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Isolation and Identification of some Fungal Species from Common carp (*Cyprinus carpio* Linnaeus, 1758) in Taqtaq District in Erbil Province, Kurdistan Region, Iraq.

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ABSTRACT

In this study, a sample of 220 common carp (*Cyprinus carpio* Linnaeus 1758) was obtained from two distinct ponds in the Taqtaq District, situated in the northeastern region of Erbil Province, Kurdistan Region, Iraq. Specifically, 98 specimens were sourced from Pond A, while 122 were collected from Pond B., between September 2021 and April 2022. The fish underwent examination to determine the presence of fungal infections. The study identified eight species of fungi through morphological analysis of colony characteristics, specific growth media, and the VITEK II compact system (Biomerieux-USA). This research marks the initial documentation of five fungal species (*Aspergillus flavus, Naganishia albidus, Penicillium vermiculatus, Cystobasidium minutum, and Sporothrix schenckii*) infecting common carp in Iraq. Additionally, three fungal species (*Aspergillus fumigatus, Aspergillus niger, and Saprolegnia parasitica*) were reported for the first time in common carp within the Kurdistan Region.

1.Introduction

Iraq's aquaculture history dates back to the mid-20th century, with the introduction of common carp (*Cyprinus carpio* Linnaeus, 1758) to the Al-Zaafaraniya fish farm in Baghdad City in 1955 (Mhaisen, 1993). However, the development of the aquaculture sector in the Kurdistan Region has been relatively recent, with the industry expanding to several local farms only in recent years (Mama & Abdullah, 2013).

C. carpio is a member of the Cyprinidae family, which is the largest group of fresh water fish in Iraq. Across fish farms and most inland water bodies in Iraq, the common carp is the greatest in amount successfully adapted shellfish. In terms of economic value and breeding characteristics*, C. carpio* is one of the most valuable fish species found in the nation's inland waters. It also plays a significant role in the culture due to the organism's voracious natural world, explosive growth, ease of maintenance in cramped areas, viability in water that ranges in temperature from 3 to 35°C, significant resistance to oxygen deprivation, resistance to disease, high fertility, and ease of reproduction and relatively tasty meat, which matures at 1-2 years of age (Mama and Abdullah, 2012; Mustafa, 2016).

A class of creatures known as fungi is mostly multicellular, nonmotile, and capable of absorbing nutrients from both living and dead things. Spores of fungi are present in every freshwater ecosystem. It is known that a number of aquatic Oomycetes fungi can harm freshwater fish (Hoole *et al*., 2001).

Both freshwater and saltwater contain fungi. Fungi typically provide an important ecological role by breaking down organic matter that has died. Fungi, however, may become an issue if fish are under stress due to malnutrition, population pressure, or overfishing. Freshwater fish infections with fungi are widespread, global, and linked to weakened immune systems. Fish gills and skin have very superficial fluffy growths that are indicative of fungal infections (Mustafa, 2016).

Some fungal recorded from Kurdistan region from *C. carpio,* Ibrahim (2011) recorded *Branchiomyces sanguinis,* Ali (2015) recorded (*Aspergillus sp., Penicillium sp. and Blastomyces sp.*) and Mustafa (2016) recorded *Saprolegnia parasitica* .

In addition to the several ways currently in use, the recently VITEK 2 method was recently concept and is now range used worldwide. This system prepares and standardizes a main inoculum, then automatically runs through all the necessary identification and antimicrobial susceptibility testing stages. (Nonhoff *et al*., 2005). The purpose objective this investigation was to separate and ascertain a fungus species from *C. carpio* that were found in ponds in the Taqtaq District of the Kurdistan Region of Iraq using morphological analysis and the VITEK 2 technology.

2.Methodology

A total of 220 *C. carpio* specimens were collected from two ponds in the Taqtaq District, situated approximately 90 km northeast of Erbil Province, Kurdistan Region, Iraq. Of these, 98 were retrieved from Pond A and 122 from Pond B, over a period spanning from September 2021 to June 2022. Local fishermen used casting and gill nets for collection, after which the live specimens were transported to the Microbiology Laboratory in the Department of Biology at the College of Education, Salahaddin University-Erbil. The fungal isolation and identification processes were then conducted using a cold box filled with pond water.

Fungal isolates were obtained from the skin, gills, and fins of the fish. The procedure involved sterilizing tools such as Bunsen burners, followed by culturing samples using sterile swabs and an inoculating swab loop. The Petri dish containing Sabouraud Dextrose agar (SDA) media was partially opened, and the microbial inoculation loop was gently applied to the surface of the articular anatomical site on the fish before streaking it on SDA. Inverted, cultured media were incubated for 48–72 hours at 37°C. Identification of fungus by preparation of slides, the material was taken from each colony and

stained with 0.05% Trypan blue in lactophenol. Olympus microscope was used to observe slides and then photographed (Iqbal and Asgher, 2013). However, Erbil International Hospital used the VITEK II compact equipment to identify yeast from common carp fish (Floris *et al*., 2021).

The VITEK 2 system reads each test every 15 minutes, enabling kinetic analysis to record fluorescence, turbidity, and colorimetric information, the optical system integrates multichannel fluorimeter and photometer readings (Garcia-Garrote *et al.*, 2000).

3.Result and Discussion

The current study isolated eight different types of fungi. The description and measurements of these fungi are given below

Aspergillus flavus Link, 1809

The fungi were isolated from the fins of *C. carpio* in pond (B), where they were found at a prevalence of 1.81% (see Table 2).

Description: Colonies typically present as dense aggregations with powdery textures, characterized by yellowish-green spores on their upper surfaces and reddish-gold on their lower surfaces. The growth rate is fast, often resulting in colonies that exhibit a downy or powdery appearance. Hyphal growth generally occurs through filamentous branching, leading to the formation of mycelium. Conidiophores lack color and have a rough texture. Both uniseriate (organized in a single row) and biseriate phialides exist. Hyphae are hyaline and septate. Asexual spores called conidia are created during reproduction (Fig. 1).

A. flavus is noted in Iraq for the first time from *Scomberooides commersonnianus* in Basrah Province (Salih *et al*., 2011). According to Mhaisen (2022), there are no known hosts for the species in Iraq anymore. Thus *C. carpio* is currently regarded as a novel host for these fungi in Iraq and present study represents the first record of *A. flavus* in Kurdistan Region.

Aspergillus fumigatus Fresenius, 1863

These fungi were found in the tail of *C. carpio* from pond (B) with a prevalence of 0.90% (see Table 2).

Description: The colonies exhibit a characteristic blue-green coloration with a suede-like texture composed of densely packed conidiophores. These colonies can grow rapidly, reaching sizes ranging from 4 to 10 mm, particularly thriving in temperatures between 37°C to 50°C. A. fumigatus, a thermophilic species, is identifiable by its green echinulate conidia measuring 2.5 to 3 µm in diameter, arranged in chains that emerge basipetally from greenish phialides typically 6 to 8 in number. Some isolates of A. fumigatus lack pigment and produce white conidia. Conidial chains are directly attached to broadly clavate vesicles in the absence of medullae (Fig. 6)

According to Mhaisen (2022), *A. fumigatus* was first documented in Iraq from *C. carpio* by Al-Darwesh (2010). Since then, there have been no additional reports of *A. fumigatus* Therefore, this represents the first recorded instance of this fungus in fish from the Kurdistan Region.

A. fumigatus is a fungal species widely distributed in various environmental niches such as soil, plant debris, and domestic dust. The fungus has the ability to generate airborne spores known as conidia. Many individuals inhale these spores regularly each day. In healthy individuals, the immune system typically eliminates these spores effectively. However, for certain individuals, inhalation of *A. fumigatus* spores can potentially result in a severe infection (Seladi-Schulman and Han, 2018).

Aspergillus niger Tieghem, 1867

These fungi were detected on the skin of *C. carpio* in ponds (A and B), with prevalences of 0.90% and 1.36%, respectively (see Table 1 and 2).

Description: Colonies are the colonies range in color from pale pink to white and have a smooth, mucoid texture. Some isolates may exhibit a rough and wrinkled appearance.

Macroscopically, the colonies range in color from cream to pale pink (Fig. 7), and most of them have a smooth, mucoid look on Sabouraud Dextrose agar at 25°C. One of the predominant species within the genus *Aspergillus.* Although it is an uncommon occurrence, some colonies have exhibited a rough and wrinkled appearance. The yeast cells are encapsulated and have a globose to ovoid shape, measuring 3.1-3.0 x 3.5-6.2 micrometers in diameter. Is opportunistic pathogen since it is isolated from healthy and clinical cases of fish. *C. carpio* showed that gills were whitish due to the presence of fungal hyphae and cotton wool like margined the caudal fin. Eyes and mouth of *C. carpio* were coverd with fungal hyphae.

This species recorded in *Scomberooides commersonnianus* for the first time in Iraq by Salih *et al*. (2011) in Basrah Province. Also, Ali (2015) isolated *A. niger* from skin, gills, fins and eyes of *C. carassius, M. mastacembelus* and *S. triostegus.* No, more hosts are known for these fungi. So, *C. carpio* regarded as new host for these fungi in Iraq, and this is first record in Kurdistan Region (Mhaisen, 2022).

***Naganishis albidus* (Saito) X. Z. Lin, F. Y. Bai, M. Groenew and Boekhut (2015)**
These fungi were isolated in the skin in Pond A, *C. carpio* exhibited a prevalence rate of 0.90% (see Table 1). The identification of this species was conducted using the VITEK 2. is detailed in Appendix 1.

Description: Colonies are cream-color to a pale pink hue, with most colonies exhibiting a smooth, mucoid texture. cells stay cream-colored, on Sabouraud Dextrose agar at 25°C for 72 h. It has been discovered that a few of the colonies are wrinkly and scratchy. On a microscopic level, *N. albidus* has an ovoid shape, cells: encapsulated yeast cells that are globose to ovoid in shape, ranging from 3.1 to 3.0 x 3.5 to 6.2 micrometers in diameter and when viewed this species also appears to have a capsule (Fig. 2). Synonyms for *Cryptococcus albidus* (Gharehbolagh *et al*., 2017).

The study of *N. albidus* from *C. carpio*, according to Mhaisen (2022), constitutes the species' first known record in Iraq.

Table (1): The distribution of fungi species on different sites of *C. carpio* from pond A.

Species of Fungi	Pond A (122 Fish)		Site of Infection
	No. Infected Fish	Prevalence %	
Aspergillus niger	2	1.63	Skin
Naganishis albidus	2	1.63	Skin
Penicillium vermiculatus	3	2.45	Gills
Sporothrix schenck	2	1.63	Gills

Table (2): The distribution of fungi species on different sites of *C. carpio* from pond B.

Species of Fungi	Pond B (98 Fish)		Site of Infection
	No. Infected Fish	Prevalence %	
Aspergillus flavus	4	4.08	Gills
Aspergillus fumigatus	2	2.04	Taill or Caudal fin
Aspergillus niger	3	3.06	Skin
Cystobasidium minutum	1	1.02	Skin
Saprolegnia parasitica	1	1.02	Skin and Gills

***Penicillium vermiculatus* Dangeard (1955)**
These fungi were isolated in the gill filaments of *C. carpio* from a Pond A, showing a prevalence rate 1.36% (see Table 1).

Description: Colonies typically exhibit rapid growth, initially appearing white and later developing into hues such as blue-green, gray-green, olive-gray, yellow, or pinkish over time, with optimal growth occurring between 20°C and 27°C within a span of three days. The thallus (mycelium) consists of extensively branched networks of multinucleated, generally colorless hyphae, each pair of cells separated by septa. The septate hyphae measure between 2 to 5 µm in diameter and give rise to both branched and

unbranched conidiophores, imparting a brush-like appearance to Penicillium species (Fig. 3). Each branch terminates in a conidiophore bearing green, spherical conidia, which serve as the primary mode of dissemination for these fungi and are crucial for their reproductive cycle.

According to Mhaisen (2022), the discovery of *P. vermiculatus* in *C. carpio* marks the first documented occurrence of this species in Iraq. Another species of this genus *P. brevicompactum* were reported in the Iraqi fish from *Scomberoides commersonnianus* by (Salih *et al*., 2011).

***Cystobasidium minutum* (Cif. and Redaelli) Yurkov, Kachalkin, H. M. Daniel, M. Groenew., Libkind, V. de Garacia, Zalar, Gouliam., Boekhout and Begerow (1928).**

These fungi were isolated in the skin *C. carpio* from Pond B exhibited a prevalence rate of 0.45% (see Table 2). The identification of this species was conducted using the VITEK 2 is detailed in Appendix 2.

Description: Colonies are orange color to red, soft, glossy, moist and sometimes mucoid. a genus of unicellular pigmented yeasts (Fig. 4),

when grown on Sabouraud's Dextrose Agar at 18-33°C. Diameter of the cell 9-12mm also this species synonyms for *Rhodotorula minuta*, catalogue of life checklist (gbif.org/ species/ 7535089, 2022).

As Mhaisen's (2022) findings, the study of *Rhodotorula minuta* from *C. carpio* is the initial account within this species in Iraq.

***Saprolegnia parasitica* Coker, 1923**

These fungi were isolated in the tail and gill filaments of *C. carpio* from Pond B, With an occurrence rate of 0.45% (see Table 2).

Description: Colonies are generally white in color, cotton-like appearance that extends outward in a circular, crescent-shaped, or whorled pattern the initially create a mass of individual hyphae; this mass of hyphae is referred to as a mycelium when it gets large enough to be seen without a microscope. Under a microscopic level, *S. parasitica* the shape and diameter of the hyphae and spores from the isolated fungi were measured (Fig. 8). Additionally, all purified cultures were assessed for both macro- and micro-morphological characteristics

S. parasitica is noted in Iraq for the first time from the eggs of *C. carpio* from Basrah Univesity Fish Farm at Al-Tannuma (Mhaisen *et al*., 1993). After that, it was reported from seven various fish hosts in the central region and south of Iraq (Mhaisen *et al.*, 2016). Also, it was reported in Kurdistan Region from *C. carpio* (Mustafa, 2016).

Saprolegnia is typically categorized as a secondary pathogen but can also function as a primary pathogen. It tends to infect fish that are stressed or have compromised immune systems (Poppe & Seierstad, 2003). Nearly all freshwater fish encounter at least one species of fungus during their lifespan (Hussein *et al*., 2001), particularly from the egg stage through mollification (Bruno & Poppe, 1994). Infection of fish by *Saprolegnia*, known as saprolegniasis, targets epidermal tissues and commonly initiates on the head or fins (Neish, 1977).

Five species of fungi were recorded for the first time in Iraq including:

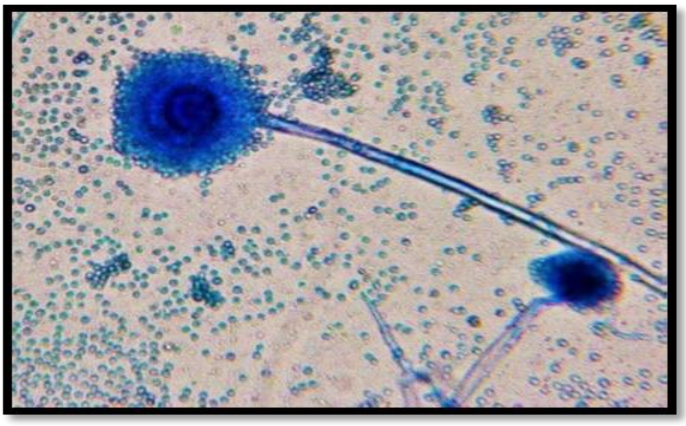

Figure (1): *Aspergillus flavus* from gills

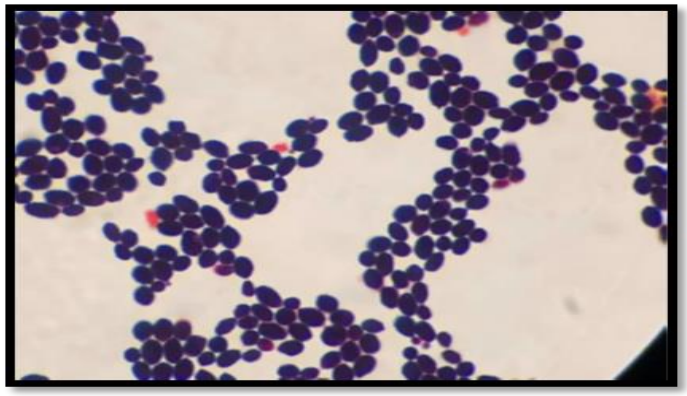

Figure (2): *Naganishia albidus* from skin

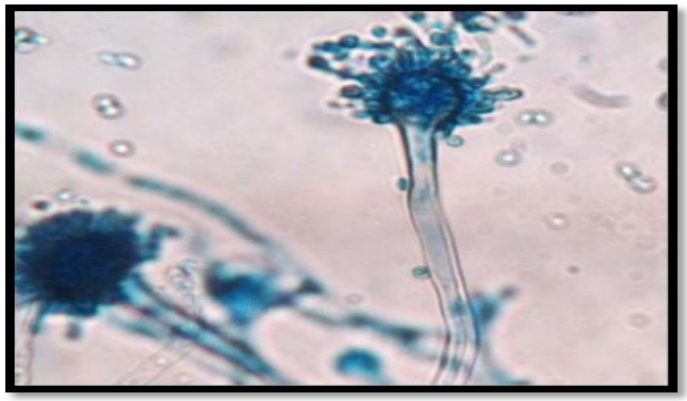

Figure (3): *Penicillium vermiculatus* from gills

***Sporothrix schenckii* Hektoen and C.F.Perkins (1900)**

These fungi were isolated in the gill filaments of *C. carpio* from the pond (A) with a rate of 0.90% (see Table 1).

Description: Colonies have a surface that is finely wrinkled, leathery to velvety, and wet. The color is originally white and may eventually turn cream or dark brown (the color of dirty candle wax). Hyphal in the natural setting or cultured at 25 °C in the lab. *S. schenckii* takes on the form of a hyphal. Filaments are visible under a microscope, and the hyphae are septate, measuring between one and two micrometers in diameter. Conidia resemble glass (hyaline) and have an oval form. They could have a dark color or be colorless. The yeast form develops into smooth white or off-white colonies under the microscope. Yeast cells have an extended cigar-shaped appearance and are 2 to 6 µm long when viewed under a microscope (Fig. 5).

As reported by Mhaisen (2022), the current detection of *S. schenckii* in *C. carpio* constitutes the first documented instance of this species in Iraq.

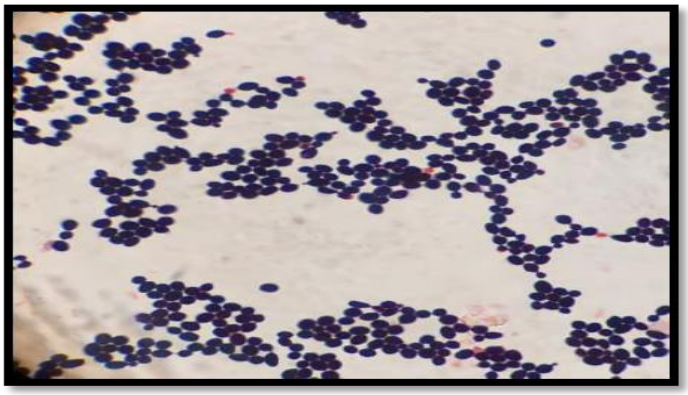

Figure (4): *Cystobasidium minutum* from skin

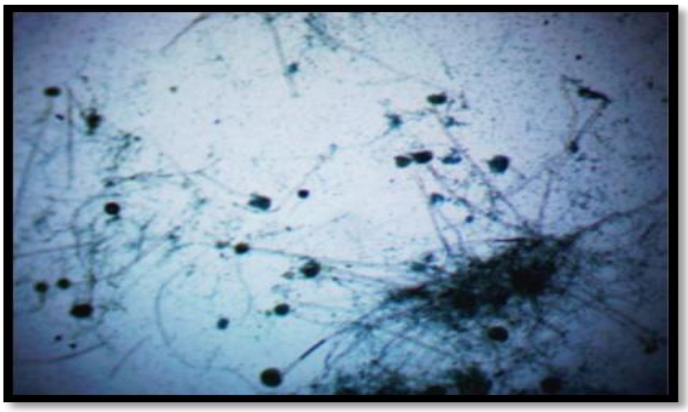

Figure (5): *Sporothrix schenckii* from gills

Three species of fungi were recorded for the first time in Kurdistan including:

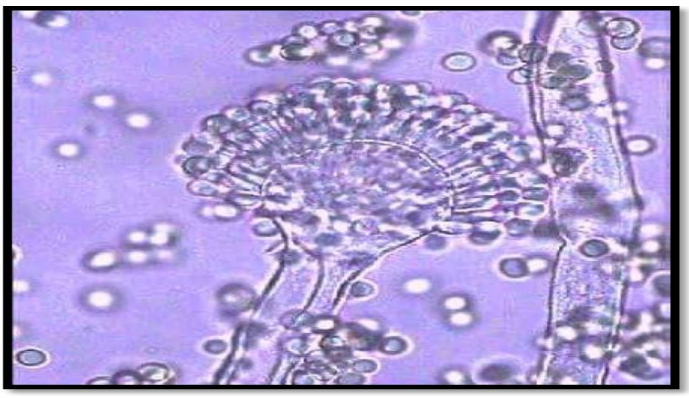

Figure (6): *Aspergillus fumigatus* from tail

Figure (7): *Aspergillus niger* from skin

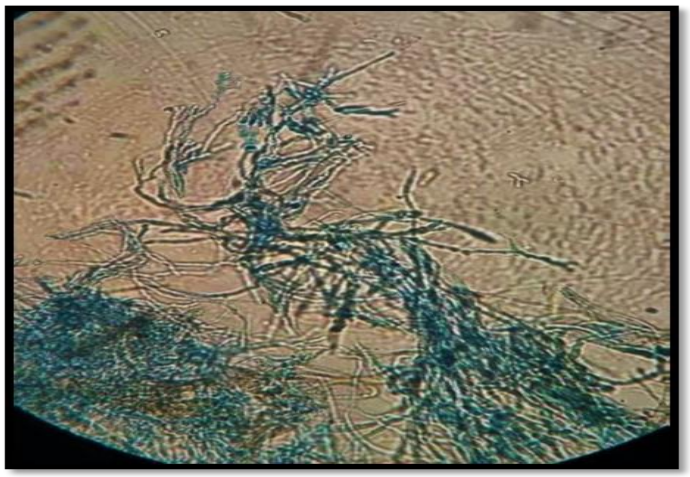

Figure (8): *Saprolegnia parasitica* from skin and gills

Conclusion

According to study findings, eight fungal species were isolated and identified from carp samples; five of these fungal species were the first to be discovered in Iraq. (*Aspergillus flavus, Naganishia albidus, Penicillium vermiculatus, Cystobasidium minutum,* and *Sporothrix schenckii*) and three fungal first new record in Kurdistan Region (*Aspergillus fumigatus, Aspergillus niger,* and *Saprolegnia parasitica*). The presence of fungul infections in different ponds with different water sources, especially in ponds with river water sources compared to well water, indicates that differences in water sources have a significant impact on fungal infections in fish, especially common carp (*C. carpio*).

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References

Al-Darweesh, A.A.R. (2010) Pathological study of endoparasitic and fungal infection in common *carp Cyprinus carpio*. M.Sc. Thesis. Coll. Vet. Med., Baghdad Univ. (Summary, In Arabic).

Ali, H.H. (2015) 'Isolation and identification of pathogenic fungi from Carp fish in Suliamania Province', *G.J.B.B.*, 4(4), pp. 356-363.

Bruno, D.W. and Poppe, T.T. (1994) '*Saprolegnia* and other Oomycetes in fish diseases and disorders', in Woo, P.T.K. and Bruno, D.W. (eds.) *Viral, bacterial and fungal infections*, volume 3. Wallingford: CABI Publishing, pp. 599-659.

Floris, R. et al. (2021) 'Isolation and identification of bacteria with surface and antibacterial activity from the gut of Mediterranean grey mullets', *Microorganisms*, 9, 2555.

Garcia-Garrote, F., Cercenado, E. and Bouza, E. (2000) 'Evaluation of a new system, VITEK 2, for identification and antimicrobial susceptibility testing of *enterococci*', *Journal of Clinical Microbiology*, 38, pp. 2108-2111.

Gharehbolagh, S.A. et al. (2017) 'First case of superficial infection due to Naganishia albida (formerly *Cryptococcus albidus*) in Iran: A review of the literature', *Current Medical Mycology*, 3, pp. 33.

Hussein, M.M., Hatai, K. and Nomura, T. (2001) 'Saprolegniosis in salmonids and their eggs in Japan', *Journal of Wildlife Diseases*, 37, pp. 204-207.

Hoole, D. et al. (2001) Diseases of carp and other cyprinid fishes.

Ibrahim, K.S. (2011) 'Isolation and pathological study of Branchiomycosis from the commercial pond of common carp (*Cyprinus carpio*) fish, in Governorate of Duhok/Iraq', *The Iraqi Journal of Veterinary Medicine*, 35(1), pp. 1-9.

Iqbal, M.N., Hafiz and Asgher, M. (2013) 'Characterization and decolorization applicability of xerogel matrix immobilized manganese peroxidase produced from Trametes versicolor IBL-04', *Protein and Peptide Letters*, 20, pp. 591-600.

Mama, K.S. and Abdullah, S.M. (2012) 'A comparative study on the parasitic fauna of the common carp *Cyprinus carpio* from Ainkawa fish hatchery (Erbil) and Lesser Zab River in Kurdistan region, Iraq', *Mesopotamian Journal of Agriculture*, 42, pp. 19-26.

Mama, K.S. and Abdullah, S.M. (2013) 'Infections of common carp *Cyprinus carpio* with ciliated protozoan parasites from Ainkawa fish hatchery in Kurdistan region, Iraq', *Proceedings of Aquaculture Europe*, Trondheim, Norway, pp. 9-13.

Mhaisen, F.T. (1993) 'A review on the parasites and diseases in fishes of ponds and farms of Iraq', *Iraqi Journal of Veterinary Sciences*, 6(2), pp. 20-27. (In Arabic).

Mhaisen, F.T. (2022) Index-catalogue of parasites and disease agents of fishes of Iraq, Unpublished.

Mhaisen, F., Ali, A. and Khamees, N. (2016) 'Checklists of protozoans and myxozoans of freshwater and marine fishes of Basrah province, Iraq', *Mesopotamian Journal of Marine Sciences*, 31, pp. 29-52.

Mustafa, S. (2016) 'Study on some disease agents of *Cyprinus carpio* L. 1758 of Fish Farm in Erbil City'.

Neish, G.A. (1977) 'Observations on saprolegniasis of adult sockeye salmon, Oncorhynchus nerka (Walbaum)', *Journal of Fish Biology*, 10, pp. 513-522.

Nonhoff, C., Rottiers, S. and Struelens, M. (2005) 'Evaluation of the Vitek 2 system for identification and antimicrobial susceptibility testing of *Staphylococcus* spp.', *Clinical Microbiology and Infection*, 11, pp. 150-153.

Poppe, T.T. and Seierstad, S.L. (2003) 'First description of cardiomyopathy syndrome (CMS)-related lesions in wild Atlantic salmon Salmo salar in Norway', *Diseases of Aquatic Organisms*, 56, pp. 87-88.

Salih, Y.A., Al-Shatty, S.M.H. and Al-Fadhly, N.K.Z. (2011) 'A study of molds and yeasts associated with Thelah fish Scomberoides commersonianus dried by solar dryer and by sunlight during different storage periods', *Journal of Basrah Research Sciences*, 37(2B), pp. 89-103. (In Arabic).

Seladi-Schulman, J. and Han, S. (2018) 'Brain overview', Accessed October.