The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(1): 114-124, 2025 www.thebioscan.com

Traditional isolation of fungal species in fish culture ponds

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DOI: https://doi.org/10.63001/tbs.2025.v20.i01.pp114-124

Keywords:

Aquaculture ponds, Water contamination, Mycotic infections, Iraq

Received on:

13-11-2024

Accepted on:

11-12-2024

Published on:

18-01-2025

ABSTRACT

Background: In Iraq, fish ponds have increasingly grown in last decade as important source of protein to maintaining of human healthy; however, contamination of water is a serious threat to aquaculture systems causing severe economic losses.

Aim: Traditional identification of the main fungal pathogens in fish ponds existed in different areas in Wasit province (Iraq)

Materials and methods: Totally, 148 water samples were collected equally from 37 fish ponds located in five main areas (Al-Kut, Al-Suwaira, Al-Aziziyah, Al-Numaniyah, and Al-Hay) in Wasit province (Iraq) during June-July (2024). Then, all samples were filtered, cultured using three different media; Sabouraud Cycloheximide Chloramphenicol Agar, Potato Dextrose Agar, and Malt Extract Agar, and the grown colonies of fungal isolates were purified and characterized morphologically and microscopically.

Results: An overall 75.68% water samples and 83.78% of fish ponds were showed contaminated with at least one fungus. Regarding study areas, fungal contamination of water samples was increased significantly in Al-Hay and decreased significantly in Al-Numaniyah and Al-Suwaira; whereas in fish ponds of study areas, significant increases of fungal contamination was shown in Al-Aziziyah, Al-Numaniyah, Al-Hay, and Al-Kut; but decreased in Al-Suwaira. Also, a total of 12 different species and genera were detected among the study samples, which involving 382 fungal colonies; in which, significant increases were reported in *Aspergillus niger* and *Penicillium* sp.; while significant decreases were seen in *Trichophyton mentagrophytres*, *Helminthosporium* sp., *Mucor* sp., and *Trichoderma* sp. when compared to values of other fungal colonies; *Cladosporium* sp., *Rhizopus stolonifer*, *Microsporium cani*, *Fusarium solani*, *Candida albicans*, and *Alternaria alternate*. Among study areas, significant increases of fungal colonies were detected in Al-Kut and reduced in Al-Aziziyah. In relation to each area, significant increases in percentage of fungal colonies were seen in *Helminthosporium* sp. in Al-Kut; *Alternaria alternate* in Al-Suwaira; *Trichophyton mentagrophytres* in Al-Aziziyah; *Trichoderma* sp. in Al-Numaniyah; and *Fusarium solani* and *Microsporium cani* in Al-Hay.

Conclusions: Our findings revealed the presence of apparent fungal contamination in fish ponds existed in Wasit province suggesting the importance of national studies to investigating and controlling the fungal contamination and their diseases to ensure continued high-quality growth of fish in aquaculture production industry.

Introduction

they can cause a range of illnesses, from skin infections to life-threating respiratory conditions (Novak Babič et al., 2017; Fisher et al., 2020). However, fungi in water can disturb the biosynthesis of native organisms and hence result in the invasion of new fungi species that make the problem even worse (Reid et al., 2019; Barros and Seena, 2022). For instance, it was approximated that 580 Indians die from water pollution associated diseases every single day (Kavitha and Dhandapani, 2018). This impact is worst where there is scarcity of clean water and also poor infrastructure of sanitation where much

The preservation of clean, uncontaminated water sources is a crucial concern for public health and environmental sustainability (Sharma et al., 2024). One significant threat to water quality is fungal contamination, which can have far-reaching consequences on human and ecosystem health (Mustafa and Hassan, 2024). Fungal contamination of water can arise from various sources including industrial waste, agriculture runoff and improperly treated sewage (Singh et al., 2022). This contamination can lead to the introduction of pathogenic fungi, which poses a serious threat to human health, as vulnerable populations are disproportionately affected (Nasim et al., 2022; Scanlon et al., 2023). In order to solve this important problem it is necessary to implement a bio-psychosocial-socio-political model of care which implies scientific evidence, interventions and practices at the national and international levels, and community approaches (Mooney et al., 2020; Maat et al., 2021; Chan et al., 2023). Some of the technological advancements like the use of smart sensors and advanced purification system may appear to have direct contribution towards detection as well as removal of the identified fungal contaminants (Wang et al., 2019; Rathi et al., 2021; Rodríguez-Hernández et al., 2022). Furthermore, the enhancement of water resource management policies and encouraging efficient and sustainable farming methods may also useful to prevent fungal-pollutants from getting introduced to the water sources (Srivastav et al., 2021; Akinsemolu et al., 2024).

Fish ponds or otherwise called aquaculture ponds are today common in many parts of the world as producers of fish for both the local and export markets (Drozdz et al., 2020; Naylor et al., 2021). Aquaculture industries all over the world have expanded tremendously within the last decades and expect to continue expanding as consumption rate of fish rises. (Delgado et al., 2003; Garlock et al., 2020). One of the primary drivers behind the rise of aquaculture and the proliferation of fish ponds is the stagnation in the production of capture fisheries which have struggled to keeping up with the growing of worldwide population and its corresponding appetite for fish products (Einarsson and Óladóttir, 2020; Maulu et al., 2024). Fish ponds, on the other hand, has demonstrated their ability to rapidly scale up yielding, making it an attractive option for meeting the world's growing protein demands (Kim et al., 2019; Boyd et al., 2020). Although several studies have been conducted, in various areas in Iraq, to investigate the fungal contaminations (Al-Musawi et al., 2021; Ghafouri and Alhamiri, 2024) or infections (Abbas et al., 2016; Khalil, 2021) in fish, the available data about the prevalence rate of these fungal infections still limited and need for supporting. Hence, this study aims to traditional identification of the main fungal pathogens in fish ponds existed in different areas in Wasit province (Iraq).

Materials and methods

Ethical approval

This study gets the official license from the Scientific Committee in the College of Veterinary Medicine (University of Wasit).

Samples

Totally, 148 samples of water were collected from 37 fish ponds (4 samples from each one) which located in five main areas (Al-Kut, Al-Suwaira, Al-Aziziyah, Al-Numaniyah, and Al-Hay) in Wasit province (Iraq) during June-July (2024). Each sample was transported within a disposable plastic container under cooled conditions, filtered, and kept into labeled glass tubes at 4°C.

Fungal isolation

As soon as possible, all collected samples were cultured using three different media; Sabouraud Cycloheximide Chloramphenicol Agar (HiMedia, India), Potato Dextrose Agar (HiMedia, India), and Malt Extract Agar (HiMedia, India) as described by other studies (Hare, 2008; Nevalainen et al., 2014; Al-Enazi et al., 2018). For diagnosis, the grown fungi were purified and characterized morphologically by observing the top and button surfaces of each colony; and then, two slides were prepared from each colony using a drop of Lactophenol Cotton Blue and examined under X40 of light microscopy (MEIJI, Japan), (Borman and Johnson, 2014; Senanayake et al., 2020; Agu and Chidozie, 2021; Knoll et al., 2023).

Statistical analysis

The *t*-test and One-Way Analysis of Variance (ANOVA) in the GraphPad Prism Software (version 8.0.1) were applied to detect significant differences between the obtained results at $p<0.05$ (Gharban et al., 2024).

Results

The findings of current study revealed that 75.68% (112/148) water samples were infected with at least one fungus; while, 24.32% (36/148) samples were negative (Figure 1). Regarding study areas, fungal contamination was increased significantly ($p\leq0.0002$) in Al-Hay (91.67%) and decreased significantly in Al-Numaniyah (62.5%) and Al-Suwaira (64.58%) when compared to the findings of Al-Kut (81.58%) and Al-Aziziyah (75%), (Table 1). Among the positive samples, values of fungal contamination were elevated significantly ($p<0.05$) in Al-Kut [55.36% (62/112)] and reduced significantly in Al-Aziziyah [2.68% (3/112)] and Al-Numaniyah [4.46% (5/112)] in comparison with the findings of Al-Hay [9.82% (11/112)] and Al-Suwaira [27.68% (31/112)], (Figure 2).

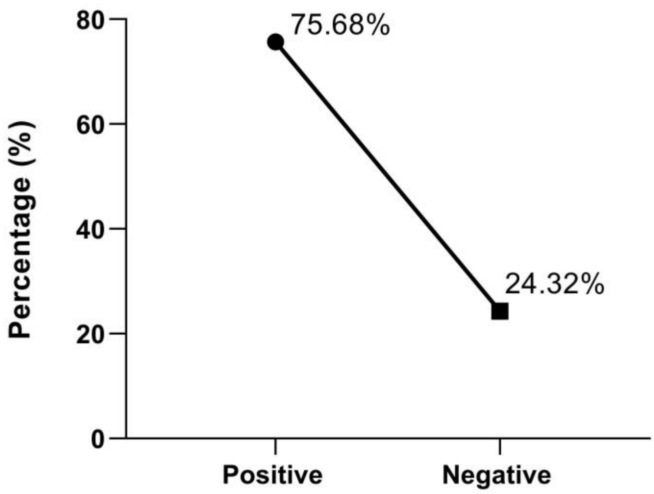

Figure (1): Total positive water samples for fungal contamination (Total No. 148)

Table (1): Association of positive contaminated samples to the total number of collected samples and study areas

Area	Total No.	Positive	
		No.	%
Al-Kut	76	62	81.58
Al-Suwaira	48	31	64.58
Al-Aziziyah	4	3	75
Al-Numaniyah	8	5	62.5
Al-Hay	12	11	91.67
p-value		0.0002 ***	
CI		60.04 to 90.09	
r^2		0.9796	

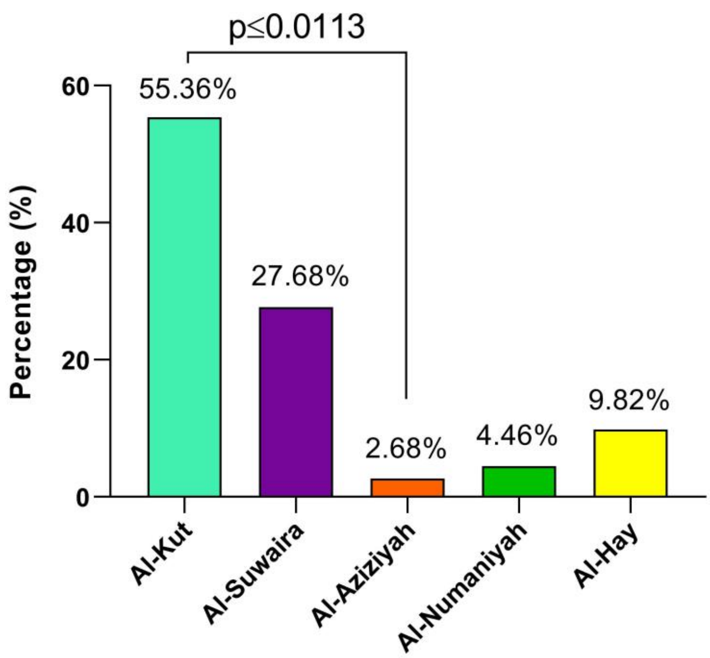

Figure (2): Association of positive contaminated samples to the total number of positive samples and study areas

Our findings showed that 83.78% (31/37) of fish ponds were contaminated with at least one fungus while 16.22% (6/37) were negative (Figure 3).

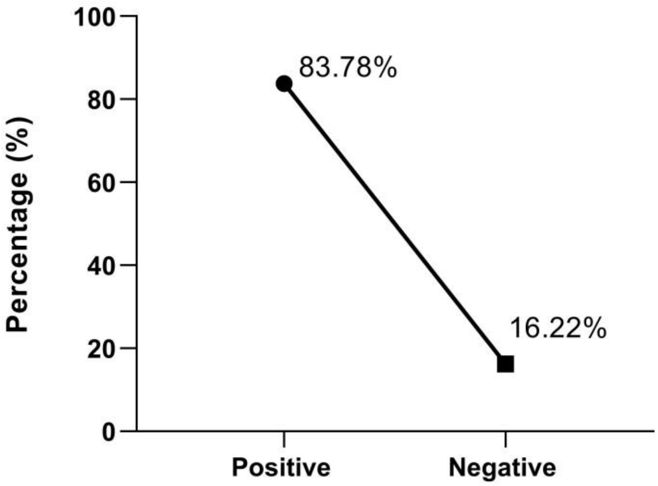

Figure (3): Total positive fish ponds for fungal contamination (Total No. 37)

observed in Al-Kut [58.07% (18/31)]; whereas the lowest values were identified in Al-Aziziyah [3.23% (1/31) and Al-Numaniyah [6.45% (2/31)] when compared to those of Al-Hay [9.68% (3/31)] and Al-Suwaira [22.58% (7/31)], (Figure 4).

Relation to the total number of fish ponds of study areas, significant highest fungal contamination was reported in Al-Aziziyah (100%), Al-Numaniyah (100%), Al-Hay (100%), and Al-Kut (94.74%); whereas, the lowest was seen in Al-Suwaira (58.33%); (Table 2). Among the positive fish ponds, significant highest contamination ($p \leq 0.0113$) was

Table (2): Association of contaminated fish ponds to the total number of fish ponds and study areas

Area	Total No.	Positive	
		No.	%
Al-Kut	19	18	94.74
Al-Suwaira	12	7	58.33
Al-Aziziyah	1	1	100
Al-Numaniyah	2	2	100
Al-Hay	3	3	100
p-value		0.0004 ***	
CI		68.03 to 113.2	
r^2		0.9688	

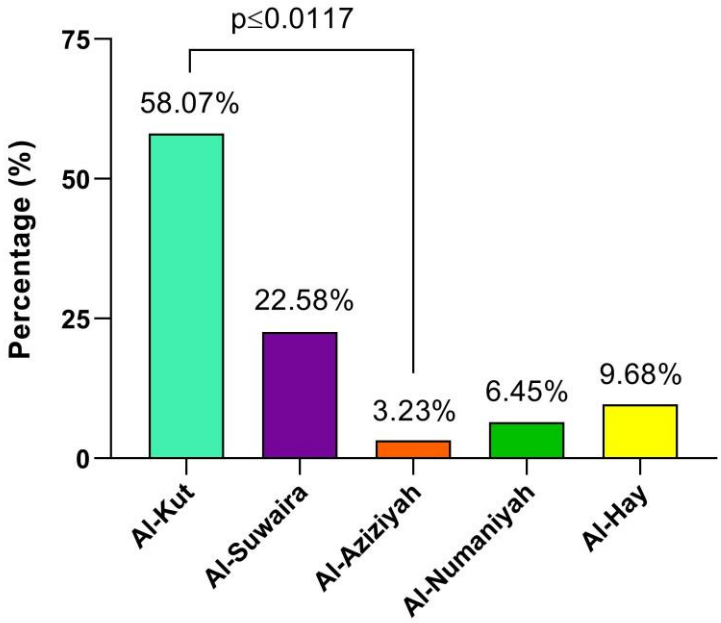

Figure (4): Association of positive contaminated fish ponds to the total number of positive fish ponds and study areas

(1.05%), *Helminthosporium* sp. (1.83%), *Mucor* sp. (2.62%), and *Trichoderma* sp. (3.93%) when compared to values of other fungal colonies; *Cladosporium* sp. (5.76%), *Rhizopus stolonifer* (6.28%), *Microsporium cani* (7.07%), *Fusarium solani* (8.9%), *Candida albicans* (9.42%), and *Alternaria alternata* (11.26%), (Table 3).

In the present study, an overall 12 different species and genera were detected among the study samples, and involving totally 382 fungal colonies; in which, significant increases (p≤0.0012) were reported in *Aspergillus niger* (22.77%) and *Penicillium* sp. (19.11%); while significant decreases were seen in *Trichophyton mentagrophytres*

Table (3): Fungal isolates detected in the present study (Total No. 382 fungal colonies)

Isolated fungi	No.	%
Alternaria alternate	43	11.26
Aspergillus niger	87	22.77
Candida albicans	36	9.42
Cladosporium sp.	22	5.76
Fusarium solani	34	8.9
Helminthosporium sp.	7	1.83
Microsporium cani	27	7.07
Mucor sp.	10	2.62
Penicillium sp.	73	19.11
Rhizopus stolonifer	24	6.28
Trichoderma sp.	15	3.93
Trichophyton mentagrophytres	4	1.05
Total	382	
p-value	0.0012 **	
CI	4.074 to 12.59	
r^2	0.6276	

compared to other areas; Al-Suwaira [19.63% (75/382)] and Al-Hay [18.06% (69/382)], (Figure 5). In addition, the fungal isolates were showed a significant variation in their existence among each study area. Significantly,

Among the study areas, the higher fungal colonies were detected significantly (p≤0.0199) in Al-Kut [40.05% (153/382) but the lowest was seen in Al-Aziziyah [9.69% (37/382)] and Al-Numaniyah [12.57% (48/382)] when and *Cladosporium* sp. (4.55%), (Table 6); in Al-Numaniyah, increases were observed in *Trichoderma* sp. (26.67%) while decreases in *Trichophyton mentagrophytres* (0%) and *Cladosporium* sp. (4.55%), (Table 6); and in Al-Hay, increases were reported in *Fusarium solani* (32.35%) and *Microsporium cani* (31.04%) whereas decreases were recorded in *Helminthosporium* sp. (0%) and *Trichoderma* sp. (0%), (Table 7). increases in percentage of fungal colonies in Al-Kut were seen in *Helminthosporium* sp. (71.43%) while decreases in *Alternaria alternate* (13.95%)], (Table 4); in Al-Suwaira increases were reported in *Alternaria alternate* (44.19%) while reduction in *Mucor* sp. (0%) and *Trichophyton mentagrophytres* (0%)], (Table 5); in Al-Aziziyah, increases were recorded in *Trichophyton mentagrophytres* (25%) whereas decreases in *Helminthosporium* sp. (0%), *Aspergillus niger* (3.45%),

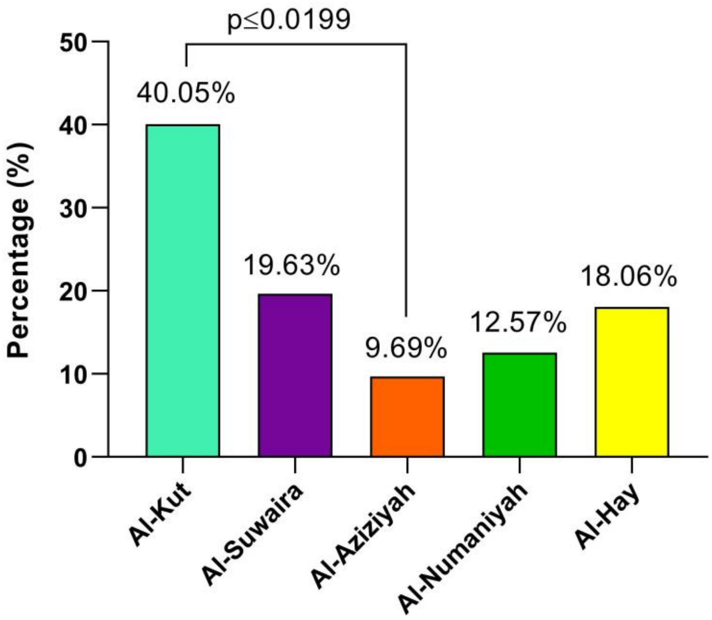

Figure (5): Association of positive fungal colonies to the study areas (Total No. 382)

Table (4): Results of positive fungal colonies in fish ponds of Al-Kut

Fungal species/genus	Total No.	Positive colonies	
		No.	%
Alternaria alternata	43	6	13.95
Aspergillus niger	87	43	49.43
Candida albicans	36	13	36.11
Cladosporium sp.	22	14	63.64
Fusarium solani	34	11	32.35
Helminthosporium sp.	7	5	71.43
Microsporium cani	27	5	18.52
Mucor sp.	10	6	60
Penicillium sp.	73	29	39.73
Rhizopus stolonifer	24	11	45.83
Trichoderma sp.	15	8	53.33
Trichophyton mentagrophytres	4	2	50
p-value		0.0001 ****	
CI		33.50 to 55.56	
r^2		0.8777	

Table (5): Results of positive fungal colonies in fish ponds of Al-Suwaira

Fungal species/genus	Total No.	Positive colonies	
		No.	%
Alternaria alternata	43	19	44.19
Aspergillus niger	87	15	17.24
Candida albicans	36	6	16.67
Cladosporium sp.	22	4	18.18
Fusarium solani	34	6	17.65
Helminthosporium sp.	7	1	14.29
Microsporium cani	27	5	18.52
Mucor sp.	10	0	0
Penicillium sp.	73	15	20.55
Rhizopus stolonifer	24	3	12.5
Trichoderma sp.	15	1	6.67
Trichophyton mentagrophytres	4	0	0
p-value		0.0007 ***	
CI		8.262 to 22.82	
r^2		0.6676	

Table (6): Results of positive fungal colonies in fish ponds of Al-Aziziyah

Fungal species/genus	Total No.	Positive colonies	
		No.	%
Alternaria alternata	43	4	9.3
Aspergillus niger	87	3	3.45
Candida albicans	36	3	8.33
Cladosporium sp.	22	1	4.55
Fusarium solani	34	2	5.88
Helminthosporium sp.	7	0	0
Microsporium cani	27	5	18.52
Mucor sp.	10	1	10
Penicillium sp.	73	13	17.81
Rhizopus stolonifer	24	2	8.33
Trichoderma sp.	15	2	13.33
Trichophyton mentagrophytres	4	1	25
p-value		0.0004 ***	
CI		5.827 to 14.92	
r^2		0.6962	

Table (7): Results of positive fungal colonies in fish ponds of Al-Numaniyah

Fungal species/genus	Total No.	Positive colonies	
		No.	%
Alternaria alternata	43	4	9.3
Aspergillus niger	87	12	13.79
Candida albicans	36	5	13.89
Cladosporium sp.	22	1	4.55
Fusarium solani	34	4	11.76
Helminthosporium sp.	7	1	14.29
Microsporium cani	27	3	11.11
Mucor sp.	10	2	20
Penicillium sp.	73	9	12.33
Rhizopus stolonifer	24	3	12.5

Trichoderma sp.	15	4	26.67
Trichophyton mentagrophytres	4	0	0
p-value		0.0001 ****	
CI		8.249 to 16.78	
r^2		0.7912	

Table (8): Results of positive fungal colonies in fish ponds of Al-Hay

Fungal species/genus	Total No.	Positive colonies	
		No.	%
Alternaria alternata	43	10	23.26
Aspergillus niger	87	14	16.09
Candida albicans	36	9	25
Cladosporium sp.	22	2	9.1
Fusarium solani	34	11	32.35
Helminthosporium sp.	7	0	0
Microsporium cani	27	9	31.04
Mucor sp.	10	1	10
Penicillium sp.	73	7	9.59
Rhizopus stolonifer	24	5	20.83
Trichoderma sp.	15	0	0
Trichophyton mentagrophytres	4	1	25
p-value		0.0003 ***	
CI		9.792 to 23.92	
r^2		0.7149	

Discussion

The global population is still increasing, and the consumption of fish also increases (Falcon et al., 2022). Fish ponds present the following opportunities over capture fisheries; environmental conditions can be controlled; fish from ponds are protected from predators; and genetically improved strains can be produced (Sievers et al., 2022; Abwao et al., 2023; Whitfield et al., 2023). Furthermore, fish ponds can also be developed and operated in a way that enhances the level of carrying capacity of natural productivity so that the degree of input supplement required is minimized in the course of operation of the aquaculture system (Ragaveena et al., 2021; Asche et al., 2022; Verdegem et al., 2023). Nevertheless, the cultivation of these ponds raises some problem, which relates to management issues with potential fungal contamination being a constant issue (Sousa Terada-Nascimento et al., 2023; Gharban, 2024).

In this study, traditional investigation of many fish ponds existed in various areas in Wasit province revealed significant variation in incidence and presence of different fungal species/genera in study areas in particularly *Aspergillus niger*, *Helminthosporium* sp., *Alternaria alternate*, *Trichophyton mentagrophytres*, *Trichoderma* sp., *Fusarium solani*, and *Microsporium* *cani*. In comparison to other conducted studies in Iraq, Abbas et al. (2016) reported that the percentage of systemic mycosis in fish was 62% which composite *Aspergillus flavus* (6%), *Blastomyces dermatitidis* (9%), *Candida krusei* (11%), *Candida pseudotropicalis* (7%), *Candida quillermondii* (9%), *Cryptococcus* sp. (19%), and *Rizipus* sp. (1%). Al-Musawi et al. (2021) showed the presence of mycotoxogenic fungi in 51 isolates at 63.8% of samples involving *Alternaria* sp. (3.92%), *Aspergillus* flavus (31.37%), *Aspergillus fumigates* (5.88%), *Aspergillus niger* (13.73%), *Fusarium* sp. (23.53%), *Penecillium* sp. (19.61%), and *Rhizopus* sp. (1.96%).

In another study, Khalil (2021) reported the presence of seven fungal species in 70% of tested samples in Mosul which including *Alternaria* (16.6%), *Aspergillus* (33%), *Blastomyces* (7.1%), *Candida* spp. (9.8%), *Penicillium* (25.6%), *Rhizopus* (5.3%), and *Saprolegnia* (2.6%). In a recent study in Khurmel pond located in Sulaimani province (Iraq), Qadir et al. (2025) reported the presence of 25 species of fungi which involved 17 zoosporic fungal species belonging to five genera (*Achly*, *Aphanomyces*, *Dictyuchus*, *Pythium*, and *Saprolegnia*) and eight conidial fungal species belonging to six genera (*Alternarea*, identify the fungal species definitely and providing more details on fungal contamination.

Acknowledgments

Not available

Conflict of interests

No.

Funding

No.

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Aspergillus, *Curvulari*, *Fusarium*, *Pencillum* and *Rhizopous*).

On the primary contributor of fungal contamination in fish ponds is the poor management of water quality (Richard et al., 2020). The unfavorable water in in aquatic production systems tends to have three-dimensionality in production (Lee et al., 2018, Fairbrass et al., 2025). Lack of appropriate water analysis and purification practices may cause accumulation of organics deposition and nutrients, development of problematic microorganisms including fungi (Mhlongo et al., 2019; Kosemani et al., 2024). Furthermore, unhygienic practices in the fish ponds may also lead to the agents and vehicles carrying fungal contaminants including the problem of improper cleaning and sterilization of equipment and improper disposal of the waste that can create conducive environment in the transmission of fungal pathogen (Lam et al., 2018; Admasu and Wakjira, 2021; Dinev et al., 2023). The stress brought by poor husbandry practices like; crowding, improper handling, and nutritional deficiency are likely to compromise the immune competence of cultures fish thus lead to fungal infection (Ciji and Akhtar, 2021; Syanya et al., 2023). Several researchers on the factors of bad management that leads to fungal infection on fish ponds need to adopt an integrated solution (Chandra, 2024; Mi et al., 2024; Onomu and Okuthe, 2024). The risk of fungal outbreaks, therefore, has to be managed through among other approaches developing and integrating effective water quality monitoring and treatment, maintaining high levels of sanitation and biosecurity, and enhancing the overall health of the cultured fishes (Opiyo et al., 2020, Manoj et al., 2022, Lateef et al., 2023).

Conclusions

Our findings revealed the high fungal contamination of fish ponds in particular in southern parts of Wasit province, with presence of several fungal species / genera suggesting the role of different ecological factors (such as physical and chemical properties of water) and management which affecting on the growth of fungus and growth of fishes. Moreover national studies appeared necessary to investigate and controlled the fungal contamination and diseases to ensure continued high-quality growth of fish in aquaculture production industry. Also, application of advance diagnostic assays such as molecular methods in combination with the morphological and traditional microscopic tools is vital to

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