First record of the pest and parasitic intermediate hostsnail Cochlicella barbara (Linnaeus, 1758) in the south -eastern Anatolia.International Journal of Agriculture, Environment and Food Sciences 4(1): 57-61.

El-Khayat, H.M.M.; Abd-Elkawy, S.; Abou- Ouf, N.; Ahmed, M. and Mohammed, W. (2018). Biochemical and histological assessment of some heavy metals on Biomphalaria alexandrina snails and fish in Lake Burullus, Egypt. Egyp J of Aqu Bio Fish., 22(3): 159- 182.

Goldani, L.Z. and Wirth, F. (2017). Animal Models and Antifungal Agents in Paracoccidioidomycosis: An Overview.Mycopathologia. 182, 633–643.

Kadhim, N.F.; Al-Amari, M.J.Y. and Hassan, F.M. (2013). The Spatial and temporal distribution of Epipelic algae and related environmental factors in Neel Stream, Babil Province, Iraq. International Journal of Aquatic Science, 4:23-32.

Khalil, M.I. (2020). Identification of *Cladosporium sp*. Fungi by in-silico RFLP-PCR. Baghdad Sci. J. Jan 2;17(1):220-6.

Mohammad, T. H. and Habeb, K.A. (2016). Survey of Keratinophilic Fungi in the Tigris River in Baghdad. B. Sc. Journal, 13(3); 449- 453.

Mohammad., T.H. (2023). A Comet Assay for *Candida glabrata* Infection and Treatment by Penicillium chrysogenum - Derived AgNPs. Iraqi Journal of Agricultural Sciences –2023:54(4):950- 956. https://www.researchgate.net/publication/ 373507404.

Nashaat, M.R.; Abbas, E.K.; Ali, E.H. and Moftin, F.S. (2015). Impact of Al-Rasheed power plant effluents on cladocera fauna biodiversity in Tigris River, Southern Baghdad. lraqi Journal of Biotechnology, 14(2): 243-254.

Noor, N. J.; Rasha, M. A. A.; Mohammad, T. H and Hussein, R. A. (2023). Synergistic Treatment of *Candida albicans* Infected Mice by AgNPs Synthesis Penicillium chrysogenum. Bionatura 1: 8 (1).

Osman, G.Y.; Mohamed, A.M.; Abdel Kader, A. and Mohamed, A.A. (2013). Biological and biochemical impacts of the fungal extract of *Aspergillus fumigatus* on *Biomphalaria alexandrina* snails infected with *Schistosoma mansoni*. Biosci., 7 (12):473-485.

Rana, A.H.A.; Al-Hayanni H.S.A; and. Shehab, Z.H. (2022). Molecular Investigation of Some Beta-lactamase Genes by PCR and DNA Sequencing Techniques in clinical *Escherichia coli*. Iraqi Journal of Science, 2022, Vol. 63, No. 10, pp: 4205-4212DOI: 10.24996/ijs.2022.63.10.7.

Saad, A.A.; Azzam, M.M.; Mostafa, B.B.; El-Said, M.K. and Hanafy, A.R. (2019). Improvement molluscicidal activity of Anagalis arvensis extracted by copper oxide nanoparticles against Biomphalaria alexandrina snails. Egy J of Aqu Biol Fish., 23(3): 27-41.

Safaei-Mahroo, B. and Ghaffari, H. (2021). The Amphibians and Reptiles of Euphratesand Tigris Basin. pp. 975-1005. In: Jawad, L.A. (ed.), Tigris and Euphrates Rivers: Their Environment from Headwaters to Mouth. Springer, Cham.

SAS. (2020). Statistical Analysis System, User's Guide. Statistical, Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.

Tamim, M.; Tahany, M.; Abdel-Rahman, M.N.M. and Abdel-Ghany, K.A. (2023). consortium effect of Jatropha curcas seed husk and its endophyte *Aspergillus niger* on biosorption of manganese and nickel from wastewater. Inter J Env Stu in press doi: 10.1080/00207233.2022.2160591.

Yu, R.; Liu, J.; Wang, Y.; Wang, H. and Zhang. H. (2021). *Aspergillus niger* as a secondary metabolite factory. Frontiers in chemistry, 8:1-12 doi: 10.3389/fchem.2021.701022.