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Onion (*Allium cepa*) improves Nile tilapia (*Oreochromis niloticus*) resistance to saprolegniasis (*Saprolegnia parasitica*) and reduces immunosuppressive effects of cadmium

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Abstract
The present study investigated the protective effects of dietary *Allium cepa* against *Saprolegnia parasitica* infections and the amelioration of cadmium-induced immunosuppression in *Oreochromis niloticus*. *Saprolegnia* isolates were recovered during an outbreak of saprolegniasis in farmed *O. niloticus* raised in a poor aquatic environment. Isolates were identified phenotypically as *S. parasitica.* Results were confirmed further by *ITS* gene sequencing. Four fish groups were kept in water with cadmium (1.5 mg/L) and fed for 30 days on a diet supplemented with crude or alcoholic extracts of *A. cepa* using two concentrations (0.5% or 1%). Positive (with Cd) and negative (without Cd) control fish groups were given the basal diet. The 96 h LC_{50} value of Cd in tilapia was (15.1 mg/L Cd). Fish exposed to Cd showed poor growth performance parameters, abnormal biochemical measurements, impaired immunological responses, and high oxidative stress indicators. Feeding tilapia on *A. cepa*-supplemented diets enhanced their growth performance (WG, SGR) and improved the nonspecific immune responses (WBCs, total protein, globulins, lysozyme, myeloperoxidase, and antiproteases). The inclusion of *A. cepa* in the diets reduced the oxidative stress (GST, SOD) and significantly decreased fish mortality after the challenge with *S. parasitica*. Dietary supplementation with *A. cepa* reduced cadmium accumulation in fish organs and up-regulated *IL-1β* and *IFNγ* levels. The most favorable benefits were obtained by the addition of 0.5% *A. cepa* extract. Our results highlight the immunostimulatory properties of *A. cepa* dietary supplementation for farmed tilapia and recommend its use prophylactically to control saprolegniasis and mitigate cadmium adverse effects.

Keywords Immunostimulation · *IFNγ* · *IL-1β* · *Saprolegnia parasitica* · Onion · Waterborne cadmium

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Introduction

Saprolegniasis is a serious disease in the aquaculture industry that causes massive fish mortality and colossal economic losses, particularly in winter (Nam et al., 2022). *Saprolegnia parasitica* (family, *Saprolegniaceae*) is one of the main causative agents of saprolegniasis and can cause massive mortality in fish and their eggs (Zahran et al. 2017; Ali et al. 2019). Members of the *Saprolegniaceae* family are ubiquitous in freshwater ecosystems and act as saprophytes, although some are detrimental fish pathogens (Sakaguchi et al., 2019). The emergence of saprolegniasis outbreaks in aquaculture is commonly linked to inferior water quality parameters and environmental stressors (Ali et al.2020).

Affected fish commonly exhibit cottony white to grey, brown masses on the skin and gills (Yanong 2003). Mechanical injuries, abrupt environmental changes, poor water quality, and aquatic pollution are the most predisposing factors of saprolegniasis in fish farms (Roberge et al. 2007).

Control of saprolegniasis requires adopting good management practices and adequate water quality parameters (Ali et al. 2019). Some disinfectants (e.g., formalin and hydrogen peroxide), sodium chloride, and boric acid have been proposed to keep the disease under control (Waterstrat and Marking 1995; Schreier et al. 1996; Ali et al. 2019). However, some of these chemicals may impact the aquatic environment, and their residues can accumulate in fish-derived products (Thanikachalam et al. 2010; Özçelik et al. 2020). Previously, Malachite green was considered one of the most effective treatments for the disease; however, it was banned in edible fish due to its carcinogenic and toxicological properties (Srivastava et al. 2004). Vaccination is a new proposed strategy for controlling saprolegniasis; nevertheless, its application in aquaculture is costly and impractical (Earle & Hintz 2014; Minor et al. 2014).

Medicinal plants and their extracts have long been considered competitive alternatives to chemotherapeutics in aquaculture as these plants contain significant amounts of biologically active compounds with immunostimulatory, antifungal, antibacterial, antioxidant, and anti-inflammatory effects (Milutinović et al. 2021; Shah et al. 2021). Extracts from pomegranate, clove, and *Thymus linearis* have been proposed as potential therapies for saprolegniasis in aquaculture (Shah et al. 2021; Mostafa & Yassin 2022). The essential oils of *Cuminum cyminum*, *Eryngium campestre*, and *Mentha piperita* have also shown antifungal activity against *S. parasitica* under in vitro conditions (Adel et al. 2020).

Enhancing fish immunity through medicinal herbs is an effective strategy for controlling aquatic animal diseases (Elgendy et al. 2016, 2021; 2022a; Ali et al. 2021). Numerous herbs have been utilized in their crud or extracted forms to stimulate fish's immune systems. Onion (*Allium cepa* L.) has been used medicinally since ancient times (Özçelik et al. 2020). *A. cepa* contains numerous bioactive compounds such as organosulfur, flavonols, ascorbic acids, carbohydrate prebiotics, and its by-products. These compounds have multiple health benefits, including anti-inflammatory, antimicrobial, antioxidative, antistress, antidiabetic, anticancer, and immunomodulatory effects, along with other nutritional benefits (Sagar et al. 2022). The health promotion outcomes relevant to feeding fish on onion-enriched diets and their resistance to some bacterial infections have been emphasized in earlier reports (Younes et al. 2021).

Recently, Egypt's Nile tilapia (*Oreochromis niloticus*) industry encountered huge economic losses due to infectious disease outbreaks (Ali et al. 2020; Abdelsalam et al. 2021; Eissa et al. 2021; Elgendy et al. 2022b). Fish reared in farms with polluted water sources are more vulnerable to numerous infections, including fungal diseases (Zahran et al. 2017;

Ibrahim 2020). Cadmium is among the toxic pollutants released into the aquatic systems and seriously threatens aquatic animal health (Bayomy et al. 2015; Elgendy et al. 2015a,b). Even trace amounts of cadmium in the aquatic environment can be toxic to cohabitant fish as it accumulates in the sediments and may subsequently be absorbed by fish (McGeer et al. 2012). Fish can uptake cadmium either directly by absorption through their gills or via food intake (Komjarova & Bury 2014). Exposure of fish to toxic levels of waterborne cadmium can enhance the production of reactive oxygen species (ROS) and cause cellular and DNA damage (Kovacik et al. 2019). Additionally, cadmium can replace other metals in proteins and enzymes, thus impairing their ability to maintain vital cell functions (Wang et al. 2021). Cadmium also disturbs thyroid hormone production, and the hypothalamus-pituitary-interrenal (HPI) axis eventually disrupts the fish's metabolism, reproductive and immune systems functions (Garcia-Santos et al. 2013). Cadmium also negatively impacts osmoregulation, growth, and fish survival (Paul and Small 2021). Cadmium can accumulate in fish tissues and may lead to organ dysfunction following the chronic exposure (Abbas et al. 2019b).

The study aimed to identify *Saprolegnia* spp. obtained from farmed Nile tilapia during an outbreak of saprolegniasis using phenotypic and genotypic characterization methods. Further, the study investigated the effectiveness of feeding tilapia on *A. cepa*-supplemented diets to alleviate the immunosuppressive effects of waterborne Cadmium and increase tilapia resistance to experimental infection with *S. parasitica*.

Material and methods

Phenotypic and molecular characterization of *S. parasitica* affecting naturally infected fish

Saprolegnia spp. were isolated during an outbreak of saprolegniasis (70% mortality) in Nile tilapia, farmed within a fish farm in ElManzala, Dakahlia Governorate, Egypt, during winter 2021. The most prominent clinical signs were visible cottony white to gray masses on the skin, fins, and gills of affected fish. Analysis of the water samples showed higher levels of cadmium, averaging about (9.89 µg/l), which exceeds the permissible limits (USEPA 1988). The other heavy metals were within permissible limits. The average oxygen and unionized ammonia levels recorded at the sampling time were 3.6 mg/L and 0.65 mg/L, respectively.

Wet mounts prepared from white masses collected from skin and gills were examined microscopically. Affected tissues from tilapia showing signs of fungal infection were aseptically excised, inoculated on Sabouraud Dextrose agar (SDA) supplemented with ampicillin (500 mg/L), and vancomycin (100 mg/L) to reduce microbial contamination as described by Ali et al. (2014) and Beckmann et al. (2020). Inoculated plates were incubated at 20 °C for 24 h in the hydrobiology department laboratory, Veterinary research institute, National Research Centre. A small plaque of the agar with an emerging hyphal tip was excised and re-inoculated onto a new SDA medium for culture purification. The purified fungal strains were identified microscopically with the methods described by Shin et al. (2017).

Purified isolates ($n=6$) were genotypically characterized by amplifying the internal transcribed spacer (*ITS*) region using the universal fungal primers *ITS*1-*ITS*4 (White et al. 1990). The extraction of genomic DNA from *Saprolegnia* isolates was performed using the DNeasy

Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The purity and concentration of extracted DNA were analyzed using the Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA), then adjusted to 50 ng/µl, and finally stored at − 20 °C until used. PCR amplification of the internal transcribed spacer (*ITS*) genes was performed using the universal *ITS* gene primers, *ITS*1: 5′-TCCGTAGGTGAACCTGCG G-3′, and *ITS*4: 5′-TCCTCCGCTTATTGATATGC-3′ as designed by White et al. (1990). PCR conditions for the *ITS* gene were as follows: preheating for 5 min at 94 °C followed by 35 cycles of denaturation (94 °C, 45 s), annealing (50 °C, 30 s), extension (72 °C, 1 min), and final elongation step at 72 °C for 7 min. The amplicons were purified from the gel using the QIAquick gel extraction kit (Qiagen, Tokyo, Japan). The amplified *ITS* genes were subjected to bi-directional Sanger sequencing with primer pairs (*ITS*1 and *ITS*4) using the Big Dye terminator Chemistry v3.1 kit (Applied Biosystems™, CA, USA). Sequencing reactions were visualized on an ABI 3730xl DNA sequencer (Applied Biosystems™, CA, USA). The editing and contig assembly of sequences were performed by BioEdit v. 7.2.5 (Hall 1999). Finally, the assembled sequences were identified using the BLAST in the GenBank database with a minimum BLAST cut-off of > 99% identity for a top match. The accession numbers were generated for six isolates after submission to the GenBank database.

The phylogenetic tree was conducted to match the *ITS* genes sequencing from the current six isolates of *Saprolegnia* spp. with the typing strains of both *S. parasitica* and *S. declina* and different isolates of *S. ferax*, *S. hypogyna*, *S. litoralis*, *S. anomalies*, *S. oliviae*, *S. bulbosa*, *S. australis*, *S. aenigmatica*, *S. furcate*, *S. terrestris*, and *S. monilifera* retrieved from the GenBank database using MEGA X (Kumar et al. 2018). The neighbor-joining tree was rooted on *Aphanomyces euteiches* strain ATCC 201,684 (AY683887), which was used as an outgroup. These factors were applied during tree construction: pattern among lineages: homogeneous; substitutions: transversions and transitions; 95% cut-off partial deletion principal; and bootstrapping with 1,000 replicates.

Assessing the 96 h LC_{50} acute toxicity assays of Cd

Healthy *O. niloticus* ($n = 84$) about (50–65 g) obtained from a fish farm in Giza governorate, Egypt, were transferred alive to the laboratory and distributed in experimental glass aquaria (50 L each) with dechlorinated tab water with aeration using aquarium air pumps, acclimatized for seven days. The temperature was kept at 26 ± 1 °C, and fish were starved for 48 h before and throughout the experiments. Fish were exposed to different $CdCl_2$ nominal cadmium concentrations of 0 (control), 10, 15, 20, 25, 30, and 35 mg/L, respectively, following the same methods described by Garcia-Santos et al. (2006). Twelve fish were equally distributed in two 50 L tanks for each tested Cd concentration. Water samples were taken at the experiments' beginning and end for Cd analysis. The experimental aquaria were observed, and dead fish were removed and recorded every 12 h. The LC_{50} value of cadmium chloride was calculated using Probit analysis following the methods described by Finney (1971).

Experimental design and samples

Diets preparation

The green onion (*A. cepa*) was bought from a local market in Cairo. The green parts of *A. cepa* were collected, washed, and left to dry in the open air. The dried onion was crushed

and ground. The alcoholic extract of *A. cepa* was prepared by soaking the crude onion powder (about 500 g) in a double volume of absolute ethyl alcohol for 5 days with shaking. The mixture was filtered, evaporated in a rotary evaporator, dried, and weighed as described by Azwanida (2015). A commercial floating fish diet (35% crude protein, 5.8% fat, 3.5% crude fibers, and 4100 kcal digestible energy) (Skretting, Egypt) was ground, mixed with 0.5 and 1% of either crude onion powder or its alcoholic extract according to Akrami et al. (2015) and Younes et al. (2021). Diets were reformed, pelleted and kept sorted at 4 °C. Diets were offered twice daily at 2% of the fish's body weight for 30 days.

Fish rearing and management

Healthy tilapia fish ($n = 360$) with an average body weight of 60–70 g were collected from a private fish farm in Giza governorate, Egypt, and left to acclimatize to the laboratory conditions for two weeks in glass aquaria (50 L each) aerated with aquarium air pumps. The average water quality parameters were examined and maintained throughout the whole experimental period as the following: 26 ± 1 °C for water temperature, 7.88 ± 0.34 mg L^{-1} for dissolved oxygen, 7.15 ± 0.01 for the pH value, and 0.012 ± 0.02 mg L^{-1} for un-ionized ammonia. Half of the aquarium's water was siphoned every other day to remove wastes and replenished with new well-aerated water from the stock tank. During the acclimatization period, fish were fed on a commercial basal diet with 35% protein (Skretting, Egypt).

Experimental set-up

After the acclimatization period, fish were distributed randomly in the glass aquaria into six groups, each containing sixty fish ($20 \times$ three replicates) as shown in supplementary Fig. 1. Fish were fed the experimental diets for 30 days at 2% of their body weight. The first four groups (G1, G2, G3, and G4) were fed on a diet supplemented with different concentrations of crude or extracted *A. cepa* as the following: crude onion 0.5% (G1), crude onion 1% (G2), onion extract 0.5% (G3), and onion extract 1% (G4). On the other hand, fish in groups 5 (positive control) and 6 (negative control) were fed on the basal diet supplemented with 0% onion. Waterborne $CdCl_2$ at the concentration of (1.51 mg Cd/ L) (1/10 of LC_{50}) was added to experimental groups (1, 2, 3, 4, and 5). Fish in group 6 were reared in normal water without cadmium (0 mg Cd/ L).

Growth performance

Fish were anaesthetized with tricaine methanesulfonate (MS-222) (Sigma). Growth performance parameters, such as weight gain (WG), specific growth rate (SGR), hepato-somatic index (HSI), gonado-somatic index (GSI), and the condition factor (CF), were calculated at the end of the experimental period following Tukmechi et al. (2011).

Blood sampling

Blood samples were collected, after anaesthetizing fish, from the caudal vein of ten fish taken randomly from each replicate. Samples for hematological analysis were obtained in tubes with ethylenediaminetetraacetic acid (EDTA). Blood samples for other analytical assays were obtained without EDTA, left to clot, and centrifuged at 1500 g for 15 min. Collected sera were kept frozen at − 20 °C for further assays.

Hematological analysis

The white blood cell counts (WBCs) were determined following the methods of Natt and Herrick (1952) using an improved Neubauer hemocytometer. The differential leukocytic count was assessed using the Giemsa stain.

Biochemical assays

The following biochemical assays were estimated using commercial kits (Spectrumdiagnostics, Egypt): Alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were used to assess liver function, kidney function (uric acid, and creatinine), lipid profile (triglycerides, and total cholesterol), and carbohydrate metabolism (glucose).

Non-specific immune parameters

Serum proteins Total protein and albumin levels (g/dl) were calorimetrically analyzed in fish sera using commercial kits (Spectrumdiagnostics, Egypt), and then globulin was estimated. The procedures were performed following the standard methods described by Wu (2006).

Myeloperoxidase content The total myeloperoxidase content in collected sera was measured according to Quade and Roth (1997). Briefly, 50 µl serum was diluted with 135 µl of Ca + 2 and Mg + 2 free HBSS (Sigma-Aldrich) in flat-bottomed 96-well microtiter plates. Then, 50 µl of 20 mM 3,3′,5,5´-tetramethylbenzidine hydrochloride (TMB, Sigma-Aldrich) and 5 mM H_2O_2 (Sigma-Aldrich) were added (both substrates of peroxidase). The reaction (color change) was stopped by adding 50 µl of 4 M sulphuric acid (H_2SO_4) after 2 min. The absorbance was read at 450 nm in a fluorimeter. Standard samples without serum were also analyzed.

Antiproteases activity Serum antiproteases were studied following methods described by Lange et al. (2001) as the percentage of trypsin inhibition (antitrypsin activity).

Lysozyme activity Lysozymes were estimated in different fish sera according to Parry et al. (1965) via the turbidimetric assay of *Micrococcus lysodeikticus* suspension (Sigma-Aldrich, 0.2 mg/ml).\

Phagocytic activity (in vitro carbon clearance assay) The phagocytic activity was determined following the methods of Spinu and Degen (1993). Blood was collected on heparin (50 IU/ml) from each fish and mixed with 6 µl of the supernatant fraction of India ink (Pelikan AG D-3000, Hanover, Germany). Samples were divided into three equal aliquots and incubated at 37 °C for 20 and 40 min, then the mixture (150 µl) was added to 2 ml saline. Samples were centrifuged at 2500 rpm for 5 min, and the supernatant was read spectrophotometrically at 535 nm, with the background taken as zero. Optical density readings were converted to a log2 scale, and the phagocytic index was taken as the negative slope of the regression of optical density (log2) on time (h).

Antioxidant activities Liver specimens collected from fish were dissected and dropped into liquid nitrogen, homogenized, and centrifuged at 9000 g for 30 min at 4 °C. The protein concentration was measured in the supernatant using bovine serum albumin (BSA) and utilized in the determination of different antioxidants: Superoxide dismutase activity (SOD) was measured according to Villa-Cruz et al. (2009) as the amount of enzyme required to inhibit 50% of Nitro Blue Tetrazolium (NBT) oxidation. Catalase (CAT) was assayed as the decomposition of hydrogen peroxide, as described by Aebi (1984). Peroxidase activity was observed by guaiacol oxidation, according to Gulcin and Yildirim (2005). The glutathione-S-transferase activity (GST) was estimated by conjugating 1-chloro-2, 4-dinitrobenzene (Sigma-Aldric) in ethanol with reduced glutathione (Sigma-Aldrich) in phosphate buffer, and the absorbance of the formed conjugate was read kinetically at 340 nm. The GST activity was calculated using a molar extinction coefficient of 9.6 mM^{-1} cm^{-1} and expressed as µmole/min/mg protein (Habig et al., 1974).

Cd accumulation analysis Liver, muscles, gonads, and gills tissues were collected from experimental fish. All samples were snap-frozen in liquid nitrogen and stored at – 80 °C until further analysis. The obtained tissues were assayed for Cd residues following Wang et al. (2020) using an AA-6300 atomic absorption spectrometer (Shimadzu, Japan). Briefly, the tissue samples were incubated in a digestion vessel overnight with 10 mL of mixed acids (HNO_3: $HClO_4$ = 4:1). The samples were dissolved completely by keeping them in a sand bath at 180 °C, placed into a volumetric flask, and subjected to atomic absorption spectrometry analysis.

Challenge experiment with *S. parasitica*

S. parasitica isolate (ON7973024) obtained from naturally infected tilapia was used in the challenge experiment. The zoospores of *S. parasitica* were produced according to the method described by Stueland et al. (2005). Briefly, bundles of growing *S. parasitica* hyphae were washed twice in autoclaved pond water (APW). They were then transferred to a glass bottle containing APW and incubated at 21 °C for 24 h for zoospores induction. Zoospore encystment was induced, and cysts were counted using a hemocytometer (Bürkertürk chamber). A total number of (n = 30) fish collected from each experimental group were utilized in the challenge experiment. Fish were subjected to "ami-momi treatment" (Hatai & Hoshiai 1993) before exposing them to *S. parasitica* spores at a concentration of 1.0×10^4 L^{-1}. Fish were observed daily for the clinical signs of saprolegniasis for 10 days. A total of 3 fish were taken and sacrificed from each experimental group at different time points, first day and the tenth day following the exposure to *S. parasitica* spores. Livers were collected in RNAlater to evaluate the expression of two immune-related genes, *IL-1β* and *IFNγ*.

Expression of *IL-1β* and *IFNγ* genes

The total RNA was extracted from fish liver samples utilizing the RNeasy mini kit (Qiagen, Germany) following the manufacturer's instructions. The Quantinova SYBR Green RT-PCR kit (Qiagen, Germany) and specific primers (Table. 1) were used for relative quantification of *IL-1β* and *IFNγ* that were normalized to *β-actin* as a housekeeping gene. The RT-PCR analysis was done in a Stepone plus instrument (Applied Biosystems) under the following thermocycler condition: 50 °C for 30 min followed by 95 °C for 5 min. The PCR

cycling was performed in 40 cycles of denaturation at 95 °C for 15 s, annealing, and extension at 60 °C for 45 s. The relative mRNA expression pattern for each gene was calculated using the comparative $2^{-\Delta\Delta Ct}$ method approved by Livak and Schmittgen (2001).

Statistical analysis

The one-way ANOVA analysis was used to determine the significant differences in the measured values (Duncan, 1955). SPSS (version 17.0 for Windows) software (SPSS Inc.) was used in statistical analyses at $p < 0.05$. In the challenge experiments, we compared the resulting survival curves among the fish groups fed on the *A. cepa* and positive controls with Kaplan–Meier survival plot using log rank (Mantel-Cox test) (Kaplan & Meier 1958). Pair-wise comparison differences between each group was considered to be significant at a *P* value of < 0.05. Statistical analyses were performed with GraphPad Prism 9 (GraphPad Software, Inc., San Diego, CA).

Results

Phenotypic and molecular characterization of *S. parasitica*

Diseased fish collected during the natural outbreak of saprolegniasis showed cotton-wool-like masses on the external body surfaces (Fig. 1a). The microscopical examination of wet mounts prepared from the skin lesions revealed the presence of non-septate hyphae with characteristic zoosporangia (Fig. 1b). After purification, white cottony growths were observed on the sabouraud dextrose agar medium (Fig. 1c).

The Basic Local Alignment Search Tool (BLAST) analysis of the *ITS* gene sequences confirmed that the six isolates were deeply embedded in the genus Saprolegnia group. The accession numbers of the *ITS* gene sequenced from the six isolates ranged from ON797302 to ON797307. The alignment of these sequences unveiled 100–99.72% similarity to *S. parasitica* strains (AY455771^{T}; FJ545238^{T}; KX494868; AB727993; KT807577; and OM275427). The intraspecies similarity was 99.57–100% for the six *S. parasitica* isolates recovered from Nile tilapia, with nucleotide differences ranging from 2 to 3 bp. The phylogenetic analysis of amplified sequences of the six *S. parasitica* isolates grouped them with known sequences of *S. parasitica* and separated from other groups belonging to *S. declina*, *S. ferax*, *S. hypogyna*, *S. litoralis*, *S. anomalies*, *S. oliviae*, *S. bulbosa*, *S. australis*, *S. aenigmatica*, *S. furcate*, *S. terrestris*, and *S. monilifera* (Fig. 2).

Table 1 Primers used in the study

Gene	Primer sequence	Reference
β-actin	F: CCACACAGTGCCCATCTACGA R: CCACGCTCTGTCAGGATCTTCA	Qiang et al. (2014)
IL-1β	F: CAAGGATGACGACAAGCCAACC R: AGCGGACAGACATGAGAGTGC	Dawood et al. (2020)
IFNγ	F: AAGAATCGCAGCTCTGCACCAT R: GTGTCGTATTGCTGTGGCTTCC	

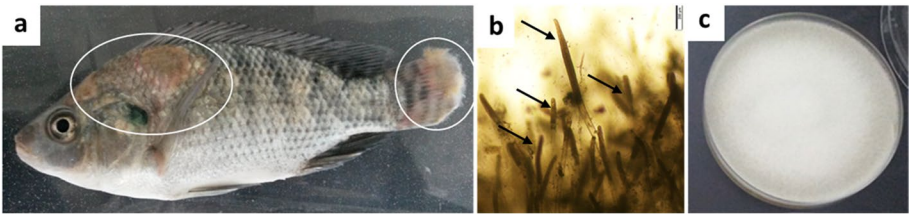

Fig. 1 **a** Naturally infected tilapia fish showing the clinical signs of saprolegniasis, white to grey patches on the external body surfaces and tail. **b** Microscopic examination of wet mount preparation of *Saprolegnia* showing characteristic aseptate hyphae and zoosporangia (arrows). **c** Purified *Saprolegnia* isolate on sabouraud dextrose agar (SDA)

96 h LC_{50} of Cd

The 96 h LC_{50} value for Cd in *O. niloticus* was (15.1 mg/l Cd). The highest fish mortality rate (91.7%) was seen in fish group exposed to the concentration of 35 mg/L Cd. Tilapia subjected to Cd at concentrations of 30, 25, 20, 15, 10, and 5 mg/L Cd showed mortality rates of 75%, 66.7%, 58.3%, 41.7%, 25%, and 16.7%, respectively.

Growth performance

Fish exposed to waterborne cadmium and fed on the basal diet (G5) displayed a significant decrease ($P < 0.05$) in WG and SGR values compared to fish not exposed to cadmium (G6). Tilapia exposure to cadmium also caused a significant reduction ($P < 0.05$) in their HSI and GSI values compared to fish which had not been exposed to waterborne cadmium, as shown in Table 2.

Tilapia fed on diets supplemented with *A. cepa* either in its crude or extracted from showed a significant increase ($P < 0.05$) in WG and SGR values compared to the positive control group without *A. cepa* treatments while still having significantly lower values ($P < 0.05$) than control negative fish not exposed to cadmium. The highest WG and SGR values were recorded in fish fed on diets with 1% crude *A. cepa*. There were no significant changes ($P > 0.05$) in HIS, GSI, and CF values in fish groups fed on *A. cepa* dietary inclusions, as shown in table.2.

White blood cells counts (WBCs) and biochemical assays

Fish exposed to waterborne Cd and fed on the basal diet showed a non-significant ($P > 0.05$) decrease in the total WBCs, granulocytes %, and lymphocytes values compared to control fish without cadmium exposure. On the other hand, monocytes % was significantly decreased ($P < 0.05$) in tilapia exposed to waterborne Cd and fed on the basal diet compared to control fish not exposed to waterborne cadmium.

Fish treated with *A. cepa* exhibited a significant increase ($P < 0.05$) in the total WBCs, with the highest increase noticed with feeding on 1% *A. cepa* extract, as shown in Table 3. Fish fed on diets with *A. cepa* extracts dietary inclusions (1% and 0.5%) displayed a significant increase ($P < 0.05$) in lymphocytes (Table 3). Treatment with *A. cepa* extracts induced a significant decrease ($P < 0.05$) in both granulocytes % and monocytes % compared to control groups, as demonstrated in Table 3.

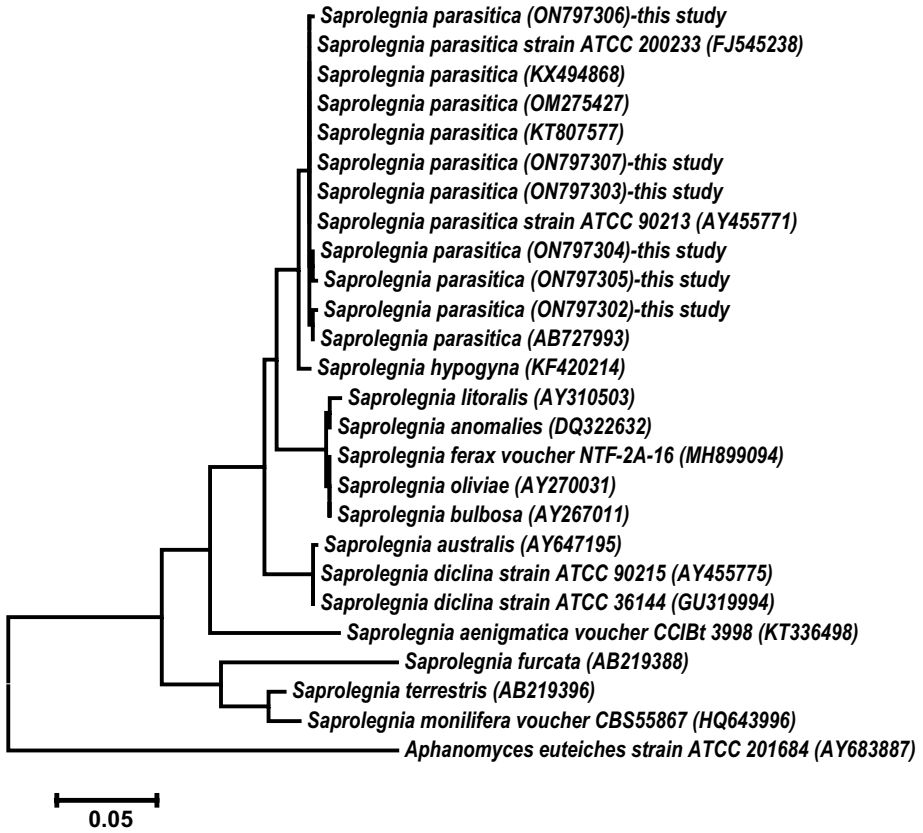

Fig. 2 The neighbor-joining phylogenetic tree showing the comparative analysis of the ITS gene sequencing of *S. parasitica* strains recovered from naturally infected *O. niloticus* and other related *Saprolegnia* spp

Tilapia exposure to waterborne cadmium caused a significant increase ($P < 0.05$) in biochemical parameters (glucose, cholesterol, triglycerides, ALP, ALT, AST, creatinine, and uric acid). On the other hand, tilapia treated with *A. cepa* had lower levels of glucose, cholesterol, triglycerides, ALP, ALT, AST, creatinine, and uric acid values than those without *A. cepa* treatments, as shown in the Table 3

Nonspecific immune parameters

Tilapia exposure to waterborne cadmium caused a significant decrease ($P < 0.05$) in most assays, including serum protein, albumin, globulin, lysozyme activity, myeloperoxidase, and antiprotease activity, with a non-significant decrease ($P > 0.05$) in the phagocytic index in comparison to fish without Cd exposure. Fish fed on diets supplemented with *A. cepa* showed an increase in serum total protein, albumin, and globulin values over those without *A. cepa* treatments. The uppermost measurements of protein and globulin were noticed in tilapia that received dietary inclusions with 0.5% *A. cepa* extract, as shown in Table.3.

Table 2 Growth performance parameters of *O. niloticus*

Parameters	Control -ve	Control + ve	Crude 1%	Crude 0.5%	Extract 1%	Extract 0.5%	*F* value
WG (g)	9.71 ± 0.53^{a}	3.46 ± 0.36^{d}	7.96 ± 0.28^{b}	6.90 ± 0.19^{bc}	5.92 ± 0.23^{c}	6.93 ± 0.37^{bc}	31.78
SGR	0.99 ± 0.08^{a}	0.38 ± 0.02^{d}	0.77 ± 0.02^{b}	0.66 ± 0.02^{c}	0.72 ± 0.01^{bc}	0.69 ± 0.03^{bc}	51.02
Hepatosomatic index (HSI)	2.17 ± 0.05^{a}	1.21 ± 0.09^{b}	1.36 ± 0.04^{b}	1.22 ± 0.08^{b}	1.21 ± 0.11^{b}	1.26 ± 0.02^{b}	25.62
Gonadosomatic index (GSI)	2.51 ± 0.03^{a}	1.52 ± 0.04^{b}	2.47 ± 0.14^{a}	1.32 ± 0.18^{b}	1.21 ± 0.08^{b}	1.23 ± 0.09^{b}	30.15
Condition factor (CF)	1.55 ± 0.20^{a}	1.40 ± 0.22^{a}	1.55 ± 0.23^{a}	1.37 ± 0.32^{a}	1.45 ± 0.24^{a}	1.63 ± 0.14^{a}	0.45

Data are expressed as mean ± standard error

Means with the same letter within the same raw are not significantly different ($P > 0.05$)

Table 3 Hematological, biochemical, antioxidants, and immunological parameters of *O. niloticus*

Parameter	Control -ve	Control + ve	Crude 1%	Crude 0.5%	Extract 1%	Extract 0.5%	*F* value
WBCs × 10^3/µl	263.75 ± 11.56^{c}	290.87 ± 9.35^{c}	345.02 ± 7.39^{b}	351.00 ± 11.04^{b}	436.42 ± 12.16^{a}	429.25 ± 16.77^{a}	50.94
Granulocytes%	25.43 ± 1.42^{a}	23.77 ± 2.14^{ab}	28.08 ± 0.86^{a}	20.38 ± 1.80^{bc}	15.97 ± 0.86^{cd}	15.49 ± 0.81^{d}	11.85
Lymphocytes %	50.80 ± 6.57^{c}	59.67 ± 5.37^{bc}	54.44 ± 1.88^{bc}	61.97 ± 1.21^{b}	71.40 ± 2.00^{a}	72.63 ± 1.85^{a}	8.52
Monocytes%	23.77 ± 1.12^{a}	16.56 ± 1.32^{b}	17.48 ± 1.19^{b}	17.65 ± 1.47^{b}	12.63 ± 2.09^{c}	11.88 ± 1.93^{c}	12.54
Total protein (mg/ml)	4.450 ± 0.09^{a}	2.372 ± 0.10^{d}	2.625 ± 0.11^{d}	3.295 ± 0.04^{c}	3.117 ± 0.07^{c}	3.612 ± 0.09^{b}	74.21
Albumin (mg/ml)	0.700 ± 0.03^{c}	0.515 ± 0.03^{d}	0.570 ± 0.07^{cd}	1.277 ± 0.04^{a}	0.573 ± 0.05^{cd}	0.958 ± 0.06^{b}	31.69
Globulin (mg/ml)	3.747 ± 0.07^{a}	1.852 ± 0.03^{d}	2.063 ± 0.06^{c}	2.010 ± 0.04^{c}	2.553 ± 0.06^{b}	2.650 ± 0.06^{b}	316.63
Glucose (mg/l)	28.24 ± 0.58^{b}	34.10 ± 0.43^{a}	25.40 ± 0.51^{c}	21.27 ± 0.29^{d}	29.72 ± 0.21^{b}	16.11 ± 0.80^{e}	147.56
Cholesterol (mg/l)	157.7 ± 5.37^{d}	237.1 ± 2.53^{a}	209.3 ± 7.38^{b}	129.8 ± 5.95^{e}	188.2 ± 2.74^{c}	145.4 ± 8.45^{de}	42.19
Triglyceride (mg/dl)	126.3 ± 4.67^{b}	163.7 ± 3.13^{a}	115.1 ± 1.86^{c}	128.3 ± 2.42^{b}	124.6 ± 3.96^{b}	115.3 ± 1.89^{c}	94.29
ALP (unit/l)	8.23 ± 0.36^{d}	15.38 ± 1.04^{a}	8.33 ± 0.36^{d}	13.27 ± 0.73^{ab}	10.40 ± 0.70^{cd}	11.68 ± 0.84^{bc}	14.65
AST (unit/l)	147.8 ± 1.26^{b}	188.1 ± 3.91^{a}	110.6 ± 1.91^{cd}	102.6 ± 1.10^{de}	100.7 ± 2.23^{e}	112.6 ± 4.54^{c}	124.80
ALT (unit/l)	35.26 ± 0.28^{b}	39.78 ± 0.64^{a}	32.27 ± 0.72^{c}	36.05 ± 0.37^{b}	34.85 ± 0.36^{b}	32.32 ± 0.59^{c}	26.72
Creatinine (mg/dl)	1.143 ± 0.056^{b}	1.342 ± 0.021^{a}	1.095 ± 0.045^{b}	0.888 ± 0.045^{c}	0.935 ± 0.030^{c}	0.865 ± 0.061^{c}	15.97
Uric acid (mg/l)	43.70 ± 1.38^{bc}	52.29 ± 2.03^{a}	45.60 ± 0.51^{b}	33.92 ± 2.16^{d}	40.41 ± 0.50^{c}	44.00 ± 1.59^{bc}	16.42
Peroxidase	0.124 ± 0.002^{f}	0.136 ± 0.002^{e}	0.160 ± 0.005^{d}	0.169 ± 0.007^{c}	0.192 ± 0.011^{a}	0.180 ± 0.005^{b}	18.58
Catalase	103.8 ± 2.50^{d}	127.4 ± 3.64^{c}	129.8 ± 2.64^{c}	133.3 ± 3.13^{c}	163.0 ± 2.90^{a}	150.1 ± 3.68^{b}	41.14
GST	0.054 ± 0.009^{b}	0.161 ± 0.006^{a}	0.020 ± 0.004^{f}	0.033 ± 0.005^{e}	0.046 ± 0.004^{c}	0.036 ± 0.006^{d}	72.51
SOD	85.47 ± 4.20^{d}	155.3 ± 4.75^{a}	131.2 ± 3.25^{b}	134.9 ± 2.04^{b}	116.3 ± 2.06^{c}	112.7 ± 1.67^{c}	56.66
Lysozyme activity (unit/ml)	244.5 ± 6.25^{c}	190.7 ± 6.25^{e}	202.6 ± 7.84^{e}	228.2 ± 4.43^{d}	300.0 ± 13.44^{b}	345.3 ± 13.57^{a}	201.37
Myeloperoxidase	2.30 ± 6.25^{a}	1.67 ± 6.25^{b}	1.37 ± 7.84^{c}	1.46 ± 4.43^{c}	1.46 ± 13.44^{c}	2.28 ± 6.25^{a}	41.07
Antiproteases %	67.88 ± 1.07^{b}	62.39 ± 1.07^{c}	64.08 ± 1.31^{c}	59.09 ± 1.46^{d}	70.72 ± 0.92^{b}	74.30 ± 0.88^{a}	33.56
Phagocytic index	0.0013 ± 0.00^{d}	0.0010 ± 0.00^{d}	0.0038 ± 0.00^{b}	0.0054 ± 0.00^{a}	0.0029 ± 0.00^{bc}	0.0018 ± 0.00^{cd}	261.83

Data are expressed as mean ± standard error

Means with the same letter within the same raw are not significantly different ($P > 0.05$)

Treatment with *A. cepa* enhanced lysozyme activity, myeloperoxidase, antiproteases, and phagocytic index values. Most measurements showed the greatest values with feeding on diets with 0.5% *A. cepa* extract, as shown in Table 3.

Antioxidant activity

Fish exposed to cadmium displayed a significant increase ($P < 0.05$) in hepatic antioxidant values (peroxidase, catalase, GST, and SOD) compared to fish not exposed to cadmium. Treatment with *A. cepa* enhanced the hepatic antioxidant activities with the highest peroxidase, and catalase activities noticed in fish fed on *A. cepa* extracts, as shown in Table 3.

Accumulation of Cd in tissues

Feeding fish with *A. cepa* dietary inclusions reduced cadmium accumulation in the liver, muscles, and gonads. The best results were achieved by feeding on a diet with *A. cepa* extracts compared to fish without *A. cepa* treatments. Cadmium levels in fish gills were not significantly affected by *A. cepa* treatments, as shown in the Table 4

Challenge experiment

Significant differences in fish survival rates were observed among all challenged groups (G1, G2, G3, and G4) compared to the positive control group (G5). The highest survival rate (77%) was recorded in fish group fed on a diet supplemented with 0.5% *A. cepa* extract followed by tilapia received a diet supplemented with 1% extract (66%). The lowest survival rate (40%) was reported in fish group received 0.5% crude *A. cepa* (Fig. 3). Experimentally infected tilapia showed clinical signs of saprolegniasis similar to those of naturally infected fish. Infections were confirmed by isolating and characterizing *S. parasitica* from succumbed fish following the same procedures performed in naturally infected tilapia.

Expression of *IL-1β* and *IFNγ* genes

IL-1β and *IFNγ* genes were upregulated at the beginning of the challenge experiment in all fish groups that received *A. cepa* dietary inclusions compared to fish fed on the basal diets and then decreased at the end of the experimental trials. The *IL-1β* and *IFNγ* were upregulated at maximum with feeding 0.5% *A. cepa* extract dietary inclusions, while the lowest

Table 4 Cadmium accumulation (ppm/wet weight) in different tissues of *O. niloticus*

Tissue	Control -ve	Control + ve	Crude 1%	Crude 0.5%	Extract 1%	Extract 0.5%	*F* value
Muscle	0.230 ± 0.02^{d}	0.600 ± 0.03^{a}	0.430 ± 0.01^{b}	0.440 ± 0.01^{b}	0.370 ± 0.01^{c}	0.130 ± 0.02^{e}	85.24
Liver	0.550 ± 0.16^{d}	21.00 ± 0.43^{a}	15.310 ± 0.82^{b}	20.290 ± 0.42^{a}	10.620 ± 1.48^{c}	17.110 ± 0.30^{b}	91.78
Gills	0.170 ± 0.02^{c}	2.760 ± 0.31^{b}	3.500 ± 0.26^{a}	3.900 ± 0.15^{a}	3.330 ± 0.12^{ab}	3.740 ± 0.18^{a}	45.78
Gonads	0.100 ± 0.01^{e}	3.770 ± 0.23^{a}	0.870 ± 0.07^{d}	1.420 ± 0.09^{c}	0.330 ± 0.06^{e}	1.880 ± 0.14^{b}	136.69

Data are expressed as mean ± standard error

Means with the same letter within the same raw are not significantly different ($P > 0.05$)

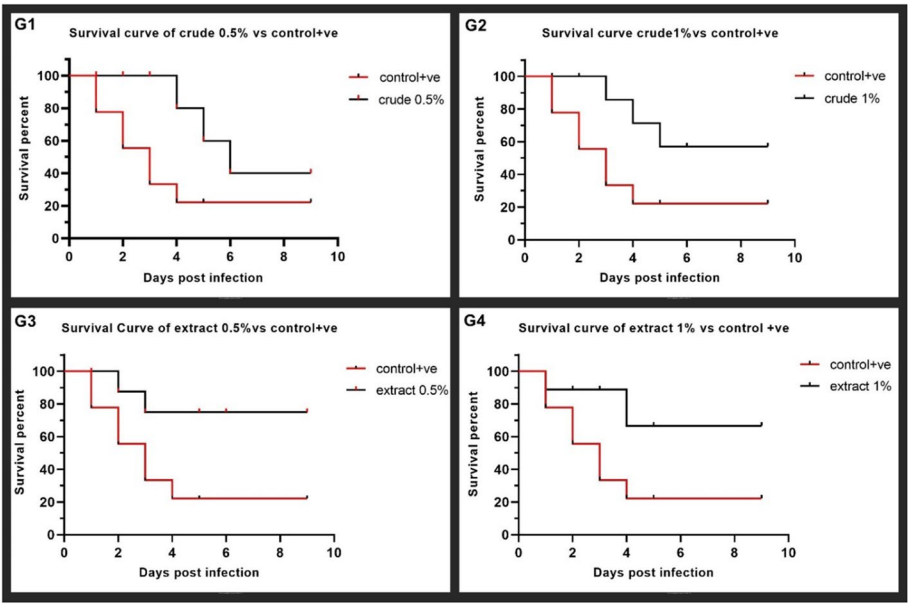

Fig. 3 Kaplan–Meier survival curves of Nile tilapia fish fed on different concentrations of *A. cepa* supplemented diets; (G1) crude 0.5%, (G2) crude 1%, (G3) extract 0.5%, and (G4) extract 1% following the experimental infection with *S. parasitica* compared to the positive control group. The results correspond to the survival percentage during 9 days post-infection (dpi). Kaplan–Meier survival data was analyzed by log-rank (Mantel-Cox) test. Pairwise comparison between each experimental group fed on *A. cepa* against the positive control group showed significant differences in survival curves ($P < 0.05$)

rate was observed in fish exposed to waterborne Cd without *A. cepa* treatments as shown in Fig. 4.

Discussion

Saprolegnia spp. are ubiquitous in freshwater environments and cause disease outbreaks when the conditions are optimal for their pathogenesis (Nam et al. 2022). Different environmental stressors in the studied farm rendered tilapia more susceptible to *S. parasitica* infections. Stressful situations can compromise the host's immune system and enhance its vulnerability to saprolegniasis (Pavić et al. 2022). Saprolegniasis outbreaks were reported in numerous Egyptian tilapia farms (El-Ashram et al. 2007; Zahran et al. 2017). The clinical examination of naturally infected Nile tilapia revealed typical signs of saprolegniasis comparable to those described in earlier studies (Ali et al. 2014; Beckmann et al. 2020). The recovered *S. parasitica* isolates revealed phenotypic characteristics similar to those reported by Mostafa and Yassin (2022).

The BLAST and phylogenetic analysis of the *ITS* gene sequences confirmed the identities of the recovered *Saprolegnia* isolates. Earlier studies reported that the *ITS* genes and the 5.8S rDNA regions are highly conserved and are very suitable for the intraspecies analysis of *Saprolegnia* spp. (Diéguez-Uribeondo et al. 2007; Zahran et al. 2017). Alignments of the ITS rDNA sequences displayed a 100–99.72% identity with the major *S. parasitica*

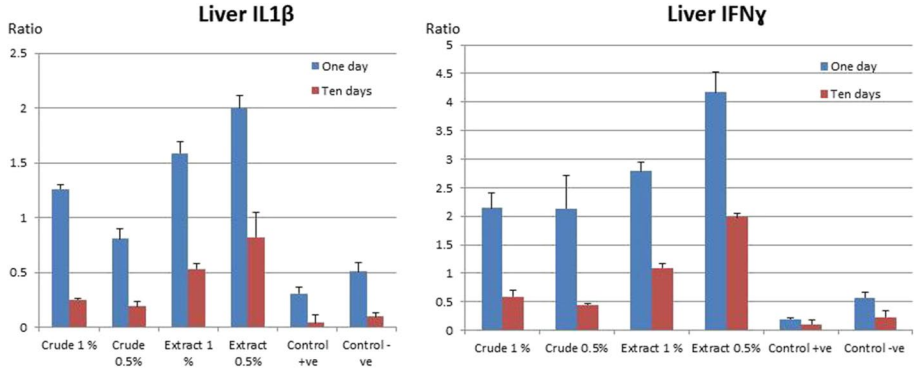

Fig. 4 Expression of IL-1β and IFNɣ genes

typing strains (AY455771^T; FJ545238^T; and OM275427). The ManS22 strain seemed to be the most diverse; in fact, the percentage of identity with the other *Saprolegnia* phylogenetic tree supported the identity of the recovered *S. parasitica* isolates.

Saprolegniasis necessitates efficient control strategies to reduce losses. Fungicides and disinfectants, including malachite green, hydrogen peroxide, and formalin, were commonly used to treat saprolegniasis in aquaculture (Ali et al. 2019). These fungicides have carcinogenic and teratogenic effects posing a concern to fish and human (Mostafa & Yassin 2022). Immunostimulation is a competitive prophylactic strategy for controlling numerous infectious diseases in aquaculture (Elgendy et al. 2016, 2021; Ali et al. 2021; Abbas et al. 2019b, c; Younes et al. 2021). The antifungal and immunostimulatory effects of onion were confirmed in many earlier studies (Khallil 2001; Kocić-Tanackov et al. 2012; Akrami et al. 2015; Younes et al. 2021). In light of these reports, the present study investigated the immunostimulatory and protective effects of *A. cepa* dietary inclusions against *S. parasitica* infection in tilapia and the amelioration of immunosuppression induced by fish exposure to waterborne Cd under the in vivo conditions.

Previous studies suggested that *O. niloticus* can tolerate high levels of waterborne Cd (Tsay & Yu 1981). The Cd 96-h LC_{50} determined in this study (15.1 mg/L Cd) was nearly similar to that reported by Garcia-Santos et al. (2006), who recorded Cd 96-h LC_{50} at (14.8 mg/l Cd). The present study confirmed that exposure of Nile tilapia to waterborne Cd significantly reduces their growth performance, which is in agreement with (Mohsen & Wafeek 2009). The lower fish WG and SGR values were restored by feeding fish on *A. cepa*–based diets.

Results demonstrated that *A. cepa*, in its crude or extracted from, has growth-promoting properties. The exact mechanisms involved in this action might be explained by the immunostimulatory and antioxidant properties of *A. cepa* bioactive compounds.

Our results are in accordance with findings reported by Younes et al. (2021), authors reported a significant increase in growth performance indicators (WG and SGR) following feeding tilapia on diets supplemented with onion. Similarly, Bello et al. (2012) reported enhancement of the WG and SGR indicators of *Clarias gariepinus* fed on diets supplemented with onion (0.5%, 1.0%, 1.5%, and 2.0%). The same authors observed a clear correlation between the quantity of added onion and the degree of growth enhancement. Akrami et al. (2015) discovered that feeding beluga juveniles, *Huso huso*, on diets supplemented with 1% onion enhanced their growth performance parameters (WG and SGR).

Additionally, Saleh et al. (2015) noticed that feeding sea bass fry on onion-based diets at 10 g/kg enhanced their growth performance, feed utilization efficiency, and fish survival. On the contrary, Cho and Lee (2012) noticed no improvement in the growth performance of olive flounder, *Paralichthys olivaceus,* with feeding onion-based diets. The growth-promoting outcomes of onions are attributed to their bioactive compounds, such as sulfur-containing compounds, cysteine sulphoxide (CSO), and S-propenyl-CSO, which have numerous health benefits (Ostrowska et al. 2004; Apines-Amar et al. 2012). *A. cepa* also stimulates beneficial microorganisms in the digestive system, such as bifidobacteria and lactobacilli, which have many health benefits (Gibson 1998). The benefits also include accelerating digestion and shortening the time needed for food to pass through the gastrointestinal tract (Platel & Srinivasan 2001).

The decrease in WBCs and lymphocytes caused by tilapia exposure to waterborne cadmium was restored by feeding fish on *A. cepa*-based diets, indicating the health-promoting outcomes of this plant. Fish that received *A. cepa* dietary inclusions displayed a significant increase ($P > 0.05$) in the total WBCs and lymphocyte counts, reflecting its immunostimulating effects, with the highest outcomes observed with feeding the *A. cepa* extracts. Results were consistent with Younes et al. (2021), who observed that the inclusion of 1% *A. cepa* extract in tilapia diets induced a non-significant increase in WBCs and lymphocytes. Results also agreed with Soliman et al. (2017), who observed a significant enhancement in WBCs and lymphocytes with feeding fish on diets enriched with onion green leaves' extracts at concentrations of 0.5 mg and 1 mg. Similar enhancement in WBCs was noticed in fish fed on diets supplemented with numerous medicinal herbs, including Moringa (Elgendy et al. 2021); curcumin (Elgendy et al. 2016); and fenugreek (Abbas et al. 2019a, b).

The prophylactic benefits of *A. cepa* were highlighted by the decrease in the indicators of metabolic indices, liver, and kidney functions upon feeding fish exposed to waterborne cadmium on *A. cepa* based-diets compared to fish exposed to waterborne cadmium without *A. cepa* treatment. Results were consistent with Akrami et al. (2015), who noticed a significant decrease in blood glucose and triglycerides levels in *Huso huso* fish fed on diets supplemented with 1% onion. However, the same authors disagreed with some of our study findings, as they reported that that ALT and ALP levels were unaffected ($P > 0.05$). Moreover, Younes et al. (2021) noticed insignificant increases in triglycerides levels in Nile tilapia fed on *A. cepa*-supplemented diets. The same authors have also reported a significant decrease in ALT and AST levels with feeding fish the dietary *A. cepa* inclusions. Cho and Lee (2012) noticed that dietary inclusions of onion caused non-significant changes in triglycerides levels in cultured *Paralichthys olivaceus* and attributed that to the wide variation of values within the same treatment. The decrease in these indicators may be relevant to the bioactive compounds of *A. cepa* (Sagar et al. 2022). On the other hand, higher AST, ALT, and ALP enzymes and creatinine in fish exposed to Cd without *A. cepa* treatments indicate liver and kidney dysfunction. The results highlighted the immunostimulatory effects of feeding tilapia on *A. cepa*-based diets and their potential to restore the immunosuppression driven by exposing fish to waterborne Cd. The total protein, globulin, lysozyme activity, myeloperoxidase, antiprotease, and phagocytic index values were improved with feeding fish exposed to Cd on the *A. cepa* dietary inclusions compared to those without *A. cepa* treatment. These indicators were significantly reduced with waterborne Cd exposure, suggesting impaired immune defense mechanisms. The greatest immunostimulatory effects of *A. cepa* were discovered when fish were fed 0.5% *A. cepa* extract. Results are supported by Younes et al. (2021), who noticed that feeding fish with *A. cepa*-enriched diets significantly improved their innate immune responses. Cho and Lee (2012) reported that feeding

P. olivaceus with dietary inclusion of 0.5% *A. cepa* boosted the lysozyme activity and lowered fish mortality after a challenge with *Edwardsiella tarda*. Apines-Amar et al. (2012) demonstrated that feeding grouper, *Epinephelus fuscoguttatus*, on onions and ginger-based diets enhanced the innate immune responses and protected fish against experimental infection with *Vibrio harveyi*. Similar enhancement of nonspecific immune responses was noticed in tilapia fed numerous medicinal herbs such as *Brassica nigra* (Abbas et al. 2016), *Moringa oleifera* (Elgendy et al. 2021), curcumin (Elgendy et al. 2016), fenugreek (Abbas et al., 2019a, b), garlic (Alam et al. 2019), and *Nigella sativa* (Elkamel and Mosaad 2012). Protease, antiprotease, and peroxidase are essential antibacterial components in fish immune-defense mechanisms that play critical roles in protection against invading pathogens (Elgendy et al. 2021). Increased blood protein and globulin levels in fish are linked to a greater innate immunological response (Al-Salahy 2002). Lysozymes stimulate phagocytosis and hinder pathogens' attachments and colonization (Magnadóttir 2006). Antiproteases also have a significant bactericidal effect against bacterial infections (Esteban 2012). The high total protein levels noticed in fish treated with *A. cepa* may be related to improved protein synthesis by the liver, as supported by Younes et al. (2021). The improvement of the immune capacity could be attributed to the bioactive compounds of *A. cepa*, such as ascorbic acids, carbohydrate prebiotics, organosulfur substances, and flavonols (Sagar et al. 2022). The reduction in the immune performance of fish exposed to Cd without *A. cepa* treatment may be linked to the damaging effects of Cd on the immune system (Chang et al. 2021).

Tilapia that received the *A. cepa* dietary inclusions also showed increased hepatic antioxidant enzymes, which may be interpreted as an attempt to overcome the resultant oxidative stress in tilapia. The enhanced antioxidant activities seen in fish treated with *A. cepa* based-diets can be attributed to the bioactive compounds of *A. cepa* (Sagar et al. 2022). The antioxidant enzymes (CAT, GSH-Px, and SOD) are the first defense against heavy metal-induced oxidative damage (Coelho et al. 2011). Similar improvement in antioxidant activities was seen earlier in fish fed on diets supplemented with onion (Akrami et al. 2015; Younes et al. 2021). Results agreed with Wang et al. (2020), where they reported that dietary supplementation with *Bacillus cereus* reversed the oxidative stress in *Carassius auratus* induced by Cd by increasing CAT and SOD antioxidant enzymes. Several studies have shown that medicinal herbs can ameliorate heavy metals' harmful effects and oxidative stress. These herbs include Egyptian leek (Authman et al. 2021), fenugreek seeds (Abbas et al. 2019a, b), and curcumin (Abbas et al. 2019c).

Fish can take up Cd from the aquatic environment through their gills or intestine, and then Cd is transferred to different tissues via the circulation (Yesilbudak & Erdem 2014). Our findings showed that feeding *O. niloticus* during the experimental exposure to waterborne Cd on diets supplemented with *A. cepa*, especially in its alcoholic extract form, can reduce Cd accumulation in fish tissues. Fish exposure to waterborne Cd increased its level in all examined organs; however, feeding fish with *A. cepa* dietary inclusions decreased Cd levels in the liver, muscles, and gonads. The decrease in Cd accumulation in fish tissues observed after feeding on *A. cepa*-based diets could be attributed to its high flavonoid content, such as quercetin, which can increase the production of metallothioneins (Weng et al. 2011; Sagar et al. 2022). These proteins have substantial protective roles in detoxifying metals in aquatic animals (Habjanič et al. 2020).

Flavonoids of *A. cepa* are powerful scavengers for harmful reactive oxygen species, free radical reaction terminators, and metal ion chelators (Rice-Evans 2001; Fang et al. 2002). The comparatively higher Cd levels seen in gills could be attributed to their constant exposure to waterborne Cd, or it could be explained as an effort by fish gills to excrete it (Langston et al. 1998). The findings are consistent with those of Abbas et al. (2021)'

they found that dietary inclusions of natural zeolite in Nile tilapia reduced the negative effects of lead acetate toxicity and decreased Pb residues in fish muscles while increasing its level in the kidneys. Chang et al. (2021) discovered that supplementing *Bacillus coagulans* SCC-19 probiotics to *Cyprinus carpio* lowered Cd residues in their tissues by removing it before it could be absorbed by the gills or intestines. Wang et al. (2020) reported that dietary inclusions with *Bacillus cereus* decreased Cd concentrations in the internal organs of *Carassius auratus gibelio*. Similarly, Yin et al. (2018) showed that dietary supplementations of *Carassius auratus gibelio* with probiotics *Bacillus subtilis* could protect fish against lead toxicity by decreasing its accumulation in fish organs.

The health-promoting and immunostimulant outcomes of *A. cepa* in the present study were evidenced by improved tilapia resistance against *S. parasitica* experimental infections in fish fed on onion-based diets compared with the non-treated fish group. High survival rates were recorded in all experimental fish groups received *A. cepa* in their diets compared to the positive control group fed on the basal diet (0% *A. cepa*). The enhanced fish resistance to *S. parasitica* infection could be attributed to the plant's bioactive compounds with their antioxidant and immunostimulatory properties, such as polyphenols, flavonoids, and quercetin (Akrami et al. 2015). Fish fed on onions enriched diets showed similar enhancements in resistance to several infections, such as *A. hydrophila* (Younes et al. 2021) and *Vibrio harveyi* (Apines-Amar et al. 2012). The high mortality rate that was observed in fish exposed to waterborne Cd without *A. cepa* supplementation may be relevant to the adverse effects of Cd on the fish immune system, as reported in previous studies (Chang et al. 2021).

In the present study, the immunostimulatory outcomes of *A. cepa* dietary inclusions in Nile tilapia were reflected by the upregulation of *IL-1β* and *IFNγ* immune-related genes following the challenge of tilapia with *S. parasitica*. Fish cytokines modulate important immunological responses in aquatic organisms, including chemotaxis, complement activation, and phagocytosis (Secombes et al. 2001; Yin et al. 2018). Interleukin-1 has humoral immune activity, modifies many host immunological responses, and regulates the production of other cytokines (Wang et al. 2006; Jiang et al. 2008). The present study findings also showed an upregulation of the *IL-1β* gene in the group exposed to waterborne cadmium without *A. cepa* treatments. Similarly, earlier studies reported significant upregulation of *IL-1β*, *IL-6*, and *TNF-α* genes in fish exposed to heavy metals pollution (Yildirim & Danabas 2014; Hossain et al. (2021). This increase can be interpreted as a body response of injured tissues to alleviate Cd-induced stress (Schoenborn & Wilson 2007; Ma et al. 2018). The upregulation of *IL-1β* and *IFNγ* in response to the *S. parasitica* challenge was the highest in fish fed 0.5% *A. cepa* extract based-diets, indicating a strong immune response to protect against *S. parasitica* infections. Similar upregulation of *IFNγ* and *IL-10* genes was noticed in Nile tilapia fed on diets supplemented with fenugreek seeds (Moustafa et al. 2020) and Quinoa after challenge with aeromonads (Ahmed et al. 2020). *IFNγ* plays a key role in the innate and adaptive immune responses against invading pathogens. It also regulates other pro-inflammatory cytokines and stimulates phagocytosis (Rosenzweig and Holland 2005; Prabu et al. 2016). Younes et al. (2021) reported a significant downregulation of *IL-1β* and an up-regulation of *TGF-β1* in the kidney of tilapia fed on onion-based diets. The changes in the expression of the studied immune genes may be relevant to onion's bioactive ingredients, such as flavonoids that have pronounced anti-inflammatory and immunomodulatory effects (Sagar et al. 2022). Cd exposure caused a significant increase in all antioxidant indicators in tilapia. Results are consistent with earlier reports indicating that Cd is a major contributor to the production of reactive oxygen species and oxidative stress in fish (Abbas et al. 2019a, b, c b; Chang et al. 2021).

Conclusion

The emergence of infectious diseases, like saprolegniasis, in aquaculture is linked to environmental stressors, bad hygiene, and pollutants in the aquatic environment. Prophylactic measures are needed to control saprolegniasis in aquaculture. Feeding tilapia on diets supplemented with some medicinal plant products such as *A. cepa* can improve the growth performance, physiological status, and antioxidative capabilities of fish and restore their immune defense mechanisms following exposure to waterborne Cd. Additionally, *A. cepa* supplementation in the fish diets reduced Cd accumulation in fish tissues. Feeding fish on dietary *A. cepa* inclusions enhanced their resistance to *S. parasitica* experimental infection and increased the expression of immune-related genes. Our study highlights the role of *A. cepa* as a feed additive to ameliorate the adverse effects of toxic metals in farmed fish while increasing their resistance to some infectious diseases.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval This study was carried out in accordance with "Guidelines for the Use of Fishes in Research" approved by the Institutional Animal Care and Use Committee, National Research Centre Egypt.

Competing interests All authors declare no competing interests.

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