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Assessment of Fungi Infection on *Clarias anguillaris* (Linnaeus, 1758) and *Oreochromis niloticus* (Linnaeus, 1758), Two Fish Species Farmed in Burkina Faso

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Abstract

Worlwide, fungi are by far the most common pathogen in fish farming. They are responsible of important economic losses in the fishery sector. However, in Burkina Faso knowledge of fungi infecting farmed fish are scarce. As a consequence, the prevalence as well as the effect of these pathogens are not known. A study was carried out on fungal infection of farmed fish. It aimed to evaluate the diversity of fungi associated with *Oreochromis niloticus* (Linnaeus, 1758) and *Clarias anguillaris* (Linnaeus, 1758) farmed in Burkina Faso. In total, 89 individuals of fish consisted of 47 specimens of *Oreochromis niloticus* and 42 specimens of *Clarias anguillaris* were collected from eleven fish farms. Swabs were taken on fish bodies and inoculated in Malt Extract Agar (MEA). The chloramphenicol was added to avoid bacterial contamination. Seventy-seven (77) fish were infected by fungi (85.51%). Four genera including Aspergillus (four species), Rhizopus (one species), Penicillium (one species) and Mucor (one species) were isolated. Small and big fish were all infected. This study shows fungi as a potential factor that impacts fish farming and suggests a need for more research on their effect on fish. Knowledge of fish parasites like fungi will allow to set effective means of fish pathology control, which once implemented will lead to an increase in fish farming productivity.

Keywords: Burkina Faso, farmed fish, fungi, pathology

1. Introduction

It has been accepted that fish represents a vital source of development, food and livelihood for humanity (Ali et al., 2022) and an important component of biodiversity (Kabré, 1997). Worldwide, they have been providing an important part of nutrients to people since ancient times and are considered as the most accessible and high nutritional value source of animal protein for much of the world's population (Hecht, 2005; Hussain et al., 2011). Global fish consumption by humans has significantly increased worldwide for over 50 years thanks to rapid population growth and factors such as developing countries' economic improvement and changes in developed countries' dietary patterns (Supartini et al., 2018). From 9 kg per year and per capita in 1961, it has exceeded 20 kg per year and per capita in recent years (FAO, 2020). In developing countries, apparent fish consumption has increased significantly, from 5.2 kg per capita in 1961 to 19.4 kg in 2017 (FAO, 2020). However, there is a critical global decline in natural fish stocks as a result of anthropogenic pressures (Limburg et al., 2011; FAO, 2016). Natural fish production supports have reached their maximum production limits (Marijani et al., 2019). In Burkina Faso, the story is not much different because the domestic supply of fish estimated at around 25,000 tons comes essentially from capture fisheries at more than 95% (FAO, 2024) and can only cover less than 20% of the national demand for fish, which is estimated to be more than 130,000 tonnes per year. The gap is filled by imports which are very costly for the country. Hence, the government like in other developing countries, encourages and promotes fish farming as an alternative to weakened captured fish (Maina et al., 2017). Some sixty public and private aquaculture units (ponds, community ponds, enclosures and floating cages) have been

created. These units produce both fish and algae (spirulina) and various production techniques are developed (FAO, 2024). These new opportunities to increase fish availability and improve the economic situation may face many economic, technical, scientific and health constraints. Generally, numerous freshwater fish species are seriously afflicted with various pathogens (Areda et al., 2019) which can impede the development of the fish culture system that is directly connected to these fish. The occurrence of diseases on farms may be a significant limiting factor for fish farming. Indeed, pathogens jeopardize fish by altering their metabolism, their reproduction, and their alimentation, by damaging their organs such as scales, gills, fins, eyes (Iqbal & Sajjad, 2013; Saleemi et al., 2020; Alam et al., 2023) as well as reducing their immune system (Namulawa et al., 2020). They can also stimulate the formation of tumoral cells on fish. In fish farming, the problem of parasite-related pathology is particularly acute as the occurrence of diseases in fish can cause significant losses in fish farms (Hossain et al., 2007; Nematollahi et al., 2012). Among pathogens, fungi are by far the most important regarding their high prevalence in fish farms (Marijani et al., 2019) and their capacity to attack all the life stages of fish from egg to adult (Iberahim et al., 2023) and cause serious losses in aquaculture (Eissaa et al., 2013; Iqbal & Saleemi, 2013; Özcan & Arserim, 2022).

In Burkina Faso, studies revealed the presence and negative effect of parasites on wild freshwater fishes (Kabre, 1997; Boungou et al., 2006; Sinare et al., 2023). However relatively, little research has been conducted on parasites and other fish pathogens of farmed fish, hence the interest of our study which was designed to evaluate the diversity of fungi associated with two fish species ((*Oreochromis niloticus* (Linnaeus, 1758) and *Clarias anguillaris* (Linnaeus, 1758)) farmed in Burkina Faso.

2. Materials and Methods

2.1 Study Site

The present study was conducted in the urban and peri-urban zones in the central part of Burkina Faso (that includes Ouagadougou and its surrounding urban zones). Eleven fish farms located in Saaba, Komsilga, Kamsoatenga, Koubri, Karpala, Zagtouli, Poedogo, Benogo, Boudgtenga and Ziga were visited. Farms in Ziga belong to the government while all other farms visited are owned by individuals.

A list of fish farmers with their respective contacts was provided by the Director of aquaculture of the fisheries department (General Directorate of Fish Resources). They were first called and an appointment was aranged. Following this first exchange, the active ones were identified and located.

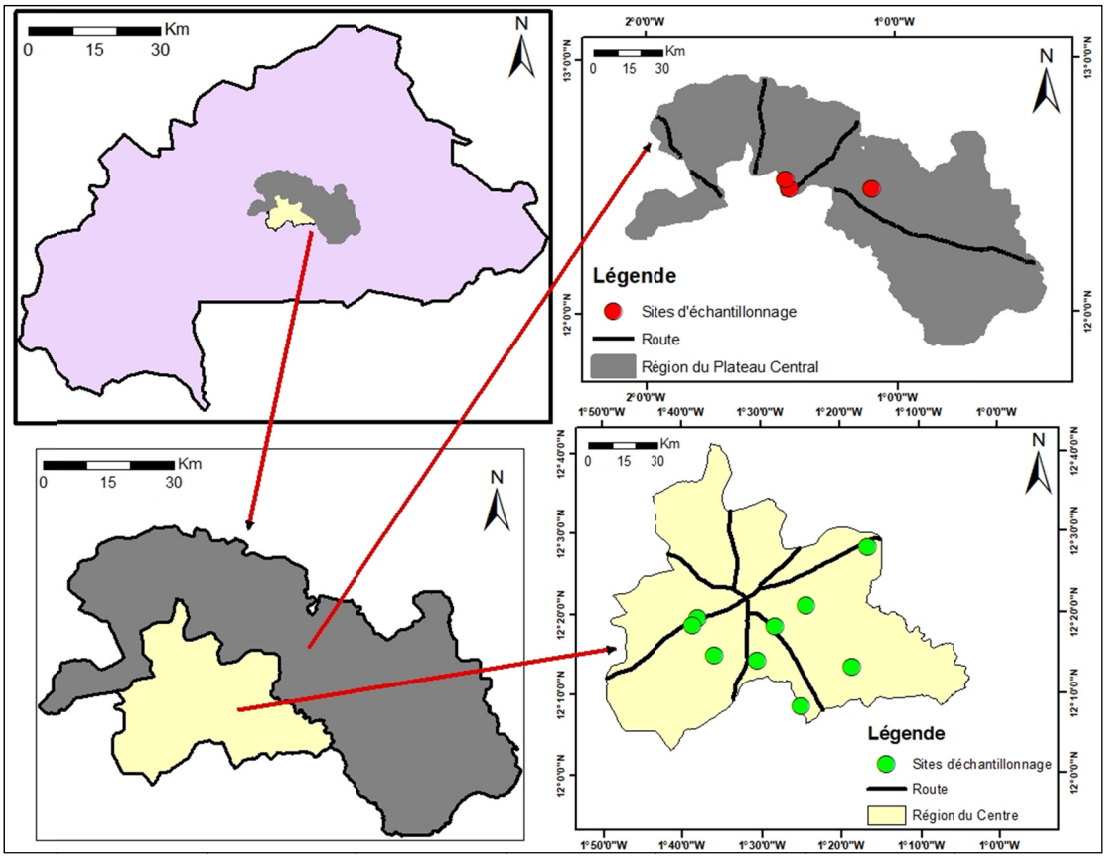

Figure 1. Map of sampling sites

2.2 Fish Sampling, Examination and Collection of Fungi

Fish farms were visited from February to April 2022. This study was conduced to make an inventory and determine the prevalence of fungi in farms. The fish species included in the current study were *C. anguillaris* and *O. niloticus.* They are the most farmed and economically important fish as they are preferred by consumers and farmers in Burkina Faso.

Fish were collected once per site. In each farm, 10-15 live specimens of different sizes were collected from 8 am to 10 am using fishing nets and then transferred into sterile polyethylene bags to avoid any external contamination. The bags were stored in ice packs and immediately brought to the Laboratory of Animals Biology and Ecology at the University Joseph Ki-Zerbo, Ouagadougou for analysis. Once in the lab, each specimen was disinfected with cotton containing 70° alcohol. Then, swabs were taken from the mouth, eyes, gills, head, fins, and the rest of the body of the fish to collect the fungi organisms. Their microorganisms were cultured according to the method of Iqbal et al. (2012). Indeed, each swab was inoculated in an agar media (Malt Extract Agar (MEA). To avoid bacterial contamination, chloramphenicol was added to each sample during the preparations and the whole was incubated at 28-30 °C. Fungal colony growth was observed after 5-7 days. Pure cultures were made by incubating part of the colony onto the same medium type for 4-5 days. Fungi were described and identified using the keys of Mathur & Kongsdal (2003), Campbell et al. (2013) and descriptions found in the literature.

All fish were weighed using a digital weighing scale and their total and standard lengths were taken with an ichthyometer and recorded.

2.3 Data Analysis

R 4.4.1 software was used for the data analysis. Biotic diversity such as fungi species richness was simply taken as the count number of species in the sample based on the presence/absence matrix. The epidemiological indices defined by Bush et al. (1997) were calculated. The overall prevalence of the fungi infection was expressed using the Equation (1). Then the occurrence rate of each fungi species was estimated using the Equation (2).

$$\text{Prevalence} = \frac{\text{Individuals number of infected fish}}{\text{Individuals number of examined fish}} \times 100 \quad (1)$$

$$F = \frac{n}{N} \times 100 \quad (2)$$

where, n: number of fish in which the considered fungi species is recorded; N: total number of examined fish.

The Chi-square Test was performed to test the relation between infection rate and fish length ($P < 0.05$ was considered significant).

For visualization, Tables, bar charts and Venn diagram were used to present fungi fauna composition. All analysis and graphs were performed in Excel and using R 4.4.1.

3. Results

Eighty-nine (89) individuals of fish consisted of 47 specimens of *Oreochromis niloticus* and 42 specimens of *Clarias anguillaris* were investigated for fungal infection. These specimens consisted of variable sizes. Regarding Clarias, their standard sizes varied between 15.0 cm and 28.8 cm and they weighted from 37 g to 343 g while Oreochromis sizes varied from 10.1 cm to 22.2 cm and they weighted between 26 g and 340 g.

Seventy-seven (77) fish were infected. That represented a prevalence of 85.51%. Single and mixed infections were observed for both species (Figure 2). However, *Orechromis niloticus* showed a higher prevalence (87.23%) and higher mixed infections (70.73%) than *Clarias anguillaris* (prevalence: 85.71%; mixed infections: 55.55%).

All fungi parasites observed and recorded in this study belonged to four genus including Aspergillus, Rhizopus, Penicillium and Mucor. In total, four Aspergillus, one Rhizopus, one Penicillium and one Mucor were recorded. Table 1 presents the occurrence of fungi species.

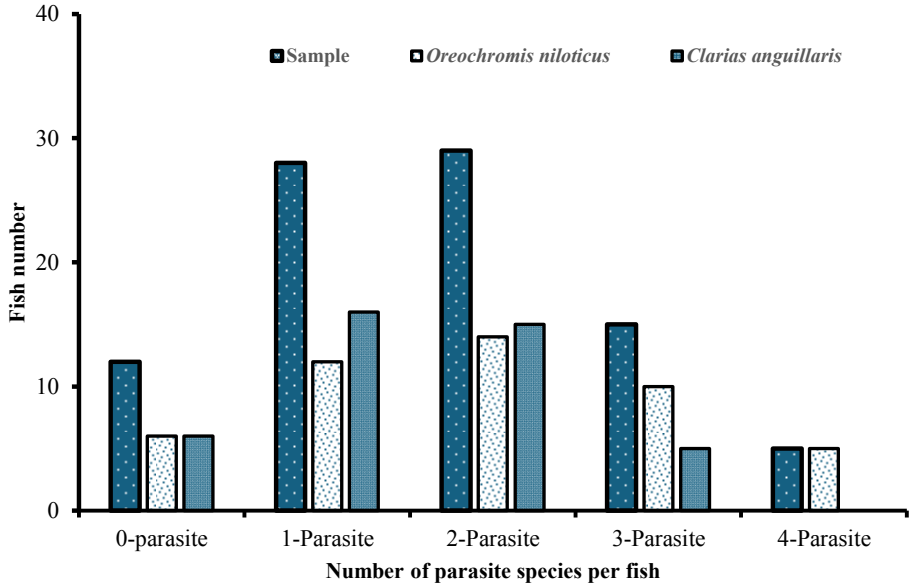

Figure 2. Overview of fungi infection on fish (0-parasite = no infection; 1-Parasite, 2-Parasite, 3-Parasite, 4-Parasite = number of fish individuals infected respectivelly by one, two, three and four fungi species)

Table 1. Occurrences of fungi in the whole sample and according to fish species

	Fungi species						
	Aspergillus niger	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus* sp.	*Rhizopus* sp.	*Mucor* sp.	*Penicillium* sp.
Occurrence on Sample (%)	48.31	16.85	39.33	35.96	5.62	19.10	4.49
Occurrence on *Oreochromis niloticus* (%)	48.94	19.15	51.06	40.43	6.38	23.40	2.13
Occurrence on *Clarias anguillaris* (%)	47.62	14.29	26.19	30.95	4.76	14.29	7.14

Figure 3 presents infection for the genus Aspergillus. Mixed infections were observed for all fungi species belonging to this genus. Single infections with *Aspergillus niger* and *Aspergillus* sp. were more important for

Clarias anguillaris (Figure 3A) while *Aspergillus fumigatus* recorded higher single infection for *Oreochromis niloticus* (Figure 3B). One specimen of *Oreochromis niloticus* was infected by the all species of Aspergillus.

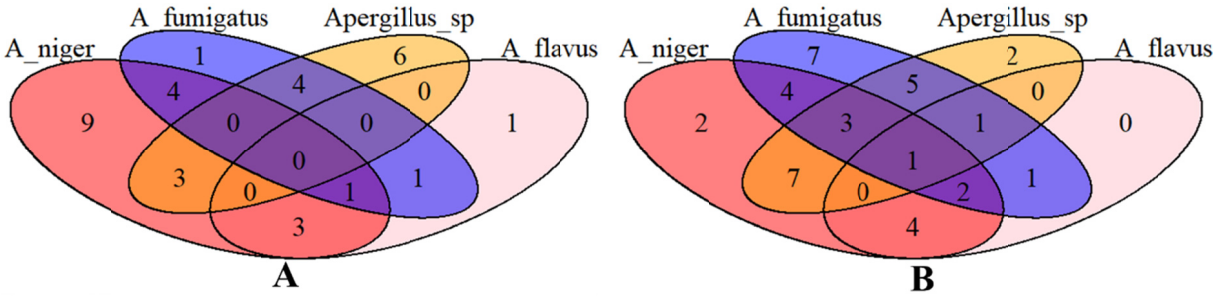

Figure 3. Occurrences of Aspergillus in *Clarias anguillaris* (A) and *Oreochromis niloticus* (B)

The variation of fungi infection in relation to fish size was investigated. For *Clarias anguilaris,* fish length categories [15-20[cm was all infected (100%). The prevalence in the other length categories was also high (more than 80%). The prevalence of fungi infections in relation to fish size showed no statistical significant difference for *Clarias anguilaris* (Chi-square = 2.21; $p > 0.05$) (Table 2). The variation of fungi infection in relation to *Oreochromis niloticus* fish size showed slightly higher prevalence (92.31%) for size categories [15-20[cm but no significant difference shown in comparison to the infection in the other size categories (Chi-square = 0.44; $p > 0.05$) (Table 3).

Table 2. Fungi infection in relation to length of *Clarias anguillaris*

	Length Group			
	[15-20[	[20-25[	[25-30[	Total
Number of examined fish	10	26	6	42
Number of infected fish	10	21	5	36
Prévalence (%)	100	80.77	83.33	85.71

Note. Chi-square = 2.21; $p > 0.05$.

Table 3. Fungi infection in relation to length of *Oreochromis niloticus*

	Length Group			
	[10-15[	[15-20[	[20-25[	Total
Number of examined fish	28	13	6	47
Number of infected fish	24	12	5	41
Prévalence (%)	85.71	92.31	83.33	87.23

Note. Chi-square = 0.44; $p > 0.05$.

4. Discussion

The fungi isolated from the fish sampled belong to four genera. They were composed of the genera Aspergillus, Rhizopus, Mucor and Penicillium. The results of this study highlighted the high presence of fungi in fish farms in Burkina Faso. Indeed, more than 80% of sampled fish were infected by at least one fungus species. Additionally, many cases of mixte infection were also noted in this study. These results are consistent with the findings of several studies conducted on fresh fish (Adamu et al., 2020) fish feed (Marijani et al., 2019; Nyamwaka et al., 2020) and dried fish (Deng et al., 2021). According to some research, the source of fungal infection may be the consumption of contaminated food in the pond (Marijani et al., 2019; Sousa Terada-Nascimento et al., 2023). In addition, these foods and large amounts of other decaying organic matter in the pond contribute to increased infection (Nadia & Nashwa, 2014). Other cited sources of fungal infection may

include non-regular maintenance of the pond, injured fish and sick fish and also poor water quality which remains one of the biggest factors in fungal growth (Nadia & Nashwa, 2014).

The high prevalence of fungi recorded in the present study highlighted a potential risk for fish farming in Burkina Faso. It also raised a lot of concern about the health safety of humans and other animals consuming fish. Indeed, some genera of fungi such as Aspergillus and Penicillium identified in this study have been known to produce mycotoxins, a metabolite that represents an ecotoxicological risk for human and animal health (Marijani et al., 2019; Danial et al., 2020). These mycotoxins can affect negatively productivity, immune and damage vital tissues and organs in animals and humans (CAST, 2003; Marijani et al., 2019; Alam et al., 2023; Sousa Terada-Nascimento et al., 2023). Also, fungal infections have been identified as one of the most serious causes of economic losses in aquaculture (Gozlan et al., 2014; Iqbal et al., 2023).

The high prevalence of fungi in this study also raised concerns about the outbreak of fungal infections in these fish and could be a public health concern. Moreover, the isolation of *A. flavus* gave an alarming message since it could predispose fish to aflatoxin contamination and expose consumers (Gonçalves et al., 2018). Indeed, aflatoxin is a toxic compound that has been incriminated previously in livestock and human beings deaths (Ghadeer & Al-Delamiy, 2012; Abdallah et al., 2022).

The genera Aspergillus was the most recorded. Their high tolerance to environmental conditions could explain their high occurrence. Many studies have confirmed a similar predominance of Aspergillus species (Marijani et al., 2019). These authors have reported that Aspergillus have ubiquitous distribution in tropical countries where they were isolated from fish feeds and their ingredients and fish themselves (Marijani et al., 2019).

5. Conclusions

Fungi of genera Aspergillus, Rhizopus, Mucor and Penicillum, were isolated from farmed fish. The presence of the genus Aspergillus and Flavus which are already incriminated in previous studies should draw the attention of consumers. This study suggests that fungi infect most of the fish farmed in Burkina Faso. Appropriate precautions should be undertaken to protect fish consumers and fish farmers. More studies on farmed fish are needed to evaluate fish infection and risk for farmers and consumers.

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Authors Contributions

KM, LLBF and RO conceived and designed the study under the supervision of AG. KM, LLBF and RO performed the fieldwork. Laboratory work was performed by LLBF assisted by KD under the supervision of ES. Data analysis and manuscript editing were done by KM assisted by LLBF, JK and AG. All the work was done under the supervision of KWN and AG. All authors have read and agreed to the published version of the manuscript.

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Data Sharing Statement

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