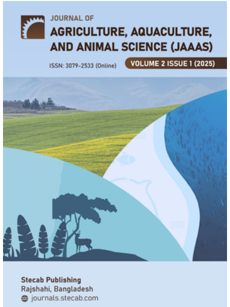

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Prevalence of Fungi Associated with Fish Cultivation Using Traditional Microscopy

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About Article

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ABSTRACT

Over the last ten years, fish ponds have become more and more popular in Iraq as a vital source of protein for human health. However, water pollution poses a major risk to aquaculture systems and may result in significant financial losses. The study aims to identify the primary fungal pathogens in fish ponds in several Wasit province (Iraq) locations using traditional methods. In Wasit province (Iraq), 37 fish ponds spread throughout five major locations (Al-Kut, Al-Suwaira, Al-Aziziyah, Al-Numaniyah, and Al-Hay) were the sites of an equal collection of 148 water samples in June and July of 2024. Following filtering, all samples were cultivated on three distinct media: Potato Dextrose Agar, Malt Extract Agar, and Sabouraud Cycloheximide Chloramphenicol Agar. The resulting fungal isolate colonies were then purified and described both visually and microscopically. Overall, 83.78% of fish ponds and 75.68% of water samples were found to be infected with at least one fungus. Regarding the study areas, there was a significant increase in fungal contamination of water samples in Al-Hay and a significant decrease in Al-Numaniyah and Al-Suwaira. In contrast, there were significant increases in fungal contamination in the study areas' fish ponds in Al-Aziziyah, Al-Numaniyah, Al-Hay, and Al-Kut, but a decrease in Al-Suwaira. Additionally, a total of 12 different species and genera were found in the study samples, which included 382 fungal colonies. In comparison to the values of other fungal colonies, such as *Cladosporium* sp., Rhizopus stolonifer, Microsporium cani, Fusarium solani, Candida albicans, and Alternaria alternate, there were notable increases in *Aspergillus niger* and *Penicillium* sp. and significant decreases in *Trichophyton mentagrophytres*, *Helminthosporium* sp., *Mucor* sp., and *Trichoderma* sp. Fungal colonies were found to have significantly increased in Al-Kut and decreased in Al-Aziziyah among the studied locations. The proportion of fungal colonies in each location increased significantly: *Fusarium solani* and *Microsporium cani* in Al-Hay; Alternaria alternate in Al-Suwaira; *Trichophyton mentagrophytres* in Al-Aziziyah; *Trichoderma* sp. in Al-Numaniyah; and *Helminthosporium* sp. in Al-Kut. Our research showed that there appeared to be fungal contamination in fish ponds in Wasit province, indicating the significance of national studies to look into and manage fungal contamination and its diseases in order to guarantee the aquaculture production industry's continued high-quality fish growth.

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1. INTRODUCTION

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 & 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 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 & Seena, 2022). For instance, it was approximated that 580 Indians die from water pollution associated diseases every single day (Kavitha & Dhandapani, 2018). This impact is worst where there is scarcity of clean water and also poor infrastructure of sanitation where much 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).

2. LITERATURE REVIEW

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 & 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).

3. MATERIALS AND METHODS

3.1. Ethical approval

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

3.2. 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.

3.3. 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 & Johnson, 2014; Senanayake *et al.*, 2020; Agu & Chidozie, 2021; Knoll *et al.*, 2023).

3.3. 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).

4. RESULTS AND DISCUSSION

4.1. 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).

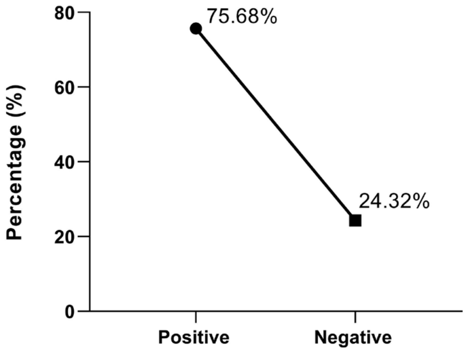

Figure 1. Total positive water samples for fungal contamination (Total No. 148)

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	

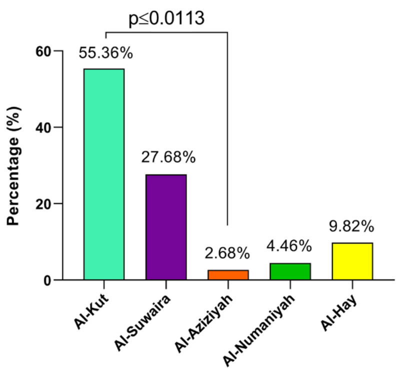

Figure 2. Association of positive contaminated samples to the total number of positive samples and study areas

The 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).

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≤0.0113) was 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).

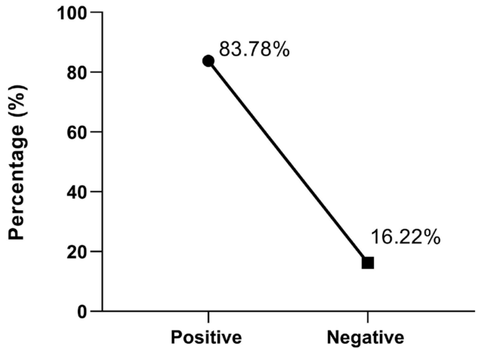

Figure 3. Total positive fish ponds for fungal contamination (Total No. 37)

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	

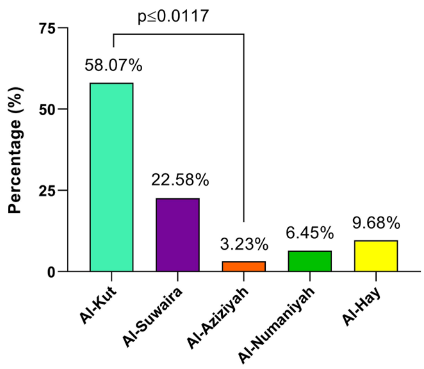

Figure 4. Association of positive contaminated fish ponds to the total number of positive fish ponds and study areas

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 ($p \leq 0.0012$) were reported in *Aspergillus niger* (22.77%) and *Penicillium* sp. (19.11%); while significant decreases were seen in *Trichophyton mentagrophytres* (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).

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

Area	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	

Among the study areas, the higher fungal colonies were detected significantly ($p \leq 0.0199$) 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 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, 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%), 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).

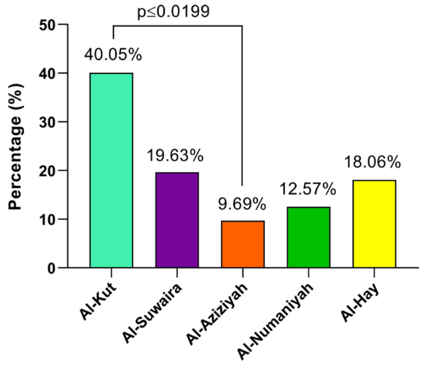

Figure 5. Association of positive fungal colonies to the study areas (Total No. 382)

Table 4. Results of positive fungal colonies in fish ponds of Al-Kut

Area	Total No.	Positive	
		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

Area	Total No.	Positive	
		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

Area	Total No.	Positive	
		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

Area	Total No.	Positive	
		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

Area	Total No.	Positive	
		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	

4.2. 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, 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 & 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).

5. 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 identify the fungal species definitely and providing more details on fungal contamination.

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