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The Nile Tilapia (*Oreochromis niloticus*) Physiological and Histopathological Biomarkers of some Fungal Infections and Proposed Silver Nanoparticles Remediation

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ABSTRACT

The Nile tilapia are susceptible to various infections originating from the aquatic environment, particularly fungal pathogens. Fungal infections are considered the second most significant cause of losses in aquaculture, especially during the cold winter months. Silver nanoparticles (AgNPs) have emerged as promising antifungal agents in aquaculture. In this study, fungi isolated from the Nile tilapia exhibiting fungal infections were identified based on their morphological characteristics. Infected fish were treated with silver nanoparticles at concentrations of 5, 10, and 15µg/ L, dissolved in the aquarium water. Histopathological and hematological analyses were conducted to evaluate the treatment effects, and the *in vitro* antifungal activity of AgNPs was also assessed. All treated fish showed signs of recovery, with the highest recovery rates observed at concentrations of 5 and 10µg/ L. These findings suggest that silver nanoparticles can be safely and effectively used as antifungal additives in aquaculture water at concentrations of 5–10µg/ L to reduce mortality caused by fungal infections, particularly during the winter season.

INTRODUCTION

Aquaculture is the rapidly expanding sector within food production and is crucial for enhancing food security, particularly in developing nations **(FAO, 2017)**. The Nile tilapia, scientifically known as *Oreochromis niloticus*, is an omnivorous fish belonging to the Cichlidae family. It is highly regarded as one of the most effective food fish both in Egypt and globally **(Moustafa & Assem, 2024)**. However, the Nile tilapia is susceptible to various infections from the aquatic environment, with fungal pathogens being a significant concern **(Mahboub, 2021)**. Fungal diseases typically arise due to poor water quality, insufficient management, injuries to the fish, or the presence of dead fish and

decaying organic matter. To maintain fish health, it is essential to mitigate these stressors **(Hashem *et al.*, 2020)**. Fungal diseases rank as the second primary cause of losses in aquaculture. Many fungi that affect fish are considered opportunistic pathogens **(Quiniou *et al.*, 1998)**, particularly in cold water during winter. Nanoparticles can be utilized in the aquaculture industry, three principal areas benefit from their application: alimentation, filters to improve water culture medium, effluents, and control of infectious diseases **(Márquez *et al.*, 2016)**. For a thousand years, silver was a precious metal for human beings; ancient civilizations used silver to maintain water quality and as an antimicrobial agent. One of the applications of silver nanoparticles (AgNPs) was disease control because of their bactericidal activity **(Gong *et al.*, 2007)**. The AgNPs are promising in aquaculture because of their antimicrobial, antifungal, and antiviral properties. AgNPs offer alternatives to conventional antimicrobial agents. Their tiny size and distinctive physicochemical characteristics boost their antimicrobial effectiveness, significantly inhibiting pathogen growth and lowering the occurrence of aquatic organisms' disease **(Khursheed *et al.*, 2023)**.

Plasmonic AgNPs from the brand NT-SNP, produced by NanoTech Egypt for the Photo-Electronics Communication Center, have been traditionally utilized to control the growth of microbes **(Wadhera & Fung, 2005)**. *In vitro* studies have shown that AgNPs possess antibacterial and antifungal properties, even against antibiotic-resistant bacteria **(Wright *et al.*, 1994; Wright *et al.*, 1999)**. Currently, silver compounds are frequently used across various industrial and sanitary applications, including the creation of synthetic materials for dentistry, the coating of catheters and surgical instruments, homeopathic remedies, treatment of burn wounds, textile treatments, and water purification **(Spencer, 1999; Klasen, 2000; Wadhera & Fung, 2005; Atiyeh, 2007; Hwang *et al.*, 2007)**. They exhibit high thermal stability, minimal toxicity to human cells, and low volatility **(Durán *et al.*, 2007)**. For size and shape analysis, transmission electron microscopy (TEM) was conducted using a JEOL high-resolution microscope at an accelerating voltage of 200kV. For optical properties, UV-Vis absorption spectra were measured with an Ocean Optics VIS-NIR Fiber optics spectrophotometer. The physical characteristics include a yellow colloidal solution that is water-soluble, with an average size of 45 ± 5nm and a spherical-like shape, as reported by the NanoTech Company.

MATERIALS AND METHODS

Preparation of the fish for experiments

The Nile tilapia (*Oreochromis niloticus*) fingerlings measuring 6.0 - 8.0cm were sourced from a local hatchery and placed in a 20L fiberglass tank under a 12-hour light and 12-hour dark photoperiod. The tanks received a constant flow of aerated dechlorinated tap water, maintained at a temperature of 25±1°C. The fish were fed a diet

containing 35% protein at a rate of 3% of their body weight daily. Feeding was halted 24 hours before the start of the experiments and right through their period **(Moustafa & Assem, 2024)**. During the experiments, the fish were transferred to 12L glass aquaria, with water changed every 24 hours using the siphon technique to minimize disruption.

Experimental design and culture system

Eight groups of fish were assigned randomly to each aquarium, with three repeats for each treatment. To evade the influence of systemic stress factors, the fish groups were redistributed at random halfway via the experiment. In each trial, one aquarium housed fish lacking fungal infections, serving as the control. Three other aquaria contained naturally fungal-infected fish, which are more commonly found in the winter. Additionally, three aquariums included fungal-infected fish treated with 5, 10, and 15µg/ L of AgNPs dissolved in the water. Every aquarium was treated as an experimental independent unit. Fish were fed *ad libitum* at 09:00, 12:00, and 15:00 hours, and fecal waste was siphoned from each aquarium daily. The experimental period lasted for two weeks for all fish groups to be tested.

Blood and tissue sample collection:

After the experiment (2 weeks), three groups of 8 fish from each diet were used to collect blood and tissues. Control fish were subjected to the same level of disturbance as those in the experimental groups. Fish were caught quickly using a hand net to minimize stress, then placed upside down. Blood was collected via incision directly into the heart using a heparinized glass pipette, with samples split for determining hemoglobin and serum analysis. Serum was separated immediately using centrifugation to avoid hemolysis and was stored at –20°C for future analyses. After blood collection, the fish were euthanized, and the liver, gills, and intestine were excised and preserved for histological examinations.

Isolation of fungi

Fungi were isolated using Potato Dextrose Agar (PDA) medium from the gills, skin, fins, and operculum. Samples were swabbed onto PDA medium, which was supplemented with 50mg/ mL of chloramphenicol to inhibit bacterial growth. Isolation was conducted in a laminar flow hood to prevent contamination. PDA plates were incubated at 28-30°C, and fungal growth was monitored over 7-14 days. Isolated fungal cultures were then sub cultured on PDA plates. The fungi were examined macroscopically and microscopically.

Morphological identification of isolated fungi

Fungal morphology was identified macroscopically by examining colony characteristics on agar plates, while micromorphological characterization was conducted

using a light microscope. Fungal strains were cultured on Czapek's yeast extract agar (CYA) as per **Pitt and Hocking (2009)**. Identification of pure fungal cultures was performed using keys and reference texts in **Pitt (1979)**, **Domsch *et al.* (2007)** and **Bensch *et al.* (2012)**. For microscopic identification, wet mounts of the fungal hyphae and spores were stained with lactophenol cotton blue and observed under an Axiostar plus trinocular research microscope from Germany. Images of stained hyphae, conidiogenous cells, and conidia were captured at a 1000× magnification using a PowerShot G6 digital camera (7.1 megapixels) made in Japan.

In Vitro antifungal Activity of AgNPs:

The agar dilution method was utilized **(Mona *et al.*, 2022)** to assess different concentrations of AgNPs (5, 10, and 15µg/ liter). Various concentrations of AgNPs were mixed into Potato Dextrose Agar and were inoculated with distinct isolated fungi, including *Penicillium crustosum*, *Aspergillus asperescens*, *Cladosporium basinflatum*, *Aspergillus versicolor,* and *Aspergillus flavus*. All inoculated plates were incubated at 30°C for three to five days.

Analytical techniques

The hemoglobin content in the blood was measured using the Diamond diagnostic hemoglobin kit. Serum glucose concentration was determined with the glucose-liquizyme GOD-PAP kit. (Spectrum, MDSS GmbH Schiffgraben 41 30175 Hannover, Germany) **(Caraway & Watts, 1987)**. Cholesterol levels in the serum were measured using the CHOD/POD method kit, **(Meiattini *et al.*, 1978)**. While triglyceride levels were determined via the GPO/PAP method kit **(Stein, 1987)**. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed using specified methods. These measurements were conducted following the manufacturer’s protocol provided by Biolabo (Maizy, France), utilizing a fully automated clinical chemistry analyzer (Mispa CCXL, AGAPPE, Cham, Switzerland).

Histopathological investigation

Three tissue samples from the gills, liver, and intestine were collected from each group, fixed in 10% formalin, and subjected to a dehydration process using progressively higher concentrations of ethanol (70 to 100%). The samples were then cleared in xylene and embedded in paraffin. Paraffin sections were cut using a Leica RM 2155 microtome from England. The sections were prepared to a thickness of 5µm.

This experiment meets all the National Institute of Oceanography and Fisheries Committee for ethical care and use of animals/ aquatic animals (NIOF-IACUC) guidelines and regulations with Ethics Committee Certificate of Approval (Serial Number NIOF F14 F 24 R 029).

RESULTS

Isolation and identification of fungi

Nine pure fungal isolates were isolated from tilapia fish and identified by morphological characterization as *Aspergillus versicolor, Penicillium crustosum, Aspergillus asperescens, cladosporium basinflatum, Aspergillus flavus, Acremonium berkeleyanum, Rhizopus oryzae,* and *Beauveria brongniartii,* as shown in Fig. (1).

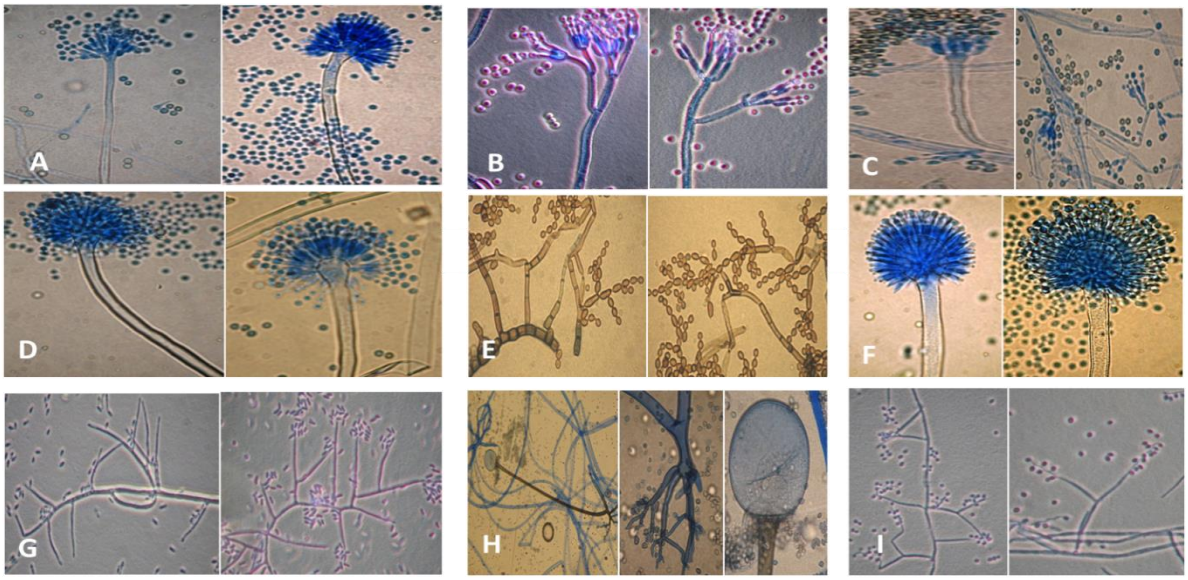

Fig. 1. Microscopic identification of fungi isolates, (A) *Aspergillus sydowii* AUMC16591: Unpigmented conidiophores terminating in biseriate conidial head producing rough-walled conidia, (B) *Penicillium crustosum* AUMC16592: Rough-walled branched conidiophores producing metulae, phialides, and chains of conidia, (C) *Aspergillus asperescens* AUMC16593: Conidiophores each with small vesicle and chains of rough-walled conidia. Small lateral heads are also produced, (D) *Aspergillus versicolor* AUMC16594: Faintly pigmented conidiophores terminating in biseriate conidial head producing chains of rough-walled conidia, (E) *Cladosporium basinflatum* AUMC16596: Darkly pigmented hyphae and basal chlamydospores bearing conidiophores and branched chains of conidia, (F) *Aspergillus flavus* AUMC16600: Rough-walled conidiophores each terminating in Biseriate conidial head producing rough-walled conidia, (G) *Acremonium berkeleyanum* AUMC16599: Hyphae, phialidic conidiogenous cells producing balls of smooth-walled ellipsoidal conidia, (H) *Rhizopus arrhizus* AUMC16598: Rhizoides, sporangiphores each with columellate sporangium producing numerous sporangiospores, branched rhizoides columella after rupturing of sporangiun and numerous sporangiospores, (I) *Beauveria brongniartii* AUMC16595: Hyphae and short branches bearing conidiogenous cells each with zigzag tip producing smooth ovate candida

In Vitro antifungal activity of AgNPs

AgNPs decreased the growth of *Penicillium crustosum, Aspergillus asperescens, cladosporium basinflatum* and *Aspergillus versicolor* (Fig. 2).

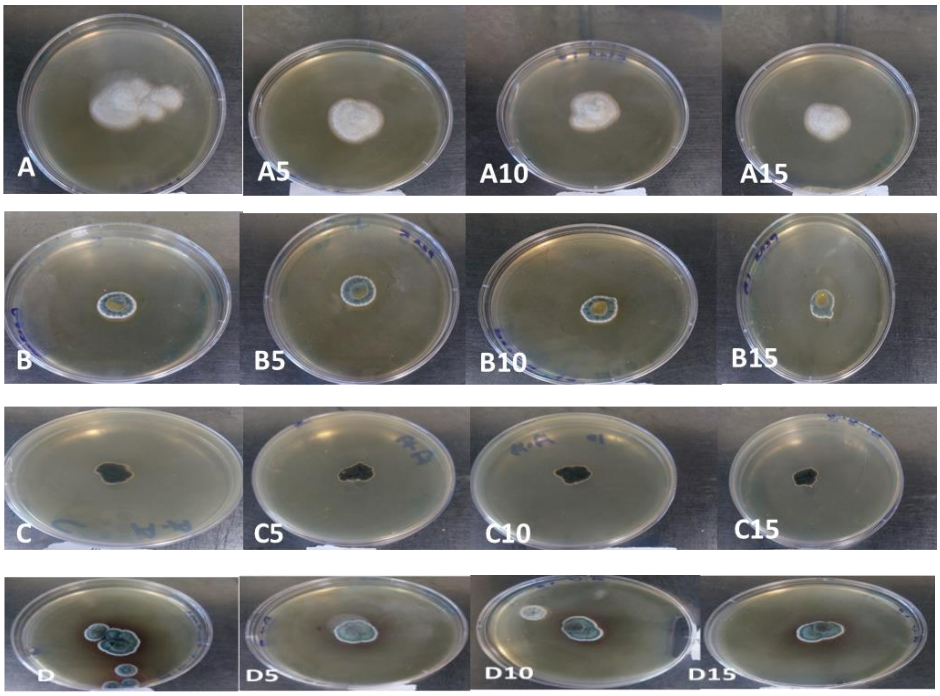

Fig. 2. Antifungal activity of AgNPs at different concentrations against *cladosporium basinflatum* (A = control, A5 = 5 µL of AgNPs , A10 = 10 µL of AgNPs and A15 = 15 µL of AgNPs), *Penicillium crustosum* (B = control, A5 = 5 µL of AgNPs , B10 = 10 µL of AgNPs and B15 = 15 µL of AgNPs), *Aspergillus asperescens* (C = control, C5 = 5 µL of AgNPs, C10 = 10 µL of AgNPs and A15 = 15 µL of AgNPs) and *Aspergillus versicolor* (D = control, D5 = 5 µL of AgNPs , D10 = 10 µL of AgNPs and D15 = 15 µL of AgNPs)

Table 1. Semi-quantitative evaluation of antifungal activity of AgNPs on different isolated fungi

Fungal species	5 µg/L AgNPs	10 µg/L AgNPs	15 µg/L AgNPs
Cladosporium basinflatum	++	++	+++
Penicillium crustosum	+	+	+
Aspergillus asperescens	+	+	++
Aspergillus versicolor	++	++	++

\+ = weak inhibition
++ = moderate inhibition
+++ = strong inhibition
A semi-quantitative evaluation of antifungal activity was conducted based on visual observation of fungal growth inhibition.

Clinical signs and postmortem findings

A) Infected group

The fish infected with fungus showed signs of appetite thrashing, instability with erratic movement, and tended to swim near the water's surface. They exhibited scale separation, bleeding in various areas of the body, mild abdominal swelling, and developed skin erosions and ulcers with tail rot (Fig. 3). There was hyperemia in the internal organs. Moreover, gills were sheltered with mucus and became pale (Fig. 3), in addition to the loss of scales and deep scattered ulcerations with necrotic myocytic cells were noticed ulcers on the region of the caudal peduncle with engorged liver and enlarged gall bladder (Fig. 3). There were fluffy, cottony growths on various parts of the Nile tilapia's body (Fig. 3). The skin of the Nile tilapia exhibited hemorrhages along with a darkening of the entire fish (Fig. 3).

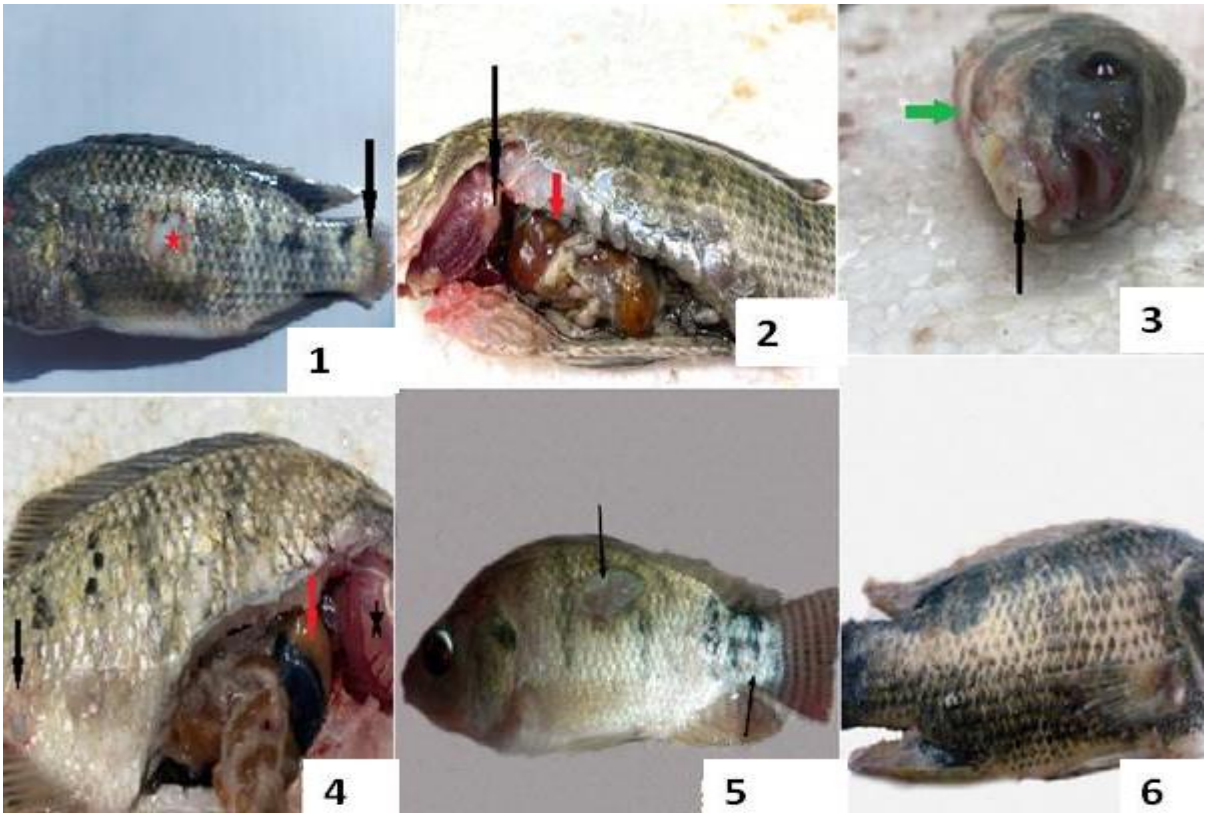

Fig. 3. (1) The Nile tilapia in the infected group with fungus showed skin ulceration and erosions (stars) with tail rot (arrow). (2) Enlarged liver (red arrow) with pale gills (black arrow). (3) Hemorrhages at the skin (green arrow) with mouth protrusion (black arrow). (4) Ulceration at skin (black arrows) with pale liver (red arrow). (5) Severe ulceration, desquamation of scales (black arrows). (6) Darkening of the skin

B) Treated groups

Nile tilapia treated with 10 and 15 µg/L of silver nanoparticles (AgNPs) exhibited moderate to severe clinical signs, whereas those treated with 5 µg/L showed only mild

clinical symptoms. Higher mortality rates were observed in the 10 and 15 µg/L treatment groups compared to the 5 µg/L group. Clinical signs included anorexia, lethargic swimming with minimal or absent escape reflexes, and respiratory distress manifested as gasping, rapid opercular movements, and aggregation near the oxygen supply. In the 5 µg/L AgNPs group, external hemorrhages were observed on the skin (Fig. 4), along with internal abnormalities such as gallbladder enlargement, pale liver, and congested gills (Fig. 4). At 10 µg/L, fish exhibited a protruded vent (Fig. 4) and internal signs of ascites (Fig. 4). The 15 µg/L group showed more severe symptoms, including skin ulceration, exophthalmia (protruding eyes) (Fig. 4), a swollen gallbladder, and the presence of greenish bile (indicated by an arrow in Fig. 4).

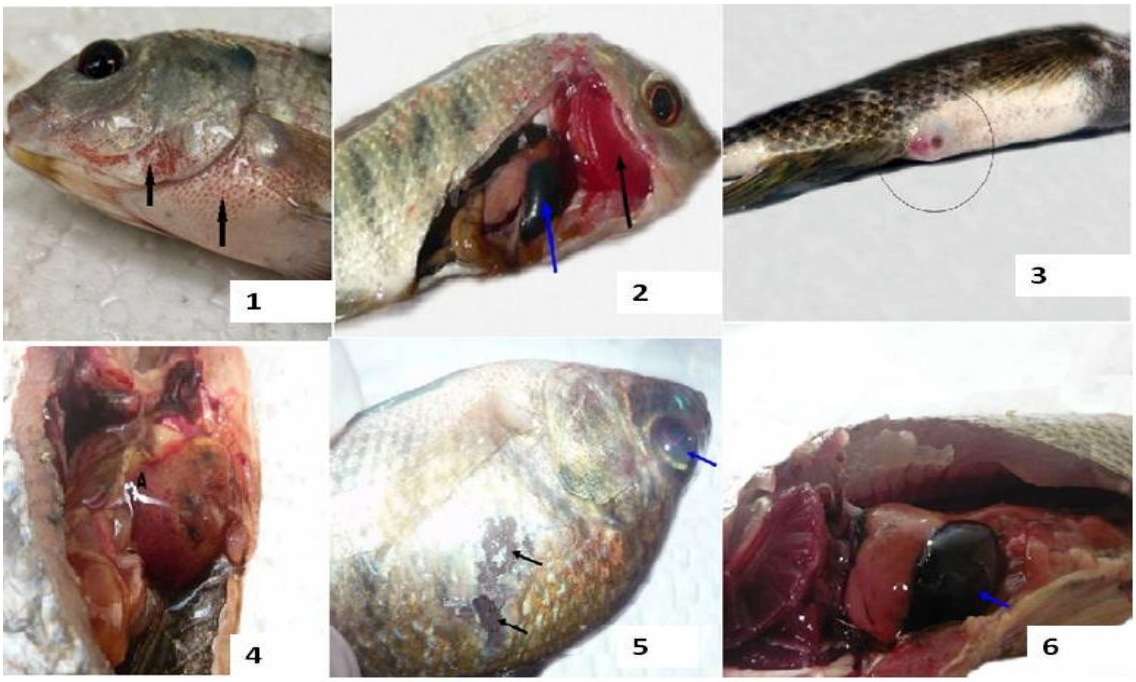

Fig. 4. (1) Treated group with 5 µg/L of AgNPs, showed multiple hemorrhagic patches (black arrows) (2) reddish gills and enlarged gallbladder (blue arrow), (3) in the treated group with 10 µg/L of AgNPs, Nile tilapia showed hemorrhagic vent, (4) ascites of internal organs. (5) In the group treated with (15 µg/L of AgNPs) showed desquamation of the scales (black arrow) with slight protrusion of the eye (blue arrow) (6) and enlarged gallbladder (blue arrow).

Histopathological findings

Infected group

The histopathological findings were evident in the gills, liver, and intestine of the infected group. Fish in the control group exhibited normal structures, without any significant microscopic lesions in the gills (Fig. 5). The liver of the control fish displayed

normal histoarchitecture, and there were no significant pathological abnormalities. The intestine of the control group showed a normal tunica mucosa, submucosa, muscularis, and serosa. (Fig. 5). In Nile tilapia infected with fungal pathogens, the gills showed lamellar telangiectasis (Fig. 5), along with focal and multifocal fusion of the secondary gill lamellae. These changes were attributed to hyperplasia of the basal epithelium of the primary lamellae, accompanied by mononuclear cell infiltrates (Fig. 5). The intestinal tissue exhibited an increased number of goblet cells (Fig. 5). In addition, the liver of infected fish displayed mild to marked activation of melanomacrophage centers (MMCs) along with signs of fatty degeneration (Fig. 5).

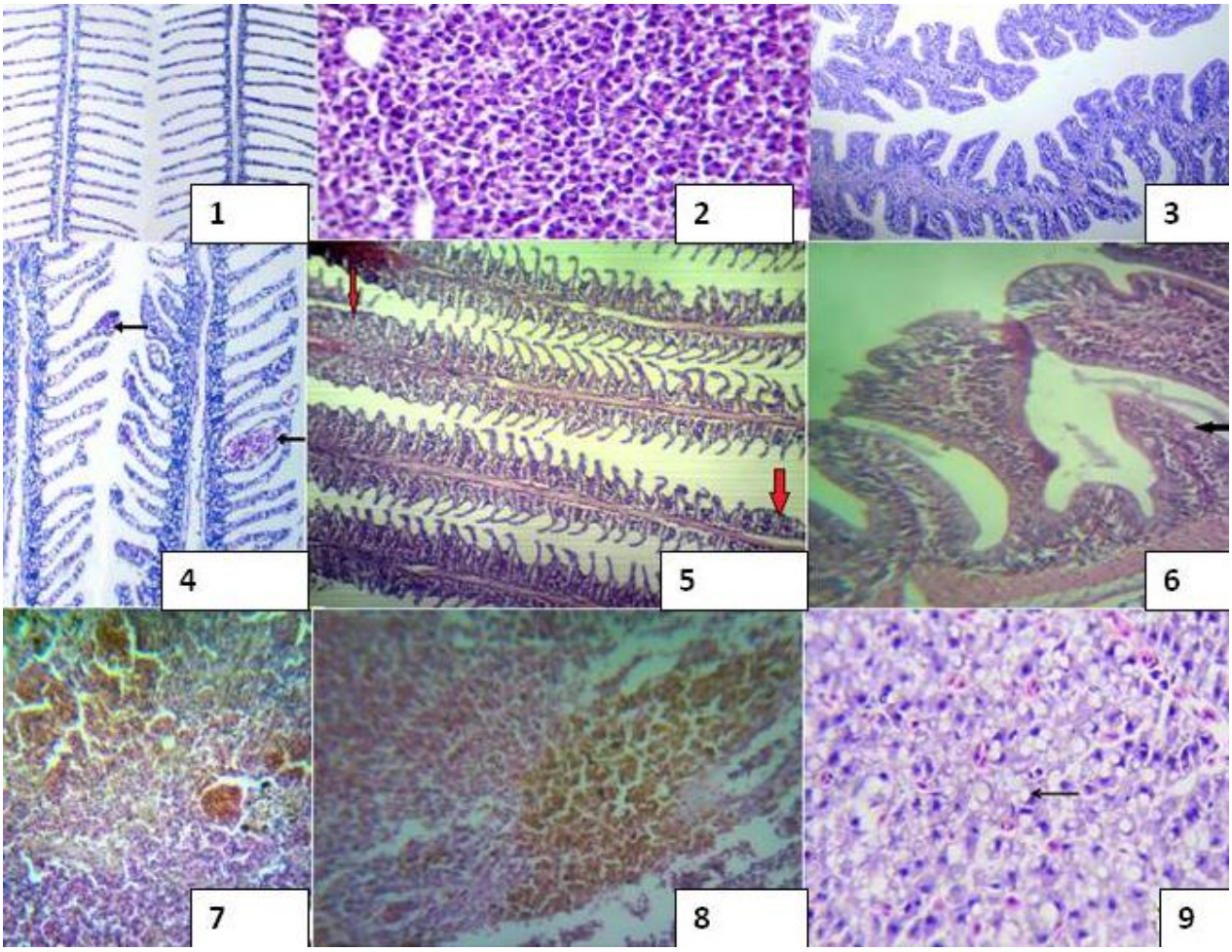

Fig. 5. (1) Normal gills (H and E: 100×), (2) normal hepatocyte and central vein in liver (H and E: 100×). (3) Normal intestinal villi (H and E: 100×). (4) In the infected group with fungus, there was lamellar telangiectasis in secondary gill lamellae (H and E: 200×), (5) multiple focal fusions in secondary gill lamellae (H and E: 200×). (6) The intestine showed activation of goblet cells (H and E: 200×). (7,8) Liver showed activation of melanomacrophage centers (H and E: 100×), (9) fat vacuoles (H and E: 100×)

In treated groups

In the treated group with 5µg/ L of AgNPs, there was an absolute fusion of secondary lamellae due to hyperplasia of basal epithelium of primary lamellae (Fig. 6). The liver showed congestion of blood vessels and fatty changes characterized by the presence of sharply outlined vacuoles (Fig. 6). The intestine showed hyperplasia of goblet cells (Fig. 6). In the treated group with 10µg/ L of AgNPs, there was a complete aggregation of inflammatory cells at the base of the primary lamellae of the gills (Fig. 7). At the same time, there was activation of melanomacrophage centers (MMCs), where some melanophores appeared seriously overloaded with dark brown melanin pigment and diffusing sharp edge outline vacuoles of hepatocytes and congestion of central veins (Fig. 7). The intestine showed activation of goblet cells and necrotic intestinal villa with inflammatory cell infiltrations (Fig. 8). In the treated group with 15µg/ L of AgNPs, there was congestion of branchial blood vessels with focal lamellar telangiectasis (Fig. 8). Moreover, there was congestion of the central vein in the liver with severe fat vacuoles (Fig. 8). The intestine showed severe activation of goblet cells (Fig. 8).

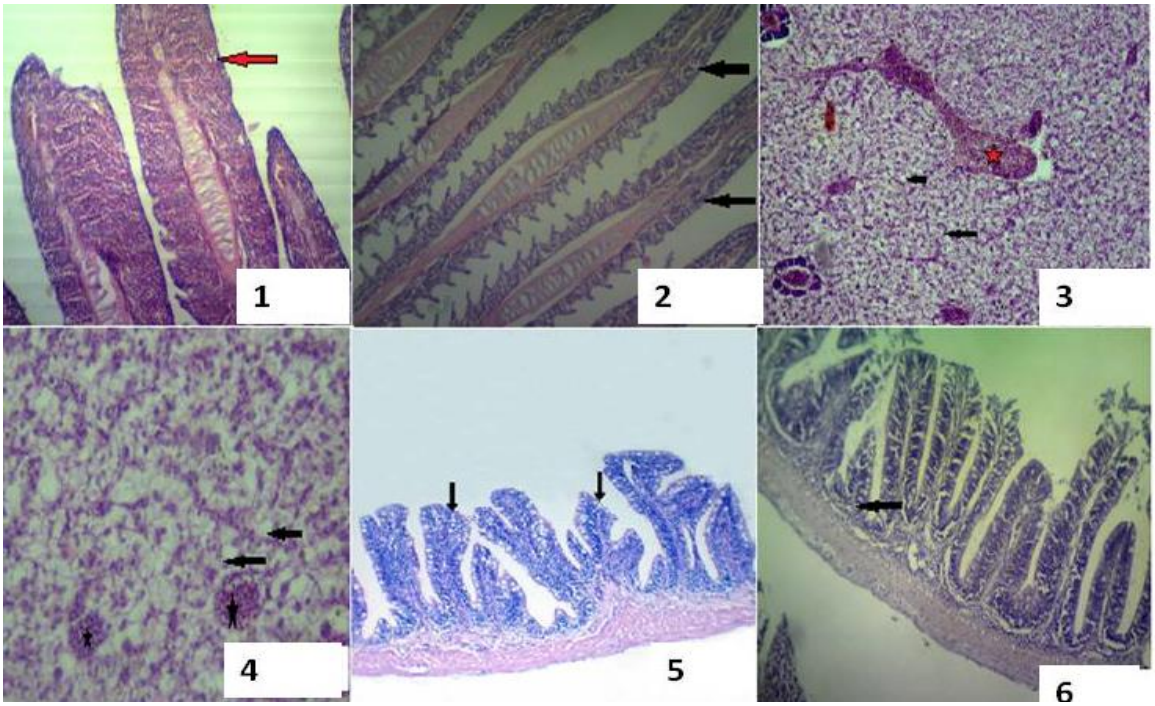

Fig. 6. (1) Treated group with (5 µg/L of AgNPs) showed complete fusion of secondary gill lamellae (red arrow) (H and E: 200×) (2) with shortening of secondary gill lamellae and fusion of secondary gill lamellae (black arrows) (H and E: 200×). (3) Liver of treated group with (5 µg/L of AgNPs congestion of central vein in liver (red star) (H and E: 100×). (4) Fat vacuoles (black arrows) with congestion of central vein (black stars) (H and E: 100×). (5,6) Activation of goblet cells in the intestine of treated group with (5 µg/L of AgNPs) (black arrows) (H and E: 200×)

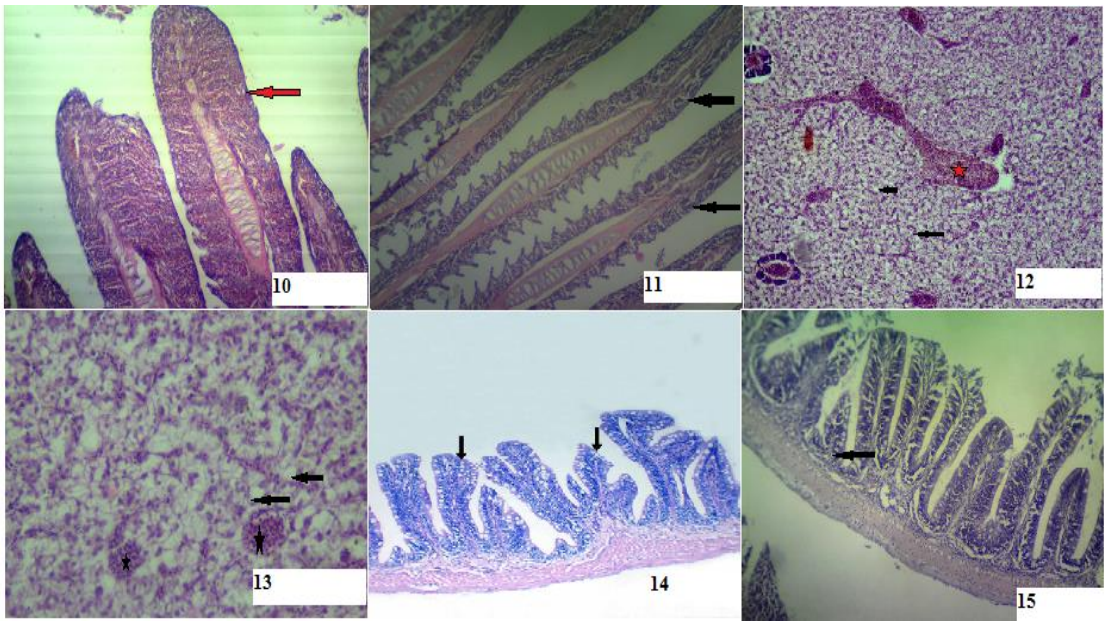

Fig. 7. (1) The treated group with 10µg/vL of AgNPs, gills showed congestion of branchial of blood vessels (stars) with severe aggregation of inflammatory cells at the base of the primary gill lamellae (arrows) (H and E: 200×). (2,v3) Activation of the melanomacrophage center where some melanophores appeared heavily loaded with dark brown melanin pigment (MMCs) (H and E: 200×). (4) Congestion of the central vein (stars) and (5) fatty change in the treated liver (black arrows, H and E: 200×). (6) The intestine suffered from necrotic intestinal villa with inflammatory cell infiltrations (arrows) (H and E: 200×).

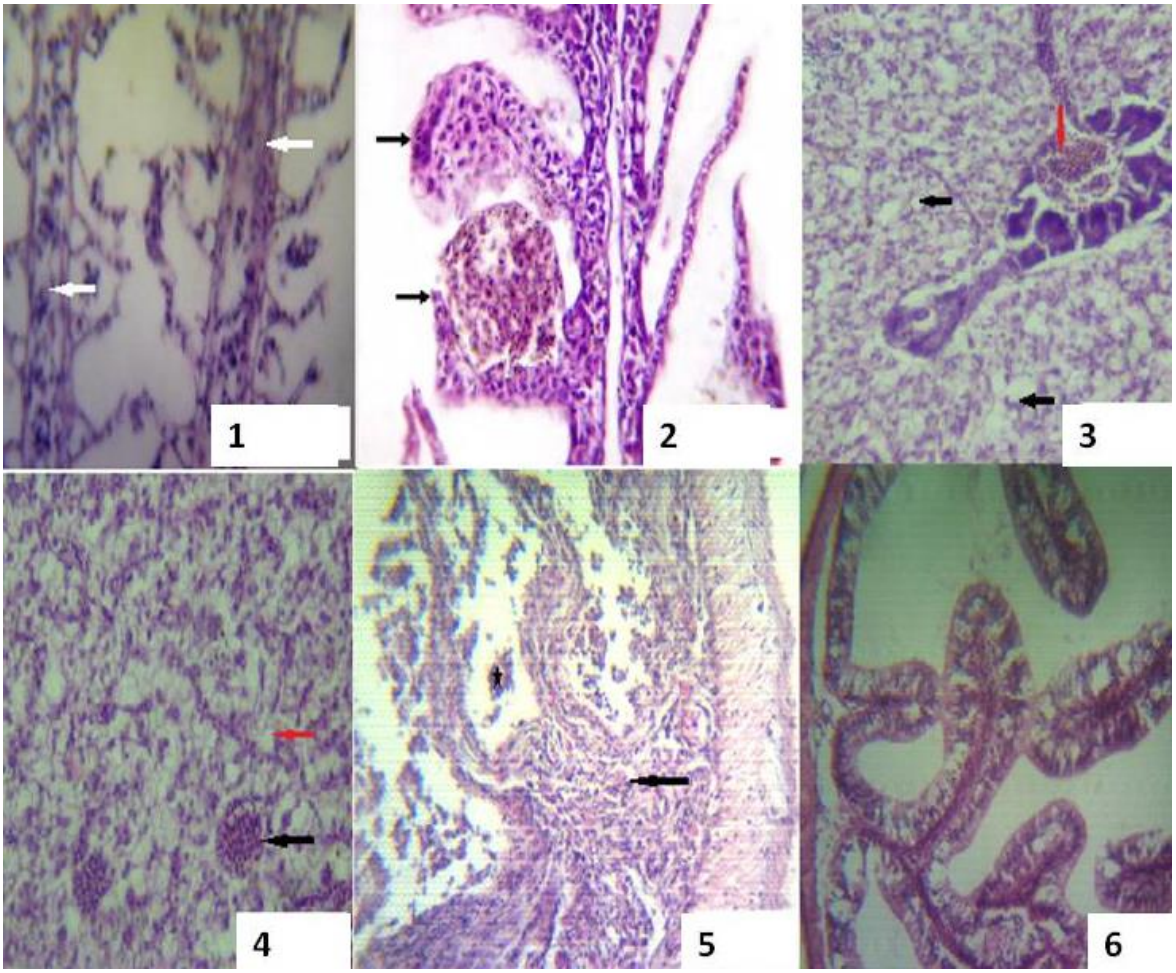

Fig. 8. (1) The treated group of the Nile tilapia with 15µg/ L of AgNPs showed congestion of branchial blood vessels of secondary gill lamellae (white arrows) (H and E: 200×), (2) presence of lamellar telangiectasis (H and E: 400×). (3,4) The liver of treated group showed congestion of central vein with fat vacuoles (H and E: 200×). (5) The intestine showed necrosis (arrow) (H and E: 200×), and (6) activation of goblet cells (H and E: 200×)

Biochemical and hematological parameters

The hematological and biochemical parameters are indicated in Table (2), where the serum glucose levels increased significantly ($P< 0.01$) at all the infected and AgNPs - treated fish showing its highest level at concentration of 15µg/ L of AgNPs.

Hemoglobin content decreased during fungal infection and AgNPs treatments at all concentrations ($P< 0.01$), except at the concentration of 10µg/ L, where there was no anemic condition (P-value 0.56) and no significant change compared to control. Cholesterol levels were significantly ($P< 0.01$) increased by fungal infection by more than two folds compared to the control. On the contrary, it decreased at AgNPs-treated groups at all concentrations ($P< 0.01$).

Table 2. Biochemical and hematological parameters of control, infected and AgNPs treated groups

Parameter	Control	Infected	5 µg/L AgNPs	10 µg/L AgNPs	15 µg/L AgNPs
Hemoglobin (g/dl)	2.9±0.1	1.5±0.2 [a, c, d]	2.6±0.3 [a, b, d, e]	2.9±0.2 [b, e]	1.5±0.1 [a, c, d]
Glucose (mg/dl)	34.3±5.0	47.9±6.0 [a, c, d, e]	49.8±5.0 [a, b, d, e]	59.3±3.0 [a, b, c, e]	70.0±5.0 [a, b, c, d]
Triglycerides (mg/dl)	135.8±6.0	112.1±8.0 [a, c, d, e]	89.3±5.0 [a, b, d, e]	133.0±6.0 [a, b, c, e]	162.9±7.0 [a, b, c, d]
Cholesterol (mg/dl)	492.5±7.0	1075±5.0 [a, c, d, e]	358.3±9.0 [a, b, d, e]	397.5±5.0 [a, b, c, e]	306.7±8.0 [a, b, c, d]
ALT (IU/L)	80.3±9.0	111.7±9.0 [a, c, d, e]	96.0±7.0 [a,b, e]	96.0±11.0 [a, b, e]	68.1±5.0 [a, b, c, d]
AST (IU/L)	112.2±9.0	129.7±7.0[a, c, d, e]	102.7 ± 8.0 [a, b, d, e]	117.5 ±7.0[a, b, c, e]	114.0 ±7.0[a, b, c, d]

Data are expressed as mean ± SEM. n = 8 for each experimental group

[a] $P < 0.01$ *vs.* control group

[b] $P < 0.01$ *vs.* infected group

[c] $P < 0.01$ *vs.* 5 µg/L of AgNPs treated group

[d] $P < 0.01$ *vs.* 10 µg/L of AgNPs treated group

[e] $P < 0.01$ *vs.* 15 µg/L of AgNPs treated group

Triglycerides decreased significantly ($P< 0.01$) at the infected and AgNPs-treated fish showing its lowest level at concentration of 5µg/ L of AgNPs, except at a concentration of 15µg/ L of AgNPs, where its level was increased significantly ($P< 0.01$).

Serum ALT increased significantly ($P< 0.01$) at the infected group, but decreased significantly ($P< 0.01$) at all the infected AgNPs-treated fish showing its lowest level at concentration of 15µg/ L of AgNPs.

Also, serum AST increased significantly ($P< 0.01$) at the infected group, but decreased significantly ($P< 0.01$) at all the infected AgNPs-treated fish compared with the infected group showing its lowest level at concentration 5µg/ L of AgNPs.

Table 3. The mortality percentage in 2 weeks

Parameter	Control	Infected	5 µg/L AgNPs	10 µg/L AgNPs	15 µg/L AgNPs
Mortality % in 2 weeks	0	90	0	10	80

The mortality percentages observed over a two-week period are presented in Table (3). No mortality was recorded in either the control group or the group treated with 5 µg/L of AgNPs. In contrast, the fungi-infected group exhibited a mortality rate of 90%. The group treated with 10µg/ L of AgNPs showed only 10% mortality, while the group treated with 15µg/ L of AgNPs experienced a significantly higher mortality rate of 80%.

DISCUSSION

The demand for fish has increased statistically, revealing the requirement for aquaculture scientists to generate high-quality fish that would reach a healthy adult size in the shortest possible time to increase production and to reduce fish and egg loss due to diseases. Several microbial diseases impact aquaculture: included among these ailments are fungal infections that result in the loss of fish and their eggs in both commercial fish aquaculture and natural ecosystems **(Meneses *et al.*, 2021)**. Fish fungal diseases cooperate with other pathogens in causing tail and fin rot in freshwater fish, especially in tilapia species. Moreover, under certain poor environmental conditions and stressors, they can produce toxic metabolites (mycotoxins), causing increased mortality, poor growth rates, and low market value **(Samira-Rezeaka, 1991)**.

The present study found that five fungal genera were isolated from diseased tilapia: *Aspergillus* spp., *Penicillium* sp., *Cladosporium* spp., *Acremonium* spp., *Rhizopus* spp., and *Beauveria* spp. They were identified macroscopically by observing colony features on an agar plate. Micromorphology was characterized using a light microscope and identified as *Aspergillus flavus*, *Aspergillus versicolor*, *Penicillium crustosum*, *Aspergillus asperescens*, *Cladosporium basinflatum*, *Acremonium berkeleyanum*, *Rhizopus oryzae*, and *Beauveria brongniartii*. **Abd El Tawab (2020)** demonstrated that diseased *O. niloticus* exhibiting eroded fins, skin redness, congested and protruding anal openings, darkened skin, and ulcers were naturally infected with *Aspergillus* sp. and *Penicillium* sp. In experimental infections with *A. flavus*, various clinical symptoms were consistent with those observed in naturally infected fish. These included yellow discoloration with skin ulcers, hemorrhagic gill and skin lesions, corneal cloudiness, fin deterioration, and abdominal swelling. *A. flavus* was the most frequently isolated species **(Mohamed *et al.*, 2017)**. *Aspergillus versicolor* was also isolated from *O. niloticus* skin **(Awad *et al.*, 2021)**. *Penicillium crustosum* was identified in ornamental fish with gill disorders **(Ebrahimi Jafari *et al.*, 2022)**. It is also known to produce mycotoxins, albeit rarely in fish feeds **(Greco *et al.*, 2015)**. *Cladosporium* spp. were associated with high mortality rates in *O. niloticus* **(Abd El-Ghany, 1998; Tasic, 2007; Oda *et al.*, 2016)**.

The wide range of applications and unique functionalities of nanotechnology make it a rapidly advancing field. Nanomedicine is being explored for various disease diagnostic, treatment, prevention, and management strategies **(Almatroudi, 2020)**.

However, limited research exists on using silver nanoparticles (AgNPs) to treat fungal infections. This study was therefore conducted to investigate the therapeutic effect of AgNPs on fungal infections in *Oreochromis niloticus*. **Zaky and Ibrahim (2017)** documented various fungal species, including *Acremonium*, in *O. niloticus* from Lake Manzala, noting potential human health impacts. *In vitro* studies showed that AgNPs inhibited the growth of *Penicillium crustosum*, *Aspergillus asperescens*, *Cladosporium basinflatum*, and *Aspergillus versicolor*. Other studies similarly reported antifungal activities of AgNPs **(Al-Zubaidi *et al.*, 2019; Azhdari *et al.*, 2020; Li *et al.*, 2022)**. AgNPs exert antimicrobial effects against both Gram-negative and Gram-positive bacterial and fungal strains **(Ghetas *et al.*, 2022)**.

In the present study, infected groups exhibited severe skin ulcerations, hemorrhages on various body parts, skin darkening, tail fin erosion with unilateral exophthalmia, scale loss, and hemorrhage on the dorsal surface, along with mild ascites. These clinical signs were comparable to those described by **El-Bouhy (2002)**. Exophthalmia may be due to exudate accumulation in the eye, leading to the condition commonly referred to as "pop-eye" **(Romalde & Toranzo, 1999)**.

The clinical signs observed in infected *O. niloticus* included respiratory distress likely due to reduced gill surface epithelium. Hyperplasia at the base of the secondary lamellae led to fusion, impairing gas exchange and resulting in erratic swimming and loss of equilibrium. Postmortem analysis revealed organ congestion, pale liver, and bloody ascitic fluid **(Zeid, 2004)**.

Fungal infection resulted in hemorrhagic patches, especially near the pectoral and dorsal fins, with skin darkening and occasional scale loss or small ulcerative sores near the caudal peduncle, consistent with **Oraic *et al.* (2002)**. The gills, essential for respiration and osmoregulation, exhibited severe lamellar fusion, necrosis of the epithelial layer, increased goblet cell activity, and leukocyte infiltration. Fungal exposure also caused rupture of pillar cells and blood pooling, resulting in telangiectasia and possible lamellar shortening or stunting due to epithelial proliferation **(Abou El-Atta & El-Tantawy, 2008)**.

Inherent immunity is the fish's primary defense and plays a key role in acquired immune responses. Leukocytes produce lysozyme (LYZ), a mucolytic enzyme found in fish plasma, mucus, and lymphoid tissues **(Ibrahim *et al.*, 2022)**. The hepatopancreas detoxifies microbial toxins. Common hepatic lesions in infected fish included vascular congestion, acute cellular swelling, and fatty degeneration **(Zhuang-lin *et al.*, 2012)**. These changes likely reflect lipid mobilization in response to stress or inflammation **(Ibrahim *et al.*, 2022)**. AgNPs showed hemolytic activity depending on dose **(Ghetas *et al.*, 2022)**, while treatment with 0.6mg/ L MS-AgNPs alleviated stress conditions

(Ibrahim *et al.*, 2022). Intestinal histopathology showed goblet cell hyperplasia from toxin exposure **(Refai, 2010)**.

Hematological parameters are vital indicators of fish health **(Hrubec *et al.*, 2000)**. Hemoglobin levels declined during fungal infection and AgNP treatment, consistent with **Moustafa *et al.* (2021)**. AgNP exposure significantly reduced WBCs, RBCs, and hemoglobin, especially in pathogen-exposed groups **(Thummabancha *et al.*, 2016)**. **Shaluei *et al.* (2013)** similarly found reduced RBCs and hemoglobin in silver carp exposed to subacute AgNP toxicity. Recovery at 10 µg/L AgNPs reversed anemia.

All infected groups had elevated liver biomarkers, but AgNP-treated fish showed reduced ALT and AST levels, supporting findings by **Ibrahim *et al.* (2022)**. Elevated enzymes in infected groups may result from fungal toxin damage **(Azimzadeh & Amniattalab, 2017)**. AgNPs improved liver biomarkers and slowed disease progression.

At 15µg/ L, AgNPs caused toxicity across hematological and histopathological parameters. This supports the concerns of **Bacchetta *et al.* (2017)** about nanoparticle toxicity due to nanoscale size and ion release. However, 5 and 10 µg/L AgNPs improved hematological markers and showed antifungal effectiveness, possibly due to AgNPs' cell-penetrating and dissolving properties **(Palza, 2015)**.

After two weeks, no mortality occurred in the control or 5µg/ L AgNP groups, and low mortality was observed at 10µg/ L. Mortality increased at 15µg/ L, likely due to nanoparticle toxicity.

AgNPs exhibited antifungal and antimicrobial properties. Treated groups showed reduced mortality and clinical improvement at 5 and 10µg/ L. This is attributed to AgNPs' antifungal effects confirmed *in vitro*. Their antimicrobial mechanism involves ROS-mediated membrane damage **(Abdel Rahman *et al.*, 2023)**. The 5µg/ L group showed optimal recovery, while 10 and 15µg/ L groups experienced increased toxicity and mortality.

CONCLUSION

According to these observations; AgNPs can be used safely as an antifungal additive to aquarium water at concentrations 5 and 10µg/ L AgNPs in aquaculture to decrease the mortality caused by fungal infections that are increased especially in winter.

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