SEASONAL SCREENING OF THE MYCOTIC INFECTIONS OF CULTURED FRESHWATER FISHES IN KAFR EL-SHEIKH GOVERNORATE

Nadia B. Mahfouz¹, Eman M. Moustafa¹*, Mohamed Kassab², Wesam H. Marzouk³

¹Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt, ²Department of of Cytology and Histology, Faculty of Veterinary Medicine, Kafrelsheikh University, ³Food safety specialist in Directorate of Health in Gharbia, Egypt

*Corresponding author, E-mail: emantarek2002@yahoo.com

Abstract: The present study was carried out to screen the predominant mycotic infections among freshwater fishes (Oreochromis niloticus and Clarias gariepinus) in Kafrelsheikh fish farms; with special focus on the seasonal incidence; as well as, the histopathological changes induced by the detected fungi. 500 specimens of freshwater fishes (400 O. niloticus and 100 C. gariepinus) were investigated for seasonal incidence of mycotic diseases. Mycological examination revealed the isolation of 2148 fungal isolates from 375 diseased and 125 apparently healthy fish samples (1828 mould and 320 yeast isolates), of which 1258 were isolated from O. niloticus and 890 isolates from C. gariepinus. Saprolegnia was the predominant among diseased fishes with highest prevalence in late autumn (10.68%, 6.96%) and winter (6.81%, 7.87%) in O. niloticus and C. gariepinus, respectively. However, Pencillium sp. and Aspergillus sp. were the most predominant fungi isolated from apparently healthy fishes. The highest prevalence of Pencillium sp. were recorded in winter, while Aspergillus showed variations between species; Aspergillus flavus, Aspergillus niger were more prevalent in summer (25.44%, 23.22%) and (26.9%, 37.44%) and Aspergillus terrus, Aspergillus fumigatus were more prevalent in autumn (5.98%, 5.67%) and (7.69%, 8.23%) in O. niloticus and C. gariepinus, respectively. Moreover, the highest prevalence of Fusarium species was recorded in spring (11.8%, 5.91%) from O. niloticus, C. gariepinus, respectively. Mucor recorded the highest prevalence in autumn (20.09%) in O. niloticus and winter (29.21%) in C. gariepinus; whileas Rhizopus was highest in summer (7.89%, 5.21%) in O. niloticus and C. gariepinus, respectively. Four genera from yeast were isolated; Candida sp. (28.44%, 36.27%), Rhodotorula sp. (36.24%, 24.51%), Cryptococcus sp. (16.97%, 20.59%) and Trichosporon Sp. (18.35%, 18.63%) in O. niloticus and C. gariepinus, respectively. The histopathological findings revealed severe degenerative changes in skin and gills with presence of fungal hyphae and spores.

Key words: Clarias gariepinus; moulds; mycotic diseases; Oreochromis niloticus; yeast

Introduction

Fish serves as an important source of human dietary protein worldwide, especially in Afri-
can countries (1). To compensate animal protein deficiency resulting from the increased interest for fish as human nourishment, fish farming is rapidly extending all over the world (2). In Egypt, the aquaculture industry provides about 77% of the total national fish production (3, 4).

Nile Tilapia (*O. niloticus*) is viewed as a standout amongst the most prevalent freshwater fishes in Egypt. It is widely cultured because of its palatability, cheap price, high growth rate, capacity to withstand pressure and infections, ability to spawn effectively and the minimal prerequisites with regard to management and energy inputs (5).

With expanding freshwater fish production movement around the world for farming, alongside enhanced ecological observing of fungal and fungal-like infections that are full degree of the effect of these pathogens on wild fish populations will soon rise as a noteworthy danger to freshwater biodiversity (6).

Serious aquaculture conditions can advance the transmission of fish diseases, particularly contagious fungal sicknesses, causing economic losses. Fungal infections are one of the primary drivers for mortalities and extraordinary financial misfortunes in cultured fishes. (7). The significance of fungal diseases in freshwater fish not halted just for frequency of mortalities but rather additionally as financial significance, such as decline growth rate, hatchability in choronic infection or by mycotoxins production by tainted organism in case of bad stockpiling feed. Fungal infections in fish are viewed as auxiliary to some other pathogen, water quality issues, poor conditions, injury (unpleasant taking care of or hostility), bacterial disease and/or parasites (8). Numerous fungi influencing fishes are considered opportunistic, assaulting the fishes only if they are stressed or immune-compromised as a result of trouble-some natural conditions, or optional to bacterial or viral diseases, or when they have lost their bodily mucus protection due to trauma or excessive handling (9). Disregarding the fungal infections significance our insight about them is still poor for two fundamental reasons: difficult distinguishing proof of pathogenic fungi and the productive development of saprophytic fungi once the fish is dead (10).

Fungi is mostly attacked due to temperature change and bad water conditions which allow excessive zoospores to grow and the ammonia which is formed by rottening of fish waste wears away the mucus that protects the skin (11). Moreover, fungi can assault fishes of all the ages and it can also forestall fruitful hatching when it invades fish eggs (12). The most widely recognized fungal infection was saprolegniosis which is the real oceanic mycotic winter freshwater fish disease, frequently impacts wild and cultured fishes (13). *Saprolegnia sp.* taints the fishes because of sudden drop of water temperature and was regularly influencing fishes exhibiting fungal skin lesions which, unmistakable as cottony-white development on the epidermis of the infected fishes (14). *Aspergillus sp.* causes systematic diseases with high death rates in fish, whereby the infections mostly occur through contamination of fish feed (15) and the pathogenesis of *Aspergillus fumigatus* and *Aspergillus niger* had been accounted in fresh water fishes by Chauhan (16).

The current study was carried out to screen the predominant mycotic infections with special focus on the seasonal incidence among freshwater fishes (*O. niloticus* and *C. gariepinus*) in Kafrelsheikh fish farms; as well as, detect the histopathological changes induced by the detected fungi.

Materials and methods

A- Materials

1. Fish

A total number of 500 examined cultured freshwater fishes; 400 *O. niloticus* and 100 *C. gariepinus*, were collected alive from different freshwater fish farms at Kaf El Sheikh Governorates along the four seasons of the year 2017. The samples were collected with an average body weight of (40±5 & 150±10 gm) for *O. niloticus* and *C. gariepinus*, respectively.

The alive collected fishes were transferred to the wet lab., Fish Diseases and Management Department, Faculty of Veterinary Medicine, Kaf El-Sheikh University, Egypt, held in well-
prepared glass aquaria supplied with sufficient amounts of dechlorinated water with continuous aeriation (17).

B- Methods

1. Clinical examination

The collected fish were examined clinically according to the methods described by McVicar (18) to detect any external changes or clinical abnormalities.

2. Postmortem examination

Postmortem examination of the internal organs was carried out on sacrificed and freshly dead fish according to Austin & Austin (19).

3. Mycological examination

A. Isolation of the fungus from diseased fishes

Mycological examination was done according to (18). Samples were taken from fish showing skin lesions using sterile dissecting needle from the skin, gills and internal organs (liver, kidney). Gathered specimens were inoculated into duplicate plates of SDA media with 500mg of cyclohexamid and 50 mg of chloramphenicol dissolved in 3 ml ethanol 95% were added to the media after autoclaving. The inoculated plates were incubated at 25°C– 30°C. For 3-5 days (20). Negative plates were not disposed before 2 weeks (21). All the positive moulds cultures examined for gross and micro morphological characteristics (22).

B. Identification of different fungi

B.1 Identification of moulds

Recognition of moulds was completed according to Refai (23). Preliminary recognition utilizing wet mount preparation of fish samples made in 10% KOH. The confirmatory test of identification was carried out using souletip technique (24). Slide culture technique was carried out on those isolates whose identification was inconclusive after staining with lactophenol cotton blue (25).

B.2 Identification of yeast

Plates of suspected specimens were analyzed microscopically for the presence of chlamydo- spores, arthrospores and blastospores (20, 23) and the plan of recognizable proof of yeasts given by Terrence (26). Rice agar media was used for identification of yeasts especillay Candida albicans by production of characteristic chlamydo- spores (23). The confirmatory distinguishing proof was carried out by germ tube test (27). Biochemical reaction using urease test was also conducted (28).

4. Histopathological Examination

Tissue specimens from the skin, gills and kidney of the infected fish samples were taken for histopathological examination (29).

Results and Discussion

The present work was applied to investigate the seasonal incidence of mycotic diseases among some freshwater fishes (O. niloticus & C. gariepinus) in Kafr El-Sheikh Governorate.

Clinical examination

The external gross lesions of the examined O. niloticus revealed darkened skin, pale body coloration, scale detachment, fins erosion, and eye opacity as shown in (Plate 1: Fig. 1, 2). These results agree with (24, 30). However, in fish naturally infected with Saprolegnia, there was scattered grayish white cotton wool-like growth on various parts of the body as well as presence of ulcerative areas in some cases as shown in (Plate 1: Fig. 3, 4); with unilateral eye cloudiness or opacity (Plate 1: Fig. 5); the result is in accordance with that of El-Atta, (31).

Fish death may be due to either blindness which consequently disable fish to feed or due to the fungal growth over gills causing suffocation. The ulcerative areas over the skin may be attributed to the lytic action of primary bacterial infection as all fungal infections are considered as secondary invader pathogen; these results agreed with many authors (8, 23, 32-36).

On the other side, the infected C. gariepinus showed skin ulceration and scattered hemorrhagic patches on the ventral abdomen and mouth (Plate 1: Fig. 6). These symptoms may be attributed to the toxins secreted by moulds and yeasts causing severe symptomatic changes that appear on the fish in the form of haemorrhagic patches, ascitis and destruction and degeneration of the gills; the result agreed with (16, 24).
P.M. lesions

The main observed postmortem lesions were liver enlargement with moderate petechial hemorrhage (Plate 1: Fig. 6& 7). This result may support that the saprolegnia is a secondary invader following systemic bacterial infection which is responsible mainly for this internal lesions due to toxins produced by fungi and yeasts that interfere with function of liver causing congestion in internal organs especially liver (2, 31).

Mycological examination

Mycological examination revealed the isolation of 2148 fungal isolates from 375 diseased and 125 apparently healthy freshwater fish samples; 1258 fungal isolates from O. niloticus and 890 isolates from C. gariepinus (from skin, gills, liver and kidney). Recognizable proof of fungi into mould and yeast revealed that the incidence of mould and yeast was marginally higher in O. niloticus (56.89%, 68.12%) in contrast with that in C. gariepinus (43.11%, 33.88%). The high frequency of mould isolates in O. niloticus agree with some authors (9, 24) and disagree with the incidence in C. gariepinus; as both of the two authors recorded high incidence of yeast isolates from catfish. This might be attributed to variable host susceptibility due to geographical distribution.

Morphological identification of isolated moulds

The colonies of Saprolgnia sp. appeared as white cotton-wool like growth on the petri dish (Plate 2: Fig. A) while, microscopically appeared as long, branched, un-septated hyphae (Plate 2: Fig. B, C). This result is in accordance with some reports (8, 31).

Pencillium sp. Colonies were white and fluffy then, turned into greenish blue in colour (Plate 2: Fig D), while microscopically, there were septated hyphae with un branched conidia possessing metule with flask-shaped stigmata forming brush appearance. (Plate 2: Fig E, F). Nonetheless, Fusarium sp. Colonies were cottyony or woolly in texture, snow white, pink-violet or blushing red in shading, with dissemination of hued colors into the switch reverse surface of the medium (Plate 2: Fig G) and microscopically, they seemed long, extended and septated hyphae from which short conidiophores climbed and sometimes branched. Two kinds of conidia were watched, a huge banana shaped, septated macroconidia and a little, round, non septated microconidia. (Plate 2: Fig H). These results are in accordance with some authors (9, 37).

On the other side, Aspergillus sp. demonstrated few varieties within the same genus. A. flavus seemed smooth with various aerial growths; the shading changes from yellow to yellowish green by aging (Plate 2: Fig I), and microscopically the conidiophores were long and thick, the vesicles were globose and the stigmata were biseriate and radiate (Plate 2: Fig J). Colonies of A. niger had black color with radiated edges with wooly texture (Plate 2: Fig K), while microscopically had extremely long, smooth and the stigmata were biseriate, minimized and radiate and the conidia were globes and smooth (Plate 2: Fig L). Colonies of A. terreus were velvety cinnamon buff to dark brown (Plate 2: Fig M), and microscopically, little hemispherical vesicle with long and smooth conidiophore (Plate 2: Fig N). Colonies of A. fumigatus have unmistakable edge with a few shades of green, surface has a powder appearance with a white overskirt was seen at the edge in the zone of dynamic development (Plate 2: Fig O), and microscopically portrayed by hyaline and particularly septated hyphae, conidiophores were long with club-molded vesicle, round conidia were conceived from single row of stigmata (Plate 2: Fig P). These results agree with some authors (9, 37).

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-septate broad hyphae. Sporangiofphores are long, might be expanded and end with bear terminal round sporangia. The spores scattered and no rhizoids are formed. Rhizopus sp. colonies were deeply cottony; white turned to gray-brown on surface with aging. Microscopically, broad hyphae
could be observed, Sporangiophores are unbranched and connect to each another by septated hyphae, large sac-like sporangia that contain sporangiospores. These results agree with some authors (9, 37).

**Morphological identification of yeast isolates**

The isolates were cultivated on Rice agar media after culturing on SDA. In the current study, four genera were identified (Candida, Rhodotorula, Trichosporon and Cryptococcus). All genera reacted positively with urease test except Candida. *Rhodotorula sp.* was identified on SDA by formation of carotenoid pigments; that vary from orange to red (light pink flat colonies). Microscopically, revealed budding of round, oval large cells when stained with Gram’s stain. On Rice agar media, showed large round blasto-conidia with absence of pseudohyphae. *Cryptococcus sp.* appeared rapidly on SDA as flat or slightly heaped shiny moist mucoid colonies with smooth edges. Its color changed from creamy at first to brown later. Microscopically, the colonies were ovoid, spherical with thick wall and mostly showed capsule with budding. On Rice agar media, no pseudo-hyphae but appeared as budding cells. *Trichosporon sp.* appeared on SDA firstly as smooth flat, or wrinkled white to creamy colonies that turned waxy with central folds surrounded by wrinkled furrows. Microscopically, appearance of hyaline mycelium which is separated and fragmented into rectangular arthrospores. On Rice agar media, *Trichosporon sp.* appeared as septated hyphae, pseudo-hyphae and arthrospores. *Candida species* colonies on SDA appeared creamy colored pasty colonies within 48-72hrs. On Rice agar media, *C. albicans* showed terminal chlamydomspores, blastoconidia and pseudo-hyphae. Other *Candida sp.* fails to produce pseudohyphae. This result is in accordance with some authors (9, 37-39).

**Incidence of moulds and yeast among different seasons and different organs**

As shown in table (1 & 2), mycological examination of 400 *O. niloticus* and 100 *C. gariepinus* revealed an incidence of several moulds including *Saprolegnia* (53, 32) isolates, *Penicillium* (152, 131), *Fusarium* (78, 38) *A. flavus* (233, 149), *A. niger* (247, 223), *A. terreus* (35,22), *A. fumigatus* (29, 15) *Mucor* (148, 136) and *Rhizopus* (65, 42) in *O. niloticus* and *C. gariepinus*, respectively.

*Saprolegnia sp.* showed the highest prevalence in late autumn (10.68%, 6.96%) and winter (6.8%, 7.8%) in *O. niloticus* and *C. gariepinus*, respectively. This may be attributed to that seasonal variation play an important role in spreading of the Saprolegnia infection among freshwater fishes where the water temperature was low. These results agree with some authors (9, 24, 37) where they mentioned that saprolegniasis occurred during the winter season and colder months of the year. The highest incidence within organs was observed to be from the skin and fins (77.4%, 65.63%) followed by the gills (22.6%, 34.4%) in *O. niloticus* and *C. gariepinus*, respectively but, not isolated from liver and/or kidney. The results are in accordance with those of many authors (8, 9, 14, 30, 31) as shown in table (2).

*Pencillium sp.* was isolated with the highest prevalence in winter (18.98% & 23.22%) from *O. niloticus*, *C. gariepinus* respectively. The highest incidence within organs was observed to be from the skin and fins (36.2%, 47.3%) followed by gills (30.9%, 20.6%) in *O. niloticus* and *C. gariepinus*, respectively. These results are similar to those reported by Ali, (37). Besides, *Penicillium sp.* could be isolated also from liver (22.4%, 13.7%) and kidney (10.5%, 18.3%). These results agree with some authors (9, 30). Different species of Pencillium were isolated with high incidence from apparently healthy fishes rather than diseased one, therefore members of this genus can be considered as saprophytes (9, 37).

*Fusarium sp.* was isolated with the highest prevalence in spring (11.8%, 5.91%) from *O. niloticus*, *C. gariepinus* respectively. The highest incidence within organs was observed to be in gills (37.18%, 34.2%) in *O. niloticus* and *C. gariepinus*, respectively. This might be attributed to the high affinity of fungal spores to high oxygen tension (37). It could be isolated from skin, fins, liver and kidney as well.
Aspergillus sp. showed some variation according to species. A. flavus was recorded all over the year and more prevalent during hot weather with high incidence during summer (25.44%, 23.22%) followed by spring (16.24%, 22.08%) in O. niloticus, C. gariepinus. The result is similar to some previous papers (9, 37, 40). A. niger was more prevalent during hot weather with high incidence during summer (26.9%, 37.44%) in O. niloticus, C. gariepinus, respectively. These results are similar to some previous papers (9, 37, 40). A. terrus was more prevalent during autumn (5.98%, 5.69%) in O. niloticus and C. gariepinus, respectively. These results are similar to some previous papers (9, 37, 40). A. fumigatus was more prevalent during autumn (7.69%, 8.23%) in O. niloticus, C. gariepinus. These results are similar to some previous papers (9, 37, 40). In the current study, the highest incidence within organs was observed to be in liver in most of Aspergillus sp. This may support the fact that Aspergillosis is a systemic disease.

Zygomycetes (Mucor and Rhizopus) are the most common fungi isolated from apparently healthy fish and diseased O. niloticus and C. gariepinus with high incidence during autumn season and these results agree with Ali, (37). Mucor species were isolated with the highest prevalence in autumn (20.09%) from O. niloticus and winter (29.21%) from C. gariepinus, respectively. The highest incidence within organs was observed to be from the skin and fins (45.3%, 46.3%) followed by gills (29.7%, 27.9%) in O. niloticus and C. gariepinus, respectively. The result is similar to those reported by Ali (37). It could be isolated also from liver (15.5%, 16.9%) and kidney (9.46%, 8.8%) in O. niloticus and C. gariepinus, respectively. These results agree with many authors (9, 37).

On the other side, Yeast isolates revealed 4 genera; Candida (62, 37), Rhodotorula (79, 52), Trichosporon (40, 19) and Cryptococcus (37, 21) isolated from O. niloticus and C. gariepinus, respectively. Yeast was also isolated with high frequency from diseased fishes rather than apparently healthy; these results came in agreement with those recorded (9). Candida sp. accounted for (28.44%, 36.27%) of the isolates and Rhodotorula sp. (36.24, 24.51%) from O. niloticus and C. gariepinus. The current results came in agreement with those recorded by Tartor et al., (39). Samples collected from skin, gills, liver, and kidney revealed that C. albicans and Rhodotorula sp. were the highest yeast isolates. These findings were supported by the view reported by (9). Cryptococcus sp. in the present study was isolated with prevalence of (16.97%, 20.59%) from O. niloticus and C. gariepinus, respectively; nearly similar results were recorded by (38) but disagree with Tartor et al., (39). Trichosporon sp. was detected to be (18.35%, 18.63%) from O. niloticus and C. gariepinus, respectively; nearly similar results were recorded by (24, 38) but disagree with Tartor et al., (39).

Histopathological findings

Histopathological examination of naturally infected O. niloticus with Saprolignia sp. revealed severe degenerative changes in the skin. Necrosis of dermis and hypodermis, the underlying dermis was edematous with degenerative changes of muscle fibers containing fragments from the fungal hyphae (Plate 3: Fig. A). Ulceration, loss of epidermis and loss of texture of scales and sometimes ulcer can be observed (Plate 3: Fig. B, C). Gills showed severe hyperplasia and hypertrophy of the epithelial lining of secondary lamellae with congestion of branchial blood vessels (Plate 3: Fig. D, E). Kidney revealed necrosis in some tubules together with peritubular fibrosis (Plate 3: Fig. F). These results agreed with many authors (11, 31, 34).
### Seasonal Screening of the Mycotic Infections of Cultured Freshwater Fishes in Kafr El-Sheikh Governorate

Table 1: Seasonal Prevalence of Mould and yeast in *O. niloticus* and *C. gariepinus*

<table>
<thead>
<tr>
<th>Yeast/Clairespira</th>
<th>Winter %</th>
<th>Winter No.</th>
<th>Spring %</th>
<th>Spring No.</th>
<th>Summer %</th>
<th>Summer No.</th>
<th>Autumn %</th>
<th>Autumn No.</th>
<th>Winter %</th>
<th>Winter No.</th>
<th>Spring %</th>
<th>Spring No.</th>
<th>Summer %</th>
<th>Summer No.</th>
<th>Autumn %</th>
<th>Autumn No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mould</strong>&lt;br&gt;Saprolegnia sp</td>
<td>28%</td>
<td>6.81%</td>
<td>28%</td>
<td>0%</td>
<td>28%</td>
<td>0%</td>
<td>28%</td>
<td>0%</td>
<td>28%</td>
<td>0%</td>
<td>28%</td>
<td>0%</td>
<td>28%</td>
<td>0%</td>
<td>28%</td>
<td>0%</td>
</tr>
<tr>
<td>Pencillium sp</td>
<td>17%</td>
<td>4.12%</td>
<td>17%</td>
<td>0%</td>
<td>17%</td>
<td>0%</td>
<td>17%</td>
<td>0%</td>
<td>17%</td>
<td>0%</td>
<td>17%</td>
<td>0%</td>
<td>17%</td>
<td>0%</td>
<td>17%</td>
<td>0%</td>
</tr>
<tr>
<td>Fusarium sp</td>
<td>65%</td>
<td>15.82%</td>
<td>65%</td>
<td>0%</td>
<td>65%</td>
<td>0%</td>
<td>65%</td>
<td>0%</td>
<td>65%</td>
<td>0%</td>
<td>65%</td>
<td>0%</td>
<td>65%</td>
<td>0%</td>
<td>65%</td>
<td>0%</td>
</tr>
<tr>
<td>Asp. flavus sp</td>
<td>83%</td>
<td>20.19%</td>
<td>83%</td>
<td>0%</td>
<td>83%</td>
<td>0%</td>
<td>83%</td>
<td>0%</td>
<td>83%</td>
<td>0%</td>
<td>83%</td>
<td>0%</td>
<td>83%</td>
<td>0%</td>
<td>83%</td>
<td>0%</td>
</tr>
<tr>
<td>Asp. Niger sp</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Yeast</strong>&lt;br&gt;Rhodotorulla sp</td>
<td>26%</td>
<td>6.33%</td>
<td>26%</td>
<td>0%</td>
<td>26%</td>
<td>0%</td>
<td>26%</td>
<td>0%</td>
<td>26%</td>
<td>0%</td>
<td>26%</td>
<td>0%</td>
<td>26%</td>
<td>0%</td>
<td>26%</td>
<td>0%</td>
</tr>
<tr>
<td>Candida sp</td>
<td>10%</td>
<td>2.43%</td>
<td>10%</td>
<td>0%</td>
<td>10%</td>
<td>0%</td>
<td>10%</td>
<td>0%</td>
<td>10%</td>
<td>0%</td>
<td>10%</td>
<td>0%</td>
<td>10%</td>
<td>0%</td>
<td>10%</td>
<td>0%</td>
</tr>
<tr>
<td>Trichosporon sp</td>
<td>7%</td>
<td>1.70%</td>
<td>7%</td>
<td>0%</td>
<td>7%</td>
<td>0%</td>
<td>7%</td>
<td>0%</td>
<td>7%</td>
<td>0%</td>
<td>7%</td>
<td>0%</td>
<td>7%</td>
<td>0%</td>
<td>7%</td>
<td>0%</td>
</tr>
<tr>
<td>Cryptococcus sp</td>
<td>9%</td>
<td>2.19%</td>
<td>9%</td>
<td>0%</td>
<td>9%</td>
<td>0%</td>
<td>9%</td>
<td>0%</td>
<td>9%</td>
<td>0%</td>
<td>9%</td>
<td>0%</td>
<td>9%</td>
<td>0%</td>
<td>9%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Total</strong>&lt;br&gt;No. %</td>
<td>411</td>
<td>3.26%</td>
<td>411</td>
<td>0%</td>
<td>411</td>
<td>0%</td>
<td>411</td>
<td>0%</td>
<td>411</td>
<td>0%</td>
<td>411</td>
<td>0%</td>
<td>411</td>
<td>0%</td>
<td>411</td>
<td>0%</td>
</tr>
</tbody>
</table>

Total No. of isolates: 1258

Table 2: Incidence of Mould and yeast in organs of *O. niloticus* and *C. gariepinus*

<table>
<thead>
<tr>
<th>Organ</th>
<th>Skin &amp; Fins</th>
<th>Gill</th>
<th>Liver</th>
<th>Kidney</th>
<th>Skin &amp; Fins</th>
<th>Gill</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mould</strong>&lt;br&gt;Saprolegnia sp</td>
<td>53%</td>
<td>41%</td>
<td>77.4%</td>
<td>12%</td>
<td>22.6%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Pencillium sp</td>
<td>152%</td>
<td>55%</td>
<td>36.2%</td>
<td>47%</td>
<td>30.9%</td>
<td>34%</td>
<td>22.4%</td>
<td>16%</td>
</tr>
<tr>
<td>Fusarium sp</td>
<td>78%</td>
<td>21%</td>
<td>26.92%</td>
<td>29%</td>
<td>37.18%</td>
<td>11%</td>
<td>14.1%</td>
<td>17%</td>
</tr>
<tr>
<td>Asp. flavus sp</td>
<td>233%</td>
<td>83%</td>
<td>35.6%</td>
<td>47%</td>
<td>20.20%</td>
<td>78%</td>
<td>33.5%</td>
<td>25%</td>
</tr>
<tr>
<td>Asp. Niger sp</td>
<td>247%</td>
<td>92%</td>
<td>37.2%</td>
<td>46%</td>
<td>18.6%</td>
<td>81%</td>
<td>32.8%</td>
<td>28%</td>
</tr>
<tr>
<td>Asp. Fu-migus sp</td>
<td>29%</td>
<td>10%</td>
<td>34.48%</td>
<td>12%</td>
<td>41.40%</td>
<td>3%</td>
<td>10.34%</td>
<td>4%</td>
</tr>
<tr>
<td>Asp. terrus sp</td>
<td>35%</td>
<td>13%</td>
<td>37.1%</td>
<td>7%</td>
<td>20%</td>
<td>10%</td>
<td>28.6%</td>
<td>5%</td>
</tr>
<tr>
<td>Mucor sp</td>
<td>148%</td>
<td>67%</td>
<td>45.27%</td>
<td>44%</td>
<td>29.73%</td>
<td>23%</td>
<td>15.54%</td>
<td>14%</td>
</tr>
<tr>
<td>Rhizopus sp</td>
<td>65%</td>
<td>42%</td>
<td>64.6%</td>
<td>23%</td>
<td>35.4%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Yeast</strong>&lt;br&gt;Rhodotorulla sp</td>
<td>79%</td>
<td>25%</td>
<td>31.6%</td>
<td>40%</td>
<td>50.6%</td>
<td>4%</td>
<td>5.1%</td>
<td>10%</td>
</tr>
<tr>
<td>Candida sp</td>
<td>62%</td>
<td>18%</td>
<td>29%</td>
<td>20%</td>
<td>32.3%</td>
<td>16%</td>
<td>25.8%</td>
<td>8%</td>
</tr>
<tr>
<td>Trichosporon sp</td>
<td>40%</td>
<td>18%</td>
<td>45%</td>
<td>12%</td>
<td>30%</td>
<td>10%</td>
<td>25%</td>
<td>0%</td>
</tr>
<tr>
<td>Cryptococcus sp</td>
<td>37%</td>
<td>0%</td>
<td>0%</td>
<td>25%</td>
<td>67.57%</td>
<td>12%</td>
<td>32.43%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Total</strong>&lt;br&gt;No. %</td>
<td>485%</td>
<td>38.55%</td>
<td>364%</td>
<td>28.93%</td>
<td>282%</td>
<td>22.42%</td>
<td>127%</td>
<td>10.01%</td>
</tr>
</tbody>
</table>

Total No. of isolates: 1258
Plate 1: (1, 2) *O. species* showing skin darkening, scale detachment and erosion of membranous part of fins. (3, 4) *O. species* showing cotton wool-like growth on various parts of the body, ulceration of skin. (5) *O. species* showing cloudy and opaque eye. (6) *C. gariepinus* showing skin ulceration, scattered hemorrhagic patches on the ventral abdomen and mouth. (7) Naturally diseased *O. niloticus* showing moderate petechial hemorrhage, dark liver enlargement. (8) A naturally examined *O. niloticus* showing threads of congestion along the surface of live

Plate 2: (A) *Saprolegnia species* with the characteristic cotton-wool like growth colony on SDA, (B, C) branched aseptic hyphae, (D, E) *Penicillium sp.* on SDA with different colour and texture, (E, F) *Penicillium sp.* showing brush-like arrangement, (G) a colony of *Fusarium sp.* on SDA with rose pigments on the center, (H) *Fusarium under light microscope* (I) *A. flavus* on SDA, (J) *A. flavus* showing characteristic typical head, (K) A colony of *A. niger* on SDA, (L) *Aspergillus niger* showing characteristic round head with black conidia, (M), Colonies of *Aspergillus terreus* on SDA, (N) *A. terreus* with small hemispherical vesicle, (O) A colony of *A. fumigatus* on SDA, (P) *A. fumigatus* with columnar head
Plate 3: Photomicrograph of skin, gills and kidney of *Oreochromis niloticus* infected with *Saprolegnia sp.* (A) Necrosis of dermis, hypodermis, edema and degenerative changes of muscle fibers (Arrow) with presence of hyphae of saprolegnia (Arrow head) x 400. (B) Ulceration & loss of epidermis and loss of texture of scales (Arrow). (C) Normal scale (Arrow) and Ulcer in neighboring (Arrow head). (D) Gills showing severe Hyperplasia & Hypertrophy of the epithelial lining of secondary lamellae with fusion in neighboring (Arrow). (E) Infection of saprolegnia at the tip of primary lamellae with congestion of blood vessels and hyperplasia & hypertrophy (Arrow head) while other part filament is normal (Arrow). (F) Infected kidney with peritubular fibrosis (Arrow) and necrosis in some tubule (Arrow head).

**Conclusion**

From the present study, it could be concluded that Saprolegnia was the predominant among diseased fishes with highest prevalence in late autumn (10.68%, 6.96%) and winter (6.8%, 7.8%) in *O. niloticus* and *C. gariepinus*, respectively. However, *Pencillium sp.* and *Aspergillus sp.* were the most predominant fungi isolated from apparently healthy fishes. The highest prevalence of *Pencillium sp.* were recorded in winter, whereas *Aspergillus* showed variations between species; *A. flavus, A. niger* were more prevalent in summer and *A. terreus, A. fumigatus* were more prevalent in autumn in *O. niloticus* and *C. gariepinus*, respectively. Moreover, the highest prevalence of *Fusarium species* were recorded in spring from *O. niloticus* and *C. gariepinus*, respectively and *Zygomycetes* (Mucor and Rhizopus) recorded the highest prevalence in autumn in *O. niloticus* and winter in *C. gariepinus*. Four genera from yeast were isolated; *Candida sp., Rhodotorula sp., Cryptococcus sp.* and *Trichosporon Sp.* The histopathological findings revealed severe degenerative changes in skin and gills with presence of fungal hyphae and spores.

**Conflict of interest**

The authors declare that no conflict of interest.
References