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Efficiency of *Persicaria salicifolia* as a natural alternative antifungal against *Aspergillus flavus* infection in *Oreochromis niloticus* from Aswan Governorate, Egypt

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Abstract

Fungal diseases in fish cause economic losses all over the world, and knowledge about them is scarce and outdated in Aswan Governorate, Egypt, making interpretation, prevention, and treatment difficult. The necessity to find a fungicide that is natural, environmentally friendly, and does not emerge drug resistance is a must. Therefore, the current study aimed to isolate and diagnose fungal infection in farmed *Oreochromis niloticus*, causing mortalities, in Aswan Governorate. During 2021, 200 fresh *O. niloticus* samples were collected from the Sahary Fish Hatchery and Aswan General Authority for Fish Resources Development fish farm. Some fish showed hemorrhagic lesions all over the body, detachment of scales, and fin erosion. Collected tissue samples were cultured on potato dextrose agar and Sabouraud dextrose agar for phenotypic characterization. Macroscopic and microscopic analyses were used to identify the isolated fungi. A total of 18 fungal species and two varieties appertaining to ten fungal genera were recovered from 48 samples out of 200 examined *O. niloticus* (24%), with *Aspergillus flavus* being the most prevalent at a rate of 25.6%. The isolated *A. flavus* was proven to be pathogenic to farmed *O. niloticus*, as by experimental infection. The natural herb *Persicaria salicifolia* had an LC_{50} value of 41.68 mg/l in exposed *O. niloticus* and was used to treat *A. flavus*-infected *O. niloticus*. It can be concluded that *A. flavus* poses a major hazard to *O. niloticus* aquaculture and can be treated with 40 mg/kg in feed or 20 mg/l in water of *P. salicifolia* for 6 days.

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Highlights

- Fungal infection in cultured *Oreochromis niloticus* in Aswan Governorate, Egypt, was explored.
- *Persicaria salicifolia* herbal extract has an LC_{50} value of 41.68mg/l in exposed *O. niloticus.*
- The extract of *P. salicifolia* was utilized as a natural alternative to traditional fungicides for treatment of *Aspergillus flavus* infection in *O. niloticus*.
- It is recommended to treat *O. niloticus* infected with *A. flavus* with 40 mg/kg in feed or 20 mg/l in water of *P. salicifolia* for 6 days.
- Further investigations are required on *P. salicifolia*'s extract as a promising fungicide for *O. niloticus* and other fish species.

Extended author information available on the last page of the article

Keywords Aquaculture and fungal diseases · *Aspergillus flavus* · Herbal medicine · *Oreochromis niloticus* · *Persicaria salicifolia* · Treatment

Abbreviations

O. niloticus	*Oreochromis niloticus*
A. flavus	*Aspergillus flavus*
P. salicifolia	*Persicaria salicifolia*
FFT	Faculty of Fish and Fisheries Technology
PDA	Potato dextrose agar
SDA	Sabouraud dextrose agar
Dc	The growth's diameter in the control plate
Ds	The measurement of the growth diameter in the treated plate
LC_{50}	50% Lethal dose
A. fumigatus	*Aspergillus fumigatus*
A. nidulans	*Aspergillus nidulans*
A. niger	*Aspergillus niger*
A. terreus	*Aspergillus terreus*
A. parasiticus	*Aspergillus parasiticus*
A. ustus	*Aspergillus ustus*
E. nidulans var. echinulata	*Emericella nidulans var. echinulata*
E. nidulans var. lata	*Emericella nidulans var. lata*
PL	Primary filaments
SL	Secondary filaments
RT	Renal tubules
H	Hepatocytes cords
PA	Pancreatic acini

Introduction

Fish aquaculture is a major source of national income in Egypt and has grown significantly over the last few decades, producing 1,585,839 tonnes in 2021 (FAO 2023; Nasr-Allah et al. 2020; Economic Affairs Sector 2021; Rossignoli et al. 2023). Egypt leads the African continent in fish farming and ranks third internationally in tilapia production behind China and Indonesia, with 971,263 tonnes produced in 2021 (Shaalan et al. 2018; FAO 2023; Economic Affairs Sector 2021).

The resilient Nile tilapia, *O. niloticus*, is one of the most often used fish species for aquaculture today, and because of its unique biological characteristics, which allow it to live in different environments and aquaculture systems, as well as scientific advances in knowledge of everything related to it, including diseases, culture methods, breeding, genetics, marketing, and consumer demand for it, all of these factors have contributed to its success (El-Sayed 2006; Hamouda and Abd Alkareem 2021; Hamouda and Younis 2021; El-Sayed and Fitzsimmons 2023).

Despite the success of Egypt's tilapia sector, it faces a number of challenges, including parasitic, bacterial, viral, and fungal diseases, which threaten the industry's long-term sustainability (Hamouda et al. 2019; Hamouda and Younis 2021; MacKinnon et al. 2023).

Aspergillomycosis is a systemic fungal disease caused by *Aspergillus* species, specifically *A. flavus* and *A. parasiticus*, which produce aflatoxins, the most hazardous and

prevalent pollutants in fish feed (Mohamed et al. 2017; Ibrahim 2020). Aflatoxin, which has hepatotoxic, nephrotoxic, neurotoxic, carcinogenic, and immunosuppressive properties in addition to hemorrhagic and causing dermatitis in humans, is produced primarily by *A. flavus* (Richard 2007; Pitt and Hocking 2009; Oliveira and Vasconcelos 2020; Zahran et al. 2020).

The potential threat posed by the emergence of drug resistance in many fish diseases, as well as the accumulation of toxic residues in fish flesh, increases the risk of environmental pollution. All the aforementioned reasons highlight the need for alternative natural solutions to control these fish pathogens (Gabriel 2019; Hamouda et al. 2019; Mostafa et al. 2020).

Hydrophytes are abundant in antimicrobial phytochemical compounds, and some of these compounds have antifungal properties (Haroon 2006; Maneemegalai and Naveen 2010; Sharma et al. 2012; Altemimi et al. 2017). *P. salicifolia*, which grows in the Nile Delta as a helophyte and geophyte, has an essential role in complementary medicine and can be used as a natural substitute for traditional fungicides (Boulos 2005; Hussain et al. 2010; Shaltout et al. 2010; Chakma et al. 2018).

So the current study was conducted to explore fungal infection in cultured *O. niloticus*, causing mortalities, in Aswan Governorate, Egypt, for the first time in Sahary Fish Hatchery and Aswan General Authority for Fish Resources Development fish farm, recording the clinical signs, postmortem lesions, and prevalence of infection. The pathogenicity and histological alterations of the most commonly isolated fungus (*A. flavus*) to *O. niloticus* were investigated. As an alternative to standard fungicides, the natural herb *P. salicifolia* was used to treat *A. flavus*-infected *O. niloticus*. The data gathered will serve as the basis for a pathogenic *A. flavus* control strategy in this region.

Material and method

Study area and collection of fish samples

Between April and October 2021, 200 fresh *O. niloticus* samples were collected at random, with body weights ranging from 11 to 100 g and total lengths ranging from 8 to 20 cm. The fish were obtained from the Sahary Fish Hatchery and the Aswan General Authority for Fish Resources Development fish farm, Aswan Governorate, Southern Egypt (Fig. 1), which had an archive of rising mortality to reach its peak and then decline. From each location, 100 fish were transported to the Faculty of Fish and Fisheries Technology laboratory at Aswan University for mycological, parasitological, and bacteriological analysis in sterile plastic bags (2/3 oxygen, 1/3 water, and fish), according to Eissa (2016).

Fish for experimental design

A total of 450 *O. niloticus*, each weighing 50 ± 5 g, were delivered to the wet lab of the Fish Diseases Department at the Faculty of Fish and Fisheries Technology from ponds separate from those showing clinical signs and mortalities of the same hatchery. Prior to the start of the experimental tests, the fish were housed for 14 days in well-prepared glass aquariums containing dechlorinated tap water with a 30-l capacity and continuous air supply at room temperature for acclimatization and inspections of random samples to ensure their clearance of any natural infection. Fish were fed twice daily at a rate of 3% of its body

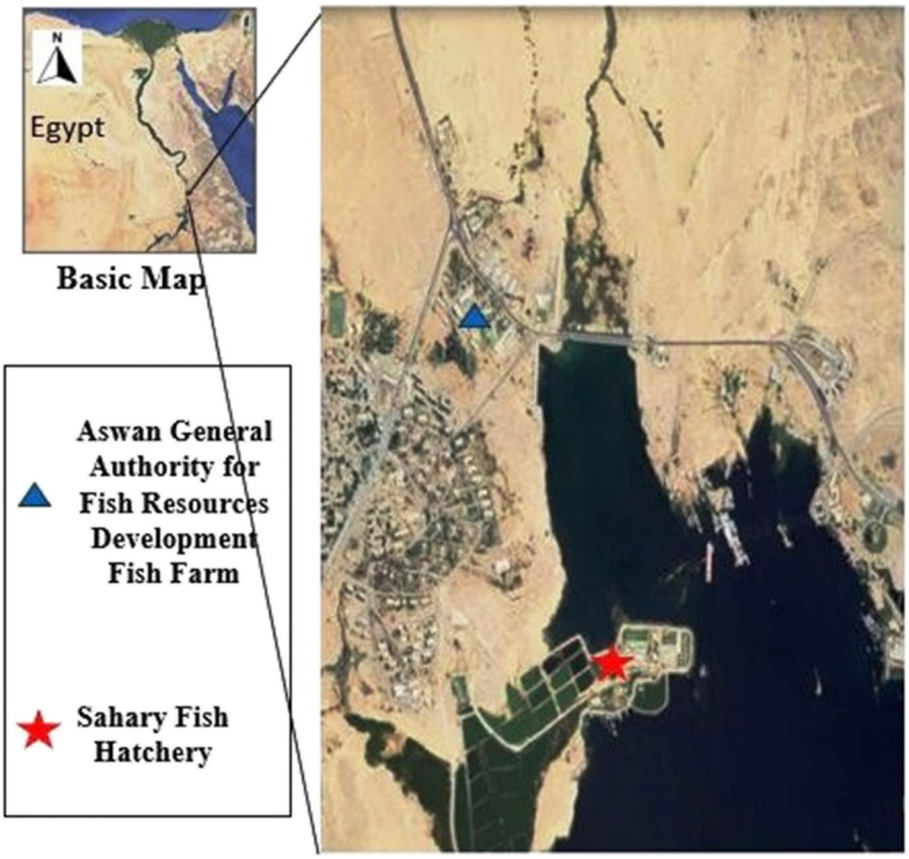

Fig. 1 Location map of Sahary Fish Hatchery and the Aswan General Authority for Fish Resources Development fish farm, Aswan Governorate, Egypt

weight. Dead fish were picked up immediately from the glass aquarium to avoid deterioration of water. Samples of fish were examined to exclude infections.

Clinical and postmortem examinations

According to Noga (2010) and the AVMA's (American Veterinary Medical Association 2020) guidelines, the fish underwent clinical evaluations and were euthanized in the lab. The collected fish were clinically examined to detect any visible changes or clinical abnormalities, and postmortem examination of the internal organs was performed.

Mycological analysis

Fish was washed in running water to remove sediments. For culturing of fungal specimens, two types of media including potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) were prepared and chloramphenicol (500 mg/l) was supplemented to each

preparation of media to avoid bacterial contamination. All of the studied fish's body surfaces were cleaned by dipping them in 1% formaldehyde for 1 to 5 min, then in 70% alcohol, then in distilled water, where they were properly rinsed (Iqbal et al. 2012). Different parts of fish including the skin, head, gills, fins, liver, and kidney were sliced. The six pieces were inoculated onto both media; isolation was performed in a laminar flow air cabinet. For each medium, three plates from each sample were used. For 5 to 7 days, the inoculated plates were incubated at 28 °C, periodically monitored, and the growing fungus species were re-cultured on Sabouraud dextrose agar with cycloheximide (500 mg/l) to inhibit saprophytic fungi (Acharya and Hare 2022). After that, fungal species were inspected and identified.

Parasitological and bacteriological analysis

The collected samples from the two areas of study were also subjected to parasitological and bacteriological examinations, according to Eissa (2016) and Austin and Austin (2012), respectively, to precisely identify the causative agent or agents of deaths.

Identification of isolated fungal species

Colony morphology, including growth appearance, growth rate, the surface colony texture and color, and the reverse side of the colonies, was observed, as well as microscopic features including fine hyphae morphology (aseptate or septate), conidia, and phialide shapes, which were also used to characterize the pure fungus according to Raper and Fennell (1965), Al-Doory (1980), and Pitt and Hocking (2009). A wet mount preparation of mold was performed by depositing a little bit of a fungus colony on a clean glass slide with a drop of distilled water, covering it with a clean cover slide, and examining it under a microscope. Staining a little bit of the periphery of a fresh colony with lactophenol cotton blue was also used to confirm the identification of isolated mold.

Pathogenicity of *A. flavus*

The spore suspensions of *A. flavus* isolated from *O. niloticus* were prepared for experimental infection according to Willoughby (1994); each pure cultures of *A. flavus* plate received 20 ml of sterile distilled water to collect the conidial material, which was then collected in sterile tubes. The suspension was filtered using two layers of sterile gauze. The conidial suspension in sterile distilled water was calculated and adjusted to be 9×10^4 spores/ml using an erythrocyte counting chamber of a hemocytometer (Refai et al. 2010; Mohamed et al. 2017). The experiment involved a total of 90 acclimated *O. niloticus*, which were divided into three equal groups ($n=30$ fish per group) and then into three replicates of ten fish each. The first group served as the control (untreated), the second group received sterile saline as a sham injection, and the third group received 0.2 ml of *A. flavus* spores (9×10^4 spores/ml) intraperitoneally. The clinical symptoms and morality rate of these fish were subsequently recorded daily for the following 7 days.

Fish that showed abnormal clinical signs were used to re-isolate the causative agent mycologically by inoculating specimens from their livers, gills, and musculatures at the injection site into SDA. Gills, liver, and kidney samples were taken from all fish groups

at the end of the experiment and kept in 10% neutral buffered formalin for histological analysis.

Preparation of *P. salicifolia* plant extract

The aquatic plant *P. salicifolia* was gathered from the banks of the Nile River in Aswan, and its extraction was done using the technique outlined by Mostafa et al. (2020).

The antifungal effect of *P. salicifolia* extract against pathogenic *A. flavus* by the Poisoned Food Method

The ethyl acetate extract of *P. salicifolia* was added to Petri dishes with molten agar at the varying concentrations (10 mg/ml, 20 mg/ml, 40 mg/ml, and 60 mg/ml) and thoroughly stirred. After an overnight pre-incubation, an inoculation can be made using a mycelia disc of *A. flavus* that is deposited in the plate's center (0.5 cm in diameter). The diameters of fungal growth in the control and sample plates are measured after additional incubation under conditions appropriate for the tested fungus, and the antifungal effect is evaluated using the method below (Singh and Tripathi 1999; Balouiri et al. 2016):

$$\text{Inhibition}(\%) = ((\text{Dc} - \text{Ds})/\text{Dc}) \times 100$$

where Dc is the growth's diameter in the control plate and Ds is the measurement of the growth diameter in the treated plate.

Toxicity bioassays of *P. salicifolia* ethyl acetate extract on *O. niloticus*

According to Essien-Ibok (2020), a toxicity experiment was conducted to evaluate the toxicity of the ethyl acetate extract of *P. salicifolia* on *O. niloticus*. In order to pinpoint the exact amount of toxicity, different *P. salicifolia* concentrations (20 mg/l, 40 mg/l, and 60 mg/l) were used in the experiment. Each of these concentrations was evenly distributed throughout 20 l of water. The experiment was carried out using 120 acclimated *O. niloticus* from the experimental fish. They were split into four equal groups, each with 30 fish. Each group was split up into three replicas, each with ten fish. As a control, the first group received no extract; the second group received 20 mg/l of *P. salicifolia*; the third group received 40 mg/l of *P. salicifolia*; and the fourth group received 60 mg/l of *P. salicifolia*. Over a period of 24 to 96 h, we monitored the fishes' clinical symptoms and mortality rates. Logistic regression was used to determine the 96 h LC_{50} (50% lethal dose).

Effect of *P. salicifolia* on *O. niloticus* infected with *A. flavus*, whether in water or feed

Six groups, each with 30 fish, were created from 180 *O. niloticus* fish. Each group was split up into three replicas, each with ten fish. Group 1 contains non-treated non-infected fish (control negative), group 2 contains infected non-treated fish (control positive); fish in groups 3 and 4 are treated with plant extracts at concentrations of 20 and 40 mg/l of water, respectively; fish in groups 5 and 6 are treated with plant extracts at concentrations of 20 and 40 mg/kg of feed, respectively. The experiment was monitored for a period of 6 days for clinical signs and mortality rates. Daily water exchanges accounted for around 20% of the aquaria's total volume; in the aquaria where plant extract was introduced to the water,

the equivalent amount of plant extract was added to replace any that was lost. The fish feed twice daily as 3% of the fish biomass. The experimental diet incorporate plant extract in this trial was a commercial normal fish diet that contained 35% crude protein, 5.8% fat, 3.5% crude fiber, and 4100 kcal of digestible energy. The ethyl acetate extract of *P. salicifolia* was added to the diet at concentrations of 20 mg/kg and 40 mg/kg before the diet was pelletized.

Histopathological examination

At the end of experiment bioassays and treatment trials, different tissue samples were cut and preserved in 10% neutral buffered formalin from the liver, gills, and kidneys of all groups. Following standard processing, the sections were cut into 5-µm-thick sections, fixed in paraffin blocks according to Suvarna et al. (2019), and stained with hematoxylin and eosin for histological analysis (Roberts 2001).

Statistical analysis

According to Duncan (1955), the statistical significance ($P < 0.05$) of the varied concentrations of the *P. salicifolia* extracts on *A. flavus* was determined using one-way analysis of variance (ANOVA) in SPSS 22.0 (SPSS version 22, SPSS Inc., IL, USA), and with the aid of the Excel program, a regression analysis was conducted to assess the association between the inhibition zone and the varied plant extract concentrations.

Results

The naturally infected fish with different fungal species showed hemorrhagic regions, scale detachment, fin erosions, and, in rare instances, ocular opacity and dark body colorations. During postmortem examinations, congested gills, enlarged gall bladder, inflated intestine, ascites, clogged kidneys and spleen, and enlarged liver with hemorrhagic spots were detected.

A total of 18 fungal species and 2 varieties appertaining to 10 fungal genera were recovered from 48 samples of 200 tested *O. niloticus*, representing a 24% overall prevalence (Table 1).

Many cases of mixed infections in the same fish were detected. Among the recovered fungi, *A. flavus* (280 colonies) were the most prevalent fungal species (Table 1).

The isolated fungal genera from different organs of *O. niloticus* were displayed, with the gills being the most affected and the kidney being the least affected (Table 2).

Phenotypic analysis of *A. flavus* colonies revealed that they appeared white on the second day of inoculation, and as they age, their color changes from pale brown to yellowish green or olive green. Under the microscope, either wet or stained with lactophenol cotton blue, the vesicles were large and spherical, the stigmata were biseriate, loose, and radiating, carrying ovoid, rough conidia, and the conidiophores were long and rough (Fig. 2).

The fish that had been intraperitoneally injected with *A. flavus* isolated from naturally infected fish to test its pathogenicity displayed the same clinical symptoms as naturally infected fish, including fin erosions, hemorrhages on the body surface, scale detachment, gill discoloration, rapid opercular movements near the aquarium's surface, sluggish movement just before death, and mild to moderate ascites. Additionally noted were liver, kidney,

Table 1 Prevalence of fungi isolated from cultured *Oreochromis niloticus* in Aswan Governorate, Egypt

Fungal genera and species	TC[a]	TC%	Prevalence (NCI)[b]
Acremonium furcatum	8	0.7	3
Aspergillus	813	74.2	48
A. flavus	280	25.6	48
A. fumigatus	86	7.9	11
A. nidulans	62	5.6	8
A. niger	245	22.3	40
A. parasiticus	31	2.8	7
A. terreus	68	6.2	13
A. ustus	41	3.7	8
Botrytis cinerea	19	1.7	8
Emericella	56	5.1	13
E. nidulans var. echinulata	25	2.3	6
E. nidulans var. lata	31	2.8	7
Fusarium solani	10	0.9	5
Mucor sp.	19	1.7	7
Penicillium islandicum	16	1.5	5
Phoma sorghina	10	0.9	5
Rhizopus stolonifer	66	6	20
Yeast	78	7.1	25
Yeast strain I	51	4.6	17
Yeast strain Π	27	2.5	8
Total fungal count	1095	100	48

[a]Total count per organ

[b]Number of cases of isolation

spleen, and intestinal congestion (Fig. 3). After 2 days of infection, the fish started to perish, and by the sixth day, mortality had reached 30%. *A. flavus* re-isolated from the organs of the injected fish. No clinical symptoms or moralities were recorded in the control or sham groups.

By parasitological and bacteriological examinations, no pathogenic parasites or bacteria were recorded from the examined *O. niloticus*.

The *P. salicifolia* ethyl acetate extract was effective against *A. flavus* in vitro (Fig. 4). Concentrations were statistically significant ($P < 0.05$), indicating that fungus inhibition increases with a corresponding increase in concentration. The extract concentrations (20 mg/ml, 40 mg/ml, and 60 mg/ml) demonstrated dose-dependent inhibition percentages (34.1%, 43.1%, and 65.9%, respectively) (Fig. 5), whereas 10 mg/ml of the extract had no activity.

In order to determine the effects of extended exposure of tilapia to the extract, a toxicity test of an ethyl acetate extract of *P. salicifolia* was conducted. Fish rose as controls in water without extract showed no signs or deaths. Fish exposed to extract concentrations of 20 mg/l or 40 mg/l did not exhibit any toxicity; however, fish subjected to a concentration of 60 mg/l had severe toxicity. In addition to having missing scales and a black appearance, the poisoned fish was struggling for air close to the aerator. All of the fish had died by the

Table 2 Prevalence of fungi isolates from different organs of cultured *Oreochromis niloticus* in Aswan Governorate, Egypt

Infected organs Fungal genera	Gills	Skin	Head	Fins	Liver	Kidney
Acremonium furcatum	0	0	0	0	3	5
Aspergillus	200	168	164	115	97	69
A. flavus	86	51	47	35	34	27
A. fumigatus	11	22	15	12	19	7
A. nidulans	9	25	10	15	3	0
A. niger	61	42	48	44	28	22
A. terreus	14	12	24	7	4	7
A. parasiticus	8	12	9	2	0	0
A. ustus	11	4	11	0	9	6
Botrytis cinerea	13	0	0	6	0	0
Emericella	6	27	11	4	5	3
E. nidulans var. echinulata	2	12	6	3	2	0
E. nidulans var. lata	4	15	5	1	3	3
Fusarium solani	0	0	10	0	0	0
Mucor sp.	0	0	6	0	7	6
Penicillium islandicum	7	6	0	3	0	0
Phoma sorghina	0	10	0	0	0	0
Rhizopus stolonifer	30	0	4	0	9	23
Yeast	11	11	32	14	6	4
Yeast strain I	6	7	22	10	4	2
Yeast strain Π	5	4	10	4	2	2
Total	260	222	227	142	127	110

conclusion of the 96-h period as they had fallen to the tank floor with raised fins as the time went. After death, the fish's postmortem examination revealed rosy gills, a gas-filled intestine, a swollen gallbladder, a hemorrhagic liver, and a congested kidney (Fig. 6). A LC_{50} value of 41.68 mg/l is established for the extract in the exposed fish (Fig. 7).

In vivo treatment with an ethyl acetate extract of *P. salicifolia* against *A. flavus* infection was performed to control the disease in *O. niloticus*. The least amount of mortality (10%) across all groups was achieved by group 6 (40 mg/kg in feed), which was followed by group 3 (20 mg/l in water) with 13.33% mortality (Table 3, Figs. 8 and 9).

According to histopathological examination conducted during the experiment bioassays and the treatment trial with ethyl acetate extracts of *P. salicifolia* on *O. niloticus*, the control group did not exhibit any abnormalities in all organs, e.g., gills and kidneys (Figs. 10 and 11A). The extract at a dosage of 20 mg/l did not exhibit any toxicity and this appeared in the normal histoarchitecture of all organs, e.g., the kidney and liver (Figs. 11C and 12A). Fish exposed to the extract concentration of 40 mg/l displayed diffuse epithelial hyperplasia of the primary lamellae (Fig. 10B). Fish subjected to a concentration of 60 mg/l of the extract showed severe toxicity as large necrotic areas in liver (Fig. 12B). Fish infected with *A. flavus* and left untreated displayed necrosis of the renal tubules associated with the presence of the fungus spores, and infiltration of inflammatory cells (Fig. 11B). Fish infected with *A. flavus* and treated with 20 mg/l of the *P. salicifolia* extract developed focal adhesion of the secondary lamellae, hyperplasia of the lamellar epithelium with infiltration of inflammatory cells (Fig. 10C), and mild to moderate hydropic degeneration of the hepatic

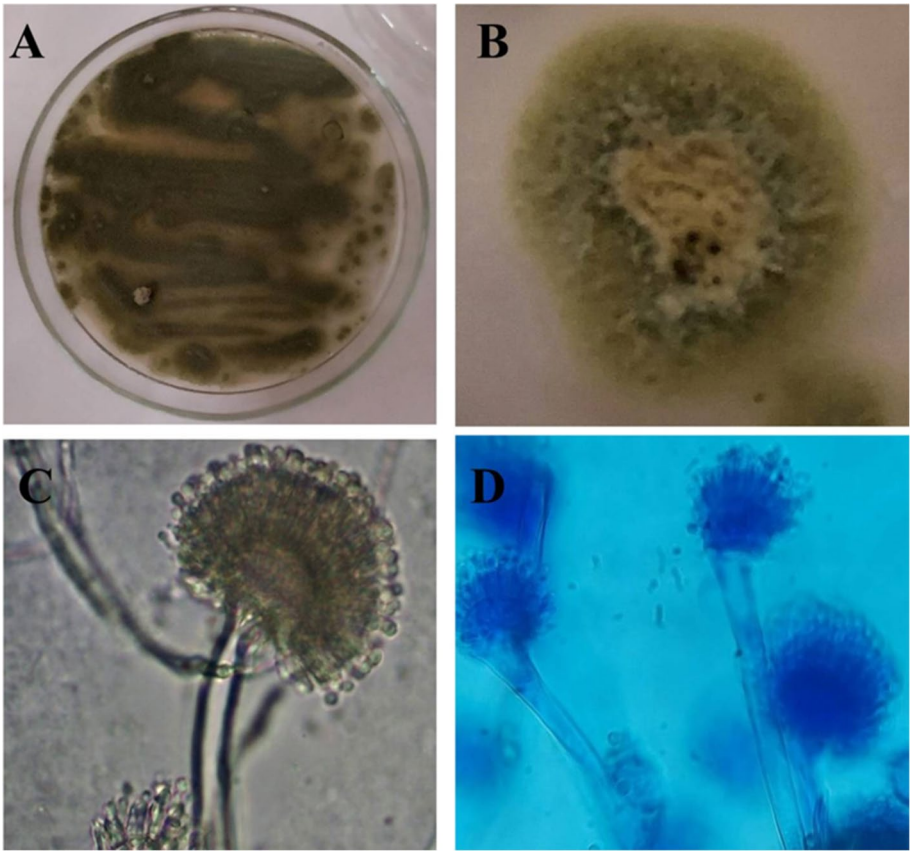

Fig. 2 **A** Yellowish-green colonies of *Aspergillus flavus* isolated from *Oreochromis niloticus* after 7 days at 28°C on Sabouraud dextrose agar (SDA). **B** Olive green colonies of *Aspergillus flavus* isolated from *Oreochromis niloticus* after 7 days at 28 °C on potato dextrose agar (PDA). **C** and **D** Light microscopic characteristics of wet-mounted (**C**) and stained with lactophenol cotton blue (**D**) specimens of *Aspergillus flavus* isolated from *Oreochromis niloticus* showing long and rough conidiophores and characteristic head with large and spherical vesicle, biseriate stigmata, and ovoid rough conidia

cells (Fig. 12C). Fish infected with *A. flavus* and treated with 40 mg/l of the *P. salicifolia* extract displayed multi-focal necrotic areas within renal tubules (Fig. 11D). *A. flavus*-infected fish that were fed the *P. salicifolia* extract at a dose of 40 mg/kg displayed focal hyperplasia of the lamellar epithelium and epithelium lifting of a few secondary lamellae, and a focal necrotic area of the pancreatic acini (Figs. 10 and 12D).

Discussion

This is the first investigation on fungal infections in cultured *O. niloticus* in Egypt's Aswan Governorate; previous studies were carried out on wild fish by El-Zayat (1988, 2000).

The clinical signs and postmortem lesions recorded during the examination of the naturally infected *O. niloticus* with fungi especially *A. flavus* could be brought on by toxic metabolites, lipase and protease enzymes produced by these fungi (Olufemi 1985;

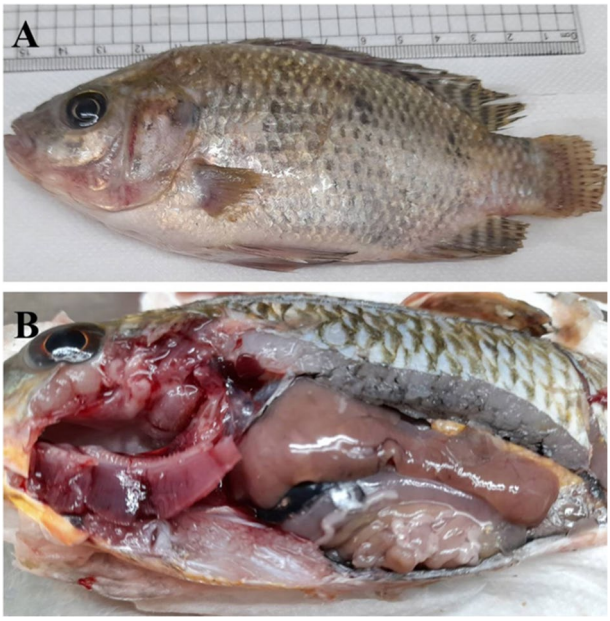

Fig. 3 *Oreochromis niloticus* experimentally infected with *Aspergillus flavus*. **A** Detachment of scales and fin erosions, especially caudal and pectoral fins. **B** Congested color of gills, liver enlargement, and distended gall bladder

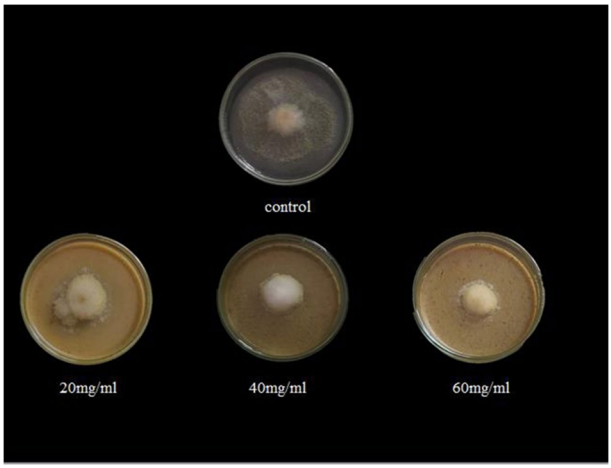

Fig. 4 The antifungal activity of *Persicaria salicifolia* extract against *Aspergillus flavus*

Moharram and El-Zayat 1989; Iqbal and Saleemi 2013; Hashem et al. 2020, Zakaria et al. 2021; Mahboub et al. 2022).

The colony morphology of *A. flavus* was exactly the same as that reported by many other prior researchers (Abd El-Tawab et al. 2020; Hashem et al. 2020; Zakaria et al. 2021; Mahboub et al. 2022). Depending on the culture media employed in their culture, the colonies of *Aspergillus flavus* and other *Aspergillus* may have different colors (Brun et al. 2001).

In our study, *A. flavus* was the most frequently isolated mold (25.6%); this result was in line with the findings of Refai et al. (2010) and Abd El-Tawab et al. (2020). The

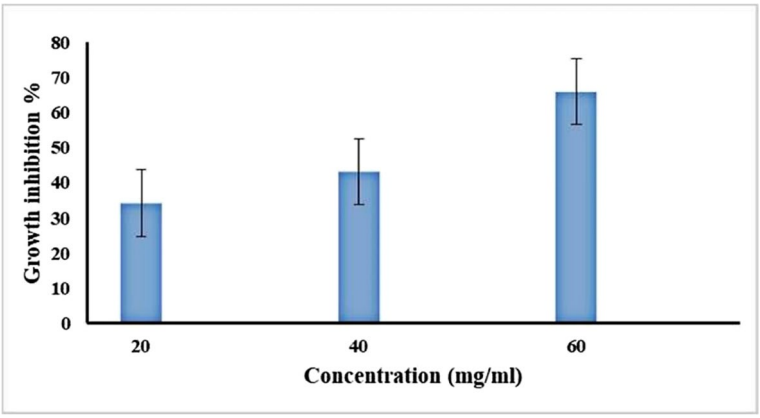

Fig. 5 Growth inhibition percentage of the pathogen *Aspergillus flavus* by *Persicaria salicifolia* plant extract at different concentrations (mg/ml)

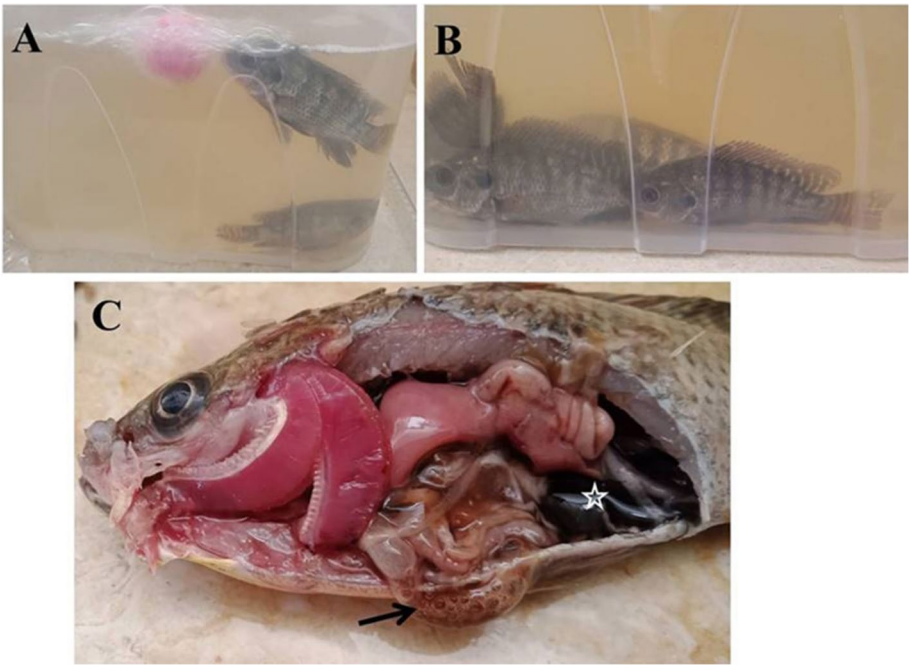

Fig. 6 *Oreochromis niloticus* exposed to 60 mg/l of *Persicaria salicifolia* extract. **A** One fish showed respiratory distress, gasping and surfacing, and the other one settled down on the aquarium floor. **B** The fish settled down on the aquarium floor with erected fins. **C** Rosy gills, a gas-filled intestine (arrow), a distended gall bladder (star), and enlarged liver

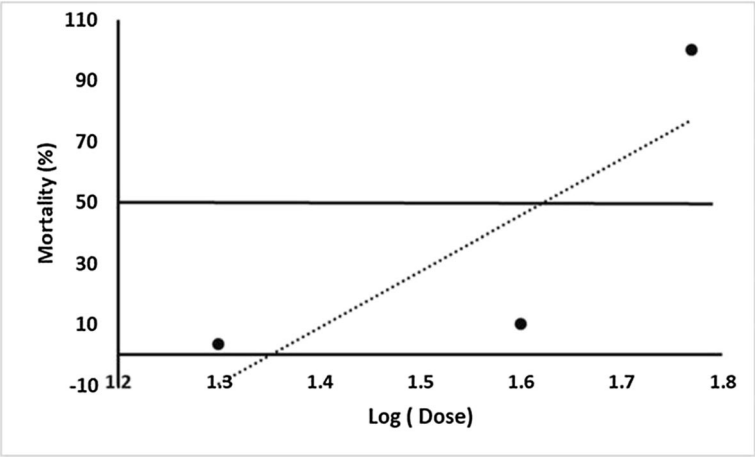

Fig. 7 Graphical illustration of the 96-h LC_{50} of *Persicaria salicifolia* by an Excel program. LC_{50} = 41.68

isolated *A. flavus* from naturally infected *O. niloticus* was proven to be pathogenic to other non-infected fish (Abdel Monem et al. 1995; Eissa et al. 2022) with 30% morality by the end of the sixth day of infection. It was then re-isolated from the morbid fish indicating its pathogenicity to *O. niloticus*. This species was believed to produce protease and aflatoxins, which may be responsible for the infection (Mohamed et al. 2017). In this regard, Oliveira and Vasconcelos (2020) found that *A. flavus* is the main producer of aflatoxins, which impair immune function and increase fish mortality (Marijani et al. 2019).

Excessive use of fungicides in the treatment of fish fungal diseases may emerge drug resistance, accumulation of toxic residues in fish flesh, and increase the danger of environmental pollution. According to Mostafa et al. (2020), fungicides are known to possess numerous carcinogenic and teratogenic properties; hence, the development of fungicides that are both effective and environmentally friendly is crucial. Therefore, *P. salicifolia* was used in this study as a natural fungicide.

The phytochemical compounds, such as glycosides, flavonoids, and phenolic acids, which have antifungal and antioxidant effects, may be the factor responsible for *P. salicifolia*'s ethyl acetate extract's antifungal activity against *A. flavus* (Haroon 2006; Abd El-Kader et al. 2012; Sharma et al. 2012; Hussein and Mohamed 2013; El-Swaify et al. 2015; El-Anwar et al. 2016). Furthermore, because flavonoids function as cell membrane stabilizers by detoxifying xenobiotics and preventing radical-induced lipid peroxidation, they are well known for their potent free radical scavenging and hepatoprotective effects (Heim et al. 2002; Kinjo et al. 2006; Wilms et al. 2008). This is consistent with Hussain et al. (2010), who reported that the crude extract of *Polygonum persicaria* leaf showed the highest activity against *A. niger* and moderate activity against *A. flavus*, and Chakma et al. (2018), who found that the methanolic extract of *P. glabra* is effective against *A. niger*. Furthermore, Maqbool et al. (2022) detected the antifungal activity of *P. Barbata* against *A. niger*, and Maksimović et al. (2023) declared that *P. amphibia* inhibited the growth of *Aspergillus* spp.

P. salicifolia extract (20 mg/l in water or 40 mg/kg in diet) significantly reduced mortality in infected fish, indicating that *P. salicifolia*'s extract treats *O. niloticus* with *A. flavus* infection.

Table 3 Treatment trial of experimentally infected *Oreochromis niloticus* with *Aspergillus flavus* using an ethyl acetate extract of *Persicaria salicifolia*

Group No	Infected with *Aspergillus flavus*	Treated with *Persicaria salicifolia* extract				Clinical signs	Mortality rate by the end of the 6th day
		20 mg/l in water	40 mg/l in water	20 mg/kg in feed	40 mg/kg in feed		
1 (negative control)	Non-infected	-	-	-	-	Normal fish	0
2 (positive control)	Infected	-	-	-	-	Appeared by the 2nd day of infection	30%
3	Infected	+	-	-	-	Appeared by the 2nd day of infection	13.33%
4	Infected	-	+	-	-	Appeared by the 2nd day of infection	16.67%
5	Infected	-	-	+	-	Appeared by the 2nd day of infection	16.67%
6	Infected	-	-	-	+	Appeared by the 2nd day of infection	10%

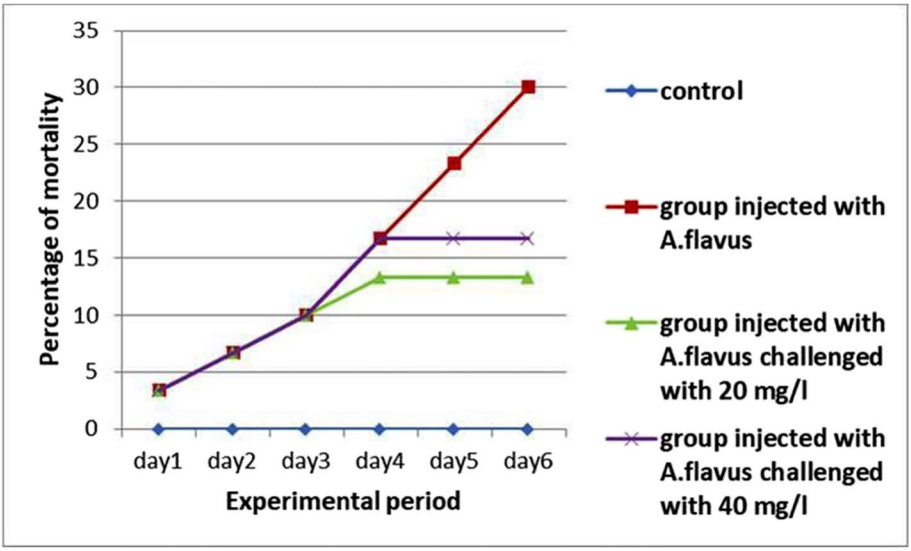

Fig. 8 Percentage of mortalities of infected *Oreochromis niloticus* with *Aspergillus flavus* through an experimental period of treatment with *Persicaria salicifolia* extract via water

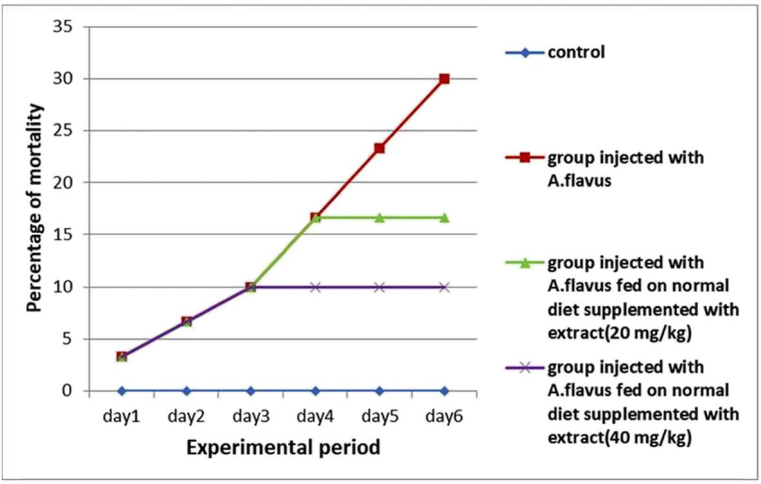

Fig. 9 Percentage of mortalities of infected *Oreochromis niloticus* with *Aspergillus flavus* through an experimental period of treatment with *Persicaria salicifolia* extract via feed

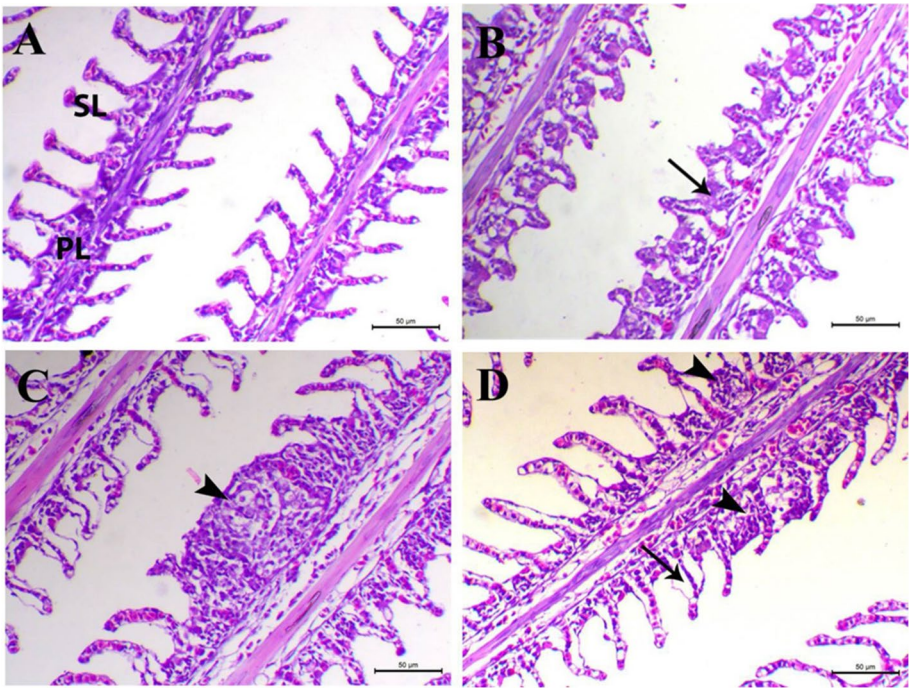

Fig. 10 Representative photomicrograph of H&E-stained gills of *Oreochromis niloticus*. **A** Control group shows normal histoarchitecture consisted mainly of primary filaments (PL) branching out into tiny secondary filaments (SL). **B** Fish group exposed to *Persicaria salicifolia* extract at concentrations of 40 mg/l shows diffuse hyperplasia of the covering epithelium of the primary lamellae (arrow). **C** Gills of a fish group infected with *Aspergillus flavus* challenged with 20 mg/l of the *Persicaria salicifolia* extract show focal adhesion of the secondary lamellae associated with hyperplasia of the lamellar epithelium and infiltration of inflammatory cells (arrowhead). **D** Gills of the fish group infected with *Aspergillus flavus* fed on a normal diet supplemented with *Persicaria salicifolia* extract at a concentration of 40 mg/kg show focal hyperplasia of the lamellar epithelium (arrowhead) and epithelium lifting of a few secondary lamellae (arrow). Scale bar = 50 µm

O. niloticus exposed to 60 mg/l of *P. salicifolia* extract shown significant toxicity despite the extract's strong fungicidal activity in vitro; this finding is consistent with the study's finding that *O. niloticus*' LC_{50} value for *P. salicifolia* was 41.68 mg/l.

The gills, kidney, and liver of *O. niloticus* infected with *A. flavus* displayed necrotic and degenerative changes in the current investigation. The fungus' toxic metabolites may be the cause of these histological alterations (Abdel Monem et al. 1995; Eissa et al. 2022).

Histopathological analyses on vital organs such as gills, kidney, and liver of *O. niloticus* infected with *A. flavus* and treated with different concentrations of *P. salicifolia*'s extract (20, 40 mg/l, or 20, 40 mg/kg) showed only minor pathological changes; however, these concentrations were still safe in comparison to 60 mg/l, which demonstrated notable cytotoxicity.

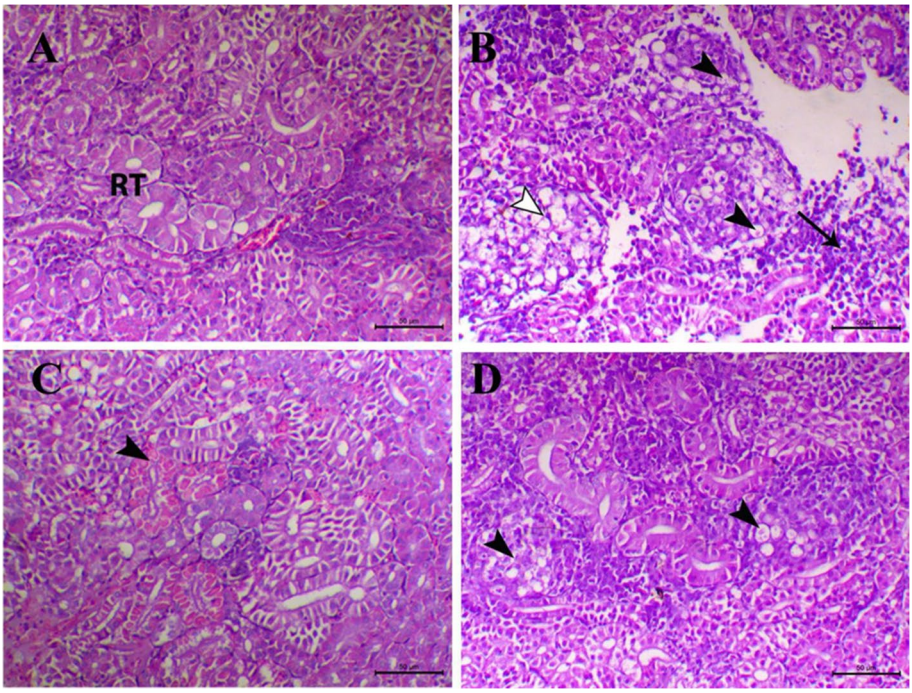

Fig. 11 Representative photomicrograph of H&E-stained posterior kidney of *Oreochromis niloticus*. **A** The control fish group shows normal histoarchitecture consisted mainly of renal tubules (RT). **B** Fish group infected with *Aspergillus flavus* shows necrosis of renal tubules (white arrowhead) associated with presence of the spores of the fungus (black arrowheads) and infiltration of inflammatory cells (arrow). **C** Fish group exposed to *Persicaria salicifolia* extract at concentrations of 20 mg/l shows normal histoarchitecture consisting mainly of renal tubules (RT). **D** Fish group exposed to *Persicaria salicifolia* extract at concentrations of 40 mg/l shows multi-focal necrotic areas within renal tubules (arrowheads). Scale bar = 50 µm

Conclusion

According to this study, the most common fungal infection in farmed *O. niloticus* in the Aswan Governorate, Egypt, is *A. flavus* (25.6%). Experimental and histological evidence confirm that *A. flavus* is pathogenic to farmed *O. niloticus*. The LC_{50} value of the herbal extract of *P. salicifolia* in exposed *O. niloticus* is 41.68 mg/l. Treatment of *O. niloticus* infected with *A. flavus* with 40 mg/kg in feed or 20 mg/l in water of *P. salicifolia* for 6 days is indicated for good survival rates and minor pathological abnormalities at these concentrations. The data gathered will serve as the basis for a pathogenic *A. flavus* control strategy in this region. Further investigations are required on *P. salicifolia*'s extract as a promising fungicide for *O. niloticus* and other fish species.

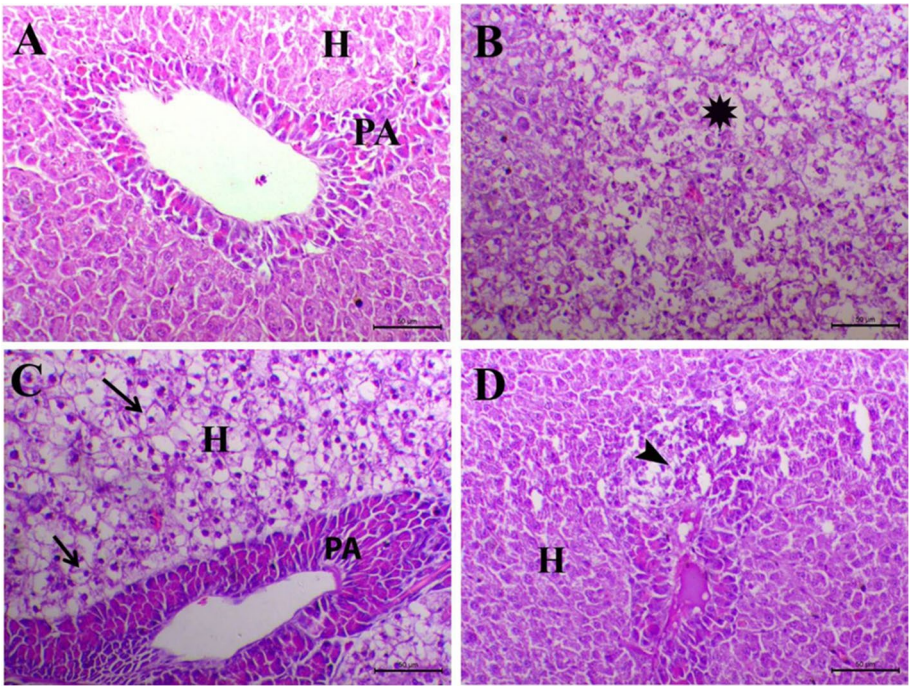

Fig. 12 Representative photomicrograph of H&E-stained hepatopancreas of *Oreochromis niloticu*s. **A** Fish group exposed to *Persicaria salicifolia* extract at concentrations of 20 mg/l shows normal histoarchitecture, where hepatocyte cords (H) and pancreatic acini (PA) surround central veins. **B** Fish group exposed to *Persicaria salicifolia* extract at concentration 60 mg/l shows large necrotic area (asterisk). **C** Fish group infected with *Aspergillus flavus* challenged with 20 mg/l of *Persicaria salicifolia* extract shows mild to moderate hydropic degeneration of the hepatic cells (arrows). **D** Fish group infected with *Aspergillus flavus* fed on a normal diet supplemented with *Persicaria salicifolia* extract at a concentration of 40 mg/kg shows focal necrotic area of the pancreatic acini (arrowhead). Scale bar = 50 μm

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Data availability The data sets used in the present study are accessible on reasonable request from the corresponding author.

Declarations

Ethical approval The Animal Use and Care Committee of the Faculty of Fish and Fisheries Technology at Aswan University in Egypt (Fac. FFT. No. 9/2021) gave its approval for the current study's standard operating procedure.

Competing interests The authors declare no competing interests.

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