JOURNAL OF ANALYTICAL SCIENCES AND APPLIED BIOTECHNOLOGY
An International Open Access, Peer Reviewed Research Journal

ISSN: 2665-8488
2023, Vol. 5, Issue 2
Pages: 139-144

Environment

DOI: 10.48402/IMIST.PRSM/jasab-v5i2.46086

Identification and Evaluation of Fungal Pathogens in Pond-Cultivated Catfish from Ketu Adie-Owe, Ado Odo Local Government Area, Ogun State (Nigeria)

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ARTICLE INFO

Received October 3rd, 2023
Received in revised form December 20th, 2023
Accepted December 20th, 2023

Keywords:
Clarias gariepinus,
Trichosporun spp.,
Malassezia,
Fusarium oxysporum,
Arthroderma quadrifidum.

ABSTRACT

Fish remains a crucial component of human nutrition, providing essential high protein and mineral content for survival. This study, conducted in Ketu Adie-Owe, Ado Odo Local Government Area of Ogun State, Nigeria, aims to isolate and comprehend fungal pathogens in pond catfish ecosystems. Three male *Clarias gariepinus* fish samples were collected from a concrete fish pond in Apena Village, Ketu, Igbesa, and transported aseptically to the Microbiology Laboratory at Crawford University for microbiological analysis. Sabouraud Dextrose Agar with chloramphenicol, to inhibit bacterial growth, was prepared for the isolation of fungi following the manufacturer's instructions. Skin and intestine samples from each fish were processed, leading to the identification of various fungal species, including yeast and mold types. Notable isolates included *Trichosporon spp.*, *Malassezia* (yeasts), *Fusarium oxysporum*, and *Arthroderma quadrifidum* (molds), each potentially contributing uniquely to the pondwater ecosystem. Understanding these isolates is important due to their potential to harm fish health, reduce marketability, and pose health risks to humans and animals. The study raised concerns about possible water contamination in the catfish pond, highlighting the risk of fish infections under such conditions. The research also underscored the ecological importance of fungi in pond catfish ecosystems, particularly in nutrient cycling and organic matter decomposition. The presence of pathogenic fungi emphasizes the need for vigilant monitoring and management to protect fish health, aquaculture productivity, and public well-being

1.Introduction:

Fish plays a complementary role in human nutrition, supplying essential high protein and mineral content vital for survival. The growing demand for fish and fisheries products in Nigeria has led to the establishment of numerous fish ponds **[1-2]**. Aquaculture, contributing over 70% of Nigeria's fish supply, is particularly prominent in Oyo State, where fish ponds vary in size from small to large commercial systems, reflecting the global trend of increasing popularity in fish pond aquaculture **[3-5]**. Various catfish species, including *Clarias anguillaris*, *Clarias gariepinus*, *Heterobranchus congifilis*, and *Heterobranchus bidorsalis*, are cultivated in these ponds, with *Clarias gariepinus* being the most extensively bred in Nigeria **[6]**.

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The cultivation of African catfish presents numerous advantages such as rapid growth, adaptability to environmental conditions, favorable sociological and physiological traits, and economic value **[7]**. It is highly recommended for fishing in Nigeria due to its low bone content, appealing flavor, and accelerated growth rate **[8]**. Ponds, with their relatively shallow depth, support a variety of aquatic organisms, distinguishing them from lakes. In Nigeria, different pond types, including earthen ponds, concrete ponds, and plastic-lined ponds, serve various purposes, including catfish rearing **[9]**.

Catfish farming in ponds has gained significant attention in Nigeria due to the high demand for catfish in the local market. The success of catfish farming relies on suitable water conditions, with temperatures ranging between 25℃ and 35℃ to promote growth and support microorganisms. Water, essential for fish ponds, can be sourced from wells, boreholes, streams, and rivers **[10]**.

The source and quantity of water are critical factors to consider when selecting a site for an aquaculture facility **[11]**. Unfortunately, few farmers analyze water samples before starting farming, which is a necessary step to ensure water quality. Water sources, including streams used directly on some catfish farms, may be contaminated with high loads of pathogenic bacteria, posing a risk to fish health, especially young fish. Microorganisms, introduced through various pathways such as organic matter decomposition, uneaten food, and fish waste, can infiltrate ponds. Contaminated water, fish, feed, equipment, and poor management practices contribute to microorganism infiltration, emphasizing the need for water quality training and the use of protected water sources **[12-13]**.

Fungi, initially classified as plants but later recognized as a distinct kingdom, play vital roles in aquatic ecosystems by cycling nutrients and decomposing organic matter. Despite their significance, research on aquatic fungi lags behind bacteria, resulting in limited knowledge about their diversity and ecological functions **[14-15]**. Fungi, distinct from plants, significantly contribute to diseases affecting aquatic and terrestrial life, thriving in various environments, including fish ponds, posing a risk to fish health and aquaculture productivity **[16]**. Aquatic environments can harbor fungal pathogens that harm stressed fish, emphasizing the importance of identifying sources and pathways of fungal spore transmission for disease control.

Fungi play pivotal roles in aquatic ecosystems, influencing carbon cycling, nutrient flow, and organic matter decomposition **[17]**. However, our understanding of aquatic fungal communities is limited, underscoring the need for extensive research to explore their patterns and ecological dynamics **[18]**. Fungal pathogens naturally occur in aquatic environments, including ponds, and stressed fish are more susceptible to these infections. Identifying predisposing factors, such as fungal spores in water, contaminated feed particles, and improper feed preservation, is crucial for disease control and maintaining fish population health in aquaculture.

Therefore, this study aims to isolate and characterize fungal species from pond catfish, shedding light on their presence, diversity, and potential impact on fish health and ecosystem dynamics.

2. Materials and methods:

2.1. Study Location:

Three male *Clarias gariepinus* samples were obtained from a concrete fish pond situated in Apena Village, Ketu, Igbesa, Ogun State, with coordinates of approximately 6° 32' 0.9672" N latitude and 3° 8' 2.9796" E longitude.

2.2. Sample Collection:

Live samples comprising a small fish (approximately 8 months old), a middle-aged fish (1.5 years old), and a matured fish (2 years old) were collected with proper authorization from the relevant authorities overseeing the pond. The live fish samples, collected directly from the pond water, were cautiously transported to the Microbiology Laboratory at Crawford University in Faith City, Ketu Adie-Owe, Lusada-Igbesa, Ogun State. To ensure the fish's well-being during transportation, a plastic container covered with a net was utilized to maintain proper airflow and prevent any potential escape or damage. The entire transportation process, from collection to arrival at the laboratory, was completed within an hour.

Earthen ponds

Concrete ponds

Plastic-lined ponds (Tarpaulin ponds)

Figure 1. Different types of ponds used for growing fish.

2.3. Sample & Media Preparation:

The workstation underwent sterilization with 70% ethanol. For media preparation, Sabouraud Dextrose Agar (SDA) was created following the manufacturer's specifications and supplemented with chloramphenicol (50 mg/L) to inhibit bacterial growth. Each fish sample's skin and intestine were individually weighed and aseptically macerated to attain a weight of 1 gram each. Subsequently, these fish tissues (skin and intestine) were placed into separate test tubes, each containing 9 ml of sterile distilled water, to establish a stock solution. This stock solution was then serially diluted up to a five-fold dilution (10^{-5}).

2.4. Isolation of Fungi:

0.1 ml from each of the skin and intestine sample dilutions (10^{-3}) was poured into Petri dishes filled with Sabouraud Dextrose Agar (SDA) as per the method described by Melaku *et al.* (2017) **[19]**. The agar plates were incubated for 3 to 7 days at a temperature of 28°C. Using a sterile inoculating loop, any identified fungal growth was subcultured onto freshly prepared SDA plates to obtain pure cultures of the various fungal isolates.

2.5. Identification and Characterization of Fungal Isolates:

To identify the isolates, a macroscopic and microscopic assessment of the isolates was conducted. Detailed macroscopic (morphological and cultural) characteristics of each isolate were recorded. Microscopic evaluation was performed using a Lactophenol-cotton blue preparation. A portion of the fungal mass was placed on a clean glass slide, stained with the mentioned pigment, and then prepared as a wet mount. After applying a cover slip, the preparation was examined under a microscope at X10 and X40 magnification to identify the fungi. Oil immersion was employed when necessary to enhance visibility under the microscope. To confirm the identity of the isolates, the results were documented and compared with the morphological traits of previously identified fungi in the Mycology atlas **[20]**.

3. Results and Discussion:

From diluent (10^{-3}), a total of 5 isolates (MFS 10^{-3}, MFI 10^{-3}, LFS 10^{-3}, SFI 10^{-3}, LFI 10^{-3}) were obtained through the process of culturing and subculturing on SDA. Following a meticulous examination of the isolate morphology and cultural characteristics, they were categorized into 3 yeasts (MFS 10^{-3}, MFI 10^{-3}, LFS 10^{-3}) and 2 molds (SFI 10^{-3}, LFI 10^{-3}).

Table 1. Morphological/Cultural Characteristics of Yeast Isolates.

Isolates	Morphological/Cultural Characteristics on SDA	Cell shape	Probable Organism
MFS 10^{-3}	Form creamy or white colonies with a wrinkled texture.	Appear as oval or elongated yeast cells.	*Trichosporun spp.*
MFI 10^{-3}	Small, cream-colored colonies with a smooth texture.	Appear as oval or round yeast budding cells.	*Malassezia*
LFI 10^{-3}	Small, creamy, or white colonies with a wrinkled texture.	Appear as oval or round yeast budding cells.	*Malassezia*

(**SFS=** Small Catfish Skin, **SFI=** Small Catfish Intestine, **MFS=** Medium Catfish Skin, **MFI=** Medium Catfish Intestine, **LFS=** Large Catfish Skin**, LFI=** Large Catfish Intestine), (SDA= Sabouraud Dextrose Agar).

Table 2. Morphological/Cultural Characteristics of Mold Isolates.

Isolates	Morphological/Cultural Characteristics on SDA	Cell shape	Probable Organism
SFI 10^{-3}	Rapidly growing colonies ranging in color from white to pink.	Long, branching hyphae.	*Fusarium oxysporum*
LFI 10^{-3}	Fast-growing colonies that appear cottony, and fluffy.	Exhibits septate hyphae	*Arthroderma quadrifidum*

(SFS= Small Catfish Skin, SFI= Small Catfish Intestine, MFS= Medium Catfish Skin, MFI= Medium Catfish Intestine, LFS= Large Catfish Skin, LFI= Large Catfish Intestine), (SDA= Sabouraud Dextrose Agar).

This study reveals a diverse array of fungal species inhabiting the pond catfish environment, encompassing both yeast and mold species. This diversity underscores the spectrum of fungal organisms associated with catfish in aquatic ecosystems.

The yeast isolates identified in (Table 1) encompass *Trichosporun* spp. and *Malassezia*. (Table 2) outlines the morphological and cultural characteristics of mold isolates, including *Fusarium oxysporum* and *Arthroderma quadrifidum*. These isolates play distinct ecological roles within the catfish ecosystem.

Identification of these yeast and mold isolates is paramount for several reasons as it sheds light on the diversity and abundance of fungal species in pond catfish, contributing to our understanding of the overall fungal ecology within this setting. Furthermore, identifying specific yeast and mold species allows for the assessment of their potential pathogenicity and associated risks to fish health, and consumers. Certain yeast and mold species may induce infections or produce toxins, which can negatively affect fish health and marketability. The presence of fungi and their mycotoxins in fish represents a significant health risk for both humans and animals **[21]**.

The diversity of fungal isolates in pond catfish underscores their possible ecological importance in preserving pond ecosystem health and balance. *Trichosporon* spp. and *Malassezia*, yeast-like fungi found in pond catfish habitats, are crucial contributors to nutrient cycling and organic matter decomposition. *Trichosporon* spp. metabolize complex organic compounds, releasing essential elements back into the environment. *Malassezia* plays a role in organic matter degradation and nutrient cycling, potentially engaging in interactions with other organisms. *Fusarium oxysporum* contributes to decomposing organic matter, particularly plant materials. This decomposition contributes to nutrient cycling and ecosystem balance. *Arthroderma quadrifidum* is involved in the decomposition of organic matter and nutrient cycling within pond ecosystems. Some strains of Arthroderma quadrifidum can also be pathogens, causing skin infections in fish. The presence of these fungi in the skin and intestines of catfish within the pond can adversely impact catfish health and production. *Trichosporon* spp. and *Malassezia* can induce skin infections in catfish, leading to skin lesions, ulcers, and scale shedding. This compromises the fish's protective barrier, making them more susceptible to external pathogens and environmental stressors. *Fusarium oxysporum* and *Arthroderma quadrifidum* in the fish's intestines can cause intestinal infections, disrupting the digestive system, and leading to poor nutrient absorption and weight loss. Fungal infections in catfish can result in economic losses within aquaculture production, reducing the market value and quality of infected fish. In severe cases, outbreaks can lead to mass mortalities, significantly impacting fish farmers financially. People in contact with infected fish or contaminated water may be susceptible to skin infections or other health issues. Fungal infections in fish also raise concerns about food safety, as improperly cooked infected fish may transmit fungal pathogens to consumers, potentially causing foodborne illnesses **[22-24]**. In contrast to the findings reported by Melaku *et al.* (2017) **[19]**, who identified four fungal genera, including *Mucor*, *Aspergillus*, *Trichophyton*, and *Penicillium*, from *Clarias gariepinus* in selected farms and dams around Zaria and its vicinity, our study has revealed a distinct set of fungal species in catfish samples, *Trichosporon* spp., *Malassezia*, *Fusarium oxysporum*, and *Arthroderma quadrifidum*. The presence of these fungi in catfish samples indicates potential water contamination, highlighting concerns regarding fish susceptibility to infections when exposed to such contaminated water. Inadequate management practices within catfish ponds, characterized by factors like poor maintenance, the accumulation of excessive decomposing organic material, the presence of unhealthy or injured fish, and other stress-inducing conditions, may contribute to the proliferation of fungi within these aquatic environments **[25]**. This study also differs from the findings of Czeczuga *et al.* (2013) **[26]**. While there are numerous reports of true fungi acting as the main infection agents in adult fish species of the *Clarias* genus from the African continent, the specific fungal species identified in our catfish samples deviate from those previously documented. *Fusarium oxysporum*, which we isolated in this study, was also identified by **[27]** in their investigation of freshwater fungi in the eggs and broodstock of *C. gariepinus* in Zaria, although the *Fusarium* species they identified was *Fusarium solani*.

This study underscores the ecological importance of fungi in pond catfish ecosystems, emphasizing their roles in nutrient cycling, organic matter decomposition, and overall ecosystem health. The presence of pathogenic fungal species demands careful monitoring and management to mitigate adverse impacts on fish health, aquaculture production, and public health in general.

4. Conclusion:

This study underscores the ecological importance of fungi in pond catfish ecosystems, emphasizing their roles in nutrient cycling, organic matter decomposition, and overall ecosystem health. The presence of pathogenic fungal species demands careful monitoring and management to mitigate adverse impacts on fish health, aquaculture production, and public health in general.

Acknowledgement

We would like to acknowledge the Microbiology Laboratory of Crawford University for providing the necessary facilities to conduct this study. The authors wish to express a deep appreciation to the fish farmers and owners for making their ponds available for us to collect our samples. The research was self-funded

Funding: This research received no external funding.
Institutional Review Board Statement: Not applicable.
Informed Consent Statement: Not applicable.
Data Availability Statement: Data is contained within the article.
Conflicts of Interest: The authors declare no conflict of interest.

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