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Isolation and Identification of *Aspergillus* sp. in Tilapia (*Oreochromis* sp.) Sold for Consumption in Bogor

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Abstract

This study investigated the prevalence of *Aspergillus* sp. in tilapia sold for consumption at Pasarean Village in Bogor District. A total of 50 fish were sampled for further investigation. The clinical signs of fish were eroded fins, gills, haemorrhages, and wounds. The presence of pathogenic fungi was confirmed through fungal isolation from the skin, gills, and cloaca and subsequent culturing on potato dextrose agar. Macroscopic and microscopic observations determined *Aspergillus* sp. The results showed that four *Aspergillus* species were identified i.e., *A. terreus* (2%), *A. flavus* (4%), *A. fumigatus* (42%), and *A. niger* (4%), with the occurrence of co-infection between *A. fumigatus* and *A. flavus* (2%); *A. fumigatus* and *A. terreus* (2%); and between *A. fumigatus*, *A. flavus*, and *A. niger* (2%) reported a total prevalence of 58%. Further study is necessary to mitigate the crucial impact of fungal diseases in aquaculture systems, leading to effective prevention and control strategies, thus ensuring the safety and sustainability of aquaculture practices in Indonesia.

Keywords: *Aspergillus* sp., aquaculture, pathogenic fungi, threat, tilapia

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INTRODUCTION

One of the essential nutrients that people require in large quantities is protein. The two main types of proteins consumed by humans are animal and plant proteins. One of the natural sources of animal protein Indonesians consume is fish (Yanestria *et al*., 2020). In 2018, Indonesians consumed 50.68 kg of fish per person per year (KKP 2019). At current prices, the Indonesian fisheries sector's gross domestic product (GDP) expanded significantly from 2010 to 2018, with growth rates as high as 13.19 percent a year. The expected value of the GDP in 2018 was US $27.293 (BI 2019). The production cost, generally less than other protein sources, is another crucial factor. Fish demand will likely dramatically rise as people become more aware of the future health benefits of eating fish. This condition will increase the number of cultivated fish to meet production demand through aquaculture (Wibowo *et al*., 2021). According to the Food and Agriculture Organization of the United Nations (FAO), aquaculture is the raising of aquatic animals and plants, including fish, mollusks, crabs, and fish. Farming typically entails some intervention to boost productivity, such as frequent stocking, feeding, and predator protection. In some areas, it also necessitates the reuse of wastewater. However, due to untreated sewage, this aquaculture-related waste-water reuse may raise questions about food safety (Fedorova *et al*., 2022). In addition, farming entails the production and transportation of raised livestock and the planning, developing, and managing of aquaculture sites, facilities, and procedures (Palma and Viegas, 2022).

Tilapia (*Oreochromis* sp.) is a genus of fish that is primarily cultured and has an essential economic impact globally. More than 100 tilapia species have been identified (Naraballobh *et al*., 2021). Tilapia is a vital source of protein in many developing countries. It is known that the three countries with the highest production of tilapia are China, Egypt, and Indonesia. Despite being a fish species with a relatively long lifespan, tilapia is

susceptible to parasitic, viral, bacterial, and fungal infections (Al-Hussinee *et al*., 2019). However, studies that reported fungal infection occurrence in Indonesian tilapias are limited.

Indonesia is the world's biggest archipelago, with 17,504 islands and a 99,093 km-long coastline. The country is located strategically between the Pacific and Indian Oceans (Ariansyach, 2017). Since fish is a substantial component of the typical person's diet and most of Indonesia's villages are situated along the shore, the nation's fisheries industry is essential to preserving national food security. The fishing sector in Indonesia benefits from both capture fishing and aquaculture. While capture fisheries encompass marine and interior waters, aquaculture includes marine, seaweed, floating net, brackish water pond, freshwater pond, cage, floating cage net, and pen fish (Sapari, 2019). West Java Province is one of Indonesia's provinces that has become the country's largest tilapia producer. In addition, Bogor City is one of the largest tilapia producers in the province. Information about fungal diseases in Tilapia in West Java, including Bogor City, has not been reported (Soetipto *et al*., 2019).

However, environmental contaminants from both natural and anthropogenic sources can potentially harm living organisms. They frequently relate to chemical, biological, and physical pollutants, and aquatic animals like fish are vulnerable to them due to bioaccumulation. Over 118 environmental contaminants related to fish have been discovered, some of which may harm human health. Humans are essential in finding and controlling environmental pollutants (Alam *et al*., 2016). Thus, this study aimed to investigate the presence of *Aspergillus* sp. found in tilapia fish sold for consumption in the Bogor District.

MATERIALS AND METHODS

Ethical Approval

This study did not require ethical approval because the researchers purchased fish sold for consumption from the aquaculture farm. However, the researchers used the most appropriate method to provide the most satisfactory result while minimising the sampled fish's pain, suffering, and distress.

Study Period and Location

The sample collection was performed between September–December 2022 at Pasarean Village, Pamijahan Sub-district, Bogor District.

Samples

The samples were 50 red tilapias. A total of 50 tilapia fish were collected in the pond using a large fish net provided by the workers in the fishing village and transferred into a bucket. The fish samples will then be collected and separated into sterile plastic, with a specific label assigned to each plastic with fish for more accessible documentation. The samples were transferred to the lab using an ice box maintained at 2°C for swabbing.

Gill, Skin, and Cloacal Cavity Swab Procedure

The procedure of collecting swab samples from skin, gills, and cloaca was carried out according to Noga (2010). The gills were visually inspected before swabbing. The inspection of the gills includes the colour. Gills in good health are bright crimson. On the contrary, unhealthy can be seen as the pale pink gills indicate anaemia, and pale tan gills indicate the production of methaemoglobin (Noga, 2010; Fikri *et al*., 2022). A sterile cotton bud was used to collect swab samples from the gills. The operculum that covered the gills was lifted, and a sterile swab was rolled around the gills following the procedures carried out by previous studies (Clinton *et al*., 2021; Salawudeen *et al*., 2017; Touhali, 2018). Skin samples were obtained by carefully swabbing the skin of each fish where skin and fin lesions occurred and throughout the body using a sterile cotton swab (Melaku *et al*., 2017; Roslan *et al*., 2023). The swabbing direction is particularly crucial when dealing with fish that have scales (Breacker *et al*., 2017). The cloacal cavity was opened, and the swab was inserted into the cloacal cavity. The residual faeces can be collected with a swab from the cloaca for 5 seconds in a circular motion (Rivera *et al*., 2018).

Inoculating Swab Samples from Gills, Skin, and Cloaca on Potato Dextrose Agar (PDA) Plates

Each gill, skin, and cloaca swab sample was swabbed on PDA (OxoidTM CM139, Thermo Fisher Scientific Inc., the United States of America) plates and incubated at 25°C for 3 to 14 days. The growing fungi were then moved to a new agar to obtain a pure fungal colony. The pure culture was repeatedly carried out until the agar was occupied by only one species of fungus (Roslan *et al*., 2023).

It is essential to minimise contamination from other microorganisms, such as bacteria, so the petri dish lid was kept as close to the base as possible. The discontinuous streaking pattern, where the inoculation loop was sterilised at the end of each quadrant before streaking over the next quadrant, helps prevent cross-contamination between quadrants (Dahal, 2022). This method was repeated on the PDA plates for all 50 tilapia fish samples from skin, gills, and cloaca.

Macroscopic Observation of PDA Agar Plates

The inoculated samples were incubated for two weeks at 25–30°C. The procedure of incubation was not performed under an incubator. The incubation of filamentous fungi can be performed at temperatures between 25ºC and 30ºC, as reported by previous studies (Chauhan *et al*., 2014; Haroon *et al*., 2014; Iqbal and Khatoon, 2019). The fungal cultures' appearance, growth rate, texture and colour of the surface colonies, and the colour of the reverse side colonies were all used to identify the fungi growing on the plates (Refai *et al*., 2010). If more than one mould colony grew on each plate, each colony was subcultured on the new PDA plate. The subcultured isolates on the agar plates were carefully observed for another 2 weeks for the mould to grow to its complete form for further identification (Hapsari, 2014).

Microscopic Observation of PDA Agar Plates

Preliminary recognition of moulds was accomplished using wet mount preparation. A little bit of fungal growth was placed on a glass slide with a drop of distilled water; the mycelial mass was teased with two dissecting needles, covered with a clean cover slide, and inspected under the microscope (Abd El Tawab *et al*., 2020). Microscopic observation was carried out by observing the morphology of the hyphae, the shape of the spores, and the location of the spores (Hapsari, 2014). In this study, two types of microscopic observations were performed to identify the species of *Aspergillus*: direct observation and tape method observation. Direct microscopic mounts or squash preparations were carried out by removing a small portion of the colony with an inoculation needle with a sterile technique, and it was mounted in a drop of lactophenol cotton blue (LPCB) stain on a clean microscope slide and covered with a coverslip. The preparations were squashed with the butt of the inoculation needle, and the excess fluid was blotted off (Campbell *et al*., 2013; Kidd *et al*., 2022; Walsh *et al*., 2018). Tape or cello tape flag preparations are an excellent method for the rapid mounting of sporulating fungi because they keep more of the reproductive structures intact. The procedure used a clear, 2 cm-wide cello tape and a wooden applicator stick to make a small cello tape flag (2 × 2 cm). Using a sterile technique, the sticky side of the flag was gently pressed onto the surface of the culture. A drop of 95% alcohol was applied to the flag. The alcohol acted as a wetting agent and dissolved the adhesive glue holding the flag to the applicator stick. A small drop of LPCB was placed on the flag onto a clean glass slide, and the applicator stick was removed and discarded; another drop of LPCB stain was added, and the slide was covered with a coverslip, gently pressed, and mopped up any excess stain (Campbell *et al*., 2013; Kidd *et al*., 2022; Walsh *et al*., 2018). The diagnostic microscope needs to have a variety of magnifications (40×, 100×, and 400×). A low-power (4×) objective was used to scan the sample. The iris diaphragm was cut down on light, boosting the contrast. In contrast, an adjustable condenser on the microscope can be lowered to improve contrast (Noga, 2010).

Data Collection and Analysis

The macroscopic and microscopic observations were compared to the reference

books to determine the fungi species and were written down for data collection (Campbell *et al*., 2013; Walsh *et al*., 2018; Kidd *et al*., 2022). Primary data for fungi identification were taken by collecting swab samples from the fish samples' gills, skin, and cloaca. A visual examination of the internal and exterior components of the diseased fish was done. Morphological and clinical characterizations were performed for isolation and identification (Noga, 2010). The primary reference source for identifying the fungal species found in tilapia was Ellis *et al*. (2007). The results of the isolation and identification of fungi on tilapia were presented in a table. The figures of fungal colonies on the agar plates and the observation of the fungi on the microscope were captured. The percentage of samples that tested positive for a particular fungus was determined by dividing that number by the total number of samples analysed (Al-Niaeem *et al*., 2015). The following equation was used to calculate the prevalence of fungus:

$$\text{Prevalence (\%)} = \frac{\sum \text{infected fish} \times 100}{\sum \text{examined fish}}$$

RESULTS AND DISCUSSION

The Tilapia Fish Samples

The fish samples came from a farming village named Pasarean, where tilapia was one of the species bred and sold for consumption. There were multiple ponds where different man-made tilapia variants that were the products of ongoing selective breeding, mainly red tilapia, and black tilapia, were cultivated at the place of sampling. Red tilapia was chosen as the primary sample for the study because it is popular among farmers and is highly sought after in many markets. The fish samples were taken from two different ponds, labelled A and B. The total number of fish samples collected was 50, of which 24 were taken from Pond A and 26 from Pond B. The average body weight of the sampled fish in Pond A was 111.7 g, whereas for Pond B, it was 46.9 g, giving an average total weight of 79.3 g. Dead fish samples were discarded immediately and were not included in the study.

Observation of the Pathological Lesions

The tilapia samples exhibited various clinical signs upon observation in the lab, including eroded tails, fins, gills, gill marbling, bleeding, wound lesions, and more (Figure 1). However, it was observed that not all samples that displayed clinical signs in their skin, gills, and cloaca were infected with *Aspergillus*. It was also noted that some samples tested positive for *Aspergillus* infection despite showing no apparent clinical signs. Of the total, 13 samples demonstrated both clinical signs and were confirmed to be infected with *Aspergillu*s. Clinical symptoms of fungal infection in fish can vary depending on the fungus type and the disease's severity. In this regard, the fish samples presented in Figure 1 visually represent the physical manifestations of fungal infection. The first image (a) shows mucous, marbling, slight erosion, and bleeding on the gills, common symptoms of *Aspergillus* infection. The second image (b) displays complete erosion on the pectoral and caudal fins, resulting from fungal or parasitic infection or physical injury. Finally, image (c) displays an apparent lesion on the chin, another possible clinical symptom of an infectious disease. Lesions were noticed on many samples, but deep wounds were not observed in any fish. These visual representations of fungal infection in fish highlight the importance of early detection and monitoring of clinical symptoms to prevent outbreaks and minimize economic and health impacts.

As mentioned in the study, the clinical symptoms observed in fish can serve as a conduit for introducing diverse pathogens, including fungal infections such as *Aspergillus*. It is important to note that these clinical symptoms, such as eroded tails and fins, wound lesions, and gill marbling, may also be caused by a fungal infection. The presence of *Aspergillus*, a common fungal pathogen, highlights the potential role of this specific pathogen in contributing to the observed clinical symptoms in this study. This study observed the link between clinical symptoms and fungal infections. The observation of clinical symptoms is crucial for the diagnosis and treatment of diseases in fish.

According to a study by Saleemi *et al*. (2020), silver carp infected with *Aspergillus* sp. exhibited comparable clinical symptoms such as scale erosions, damaged fins, and lesions. Haroon *et al*. (2014) also reported fin erosions, haemorrhages, and body surface lesions from freshwater ornamental fish infected by fungi. A study conducted by Podeti and Benarjee (2016) revealed similar symptoms observed in fish infected with fungi, such as damaged fins, erosions, and skin lesions, and identified the presence of *A. fumigatus* and *A. niger* infections in *Channa striatus* (Bloch) among other species of different genera.

Macroscopic and Microscopic Observation of Isolated Fungi

Two weeks after incubation at 25–30 ºC, macroscopic and microscopic observations of the cultured fungi were carried out. Previous literature showed that macroscopic and microscopic are essential for identifying fungal species, including *Aspergillus* sp. The macroscopic observation was carried out by observing the colour of the colony surface, the colony colour from the reverse, the texture, and the topography. The suspected *Aspergillus* sp. from macroscopic observation is confirmed through further microscopic observation. In microscopic observation, the *Aspergillus* structures that should be observed for identification are septate-hyphae, vesicle, conidia, conidiophore, metulae, phialides, and foot cells. According to macroscopic and microscopic observations, this study reported four species of *Aspergillus* from tilapia, namely *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*. The aspergillum-like conidial bearing structure, or conidial head, primarily defines the genus' microscopic traits. The development of conidiophores with enormous stipes and inflated apices, or vesicles, distinguishes the genus *Aspergillus*. Vesicles are typically spherical; however, other fungal species have elongated vesicles or vesicles that are less obviously inflated. Vesicles typically bore metulae and phialides, sometimes known as crowded phialides, simultaneously (Li Yee and Zakaria 2018).

Aspergillus niger

In this study, *A. niger* colonies in PDA agar were observed as black on the surface with a reverse of tan or green. The texture was observed as powdery, and the topography was observed as flat (Table 1). The analysed sample depicted in Figure 2 was identified as *A. niger*, one of the most widespread and easily recognisable species of *Aspergillus*. Macroscopic observation of the isolated sample on the PDA plate (Figures 2A and 2B) revealed a black powdery surface with tan in the centre, surrounded by a green circular colony with yellowish white in reverse. Further microscopic observation was conducted using an LPCB stain, showing the vesicle, conidia, phialides, and conidiophores (Figures 2C and 2D). The biseriate phialides radiated around the entire vesicle, and the globose conidia were abundant and blackish brown. The vesicle was globose and had a colourless to yellowish-brown hue, while the smooth conidiophores were colourless. These observations provide a comprehensive understanding of the morphological features of *A. niger* and further validate its identification in the analysed sample. Based on Figure 2, the identification of fungi related to the description of *A. niger*; therefore, *A. niger* was confirmed.

The characteristics of *A. niger* observed in this study agreed with those of other studies (Campbell *et al*., 2013; Kidd *et al*., 2022; Walsh *et al*., 2018). Macroscopically, this study observed the dark brown to black conidial heads covered by a compact white or yellow basal felt. The conidial heads are globose, large, dark brown to black, with a diameter of 3 mm by 15 to 20 µm. The conidiophore stipes were smooth-walled, hyaline, or turned black toward the vesicle. The phialides are carried on brown, frequently septate metulae, and are biseriate with the conidial heads. The conidia have a rough-walled, globose to subglobose shape, measuring 3.5 to 5 µm in diameter.

Table 1. Macroscopic characteristics of *Aspergillus* isolates on tilapia

Code of Sample	Species of Fungi	Colony			
		Colour	Texture	Reverse colour	Topography
A1K	*A. flavus*	Gr	V	T	Flat
A1K	*A. fumigatus*	Dg	V	T	Rugose
A2K	*A. fumigatus*	Dg	P	Lg	Rugose
A4Kulit	*A. flavus*	Gr	P	T	Rugose
A5K	*A. fumigatus*	Dg	P	T	Flat
A7K	*A. niger*	Bl	P	Gr	Flat
A8I	*A. fumigatus*	Dg	V	T	Rugose
A9I	*A. niger*	Bl	P	T	Flat
A14I	*A. terreus*	Db	V	T	Flat
A15I	*A. fumigatus*	Gr	P	Lg	Rugose
A19Kulit	*A. fumigatus*	Dg	Vp	T	Flat
A22Kulit	*A. fumigatus*	Dg	C	Gb	Rugose
A22Kulit	*A. fumigatus*	Dg	V	Gr	Flat
B3K	*A. fumigatus*	Dg	V	T	Rugose
B5Kulit	*A. flavus*	Lg	P	T	Flat
B6I	*A. terreus*	Db	V	T	Flat
B6Kulit	*A. fumigatus*	Dg	V, P	Lg	Rugose
B7Kulit	*A. fumigatus*	Dg	V	T	Flat
B8K	*A. fumigatus*	Dg	P	Lg	Rugose
B9Kulit	*A. fumigatus*	Dg, Wb	V	T	Rugose
B10Kulit	*A. fumigatus*	Dg	P	Lg	Rugose
B10Kulit	*A. fumigatus*	Dg	V, P	T	Rugose
B13I	*A. fumigatus*	Dg	Vp	Lg	Rugose
B13Kulit	*A. fumigatus*	Dg	V	Lg	Flat
B14K	*A. fumigatus*	Dg	V	T	Rugose
B14I	*A. fumigatus*	Gr	V, P	Gr	Flat
B15K	*A. fumigatus*	Dg	V	T	Flat
B15K	*A. fumigatus*	Dg	V	T	Rugose
B16K	*A. fumigatus*	Dg	V	T	Flat
B18K	*A. fumigatus*	Gr	V	Lg	Flat
B19Kulit	*A. fumigatus*	Dg	V, P	Lg	Rugose
B21Kulit	*A. fumigatus*	Dg	V	T	Rugose
B22K	*A. fumigatus*	Dg	V	T	Flat
B23Kulit	*A. fumigatus*	Dg	V	Lg	Rugose
B24I	*A. fumigatus*	Dg	V	T	Flat
B24Kulit	*A. fumigatus*	Dg	V	Lg	Flat
B24K	*A. flavus*	Dyg	V	T	Flat
B24K	*A. niger*	Black	P	T	Flat
B25Kulit	*A. fumigatus*	Dg	V, P	Lg	Rugose

The code of sample "A" represents the source of the fish sample from pond A, and the code of sample "B" represents the source of the fish sample from pond B. The letter "K" represents cloaca; the letter "I" represents gills; and "Kulit" represents skin, wound, or fins. Colour code represents (Gr) green; (Dg) dark green; (Bl) black; (Lg) light green; (Db) dark brown; (Wb) white border; (Dyg) dark yellowish green. Texture code represents (V) velvety; (P) powdery; (Vp) velvety patches; (C) cottony. Reverse colour code represents (T) tan; (Gr) green; (Lg) light green; (Gb) greenish brown.

Table 2. Number of samples infected with single and multiple *Aspergillus* sp.

Species of Fungi	Code of Sample Isolate(s)	Frequency	Prevalence (%)
▪ *A. fumigatus*	A2, A5, A8, A15, A19, A22, B3, B7, B8, B9, B10, B13, B14, B15, B16, B18, B19, B21, B22, B23, B25	21	42
▪ *A. flavus*	A4, B5	2	4
▪ *A. terreus*	A14	1	2
▪ *A. niger*	A7, A9	2	4
▪ Coinfection of *A. fumigatus* and *A. flavus*	A1	1	2
▪ Coinfection of *A. fumigatus* and *A. terreus*	B6	1	2
▪ Coinfection of *A. fumigatus*, *A. flavus* and *A. niger*	B24	1	2
Total	58	29	58

Table 3. *Aspergillus* sp. prevalence based on organ predilection

Aspergillus species	Skin		Gills		Cloaca	
	Infected	Prevalence (%)	Infected	Prevalence (%)	Infected	Prevalence (%)
A. fumigatus	12	24	5	10	8	16
A. flavus	2	4	-	-	3	9
A. terreus	-	-	2	4	-	-
A. niger	-	-	1	2	2	4
Total	14	28	8	16	13	29

Figure 1. The clinical symptoms of tilapia from Pasarean Village. Shown by green circles: (a) Marbling of gills; (b) Eroded tail and fins; (c) Wound lesion at the chin.

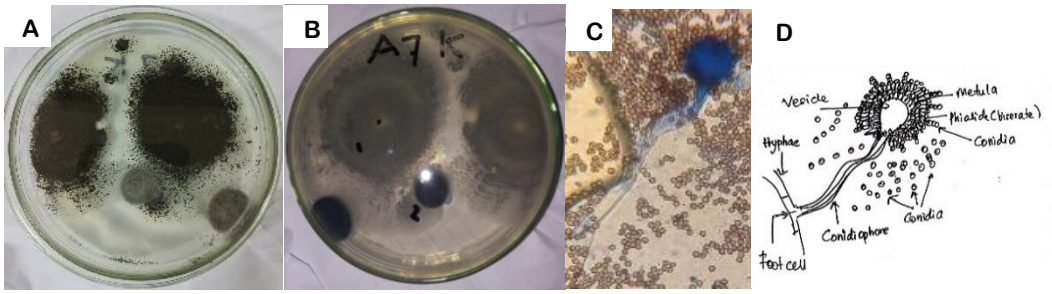

Figure 2. Macroscopic and microscopic observation of *A. niger*. (A) macroscopic morphology of *A. niger* from the surface; (B) macroscopic morphology of *A. niger* from the reverse; (C) microscopic image of *A. niger* (magnification 400×); (D) labelled microscopic drawing of *A. niger*.

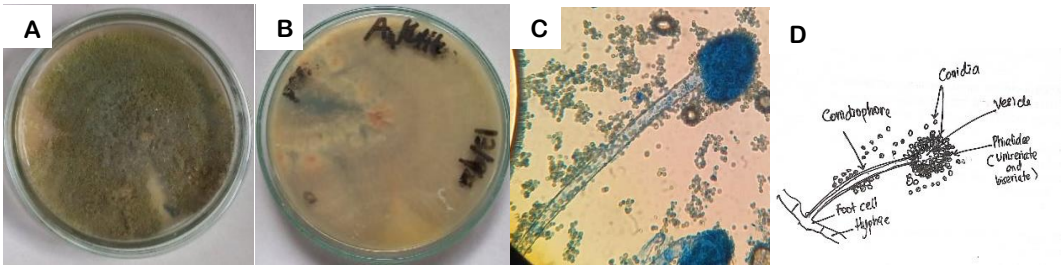

Figure 3. Macroscopic and microscopic observation of *A. flavus*. (A) Macroscopic morphology of *A. flavus* from the surface; (B) macroscopic morphology of *A. flavus* from the reverse; (C) microscopic image of *A. flavus* (magnification 400×); (D) labelled microscopic drawing of *A. flavus*.

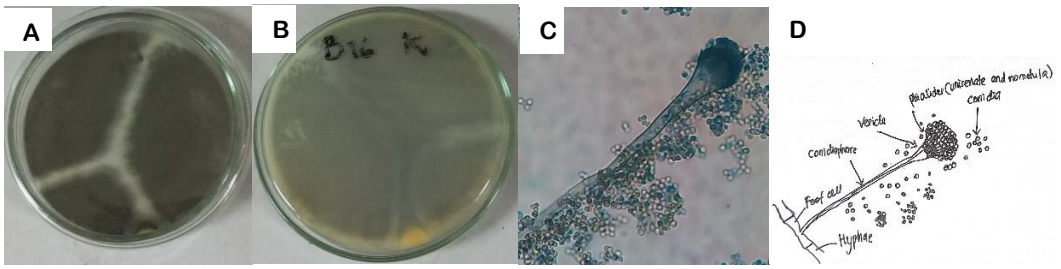

Figure 4. Macroscopic and microscopic observation of *A. fumigatus*. (A) Macroscopic morphology of *A. fumigatus* from the surface; (B) macroscopic morphology of *A. fumigatus* from the reverse; (C) microscopic image of *A. fumigatus* (magnification 400×); (D) labelled microscopic drawing of *A. fumigatus*.

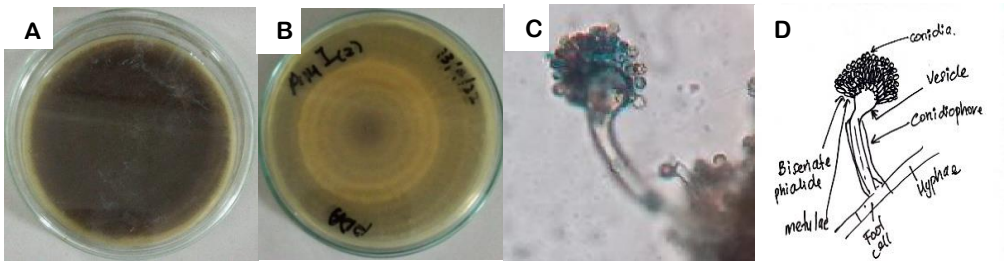

Figure 5. Macroscopic and microscopic observation of *A. terreus*. (A) Macroscopic morphology of *A. terreus* from the surface; (B) macroscopic morphology of *A. terreus* from the reverse; (C) microscopic image of *A. terreus* (magnification 400×); (D) labelled microscopic drawing of *A. terreus*.

Aspergillus flavus

In this study, *A. flavus* colonies in the PDA agar were observed as green, light green, and dark yellowish green. The reverse colour was observed as tan, and the texture was observed as powdery or velvety. The topography was observed as flat or rugose (Table 1). The image presented in Figure 3 was identified as *A. flavus*, another widely spread species of *Aspergillus*. Upon macroscopic observation of the isolated sample on the PDA plate (Figures 3A and 3B), it was found to have a velvety light greenish-yellow surface with a deep greenish-yellow to tan-like reverse colour. Further microscopic observation was conducted using an LPCB stain, showing the globose-shaped vesicle (Okayo *et al*., 2020). The conidia were smooth, globose, and slightly tan to colourless and were observed in abundance. The phialides were observed to be uniseriate and biseriate. The conidial heads are mostly biseriate and radiate (Figures 3C and 3D). Based on the features observed, the fungus is identified as *A. flavus*.

The *A. flavus* colonies observed in this study appeared to have a granular and flat appearance and frequently displayed radial grooves. The

colony's visual characteristics were similar to previous studies (Arafat *et al*., 2022; Kidd *et al*., 2022). The colour of the colonies was observed as dark green from the surface and tan from the back. The conidial heads are mostly biseriate and radiate, forming loose columns of around 300–400 µm in diameter. Conidiophores are hyaline and coarsely roughened, and the conidiospores are noticeably echinulate, globose to sub-globose, and measure between 3–6 µm in diameter. The distinctive features of *A. flavus* include expanding yellow-green colonies, rough-walled stipes, mature vesicles carrying phialides (uniseriate and biseriate) on every surface, and noticeably echinulate conidia (Arafat *et al*., 2022; Kidd *et al*., 2022).

Aspergillus fumigatus

This study observed *A. fumigatus* colonies in PDA agar as green, dark green, or dark green with a white margin. The reverse colours were tan, light green, greenish-brown, or green. The texture was observed as velvety, powdery, velvety patches, cottony, or a mix of velvety and powdery. The topography was observed to be rugose or flat (Table 1). The image presented in Figure 4 was identified as *A. fumigatus*. The macroscopic observation of the isolated sample on the PDA plate showed a green powdery surface with a white to yellow, more to a tan-like reverse colour, a typical characteristic of *A. fumigatus* (Figures 4A and 4B). Under microscopic observation using LPCB stain, the uniseriate phialides were observed along the top of the flask-shaped vesicle, an essential characteristic for identifying *A. fumigatus*. The conidial heads were observed to be dark green. The conidia are bluish-green and globose in shape, and the conidiophores are smooth-walled (Figures 4C and 4D). These distinct features match the description of *A. fumigatus*. Colonies of *A. fumigatus* typically display a distinctive blue-green surface.

The morphological description of the *A. fumigatus* colonies on PDA resembled the findings from a study by González-Ramírez *et al*. (2016). According to González-Ramírez *et al*. (2016) study, the colony structure of *A. fumigatus* exhibited velvety characteristics with a flat surface. Furthermore, the surface of the colonies appeared greenish-grey, while the reverse side showed a similar coloration. This study also highlighted the consistency in the visual appearance of the colonies on PDA. The texture of the colonies was suede-like and made of a dense felt of conidiophores. The colour of *A. fumigatus* colonies on the surface was dark green, while the reverse showed a tan colour. Conidial heads are columnar and uniseriate, measuring up to 400 × 50 µm but frequently considerably shorter and smaller. Short, smooth-walled conidiophore stipes feature conical terminal vesicles that sustain a single row of phialides on the upper two-thirds of the vesicle. Conidia are generated in long chains in a basipetal succession and range in size from globose to subglobose (2.5–3.0 µm in diameter), are green, and have a rough or echinulate wall. Truly a universal mould, *A. fumigatus* has been discovered everywhere and on every imaginable kind of substrate. It is a significant human and animal pathogen. Uniseriate and columnar conidial heads are the crucial characteristics for identification, along with phialides confined to the top two-thirds of the vesicle and curved to be parallel. Based on Figure 4, the identification of fungi related to the description of *A. fumigatus*; therefore, *A. fumigatus* was confirmed (Kidd *et al*., 2022).

Aspergillus terreus

In the current study, the *A. terreus* colonies in the PDA agar were observed as dark brown with a reverse colour of tan. The texture was observed as velvety, and the topography was observed as flat (Table 1). The image presented in Figure 5 was identified as *A. terreus*. Macroscopic observation of the isolated sample on the PDA plate (Figures 5A and 5B) revealed a cinnamon-brown surface with a white to tan-like reverse colour. Further microscopic observation was conducted using LPCB stain, showing the vesicle, conidia, phialides, and conidiophores. The conidial heads were observed to be compact and biseriate. The conidiophores were smooth and colourless. The vesicles appear to be hemispherical, with the upper half to two-thirds

covered by phialides (sterigmata), and the phialides are seen to be in two series, giving a crowded appearance. The conidia appear to be smooth, small, and globose to slightly elliptical in appearance. They have a compact cinnamon to orange-brown shade (Figures 5C and 5D).

Typical colonies of *A. terreus* are suede-like, cinnamon-buff to sand brown in colour, with a yellow to deep dirty brown reverse. Conidial heads are small, columnar, biseriate, and can measure up to 500 × 30–50 µm in diameter. Hyaline stipes on conidiophores have smooth walls. Conidia are smooth-walled, hyaline to slightly yellow, and globose to ellipsoidal (1.5–2.5 µm in diameter) (Kidd *et al*., 2022). Observing cinnamon-brown cultures and conidial heads biseriate with metulae of the phialides are some crucial characteristics in identification. Based on Figure 5, the identification of fungi related to the description of *A. terreus*; therefore, *A. terreus* was confirmed.

Prevalence of *Aspergillus*

The overall prevalence of aspergillosis among the 50 sampled tilapias in this study is 58% (29/50). However, a study by Al-Niaeem *et al*. (2015) reported a lower prevalence of *Aspergillus* sp. (49%) in tilapia compared to the present study. This study reported both single and co-infections of *Aspergillus*. *A. fumigatus* had the highest prevalence of 42% (21/50), whereas *A. flavus* had a prevalence of 4% (2/50), *A. terreus* had a prevalence of 2% (1/50), and *A. niger* had a prevalence of 4% (2/50), as recorded. Co-infection of *A. fumigatus* and *A. flavus* was observed to have a prevalence of 2% (1/50), co-infection of *A. fumigatus* and *A. terreus* was observed to have a prevalence of 2% (1/50), and co-infection of *A. fumigatus*, *A. flavus*, and *A. niger* was reported with a prevalence of 2% (1/50) (Table 2).

The results of this study, as shown in Table 3, indicate the distribution of *Aspergillus* sp. in various organs, namely the skin, gills, and cloaca. It was found that *Aspergillus* sp. was more commonly present on the skin compared to the gills and cloaca. *A. fumigatus* was isolated from all three locations, namely the skin, gills, and cloaca, with the highest occurrence observed on the skin. *A. flavus*, on the other hand, was only found on the skin and cloaca, with the highest occurrence noted on the cloaca. *A. terreus*, however, was exclusively isolated from the gills. Additionally, *A. niger* was found in both the gills and cloaca, with a higher number observed in the cloaca. *A. fumigatus* can be detected in different organs of each fish. For instance, in samples B13 and B24, *A. fumigatus* was isolated from skin and gill samples. In addition, *A. fumigatus* can be detected in gills and cloaca samples from sample B14.

A significant portion, precisely 24%, of *A. fumigatus* was prevalent on the skin in this study, while the gills exhibited a prevalence rate of 10%. On the other hand, the cloaca demonstrated a prevalence rate of 16% for *A. fumigatus*. In contrast, *A. flavus* displayed a lower prevalence rate of 4% on the skin, with no prevalence observed on the gills but a 9% prevalence rate on the cloaca. *A. terreus*, however, exhibited a fungal prevalence of 4% exclusively on the gills, with no presence on the skin or cloaca. As for *A. niger*, a prevalence rate of 2% was detected on the gills, whereas a 4% prevalence rate was identified on the cloaca, but no presence was noted on the skin (Table 3).

The prevalence of *A. fumigatus* infection in this study was higher (9.1%) compared to the prevalence of *A. fumigatus* infection in tilapia (Younis *et al*., 2019). However, in the same study, *A. niger* (27.3%) and *A. flavus* (18.2%) were reported to be higher than in this study. In the study conducted by Saleemi *et al*. (2020), several species of *Aspergillus* were observed in carp, including *A. terreus* (8.40%), *A. flavus* (17.06%), *A. fumigatus* (24.02%), and *A. niger* (50.52%). The previous study revealed that the carp's fins exhibited the highest infection rate at 39.10%. Conversely, the intestine showed the lowest infection rate, with only 6.59% affected. The prevalence of *A. fumigatus* in this study was higher (42%), compared to the study by Saleemi *et al*. (2020) (24.02%), while the prevalence of *A. niger* was lower (4%) compared to their study (50.52%). Saleemi *et al*. (2020) reported a higher incidence of *Aspergillus* infection in fins (39.1%),

skin (16.38%), and gills (12.7%) than other organs, i.e., the intestine (6.59%), heart (8.65%), liver (8%), and kidney (8.54%). Similarly, the current study observed that *Aspergillus* sp. had a higher prevalence in the skin and gills. These findings suggest potential variations in the distribution and manifestation of *Aspergillus* infections among different fish species, emphasizing the importance of considering the specific host and anatomical factors when studying fungal infections in aquatic organisms.

According to Lesmana *et al*. (2021), their study isolated and identified *A. flavus* and *A. niger* as the only types of *Aspergillus* sp. among various other fungal genera in tilapia and catfish. In contrast to their findings, the current study identified four different species of *Aspergillus*, indicating a greater diversity within this group of tilapias. This discrepancy in the number of species detected highlights the potential variations in fungal composition. It emphasizes the need for further exploration and comprehensive studies to fully understand the range of *Aspergillus* species in different environments.

Co-infections refer to infections caused by two or more genetically distinct pathogens, where each pathogen individually poses a risk to the host's health and their presence together exacerbates the harm caused (Kotob *et al*., 2016). However, a limited study has been published on co-infection between two fungal species in a sample. Mycosis in fish is opportunistic and might have a relationship with secondary invaders alongside other infectious pathogens (bacteria, viruses, or parasites) or environmental changes that cause fish stress (Abdel-Latif *et al*., 2020). However, some fungi can induce infection as the primary agent in some instances (Chauhan *et al*., 2014; Salter *et al*., 2012; Yanong, 2003). Nonetheless, there have been reports of co-infection between fungi and bacteria (Cutuli *et al*., 2015; Eissa *et al*., 2013; Oda *et al*., 2016). A previous study reported co-infection of *Aeromonas hydrophila* and *Saprolegnia parasitica* in sea bass with 18% mortality (Dinçtürk *et al*., 2018).

Younis *et al*. (2019) conducted a study and found slightly similar results, whereby the skin had a significantly high occurrence of *A. niger* (50%), *A. flavus* (50%), and *A. fumigatus* (50%). Similarly, the gills had a prevalence of *A. niger* (27.8%), *A. flavus* (25%), and *A. fumigatus* (33.3%). The detection of fungal agents in fish intended for consumption in this study raises the possibility that mycosis is a severe illness. The pathological lesions and disease conditions observed in the fish may have been caused by the fungi discovered in this study, or they may have developed because of a bacterial or viral infection. Fungal diseases are a primary concern in aquaculture and have been linked to significant economic losses and potential health risks for consumers of contaminated seafood. Therefore, the findings from this study provide essential information that can contribute to the early detection and prevention of fungal outbreaks in fish farms. The fungal isolates in this study from the genus *Aspergillus* sp. are *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*. The fungi *Aspergillus* sp. were the predominant isolates from this observation, and their isolation is noteworthy because *Aspergillus* sp. have been linked to disease outbreaks in fish culture (Salawudeen *et al*., 2017). However, the presence of *Aspergillus* in fish can originate from several factors, such as contaminated feed (Marijani *et al*., 2019), contaminated water (Iqbal and Saleemi, 2013; Obire and Vincent, 2017), and environments such as diseased fish and fish carcasses (Patel *et al*., 2018; Safira *et al*., 2023). Using contaminated feed could be the source of fungal infection in aquaculture fish.

Feed contamination by fungi differs in a variety of geographic regions, temperature, sanitary conditions, and moisture (Mohamed *et al*., 2017; Fikri *et al*., 2023). If fish ingest this contaminated feed, it can have severe negative consequences, resulting in mass mortality (Mohamed *et al*., 2017). The factors enhancing the fungal infection hazard through feed comprise a humidity level greater than 62%, an environmental temperature of 27°C, and a moisture level in the feed greater than 14%. Fungal concentrations in feed rise during feed

storage at higher humidity levels (Iqbal and Sajjad 2013). Iqbal and Saleemi (2013) isolated *Aspergillus* sp. from pond water, which showed contamination of these fungal species. Free-living fungal species collected from fish farm water, such as *A. fumigatus*, are the most common fungal species that cause diseases in humans with an immunity deficiency (Sousa Terada-Nascimento *et al*., 2023). A previous study reported that *Aspergillus* is the main fungal species isolated from pond waters. The entrance of these fungi from the environment to the pond waters has been reported through dead plants, which are thought to endure for a long time (Obire and Vincent 2017). Inappropriate pond management, a considerable amount of decomposing organic matter, sick or injured fish, and other stressful conditions are all possible sources of fungi in the study sites (Patel *et al*., 2018). Another study reported the possibility of introducing fungal agents from the poultry units to the ponds by the attendants because all the farms practiced mixed farming. So, it is easy for these isolates to be spread either by the attendants or by the wind dispersing the spores. The isolation of some of these isolates from fish in this study might make their consumption hazardous to human health (Salawudeen *et al*., 2017).

The primary causes of infection consist of fungal spores transported in the water and waste found on the bottoms of ponds. The affected fish may appear dull and exhibit behaviours such as gasping for air at the water's surface, also known as piping. As the tissue deteriorates and disintegrates, the spores are released into the water and spread to other fish (Patel *et al*., 2018). The challenging environmental conditions in fish farms during fish rearing can significantly stress the fish, making them more vulnerable to various pathogens such as parasites, bacteria, fungi, and viruses. When the fish's skin loses its protective mucus layer or lacks mucous, it becomes exposed, allowing fungal spores to develop and further increasing its susceptibility to fungal attacks. The injuries in fish are typically caused by factors like high stocking density, inadequate water quality, excessive organic manure, and other environmental elements (Iqbal *et al*., 2014). In the case of gill infections caused by *Aspergillus*, it can damage the fish's secondary lamellae, potentially leading to respiratory difficulties for the fish (Saleemi *et al*., 2020). When the gill tissue of fish becomes infected, it displays a striped or marbled pattern with pale areas, indicating the presence of infected and dying tissue (Patel *et al*., 2018).

This study documented the presence of *Aspergillus* in three distinct organs i.e., gills, skin, and cloaca. The results agreed with previous studies that reported fungal infection in these organs (Abd El Tawab *et al*. 2020). In addition, other studies have reported *Aspergillus* infection in other organs, such as the liver and kidneys (Abd El Tawab *et al*. 2020). Abd El Tawab *et al*.'s (2020) isolated *A. flavus* and *A. niger* from the samples. Aspergillosis, sometimes called aspergillomycosis, is a fungal disease caused by *Aspergillus* sp. that has the potential to impact humans (Elad and Segal, 2018) and a diverse range of terrestrial (Seyedmousavi *et al*., 2015) and aquatic animal species (Chauhan *et al*., 2014; Iqbal and Saleemi, 2013; Safira *et al*., 2022). The terrestrial animals reported to be infected by *Aspergillus* include cats, dogs, birds, horses, cattle, and non-human primates (Seyedmousavi *et al*., 2015). In addition, aquatic animals reported to be contracted with aspergillosis include marine mammals and fish (Seyedmousavi *et al*., 2015). Respiratory complications are common in humans, particularly those with compromised immune systems or pre-existing lung conditions. The specific manifestations of aspergillosis in animals can vary depending on the species, but it poses a significant health risk. A study by Sudipa *et al*. (2023) reported *A. fumigatus* and *A. niger* from 15 samples of free-roaming dogs in Bali, with a prevalence of 53.33% infection. This condition is because *Aspergillus* is found in the environment.

CONCLUSION

In conclusion, this study revealed a significant presence of *Aspergillus* sp. infections among tilapia in Pasarean Village, highlighting the detrimental impact of fungal pathogens on

aquaculture. Aspergillosis, a systemic fungal infection caused by *Aspergillus* sp., poses a significant threat to the widely cultured tilapia species in the region. The high prevalence (58%) of *Aspergillus* sp. infection among the sampled tilapia population was reported. Among the 50 tilapia samples evaluated, 29 were detected with *Aspergillus* sp. infections, with *A. fumigatus* being the most prevalent species (42%) of the infected samples. Other identified species included *A. flavus* (4%), *A. niger* (4%), and *A. terreus* (2%), with the occurrence of co-infection between *A. fumigatus* and *A. flavus* (2%), *A. fumigatus* and *A. terreus* (2%), and between *A. fumigatus*, *A. flavus* and *A. niger* (2%). Notably, *A. fumigatus* displayed the highest prevalence, while *A. terreus* exhibited the lowest prevalence among the suspected *Aspergillus* sp. infections. Furthermore, the study found that *Aspergillus* sp. was more commonly present on the tilapia's skin compared to the gills and cloaca. These findings emphasized the effectiveness of disease management strategies to mitigate the impact of *Aspergillus* sp. infections and safeguard the sustainability of tilapia aquaculture in Pasarean Village and similar regions.

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AUTHORS' CONTRIBUTIONS

NGB: Conceptualized and designed the study. NGB and BJW: Validation, supervision, and formal analysis. KA and NGB: Performed sample collection. KA: Performed sample evaluation under the supervision of NGB. KA and NGB: Performed the statistical analysis and the preparation of tables and figures. KA: Drafted the manuscript. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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