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Identification of *Cryptococcus laurenti* from infected *Cyprinus carpio* in Fish Culture in Erbil - Iraq

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ABSTRACT

This study presents the first isolation of the fungus *Cryptococcus laurentii* from common carp *Cyprinus carpio* in fish ponds in Zrarati area. Erbil City, Kurdistan Region of Iraq. A total of 98 common carp specimens were collected from ponds in the region and examined for fungal infestations from September 2023 and by the end of April 2024. The identification of the fungal isolates was conducted using morphological analysis and the VITEK 2 compact system from Erbil International Hospital. The discovery of *C. laurentii* in common carp in Iraq signifies a novel finding, shedding light on the fungal diversity present in fish populations and aquaculture environments. The present research underscores the importance of monitoring and managing fungal infections in fish to ensure the health and sustainability of aquaculture practices in the region. The present record of species of fungi of *C. laurentii* is considered the first record in Iraq since no previous were seen.

1.Introduction

Among freshwater fish, *C. carpio* is a member of the Cyprinidae family, the largest fish group in Iraq. The common carp is the most prevalent and well-established species in fish farms and most inland water bodies across Iraq (Mala and Abdullah, 2022). *C. carpio* is one of the most valuable fish species in the nation's inland waters, considering its economic significance and breeding characteristics. It also plays a key role in aquaculture due to its voracious appetite, rapid growth, ability to thrive in confined spaces, strong resistance to oxygen deprivation and diseases, adaptability to water temperatures ranging from 3 to 35°C, high fertility, ease of reproduction, and relatively tasty meat, which matures in 1-2 years (Mojer and Al-Dubakel, 2024). The expansion of fish farming has also brought about concerns regarding fish health. Bacterial hemorrhagic septicemia, lernaeasis, saprolegniasis, and anoxia are among the most common fish diseases affecting freshwater pond species (Al Sulivany *et al*., 2024). The most widely farmed commercial freshwater fish in Iraq is the Common Carp, which is highly sensitive to a variety of water-borne pathogens (Elsayed et al., 2024).

Fungal infections in fish usually occur as secondary infections due to factors such as water quality problems (low pH, temperature, water contaminated organic matter), poor condition, trauma, high stock density, poor nutritional status, stress, dead fish, bacterial diseases and parasite infestations. However, fungal pathogens of high pathogenicity can also be the primary cause of disease (Kaydu and Yardımcı, 2024). Fungal infections can be divided into external and internal (systemic) fungal infections. It has been observed that external fungal agents infect fertilized eggs and cause larval deaths and economic losses. Some fungal species are also known to proliferate in poorly stored feed, produce mycotoxins and cause toxicity (Yanong, 2003); (Patel *et al*., 2018); (Sarkar *et al*., 2022). Fungal spores are present in all freshwater ecosystems (Al-Qaissi *et al*., 2022). Several species of aquatic Oomycetes fungi are known to be harmful to freshwater fish (Tandel *et al*., 2023). Fungi comprise a group of non-motile, non-photosynthetic, primarily multicellular organisms that derive nutrients from living or dead organisms (Özcan and ArserİM, 2022).

Cryptococcosis is a fungal infection caused by *Cryptococcus neoformans* and *Cryptococcus gattii* (Taha *et al*., 2024). Fish can become infected by ingesting fungal spores, fish may show respiratory distress, lethargy, skin ulcers and abnormal swimming behavior. Internal organs, particularly the brain and lungs may exhibit granulomas and necrosis (Alam *et al*., 2023). Furthermore, it is widely acknowledged that this particular entity serves as a reservoir for zoonotic yeasts and various other dangerous fungi, the two most common species of *Cryptococcus, C. neoformans*, and *C. gattii*, cause the disease, in recent decades other species of *Cryptococcus* including *C. laurentii, C. albidus, C. uniguttulatus, C. luteolus, C. adeliensis* yeast have been known to cause infections in humans and animals, encapsulated yeasts of the genus Cryptococcus are commonly found in nature (Rajab and Ramadan, 2024).

Although *C. laurentii* is less commonly reported compared to other *Cryptococcus* species. Because *C. laurentii* infections in fish are less common compared to other fungal pathogens, they can occur, particularly in environments where the fungus is present (Perfect, 2015). The aim of the present work is detecting and classify the pathogenic fungi that infesting *C. carpio* found in ponds in the Zrarati Qr. from Erbil City. Using morphological analysis and VITEK 2 compact system methods. The study showed first presence of *C. laurentii*.

2.Material and Methods

1.2. Study Area

The Zrarati area predominantly falls within the boundaries of Erbil-Kurdistan Region, Iraq. Geographically, it is positioned between coordinates 11° to 36° N and 2° to 42° D E, situated to the northwest of Bahrka district (Fig. 1). Notably, the area extends to the Great River, commonly referred to as the Badinan River, as indicated on relevant maps (Gardi, 2017).

2.2 Fish Sampling

A total of 98 *C. carpio* that their weight between 0.5 – 2kg, specimens were collected from five ponds. The duration starting from September 2023 and by the end of April 2024 through the use of cast nets and gill nets by local fishermen, then the fish samples, containing pond water for fungi isolation and identification, were placed inside a cool box and transported to the Salahaddin University- Erbil, College of Education, Department of Biology, Microbiology laboratory.

Fungi Growth Media and Stain

Sabouraud Dextrose Agar is used to isolate, culture, and maintain a variety of fungal and yeast species, including both pathogenic and non-pathogenic types. The medium is prepared by dissolving 65 g/L of the powder in distilled water with gentle heating, subsequently, sterilized in an autoclave at 121°C for 15 minutes. After sterilization, 50 mg/mL of chloramphenicol is added to the solution. (Sherif and Abdel-Hakim, 2016). Lactophenol cotton blue staining solution is a mounting medium and a staining solution employed to prepare slides for microscopic analysis of fungal specimens. Fungal elements are stained intensely blue (Dabban *et al*., 2024).

3.2 Fungal Isolation and Identification

For separation of fungi, specimens were taken from skin, gills, and fins of fish using sterile swabs and then inoculated on Sabouraud Dextrose Agar (SDA). A laminar flow air cabinet was used for fungi isolation to avoid contamination. After 2-4 days of incubation of agar plates in 28-30 °C fungal growth was observed and grown culture was kept until seven days (El-Zayat *et al*., 2024). For the slide preparation, a sample from each growth colony was collected and colored with 0.05% Trypan blue solution in lactophenol. Permanent slides stained with methylene blue were then observed under an Olympus microscope and photographed (Al-Jumaa *et al*., 2024).

The identification of fungal isolates from common carp was conducted using the VITEK 2 Compact system (Biomerieux, France) at Erbil International Hospital (Floris *et al.*, 2021).

The VITEK 2 system performs kinetic analysis by taking readings of tests are carried out every 15 minutes, and the optical system features a multichannel fluorimeter and photometer to monitor fluorescence, turbidity, and colorimetric signals (Vittorakis *et al*., 2024).

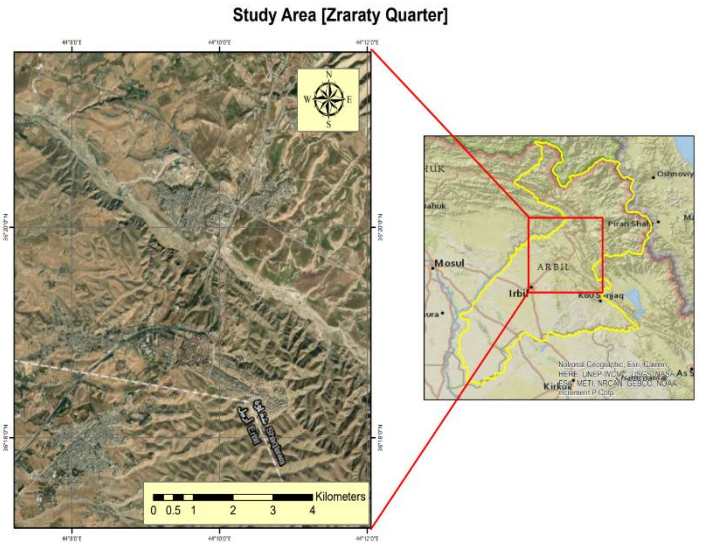

Figure 1. [A] Map of Kurdistan Region. [B] Map of Zraraty

3. Result and Discussion

A total of 98 common carp *C. carpio* were collected between September 2023 and the end of April 2024, from five different ponds in Zrarati area, near Erbil-Kurdistan Region, Iraq. From the total fish that collected 33 fish infected with fungi *C. laurenti* during different months. The prevalence of *C. laurentii* infections in common carp (*C. carpio*) as reported in the study reveals important trends and insights into the dynamics of fungal infections in aquaculture. Here's a more detailed discussion on the prevalence of infection as shown in (table 1):

Table 1: Monthly fluctuations of the infection of *Cryptococcus laurentii* from *C. carpio* from Erbil province

Month	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Fish Examined	Fish infected	Prevalence%
Sep	4	3	5	2	4	18	7	38.88%
Oct	3	2	3	4	3	15	5	33.33%
Nov	1	zero	4	3	2	10	2	20%
Dec	1	4	zero	zero	4	9	3	33.33%
Jan	2	zero	1	3	2	8	zero	0%
Feb	3	1	2	zero	zero	6	zero	0%
Mar	zero	1	2	7	3	13	7	53.84%
Apr	1	4	3	5	6	19	9	47.36%
Total						98	33	33.67%

Overall Infection Rate: The study found that out of 98 common carp specimens collected, 33 fish were infected with *C. laurentii*, resulting in an overall infection rate of approximately 33.67%. This indicates a significant presence of this fungal pathogen in the sampled fish population, suggesting that *C. laurentii* may be a relevant concern for fish health in the region.

Monthly Variations: The data showed notable monthly fluctuations in infection rates, with the highest prevalence occurring in March (53.84%) and a significant drop to 0% in January and February. This seasonal variation suggests that environmental factors, such as temperature and humidity, play a critical role in the proliferation of *C. laurentii*. The increased humidity and warmer temperatures in March likely create favorable conditions for fungal growth, while the colder months may inhibit its development.

The study also highlighted differences in infection rates across five different ponds. For instance, certain ponds exhibited higher infection rates during specific months, indicating that localized environmental conditions and management practices may influence the prevalence of fungal infections. This variability underscores the importance of site-specific monitoring and management strategies in aquaculture.

The taxonomy of *Cryptococcus laurentii* is as follows:

- Kingdom: Fungi
- Phylum: Basidiomycota
- Class: Agaricomycetes
- Order: Tremellales
- Family: Tremellaceae
- Genus: Cryptococcus
- Species: *Cryptococcus laurentii* (Kuff.) C.E.Skinner (Ke Wang, 2023)

Cryptococcus laurentii is a yeast-like fungus belonging to the genus Cryptococcus The colonies of *C. laurentii* were described as cream-colored to pale pink, with a majority of smooth colonies exhibiting a mucoid appearance. Some colonies were noted to be wrinkly and scratchy. On Sabouraud Dextrose agar at 25°C for 72 hours, the cells of *C. laurentii* remained cream-colored (Fig. 2). Morphology: it appears as a yeast with round-to-oval cells that reproduce by budding. Under the microscope, these cells typically show a distinctive halo or capsule surrounding them, ranging from 2-5 x 3-7 µm in size. The cells were encapsulated budding yeast, gathering in chains of 3-4. This fungus is facultative alkaliphile and psychrophilic. Additionally, *C. laurentii* produces a yeast killing factor (Fig. 3). According to (Mhaisen, 2024) no additional records of this species were reported in the study for *C. laurentii*. So, this represents the first recorded instance of this fungal species in fish from Iraq. The result of VITEK 2 was finished fitting to (Tshabuse *et al*., 2022). The micros coped culture identify when supported by VITEK 2 test (Table 2), that showed referring to *C. laurentii* according to data base of (Biome Rieux, 2023).

1.3 The signs and symptoms

In *C. carpio* include the appearance of grayish-white growths on the surface and fins, along characterized by erosion and hemorrhaging on the external surface (Fig. 4). If *C. laurentii* were to be present in fish, it could potentially cause opportunistic infections, particularly in stressed or weakened fish and fungal infections can be secondary to other issues, such as injuries, parasitic infections, or bacterial infections (Admasu and Wakjira, 2021).

Symptoms in infected fish can include, skin lesion or ulcer developed on the skin, respiratory distress difficulty breathing or gasping at the surface, lethargy reduced activity and abnormal swimming behavior, swelling abdominal swelling or distension and fungal growth visible fungal growths, often in the gills or on the skin (Fish *et al*., 2024). In fish, post mortem examination may reveal granulomatous lesions in the brain, kidneys and other organs (Alam *et al*., 2023).

Figure 2. *Cryptococcus laurentii* on Sabouraud Dextrose Agar (SDA)media

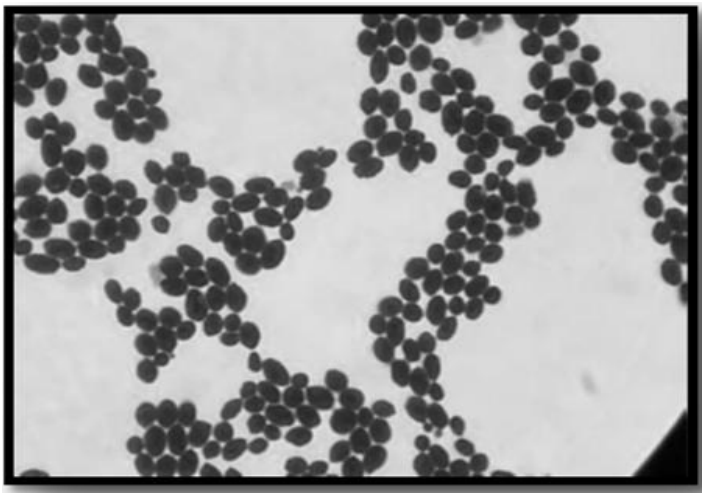

Figure 3. *Cryptococcus laurentii* from skin under microscope

Figure 4. *Cryptococcus laurentii* from skin and fins of *C. carpio*

2.3 Control and Prevention

Fungal spores are present everywhere, and poor water quality and stress in fish are two main factors that contribute to the disease outbreak in aquaculture system. Other factors contributing to fungal infections are; poor hygiene, low immunity and high amount of decomposing organic matter at the pond bottom (Mehta *et al*., 2024).

Maintain good water quality, ensure the aquatic environment (such as aquariums or ponds) has proper sufficient filtration regularly to reduce the buildup of organic matter, which can encourage fungal growth (Yusoff *et al*., 2024). Maintaining appropriate pH levels and temperature ranges suitable for the specific fish species can help prevent stress, making them less susceptible to infections. Preventing overcrowding because overcrowded conditions can stress fish, reducing their immune system's effectiveness(Chandra, 2024). Stress can also create an environment that favors the growth of opportunistic pathogens like fungi. Clean aquarium or tank equipment (such as nets, filters, and decorations) regularly to minimize contamination from pathogens like *Cryptococcus* that might be present in the environment (Qadir *et al*., 2025).

Table 2: Species identification of *Cryptococcus laurentii* isolated by the VITEK® 2 Compact system

Biochemical Details																	
3	LysA	-	4	lMLTa	-	5	LeuA	+	7	ARG	+	10	ERYa	-	12	GLYLa	-
13	TyrA	+	14	BNAG	+	15	ARBa	-	18	AMYa	+	19	dGALa	-	20	GENa	+
21	dGLUa	+	23	LACa	-	24	MAdGa	-	26	dCELa	+	27	GGT	-	28	dMALa	+
29	dRAFa	+	30	NAGA1	-	32	dMNEa	-	33	dMELa	-	34	dMLZa	+	38	lSBEa	-
39	lRHAa	-	40	XLTa	+	42	dSORa	+	44	SACa	+	45	URE	+	46	AGLU	-
47	dTURa	-	48	dTREa	+	49	NO3a	-	51	lARAa	+	52	dGATa	+	53	ESC	+
54	lGLTa	+	55	dXYLa	+	56	LATa	-	58	ACEa	+	59	CITa	-	60	GRTas	+
61	lPROa	+	62	2KGa	+	63	NAGa	-	64	dGNTa	(-)						

Conclusions

The article presents a significant advancement in the understanding of fungal infections in aquaculture, specifically highlighting the first isolation of *Cryptococcus laurentii* from common carp (*Cyprinus carpio*) in the Zrarati area of Erbil City, Kurdistan Region of Iraq. This finding underscores the need for increased surveillance and research into fungal pathogens affecting fish populations, as they can have serious implications for aquaculture health and productivity. The study emphasizes the importance of identifying and characterizing fungal infections to develop effective management strategies in aquaculture. It also calls for further research to explore the ecological and health impacts of *C. laurentii* on fish and the potential zoonotic risks it may pose to public health. Overall, the article contributes valuable insights into the field of aquatic mycology and highlights the necessity for ongoing monitoring and research to ensure sustainable aquaculture practices in the region.

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