Insight into Isolation, Identification and Antimicrobial Sensitivity of some Moulds Isolated from Fresh Water Fishes

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Abstract | The expanding recurrence of invasive fungal infection and the high mortality associated with dispersed fungal disease have highlighted the requirement for fast recognizable proof of infectious molds. The current study was conducted to identify the most prevalent mycotic diseases among cultured freshwater fishes with special focus on isolation, molecular identification and antimicrobial sensitivity testing. A total number of 300 examined cultured freshwater fishes; 200 Oreochromis niloticus (120 apparently healthy and 80 showing clinical signs) and 100 Clarias gariepinus (30 apparently healthy and 70 showing clinical signs), were collected alive from five freshwater fish farms at Kafr El Sheikh Governorate all over the year 2018. Clinically, examined Oreochromis niloticus revealed scale detachment, fins erosion, pale body coloration and eye opacity particularly in fish naturally infected with Aphanomyces, Aspergillus, Pencillium and Fusarium; however, infected Clarias gariepinus showed scattered hemorrhagic patches on the ventral abdomen. Mycological examination revealed the isolation of 900 fungal isolates; 570 fungal isolates from Oreochromis niloticus and 330 isolates from C. gariepinus. Aphanomyces invadans was the predominant fungi among diseased fishes mainly from skin, fins and gills. However, Aspergillus flavus, Aspergillus niger and Alternaria sp. were the most predominant fungi isolated from apparently healthy fishes mainly from internal organs. Molecular identification was performed using inter-transcribed spacer (ITS) gene for the most prevalent isolates among either diseased or apparently healthy fishes. Developed PCR assay specific primer detect not only Aphanomyces invadans showing clear bands at 569 bp molecular weight, but also Aspergillus flavus and Aspergillus niger showing clear bands at 595 and 600 bp molecular weight, respectively. Anti-fungal sensitivity test revealed that all mould isolates are sensitive to Nystatin.

Keywords | Clarias gariepinus, Fungi, Identification, Oreochromis niloticus, PCR

INTRODUCTION

Fish represents the most important source of human dietary protein worldwide, especially in African countries (Hussain et al., 2011; Rubbani et al., 2011). Natural fisheries are dynamically exhausted and the aquaculture wind up imperative intends to compensate the lack; providing a valuable and fundamental protein source for human consumption (Fletcher et al., 1999; Dawood and Koshio, 2019). Aquaculture contributes more than half of the total fish production in the world and about 77% of the total Egyptian fish production (Subasinghe and Soto, 2009; Mur, 2014).

Nile Tilapia (Oreochromis niloticus) and African catfish (Clarias gariepinus) are the most prevalent freshwater fishes...
in Egypt because of their rapid growth rate with excellent meat quality, market demand, resistance to diseases and ability to withstand adverse environmental conditions (Goda et al., 2007; Amin et al., 2019; Dawood et al., 2019a, b).

The intensification and extension of the aquaculture industry are subjected to several ecological stressors (Awad and Awaad, 2017). It was accounted that disease is a final product of a complex collaboration of the host, its environment and the pathogen itself where the environmental stress is viewed as a vital inciting element regarded as a crucial inducing factor causing various diseases (Duan et al., 2017; El-Deep et al., 2019; Vicente et al., 2019). Adverse aquaculture conditions can enhance fish diseases transmission, especially infectious fungal diseases, resulting in limited fish production and subsequent financial losses (Faruk et al., 2004; Rodolphe et al., 2014; Ashour et al., 2017).

The importance of fungal diseases in freshwater fish not stopped just for excessive mortalities but also as economic issue, such as growth rate depletion, decreased hatchability especially in chronic infection or by mycotoxins creation by polluted organism in case of bad stocking feed. Fungal infections in fish are viewed as secondary cause of disease; fungi affecting fishes are opportunistic and attack the fishes only if they are stressed or immune-compromised. Primary causes may be attributed to temperature change, water quality problems, poor conditions, skin injury as a result of rough handling, bacterial disease and/or parasites (Verma, 2008; Chauhan et al., 2014; Noor El-Deen et al., 2018). In spite of the fungal infections importance, the available knowledge about them is still poor for two basic causes: difficult identification of pathogenic fungi and the growth of saprophytic organisms once the fish is dead (Bassiouny et al., 2019).

The most common widely recognized fungal infection was the pathogenic oomycetes; which belong to the order Saprolegniales, including the genera Saprolegnia, Achlya and Aphanomyces. Saprolegniosis has significant impact on freshwater ecosystems by infecting different host organisms and their eggs (Kiesecker et al., 2001; Fernández-Benéitez et al., 2008; Ruthig 2009; Eissa et al., 2013). Some species of Aphanomyces invadans are regularly in charge of serious disease outbreaks in fish farms and in the common habitat causing enormous economic losses by threatening industry generation targets and long-term viability (Sarowar et al., 2013; Van West and Beakes, 2014; Iberahim et al., 2018; Sarowar et al., 2019). The World Organization for Animal Health (OIE) listed that this disease has been responsible for large-scale mortalities of farmed and wild fish in more than 20 countries across four continents (Pradhan et al., 2008; Iberahim et al., 2018). In Egypt, Aphanomyces invadans was isolated from naturally infected Striped and thin lip grey mullet (Shaheen et al., 1999), and African catfish “Clarias gariepinus” (Amany et al., 2004).

Aphanomyces sp. causes systematic diseases with high death rates in fish, whereby the infections mostly occur through contamination of fish feed (Urquhart et al., 2016) and the pathogenesis of Aphanomyces fumigatus and Aphanomyces niger has been reported in fresh water fishes by Chauhan (2013). Fusarium is additionally viewed as imperative fish pathogens influencing marine fish and shellfish (Hatai, 2012).

The current study was conducted to detect the predominant mycotic infections with special focus on isolation, molecular identification and antimicrobial sensitivity testing for most dominant isolated fungi from cultured freshwater fishes (O. niloticus and C. gariepinus) in Kafr El Sheikh fish farms.

MATERIALS AND METHODS

Materials

Fish
A total number of 300 examined cultured freshwater fishes; 200 O. niloticus (120 apparently healthy and 80 showing clinical signs) and 100 C. gariepinus (30 apparently healthy and 70 showing clinical signs), were collected alive from five freshwater fish farms at Kafr El Sheikh Governorate all over the year 2018. The samples were collected with an average body weight of (40±5 and 150±10 gm) for O. niloticus and C. gariepinus, respectively.

The alive collected fishes were transferred to the lab., held in well-prepared glass aquaria supplied with sufficient amounts of dechlorinated water with continuous air supply (Innes, 1966; Langdon and Jones, 2002).

Methods

Clinical and postmortem examination
The gathered fish were clinically inspected to identify any external changes or clinical abnormalities (McVicar, 1982). Postmortem examination of the internal organs was carried out on sacrificed and freshly dead fish according to Austin and Austin (2012).

Mycological examination

Isolation of the fungus from diseased fishes
Using sterile dissecting needle, samples were taken from the skin, gills and internal organs (liver, kidney) of the collected fishes. Mycological examination was conducted according to (Noga, 1993). Gathered specimens were inoculated into Sabouraud’s dextrose broth tubes (SDB) and subsequently, inoculated into duplicate plates of Sabaroud’s dextrose agar (SDA) media with addition of chloramphenicol (50 mg/mL) to the media after autoclaving; to inhibit bacterial growth. The inoculated plates were incubated at 25±2°C for 4-7 days (Whitman, 2004). Aphanomyces sp. colonies were re-isolated on Glucose peptone yeast extract agar medium.
To amplify the DNA, a PCR reaction was prepared by adding 6 μL of extracted DNA, 1 μL of forward and reverse primers (20 pmol), 12.5 μL Emerald Amp GT PCR master mix (2x premix) and sterile water up to a final volume of 25 μL. The PCR reaction was carried out in thermal cycler (Eppendorf, Hauppauge, New York) and included an initial cycle at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec., 56°C for 40 sec., 72°C for 50 sec., and a final elongation step of 72°C for 10 min. Subsequently, PCR amplicons were separated and analyzed by gel electrophoresis by loading products onto a 1.5 % agarose gel supplemented with 1% ethidium bromide (50 ng/mL) and visualizing bands using an ultraviolet lamp at a wavelength of 300 nm.

**Antifungal Sensitivity Testing for Isolated Moulds**

Antifungal sensitivity test was conducted using Mueller-Hinton agar media supplemented with 2% glucose, providing a suitable growth for most yeasts, and 0.5 mg/L methylene blue dye medium (enhances the zone edge definition) limiting the trailing impact.

The pH of the medium should be in the range of 7.2 and 7.4 after gelling and the agar should be 4 cm high. The inoculum is standardized to 0.5 McFarland using a densitometer and plates should be incubated at 35 °C for 24 h; some strains show inadequate growth and may need 48 h of incubation. Two main groups of antifungals are used in the clinical setting to treat fungal infections: polyenes represented by (Amphotericin B, Nystatin,) and azoles with several derivatives such as itraconazole, fluconazole, voriconazole, clotrimazole, fluconazole (Humid).

**Results and Discussion**

Mycotic diseases of fish, caused by oomycete pathogens, are a real threat to the sustainability of a developing aquaculture industry. The increasing frequency of invasive fungal infection and the high mortality associated with disseminated fungal disease have highlighted the need for rapid identification of infectious molds from clinical samples.

**Clinical and Postmortem Examination**

The external gross lesions of the examined *O. niloticus* revealed scale detachment, fins erosion, pale body coloration and eye opacity particularly in fish naturally infected with Aphanomyces, Aspergillus, Pencillium and Fusarium. Scale detachment and fins erosion may be due to the lytic activity of primary bacterial infection as all fungal infections are considered as secondary invader pathogen; these results concurred with many authors (Refai, 1987; Bruno, 1994; Noor El-Deen et al., 2018). Fin erosion and eye opacity were mainly observed in diseased fish infected with...
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However, the infected *C. gariepinus* showed scattered hemorrhagic patches on the ventral abdomen; this may be credited to toxins discharged by moulds causing extreme symptomatic changes in the form of haemorrhagic patches, the result agreed with (Diab, 2006; Bassiouny et al., 2019). On the other hand, the main characteristic postmortem lesions were liver enlargement with moderate petechial hemorrhage.

### Mycological Examination

Mycological examination revealed the isolation of 900 fungal isolates from 120, 70 fishes showing clinical signs and 80, 30 apparently healthy *O. niloticus* and *C. gariepinus*, respectively. As shown in Table 1 and Table 4, 570 fungal isolates were isolated from *O. niloticus* and 330 isolates from *C. gariepinus* (from skin, gills, liver and kidney) with incidence percent of (63.3% and 36.7%) among *O. niloticus* and *C. gariepinus*, respectively. The high recurrence of mould isolates in *O. niloticus* concurs with Diab (2006), Refai et al. (2010) and Bassiouny et al. (2019).

Table 1: Total prevalence of moulds infections among *O. niloticus* and *C. gariepinus*

| Fish species               | No. of examined fish | No. of diseased fish | No. of apparently healthy fish | No. of mould isolates | No. per cent-age |
|---------------------------|---------------------|----------------------|-------------------------------|-----------------------|-----------------|
| *O. niloticus*            | 200                 | 120                  | 80                            | 570                   | 63.3%           |
| *C. gariepinus*           | 100                 | 70                   | 30                            | 330                   | 36.7%           |
| Total                     | 300                 | 190                  | 110                           | 900                   | 100%            |

### Morphological Identification of Isolated Moulds

The morphological identification of moulds revealed the presence of different moulds including: *Aphanomyces* sp., *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* sp., *Fusarium* sp., *Mucor* sp. and *Alternaria* sp. The most prevalent isolated fungi was *Aphanomyces* sp. (162 isolate representing 28.4%) (100 isolates representing 30.3%) mainly from skin, fins and gills from *O. niloticus* and *C. gariepinus*, respectively showing the highest incidence among diseased fishes. However, the most prevalence isolated fungi from apparently healthy fishes were *Aspergillus flavus* (55, 34), *Aspergillus niger* (60, 26), *Mucor* sp. (29, 25) and *Alternaria* sp. (28, 10) mainly from internal organs of *O. niloticus* and *C. gariepinus*, respectively; the results concur with previously investigated data (Fafioye et al., 2008; Refai et al., 2010; Bassiouny et al., 2019). Refai et al. (2010) has characterized *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. as normal mycoflora and these species may be considered as opportunistic pathogens as many of these genera possess virulence factors which enable them to cause disease especially under unfavorable conditions.

As shown in (Tables 2A, 2B), mycological examination demonstrated the incidence of *Aphanomyces* sp. (143, 19), *Aspergillus flavus* (26, 55), *Aspergillus niger* (19, 60), *Penicillium* sp. (24, 28), *Fusarium* sp. (18, 11) *Mucor* (50, 29) and *Alternaria* sp. (60, 28) in diseased and apparently healthy *O. niloticus*, respectively. On the other side, mycological examination of *C. gariepinus* demonstrated the incidence of *Aphanomyces* sp. (80, 20), *Aspergillus flavus* (15, 34), *Aspergillus niger* (25, 26), *Penicillium* sp. (10, 20), *Fusarium* sp. (7, 3) *Mucor* (15, 25) and *Alternaria* sp. (40, 10) in diseased and apparently healthy *C. gariepinus*, respectively (Table 3A, 3B).

Table 2A: Incidence of moulds isolated from different organs of diseased *O. niloticus*.

| Moulds                | *O. nilotica* | No. | No. | No. | No. |
|-----------------------|---------------|-----|-----|-----|-----|
|                       | Skin & fins   | Gills | Liver | Kidney |
| *Aphanomyces* sp.     | 143           | 109  | 34  | 0   | 0   |
| *Aspergillus flavus*  | 26            | 0    | 26  | 0   | 0   |
| *Aspergillus niger*   | 19            | 0    | 15  | 4   | 0   |
| *Penicillium* sp.     | 24            | 12   | 8   | 4   | 0   |
| *Fusarium* sp.        | 18            | 0    | 18  | 0   | 0   |
| *Mucor* sp.           | 50            | 25   | 17  | 6   | 2   |
| *Alternaria* sp.      | 60            | 35   | 21  | 4   | 0   |
| Total                 | 340           | 181  | 98  | 55  | 6   |

Table 2B: Incidence of moulds isolated within different organs of apparently healthy *O. niloticus*.

| Moulds                | *O. niloticus* | No. | No. | No. | No. |
|-----------------------|---------------|-----|-----|-----|-----|
|                       | Skin & fins   | Gills | Liver | Kidney |
| *Aphanomyces* sp.     | 19            | 11   | 8   | 0   | 0   |
| *Aspergillus flavus*  | 55            | 0    | 50  | 5   | 0   |
| *Aspergillus niger*   | 60            | 8    | 11  | 13  | 28  |
| *Penicillium* sp.     | 28            | 12   | 8   | 5   | 3   |
| *Fusarium* sp.        | 11            | 0    | 11  | 0   | 0   |
| *Mucor* sp.           | 29            | 13   | 13  | 3   | 0   |
| *Alternaria* sp.      | 28            | 10   | 8   | 6   | 4   |
| Total                 | 230           | 54   | 59  | 77  | 40  |

The colonies of *Aphanomyces* sp. appeared white and transparent with velvet surface (become heavy and opaque at 7th day); while, microscopically by wet mount appeared as branched non-septated hyphae and when stained with lactophenol cotton blue, gave non septated, y- shape branched hyphae with cytoplasmic organelles (Figure 1).
The result concurs with that reported by Abd El-Latif (2003), Afzali et al. (2013) and Youssuf et al. (2017).

Table 3A: Incidence of moulds isolated from different organs of diseased C. gariepinus.

| Moulds               | C. gariepinus | Skin & fins | Gills | Liver | Kidney |
|----------------------|---------------|-------------|-------|-------|--------|
|                      | No. | No. | No. | No. | No.    |
| Aphanomyces invadans | 80  | 55  | 25  | 0   | 0      |
| Aspergillus flavus   | 15  | 0   | 0   | 15  | 0      |
| Aspergillus niger    | 25  | 5   | 3   | 10  | 7      |
| Pencillium sp.       | 10  | 5   | 2   | 3   | 0      |
| Fusarium sp.         | 7   | 0   | 7   | 0   | 0      |
| Mucor sp.            | 15  | 10  | 5   | 0   | 0      |
| Alternaria sp.       | 40  | 8   | 13  | 13  | 6      |
| Total                | 192 | 83  | 55  | 41  | 13     |

Table 3B: Incidence of moulds isolated from different organs of apparently healthy C. gariepinus.

| Moulds               | C. gariepinus | Skin & fins | Gills | Liver | Kidney |
|----------------------|---------------|-------------|-------|-------|--------|
|                      | No. | No. | No. | No. | No.    |
| Aphanomyces invadans | 20  | 13  | 7   | 0   | 0      |
| Aspergillus flavus   | 34  | 0   | 0   | 30  | 4      |
| Aspergillus niger    | 26  | 4   | 4   | 6   | 12     |
| Pencillium sp.       | 20  | 10  | 7   | 3   | 0      |
| Fusarium sp.         | 3   | 0   | 3   | 0   | 0      |
| Mucor sp.            | 25  | 10  | 7   | 8   | 0      |
| Alternaria sp.       | 10  | 2   | 4   | 4   | 0      |
| Total                | 138 | 39  | 32  | 51  | 16     |

Interestingly, Aspergillus species exhibited some variation within the same genus. Aspergillus flavus (A. flavus) appeared velvety with numerous aerial growths; the color changes from yellow to yellowish green by aging; while microscopically, the conidiophores were long and rough, the vesicles were rounded and large and the strig mata were biseriate, loose, and radiate giving rise to ovoid rough conidia (Figure 2). Colonies of Aspergillus niger (A. niger) had black color with radiated edges with wooly texture, while microscopically had extremely long, smooth and yellowish conidiophores, vesicles were very large and globes while the strig mata were biseriate, compact and radiate and the conidia were globes and smooth (Figure 3).

Pencillium sp. colonies white and friable transformed into greenish blue in colour, while microscopically, septated hyphae with unbranched conidiophores possessing metula with flask-shaped strig mata and the metulae bear unbranched chain of conidia; the entire structure form brush appearance (Figure 4). Regardless Fusarium sp., colonies were cottony or wooly in texture, snow white, pink-violet or rosyl-red in color, with scattering of colored pigments into the reverse surface of the medium; while microscopically, they appeared long, branched and septated hyphae from which short conidiophores rose singly or in groups, and sometimes branched. Two types of conidia were observed, a large banana-shaped, septated macroconidia and small, round, non-septated microconidia (Figure 5).

Colonies of Mucor sp. appeared fast-growing, white-to-gray cotton candy, became dark with time and fills the petri dish with fluffy mycelium, and microscopically, non-septated wide hyphae. Sporangiophores are long, may be branched and end with bear terminal round sporangia. The
sporangia have a thin wall which when mature dissolves (or is disrupted) to discharge round or somewhat ellipsoidal sporangiospores (4-8μm diameter). With the spores dispersed. No rhizoids are formed (Figure 6).

**Figure 4:** Penicillium sp. (A and B) Petri dish on SDA with different colour and texture. (C) hyphae stained with LCB showing characteristic brush-like arrangement.

**Figure 5:** Fusarium sp. (A) Petri dish Surface side (B) Petri dish reverse side (C) hyphae stained with LCB showing characteristic snow white colonies with rose pigment in the center.

**Figure 6:** Mucor sp. (A) Petri dish...Surface side (B) Petri dish reverse side (C) hyphae stained with LCB showing characteristic round sporangia.

Alternaria sp. revealed grayish brown to green color colonies; while microscopically appeared as septated hyphae with dark branched condidiophores creating at least two expanding tree-like with oval conidia having velvety dull gray colour (Figure 7). The observed colonies of all isolated moulds, in the current study, are in accordance with previous reports (El-gamal, 2000; Ali, 2007; Refai et al., 2010; Bassiouny et al., 2019).

**Figure 7:** Alternaria sp. (A) SDA Petri dish Surface side showing grayish brown to green color (B) Petri dish reverse side (C) hyphae stained with LCB showing characteristic septated hyphae with dark branched condidiophores producing two or more branching tree-like with oval conidia having velvety dull gray colour under microscope.

**Molecular Identification**

Molecular identification (Polymerase Chain Reaction) using inter-transcribed spacer (ITS) gene, which is a universal gene, is one of the most important approaches to distinguish between different fungal infections (Eissa et al., 2013). Therefore, in the current study, molecular identification was performed using inter-transcribed spacer (ITS) gene for the most prevalent among either diseased or apparently healthy fishes. Three Aphanomyces invadans isolates from diseased C. gariepinus and two isolate of Aspergillus flavus and Aspergillus niger isolates from apparently healthy O. niloticus were used. Developed PCR assay specific primer detect not only Aphanomyces invadans showing clear bands at 569 bp molecular weight (Figure 8), but also Aspergillus flavus and Aspergillus niger showing clear bands at 595 and 600 bp molecular weight, respectively (Figure 9). The current obtained result is nearly similar to Oidtmann et al. (2008) and Youssef (2017) where Aphanomyces invadans produced a clear band at 564 bp and 600 bp molecular weight, respectively. On the other hand, Afzali et al. (2015) reported that the result of PCR assay of Aphanomyces invadans showed clear band at 400 bp. These variations may be attributed to various tested primers sequences and PCR protocol as a result of the different amplification cycles (Oidtmann et al., 2008; Afzali et al., 2015; Youssef, 2017). On the other hand, the result of PCR assay of Aspergillus flavus and Aspergillus niger was exactly similar to Henry et al. (2000) where Aspergillus flavus and Aspergillus niger produced a clear band at 595 bp and 599 bp, respectively.

**Antifungal Sensitivity Test**

Antifungal susceptibility testing methods was applied to distinguish antifungal resistance and to decide the best treatment for a particular fungus. Clinical microbiology relies on these techniques to select the agent of choice for a fungal infection, and to know the local and the global epidemiology of antifungal sensitivity.

Anti-fungal sensitivity test was conducted using the disc diffusion method for the isolated strains. As demonstrated in Table 5, all mould isolates are sensitive to Nystatin and the majority of them were resistant to Voriconazole. The results agree with Ebaied (2014) where it was investigated that Aspergillus flavus, Aspergillus niger, Penicillium and Alternaria were sensitive to Nystatin.
Table 4: Incidence of Moulds isolated from *O. niloticus* and *C. gariepinus*.

| Isolates            | *O. niloticus* | *C. gariepinus* | Total isolates | % in relation total isolate No. |
|---------------------|----------------|----------------|----------------|---------------------------------|
|                     | No. %          | No. %          | No. %          | No. %                           |
| *Aphanomyces invadans* | 162 28.4%    | 100 30.3%      | 262            | 29.1%                           |
| *Asperigillus flavus* | 81 14.2%      | 49 14.8%       | 130            | 14.4%                           |
| *Asperigillus niger*  | 79 13.9%      | 51 15.5%       | 130            | 14.4%                           |
| *Pencillium sp.*     | 52 9.1%       | 30 9.1%        | 82             | 9.1%                            |
| *Fusarium sp.*       | 29 5.1%       | 10 3.0%        | 39             | 4.4%                            |
| *Mucor sp.*          | 79 13.9%      | 40 12.1%       | 119            | 13.2%                           |
| *Alternaria sp.*     | 88 15.4%      | 50 15.2%       | 138            | 15.4%                           |
| Total Moulds         | 570 100%      | 330 100%       | 900            | 100%                            |

Figure 8: Agarose gel electrophoresis for PCR products representing amplification of 569 bp of inter-transcribed spacer (ITS) gene in *Aphanomyces invadans*. Lane 1: 100 bp DNA Ladder. Pos.: is control positive; Neg.: is control Negative; Lane 1, 2 and 3: positive sample.

Table 5: Results of Antifungal susceptibility test of Moulds isolated from fresh water fish.

| Mould species | NS (10) | AP (100) | CC (10) | IT (10) | FLU (10) | VRC (10) |
|---------------|---------|----------|---------|---------|----------|----------|
| *Aphanomyces sp.* | S       | S        | S       | R       | R        |          |
| *Asperigillus flavus* | S       | R        | S       | S       | R        |          |
| *Asperigillus niger*  | S       | S        | R       | S       | R        | S        |
| *Pencillium sp.*     | S       | R        | R       | R       | R        |          |
| *Fusarium sp.*       | S       | S        | S       | S       | S        | R        |
| *Mucor sp.*          | S       | S        | S       | S       | S        | S        |
| *Alternaria sp.*     | S       | S        | S       | S       | S        | S        |

NS: Nystatin (10mcg/disc) IT: Itraconazole (10mcg/disc); AP: Amphotericin B (100mcg/disc) FLU: Fluconazole (10mcg/disc); CC: Clotrimazole (10mcg/disc) VRC: Voriconazole (10mcg/disc); S: sensitive R: resistant.

CONCLUSION

From the current study, it could be concluded that *Aphanomyces invadans* was the predominant fungi among diseased fishes mainly from skin, fins and gills. However, *Asperigillus flavus*, *Asperigillus niger* and *Alternaria sp.* were the most predominant fungi isolated from apparently healthy *Oreochromis niloticus* and *Clarias gariepinus* mainly from internal organs. In brief, although most fungi are considered to be normal mycoflora, but they still can cause infections to fishes. Therefore, it is suggested that proper health management practices should be adapted while rearing fish, so that chances of fungal infection can be minimized.

AUTHORS CONTRIBUTION

All authors contributed equally.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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