Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

A Study of the Effect of Selenium Nanoparticles on *Aeromonas* Resistance in Nile Tilapia Fish

Safaa M. Elmesalamy[1], Randa M. Khallaf[2], Azza Z. Refaie[3], Sahar S. Abd El-Hamied[4] and Omnia A. Elewasy[3]

[1]*Biochemistry, Toxicology and Feed Deficiency Dept., Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Zagazig Branch, Egypt.*
[2]*Biochemistry, Toxicology and Feed Deficiency Dept., Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Dokki, Giza, Egypt.*
[3]*Bacteriology Department, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Zagazig Branch, Egypt.*
[4]*Pathology and Clinical Pathology Department, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Zagazig Branch, Egypt.*

Abstract

Aeromonas hydrophila is a significant pathogen affecting warm-water fish, particularly Nile tilapia, leading to various bacterial illnesses. This study investigates the limitations of antibiotics in treating *A. hydrophila* infections and explores selenium nanoparticles (Se-NPs) as a potential alternative treatment. The research was conducted in Sharkia governorate, Egypt, where a high prevalence (92%) of *Aeromonas* spp. was found among isolated strains, all exhibiting multidrug resistance to 11 out of 13 antibiotics tested. In a 35-day feeding trial involving 120 Nile tilapia, fish were divided into four groups: a control group, a group receiving a basal diet supplemented with Se-NPs, a positive control group infected with *A. hydrophila*, and a group receiving Se-NPs while also infected. Results indicated that *A. hydrophila* infection led to significant alterations in hematological and biochemical profiles, including increased levels of liver enzymes (ALT, AST, ALK, GGT), urea, creatinine, and uric acid, alongside decreased total protein, albumin, globulin, and antioxidant enzyme levels. However, dietary supplementation with Se-NPs significantly improved these parameters, enhancing antioxidant activity and immune response in Nile tilapia. Furthermore, Se-NPs mitigated the pathological effects caused by *A. hydrophila* infection, suggesting their potential as an effective treatment strategy for managing bacterial diseases in aquaculture.

Keywords: *Oreochromis niloticus,* Selenium nanoparticles, *Aeromonas hydrophila,* hematology. Antioxidants, Phagocytic percent.

Introduction

One of the animal food production sectors with the greatest rate of growth in the world is aquaculture, which significantly boosts both economic growth and global food security [1]. The Nile tilapia, (*Oreochromis niloticus*), is a common species of freshwater fish because of its rapid growth, ability to adapt to a variety of environmental circumstances, and high demand from consumers [2]. However, intense aquaculture operations frequently expose fish to stress and opportunistic bacterial infections, resulting in substantial economic losses. *Aeromonas* species, notably *A. hydrophila*, are recognized as the primary.

Causal agents of motile Aeromonas septicemia (MAS), characterized by hemorrhages, fin rot, ulcers, and high mortality rates [3]. Bacterial infections in fish are frequently associated with oxidative stress, which is caused by a surplus of reactive oxygen species (ROS) that overwhelm the antioxidant defense systems. Oxidative damage can harm key organs, including the liver and kidneys, affect protein metabolism, and weaken immunological responses [4]. Thus, nutritional measures aimed at increasing antioxidant capacity and immune defense are

*Corresponding authors: Safaa M. Elmesalamy, E-mail: drsafaamelmesalamy@gmail.com Tel.: 01062268081
(Received 31 August 2025, accepted 02 November 2025)
DOI: 10.21608/ejvs.2025.419094.3095

regarded as useful complementary approaches to disease control in aquaculture. In this setting, there is a critical need for environmentally benign, effective antibiotic alternatives that can improve disease resistance and immune response in fish. An interesting solution is to use nanotechnology, specifically nano-selenium (Se-NPs) as a food supplement in aquaculture.

Selenium (Se) is an essential trace element that aids the antioxidant defense system. It is largely a component of selenoenzymes such as glutathione peroxidase (GPx), which guards against oxidative cell damage [5]. Selenium has been shown to enhance immune responses and disease resistance in fish, in addition to its antioxidant properties [6]. However, the bioavailability of traditional selenium sources is frequently limited, and excessive supplementation may be harmful [7].

Nano-selenium, a unique form of selenium with particle sizes in the nanometer range, has received interest in aquaculture nutrition due to its better bioavailability, lower toxicity, and robust biological activity compared to inorganic and organic selenium forms [8]. Recent studies have revealed that dietary nano-Se can enhance growth, boost antioxidant status, and protect against bacterial and environmental stresses in fish [9, 10].

However, there has been little investigation into its therapeutic efficacy against Aeromonas infections in Nile tilapia, notably in terms of liver and kidney function, protein metabolism, and antioxidant responses. Therefore, the current work was conducted to study the protective effects of dietary Se-NPs on liver and kidney biomarkers, serum protein profile, and antioxidant status in Nile tilapia experimentally infected with *Aeromonas* spp. The findings aim to shed light on the potential application of nano-selenium as a feed additive in aquaculture to enhance health and resistance to diseases.

Material and Methods

Fish

An earthen fish farm at the Central Laboratory for Aquaculture Research in Abassa, Sharkia province, Egypt, donated 120 healthy male Nile tilapia weighing 30 ± 1.8 g to the Animal Health Research Institute's Zagazig branch. The fish were transported in plastic bags containing water. Fish were acclimatized for two weeks in glass tanks (100 × 40 × 40 cm) with 100 liters of dechlorinated tap water. The tanks were aerated using an electric air pump, and heaters were utilized to keep the natural photoperiod temperature of 26°C. The water was maintained at 6.5±0.5, 7.1±0.8, 0.003, 0.31, and 0.42 mg L-1 for dissolved oxygen, pH, ammonia, nitrate, and nitrite levels. A basal diet containing 30% protein was administered twice daily at a rate of 4% of body weight for the duration of the two-week adaptation period.

Selenium nanoparticles

Mint leaf extract was used as a stabilizing and reducing agent in an environmentally friendly reduction procedure to create green selenium nanoparticles. After being thoroughly cleaned with deionized water and chopped into small pieces, fresh mint leaves were boiled for 20 minutes at 80 degrees Celsius in 100 milliliters of distilled water. Whatman filter paper was used to filter the extract, which was then stored for later use at 4°C. Mint extract was added dropwise to a sodium selenite (Na2SeO3) solution while being continuously stirred at room temperature to create the selenium nanoparticles. As soon as selenium nanoparticles were formed, the reaction mixture's color changed from colorless to brick red. For four hours, the mixture was constantly agitated to guarantee full reduction and stabilization. To obtain selenium nanoparticle powder, the resultant nanoparticles were collected by centrifugation at 10,000 rpm for 15 minutes, repeatedly cleaned with distilled water and ethanol to get rid of any materials that hadn't reacted, and then dried in a vacuum oven at 60°C for 12 hours [11-14]. UV-visible spectra and XRD patterns were used to identify the produced Se-NPs and determine their size, shape, and morphology [13, 15, 16 & 17].

Experimental Design

Four groups of thirty fish each were created from 120 freshwater Nile tilapia. The fish were randomly divided into two replicates of four groups, 15 fish each. Each group received one of the treatments indicated below:
Group 1 (G1): Fish were fed a baseline diet (30% crude protein) for 35 days and served as the control.
Group 2 (G2): Fish were fed the base diet supplemented with Nano-selenium 0.3 mg/kg according to [18] from day one to the completion of the experiment (35 days).
Group 3 (G3): Fish were fed a basal diet from day one to the completion of the trial (35 days) and artificially infected with *Aeromonas hydrophila* on day 21 of the experiment. Each fish received a 0.5 ml intraperitoneal injection of 1×107 CFU and served as a positive control, following [19].
Group 4 (G4): fish were fed a basal diet supplemented with Nano-selenium 0.3 mg/kg from day one to the completion of the experiment (35 days) and artificially infected with *Aeromonas hydrophila* on day 21 of the experiment.

For 35 days, each group's fish were given 3% of their body weight twice a day. During the acclimation stage, half of the water was changed daily to avoid the buildup of feces. The test included an analysis of the water quality measures. Collecting samples after 35 days from all groups, 14 days after infection in groups 3 and 4.

Bacterial Isolation

Seemingly healthy and diseased Nile tilapia were collected from the market place in Sharkia Governorate (muscles, fins, intestine, gills, and skin) during the summer period (2024). Fish specimens were promptly and aseptically conveyed to the lab of the Microbiology Department, Animal Health Research institute. All fish exhibited indications of hemorrhages around the operculum region (circle) and ulceration on the tail fin.

Conventional identification

The specimens were cultivated in nutrient broth (Oxoid), and kept aerobically for 18–24 hours at 37°C. After being streaked across Aeromonas agar base medium (Hi-medium, India). A single colony from the agar was used to make a smear stained with Gram stain and then morphologically observed under a microscope [21].

Re-isolation of A. hydrophila was made from all groups

Molecular identification of A. hydrophyla

The QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was used to extract DNA from samples, with some adjustments made based on the manufacturer's instructions. Metabion (Germany) provided the primers (Table 1).

PCR amplification

A 25 µl reaction comprising 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer at a concentration of 20 pmol, 5.5 µl of water, and 5 µl of DNA template was used to use primers. An Applied Biosystem 2720 heat cycler was used to carry out the process.

Analysis of the PCR Products

A 1.5% agarose gel was used for electrophoresis of the PCR product. A gel documentation system (Alpha Innotech, Biometra) took pictures of that, and a computer was used to analyze the data.

Sensitivity assay

Examinationof Antimicrobial activity of A. hydrophyla for Se NPs

The disc diffusion approach utilized. Sterile discs (0.6mm diameter) of Whatman No. 2 filter paper were loaded with 100 µL/disc of Se NPs at a concentration of 10000 µg/ml and positioned on Muller Hinton agar plates inoculated with bacterial cultures, generated inoculum equivalent to 0.5 McFarland (1.5 x 10 8 CFU). Petri plates incubated at 37 degrees Celsius for 24 hours, and the inhibitory zone was properly quantified [23].

Antimicrobial susceptibility testing of Aeromonas spp for antimicrobial agents

Using the disc diffusion method and the CASFM recommendations, all of the isolates were evaluated for resistance to 13 antimicrobial drugs (Table 2). [24] regarding its susceptibility to antimicrobials [(Oxoid, UK). Some colonies were moved to a tube containing 5 ml of sterile physiological saline and matched with a 0.5 McFarland standard tube (1.5 x 10 8 CFU). Antimicrobial discs were put on the Muller Hinton agar after the bacterial culture had been scattered onto the plates surface. Afterward, incubation at 37°C for 24 hours. The outcomes were interpreted by CLSI [25].

Sampling

After a 24-hour fast, five fish from each group were chosen at the end of the experiment and given a 5-minute anesthesia with buffered tricane-methane sulfonate (E10521-10G, Sigma-Aldrich) at a dosage of 100 mg/L [26]. Blood samples were obtained from caudal blood vessels and placed in a 1-mL sterile syringe with 0.2 mL of EDTA for hematological parameter evaluation, heparinized tubes for phagocytic test, and dry, clean centrifuge tubes for serum separation. After 15 minutes at ambient temperature, the tubes were centrifuged for 10 minutes at 3000 rpm.

Hematological studies:

A Neubauer's hemocytometer was used to manually estimate the total erythrocyte count (RBC × 10^6/µl) and total leukocyte count (WBC × 10^3/µl), with Hayem solution serving as the diluent [27]. The cyanmethemoglobin technique, [28] was used to compute hemoglobin (Hb), and the hematocrit % was determined using a microhematocrit centrifuge [29]. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated [30].

Biochemical studies

Commercial kits and established protocols were used to determine the serum biochemical parameters. Using spectrophotometric kits (Biomed Diagnostic, Egypt), the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured [31, 32]. The activities of alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were examined [33]. The concentrations of albumin (ALB) and total protein (TP) were determined [34], and the difference between the ALB and TP values was used to compute globulin (GLOB) [35].

Renal function markers were analyzed following standard protocols. Serum creatinine concentration was determined according to the colorimetric method [36]. Urea levels were measured [37], while uric acid concentration was estimated according to the enzymatic procedure [38].

Antioxidant enzyme activities were evaluated to monitor oxidative stress responses. Superoxide dismutase (SOD) activity was measured using both an ELISA kit (Catalog No. CSB-E08555r, Cusabio Biotech Co., China) and the NADH oxidation method [39]. Glutathione peroxidase (GPx) activity was determined [40], and catalase (CAT) activity was assayed [41].

Phagocytic % and phagocytic index

By determining the percentage of macrophages in a random sample of 300 macrophages that house intracellular yeast cells and expressing the result as a percentage of phagocytic activity, phagocytosis was evaluated. The phagocytosis assay will be computed [42] using the following equations:

Phagocytic activity is calculated as yeast-containing macrophages divided by the total number of macrophages x 100.

Phagocytic index equals the number of yeast cells phagocytized divided by the number of phagocytic cells.

Statistical analysis

The One-Way Analysis of Variance (ANOVA) test was performed to statistically analyse the data. SPSS 14.0 (2006) was used to perform the Duncan test on data reported as mean ± standard error (SE). Statistical significance was defined as $p < 0.05$.

Results

Phenotypic and genotypic identification of the bacterial isolate

Aeromonas spp detected with an incidence rate of 92% of the bacteriologically tested samples. *Aeromonas* spp showed round, smooth colonies with 2-3 mm size and opaque green with a dark center on Aeromonas medium base (Fig. 1). Under the microscope, it revealed gram-negative, straight rods bacteria with rounded ends (bacilli to coccobacilli shape), motile by a single polar flagellum. Confirmation by molecular technique observed the existence of *A. hydrophila* 16S rRNA gene utilizing a specific primer for species identification yielding amplicon size 685 bp (Fig. 2).

Re-isolation of the inoculated bacterial isolate was obtained from the experimentally infected fish(G3,G4) and the culture and molecular examination of the re-isolated *A. hydrophyla* revealed the same phenotypic and genotypic characteristics of the inoculated one, while re-isolation of *A. hydrophyla* from G1,G2 show negative results.

Antimicrobial susceptibility findings

A surprisingly elevated proportion of resistance to diverse antimicrobial agents and development of MDR among the *A. hydrophila* strains was observed (Fig. 3 a). All isolated strains demonstrated 100% resistance to all used antibiotics with the exception of Gentamicin, they showed 66.6% resistance, and 33.3% were sensitive and for Neomycin, 75% of isolates were resistant, whereas 25% were intermediate (Table 3).

On the other hand, findings of disc diffusion technique used to determine the zone of inhibition after exposure of *A. hydrophila* to Se NPs showed inhibition zones ranged from11mm: 28mm diameter (Fig. 3 b).

Hematological results

The results, shown in Table (4), revealed a significant rise in total erythrocytic count, hemoglobin content, and packed cell volume percent in non-challenged groups supplemented with Se-NPs (group 2) compared to the control group. However, after *A. hydrophila* infection, there was a substantial decrease in RBC count, hemoglobin concentration, and hematocrit %, as well as a significant increase in MCV in group 3, compared to the control. Group 4, challenged with *A. hydrophila* and supplemented with Se-NPs, showed an improvement in all hematological parameters compared to the challenged non-treated group (G 3).

Moreover, the Se-NPs supplemented group (G2) showed significant increase in total leukocytic, neutrophilic, lymphocytic, and monocytic counts compared to the control group as recorded in Table (5). Infected group with *A. hydrophila* revealed changes in leukogram represented by a significant increase in total leukocytes, neutrophils and monocyte count with a significant decrease in lymphocytic count compared with groups 1 and 2. Eosinophils and basophils counts revealed no significant difference between all experimental groups

Biochemical results

The results in Table (6) demonstrated that the Se-NPs supplemented group (G2) had no significant differences in liver or kidney functions when compared to the control. Aeromonas-infected group (G3) demonstrated significant increases in ALT, AST, ALK, GGT activity, urea, creatinine, and uric acid levels, linked with a significant drop in total protein, albumin, and globulin levels compared to either the control group or the Se-NPs-treated group.

Oxidative stress responses

As shown in Table (7), SOD, CAT, and GPx activity were highest in G2 and lowest in G3 (infected control) when compared to control, although G4 exhibited considerably enhanced SOD, CAT, and GPx activity relative to G3 but did not reach the levels of G1 or G2.

Phagocytic Activity

Phagocytic percentage and phagocytic activity increased significantly in all experimental groups compared to the control, with the greatest levels in groups 3 and 4, as shown in Table (7).

Discussion

Fish is one of the most fundamental foods because of its great nutritional value, delicious flavor, and ease of digestion. However, it acts as a source of many harmful bacteria, particularly Aeromonas spp., which may pose public health risks. Fish bacterial contamination is blamed on a variety of sources, including water, soil, and handlers. Poor handling during catching, transporting, and freezing can lead to fish contamination from the environment. These bacteria are linked to a variety of human ailments, making aquaculture products a possible danger factor for customers [43].

The present study explored the prevalence of *Aeromonas* spp. in *Tilapia nilotica* fish in Egypt. High proportion (92%) of the examined fish contaminated with *Aeromonas* spp, perhaps during storage, transit or merchandising, these findings concur in some measure with studies conducted in Turkey [44] and Malaysia [45], in which *Aeromonas spp*. was isolated in a high percentage of 82.8% and 69% of fish samples respectively. The 16S rRNA gene provides an effective molecular identification and a quick technique to assess the identity of *A. hydrophila* [46].

The current study has proven the existence of MDR *Aeromonas spp*. Also, the high MDR indices of *Aeromonas spp* were detected in Nile tilapia broodstock [47].

A. hydrophila was highly resistant to the thirteen antimicrobial agents used in the present investigation. The isolates were 100% resistant to all used antibiotics (DO_{30}), ($SXT_{1.25\ /23.75}$), (CIP_5), (C_{30}), (E_{15}), (S_{10}), (K/30), (AX_{25}), (NA_{30}), (CT_{10} (FEP_{30}) , except for Gentamicin(CN_{10}), they showed 66.6% resistance and 33.3% were sensitive and Neomycin (N_{30}) 75% of isolates were resistant while 25% were intermediate, similar results were obtained by another study in Egypt. As *A. hydrophila* isolates were resistant to erythromycin and streptomycin in percentages of (100% each) but it Conflicts with our findings as it was sensitive to ampicillin (80%) and gentamicin (60%).

In the current study, results of the disc diffusion technique applied after exposure of *A. hydrophila* to Green selenium nanoparticles synthesized from mint leaf extract, Se NPs showed inhibition zones ranged from 11mm : 28mm diameter. Similarly, the Se NPs synthesized from Blumea axillaris plant inhibited *Aeromonas species* with an inhibition zone size of 1.8 cm (stem) and 2.6 cm(root). Also, it inhibited *A. hydrophila* (moderate), with inhibition zone size of 2.8 cm (stem) and 3.0 cm (root), whereas it was 1.0 cm (stem) and 1.5 cm(root) against *A. hydrophila* (virulent) y [48].

Se-NPs exhibited efficacy as antimicrobial agent at a concentration of 10000µg/ ml via disk diffusion technique, against *A. hydrophila* in our study. Also, antibacterial activity of SeNPs was confirmed with different concentrations (5000, 4000, 3000, 2000, 1000, 900, 800, 700, 600, 500, 400) µg/mL which inhibited the growth of many pathogenic Gram-negative and Gram-positive bacteria (*S. typhi, E. coli, P. aeruginosa,* and *S. aureus*) by agar well diffusion [49].

The antimicrobial action of Se-NPs was reliant concentration As the Se-NPs succeeded to displayed a high antimicrobial action against *B. subtilis* and *E. coli* at 100 µg mL^{-1} followed by concentrations of 75, 50, and 25 µg mL^{-1} [50]. In the same vein the greatest antibacterial activity of Se-NPs against *B. subtilis, S. aureus, P. aeruginosa, E. coli, C. albicans, C. tropicalis, C. glabrata,* and *C. parapsilosism* was gotten at the concentration of 400 µg mL^{-1} and lessened at low at 200 µg mL^{-1} [51].

Blood parameters can reveal important information about an organism's internal health. The current study discovered that Se-NP supplementation enhanced the blood indices of *O. niloticus* fingerlings. A prior study demonstrated enhanced haematology in juvenile common carp, indicating a better health profile for fish fed with Se-enriched diets [52]. Similarly, it was found that Se-NPs supplementation raised all hematological markers, which coincide with our results [53]. Additionally, Nile tilapia fed dietary Se-NPs (0.8mg/kg) had higher levels of HCT, Hb, RBCs, and WBCs than tilapia fed bulk-selenium or a control diet [54]. These findings are related to the regulation of metabolic rate induced by Se-NPs, as the higher the number of RBCs and Hb content, the greater the availability of oxygen in human tissues [55]. Selenium's potent antioxidant activity [56] may increase RBC membrane balance and life span by protecting them from damaging oxygen-free radicals that cause anaemia, avoiding membrane rupture, cell hemolysis, and degeneration, and ensuring erythrocyte health and integrity [57].

A. hydrophila infection resulted in a considerable decrease in RBCs count, Hb concentration, and Hct% which can be attributable to the hemolytic activity of β-hemolysin. This hemolysin can cause RBC lysis, resulting in anemia, as previously documented [58].

The substantial fall in haemoglobin levels reported after the infection could be owing to a higher rate of RBC breakdown by pathogenic bacteria and/or a slower rate of RBC production [59]. Hemoglobin content falls due to RBC enlargement and inadequate haemoglobin mobilization by the spleen and other haematopoiesis organs [30]. In the

current investigation, the considerable fall in hemoglobin following fish infection could be due to severe anaemia. The anaemic reaction could be caused by the loss of intestinal cells that produce vitamin B12, which is utilized in the formation of the haemoglobin portion of red cells, haemodilution, or disturbance in erythrocyte development [60]. Furthermore, septicemia caused by the infection may result in RBC lysis due to the action of bacterial enterotoxins. The observed variations in mean corpuscular volume (MCV) indicate erythrocyte swelling, which reflects macrocytic anemia. The increase in MCV could be attributable to erythrocyte enlargement caused by hypoxic circumstances, disturbed water balance, or osmotic stress [61].

In the current study, WBCs revealed an increasing trend in the Se-NPs supplemented group (G 2), which could be attributed to the fish's enhanced health profile, which includes an increased immune response and various cell-mediated physiological activities. These alterations were attributed to a powerful antioxidant component of selenium that increased the lifespan of blood cells [62]. In fact, diets enriched with Se-NPs significantly enhanced fish cell-mediated immunity by long-term Se supplementation, as seen by rising WBC numbers in fish [63]. The current study found that the number of neutrophils, lymphocytes, and monocytes increased considerably ($P < 0.05$) in group 2 compared to the control. The current findings are consistent with those of another researcher, who demonstrated that in *O. niloticus*, selenium alters and controls the expression of immune-regulated selenoprotein [18].

Leukocytes play a protective function against pathogenic bacterial infections by activating the nonspecific defense system. An increase in leukocyte cells promotes faster recovery from *A. hydrophila* infection [64]. Total leukocytes increased following *A. hydrophila* infection, indicating a fish body defense response against existing antigens [65]. This finding is consistent with prior research, which found that WBC levels rose in tilapia infected with *A. hydrophila* [58]. WBCs are the primary component of the body's front lines of defense, and their numbers rapidly increase when an infection occurs. Increased WBC count in sick fish may serve as a protective barrier against pathogenic infestation [66].

Fish injected with *A. hydrophila* had considerably fewer lymphocytes than those not infected ($P < 0.05$). Decreases in lymphocytes following *A. hydrophila* infection of the Nile tilapia has been linked to cell re-trafficking to lymphoid tissues, resulting in their clearance from the blood stream [67].

Tilapia's monocyte and neutrophil levels increased during the challenge test. This is the fish body's attempt to absorb antigens that enter it, as seen by an increase in the phagocytosis index following infection with *A. hydropila.* Neutrophils and monocytes are important components of the fish's innate immune system, especially in the elimination of foreign substances [68].

Neutrophils and monocytes are professional phagocytes that consume and destroy infections by phagocytosis [69]. They are engaged in the identification and clearance of foreign entities such as pathogens and cellular debris, which helps to maintain tissue integrity and homeostasis [70]. Fish have a sophisticated innate immune response, with neutrophils and macrophages playing critical roles in pathogen identification, killing, and the initiation of adaptive immune responses [71].

The increase in Hb concentration and RBC count in group 4 treated with Se-NPs could be attributed to Selenium antioxidant activity, which protects the Hb molecule and the erythrocyte membrane from oxidative damage while also restoring erythropoiesis [56]. The increase in leukocytic and lymphocytic counts may be attributed to the immunostimulant effect of Se-NPs, which promotes leukocyte proliferation, the antibacterial efficacy of selenium in neutralizing infection, and the preservation of the leukocyte redox status [72].

The current study found that Aeromonas infection in Nile tilapia caused significant changes in liver function biomarkers, as revealed by significantly higher serum ALT and AST levels in the infected group compared to the control and Se-NPs treated groups. This rise reflects hepatic damage and leaking of these enzymes into the bloodstream due to structural and functional degradation of the liver tissue, a common reaction to bacterial septicemia and endotoxin exposure [73]. The observed non-significant reduction in ALT, along with a significant decline in AST activity in group 4, suggests a hepatoprotective role of nano-selenium, likely mediated through its antioxidant capacity and free radical scavenging properties [9].

Similarly, serum ALP and GGT activities were significantly raised in infected fish, indicating cholestasis and biliary epithelial damage linked with Aeromonas-induced hepatic injury [74] Nano-selenium treatment considerably lowered both values, indicating enhanced hepatobiliary function and membrane stabilization, similar with prior studies on selenium-mediated protection in fish challenged with bacterial pathogens [75]

The protein profile analysis revealed that Aeromonas infection dramatically lowered total protein, albumin, and globulin levels, indicating poor protein synthesis and potential protein loss by tissue exudation during systemic inflammation [62]. Globulin reduction also suggests a suppressed humoral immune response. A decreased humoral immune response is also shown by a decrease in

globulin. Although values were still lower than those of uninfected controls, the addition of nano-selenium to infected fish greatly improved these parameters when compared to the infected group. The enhancement of immune cell function and protein production by selenium may be the cause of this improvement [76].

In terms of kidney function, fish infected with Aeromonas had higher levels of serum urea, creatinine, and uric acid, indicating renal impairment and decreased clearance capacity, most likely as a result of glomerular and tubular damage brought on by bacterial toxin [77]. Urea, creatinine, and uric acid levels were all markedly reduced after receiving nano-selenium, indicating nephroprotection via anti-inflammatory and antioxidant properties[78].

Antioxidant research revealed that infected fish had considerably lower levels of SOD, CAT, and GPx activities, indicating severe oxidative stress. This is consistent with the bacterial infection's increased production of reactive oxygen species (ROS), which overwhelms the body's built-in antioxidant defenses [4]. SOD, CAT, and GPx activity were all markedly restored by nano-selenium therapy. These results are consistent with the known function of selenium as a crucial part of selenium-dependent enzymes such as GPx, which aid in ROS detoxification and oxidative damage prevention [10]. Moreover, selenium nanoparticle-supplemented diets for European sea bass (*Dicentrarchus labrax*) reduce oxidative stress and increase the activity of oxidative enzymes [79]. Furthermore, when fish were exposed to pathogens that cause disease, the addition of selenium nanoparticles enhanced their cellular defenses against oxidative stress and increased their antioxidant defenses [80].

Selenium is a structural and functional component of GPx as well as a cofactor [81]. As a result, GPx activity may serve as a possible indicator of dietary Se supplementation and aid in the reduction of oxygen free radicals and detoxification of lipid hydroperoxides [82]. Furthermore, the use of dietary Se-NPs that promote glutathione formation could greatly reduce oxidative stress [83].

According to some studies, nanomaterials have gained popularity in fish farming because of their beneficial qualities (disease resistance and immunostimulant) and lower toxicity when compared to their organic and/or inorganic sources [84]. Furthermore, Se-NPs' immune-stimulant, antioxidant, bioavailable, and low toxicity properties may lessen the bacterial challenge in O. niloticus [85].

One of the key defense processes in the fish immune system is phagocytosis, which occurs when macrophages and neutrophils ingest and remove pathogens. Phagocytes are white blood cells that help the innate immune system engulf and remove invading particles. Phagocytes' ability to affect other immune components in the blood and tissue allows them to act as a link between innate and adaptive immunity. Fish phagocytes are mostly composed of macrophages, monocytes, granulocytes, and dendritic cells, but antibody-producing cells (B cells) have also been shown to have bactericidal and phagocytosis activities [86]. Fish phagocytes contribute to the engulfment of harmful bacteria, which reduces bacterial pathogenic movement, destroys pathogenic bacterial cells, and thereby reduces bacterial infection in the host [887].

Selenium is a component of selenoprotein, which can boost serum protein production, therefore taking Se-NPs as a dietary supplement may improve non-specific immunity [88].

The phagocytic index and phagocytic percentage were observed to significantly rise with Se-NPs supplementation in the present investigation. Our findings are supported by previous work [89], which reported an increased lysozyme level, pro-inflammatory cytokines (TNF-α and IL-1β), and macrophage activity after supplementing Nile tilapia with nano-Se. Dietary Se nanoparticles (1-2 mg/kg) improved serum lysozyme and respiratory burst activity in Nile tilapia. Additionally, Nile tilapia given dietary Se nanoparticles (1-2 mg/mg) demonstrated increased phagocytic and lysozyme activity, phagocytic index, globulin, and immunoglobulin M, according to [90]. Furthermore, the total serum protein globulin, phagocytic index, phagocytic, and lysozyme activity of European seabass were increased by dietary Se nanoparticles (0.5-1 mg/kg) [91].

Bacterial infections such as *A. hydrophila* can trigger an increase in phagocytic activity as a first line of defense against pathogen transmission in the fish body. Detection of pathogen-associated molecular patterns (PAMPs) by immune cell receptors results in the production of proinflammatory cytokines and the activation of phagocytes, which is the cause of this increase [92].

Conclusion

Overall, the findings indicate that nano-selenium supplementation in Aeromonas-infected Nile tilapia reduces hepatic and renal damage, improves protein metabolism, and boosts antioxidant levels. This protective benefit is most likely due to selenium's dual role in improving immunological function and combating oxidative stress, which supports organ function and overall health in diseased fish on the bacteriological level (Se NPs) exhibited excellent antibacterial activity for MDR *A. hydrophila* isolates, so it can considered as an appropriate and environment-friendly therapeutic measure. Finally, it recommended that additional research conducted on the antimicrobial susceptibility tests for *Aeromonas.*

Acknowledgments

Many thanks to A.M. Abdelghany, Professor of Applied Spectroscopy, Dean of Physics Research Institute, National Research Centre, Giza, Egypt for preparation selenium nano particles

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This work reviewed and approved by the ZUIACUC committee at the Faculty of Veterinary Medicine, Zagazig University, Egypt. Approval number: ZU IACUC/2/F/20/2025.

TABLE 1. Oligonucleotide primer sequences, its product size and cycling conditions during PCR [22].

Gene	Primers sequences	Product size (bp)	Amplification (35 cycles)				Final extension	Reference
			Primary Denaturation	Secondary denaturation	Annealing	Extension		
A.hydrophila 16S rRNA	GAAAGGTTGATGCCTAATACGTA CGTGCTGGCAACAAAGGACAG	685	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**22**

TABLE 2. Breakpoints of the disk diffusion methods used to determine the antimicrobial susceptibility of *Aeromonas spp.*

Antimicrobial agent	Symbol	Disc potency (µg)	Resistant S	Intermediate I	Sensitive R
Doxycyclin	DO	30	≥14	11-13	≤10
Trimethoprimsulfa- methoxazole	SXT	1.25 /23.75	≥19	-	<16
Ciprofloxacin	CIP	5	≥27	-	<24
Chloramphenicol	C	30	≥18	13–17	12
Gentamicin	CN	10	≥18	16–17	<16
Neomycin	N	30	≥23	13-16	<13
Streptomycin	S	10	≥15	12-14	≤11
Erythromycin	E	15	-	-	-
Kanamycin	K	30	≥18	14-17	≤13
Amoxicillin	AX	25	≥19	16–18	<16
Nalidixic acid	NA	30	≥20	15–19	<15
Colistin	CT	10	Not available	Not available	Not available
Cefepime	FEP	30	≥27	-	<24

zone diameter breakpoints, according to the Committee for Antibiogram of the French Society of Microbiology [26]

TABLE 3. Antimicrobial sensitivity test results of *A. hydrophila* obtained with disc diffusion method

Antimicrobial agent	Symbol	Disc potency (µg/ Disc)	Isolates %		
			S	I	R
Doxycyclin	DO	30	-	-	100
Trimethoprimsulfa- methoxazole	SXT	1.25 /23.75	-	-	100
Ciprofloxacin	CIP	5	-	_-	100
Chloramphenicol	C	30	-	-	100
Neomycin	N	30	--	25	75
Streptomycin	S	10	-	-	100
Erythromycin	E	15	-	-	100
Kanamycin	K	30	-	-	100
Amoxicillin	AX	25	-	-	100
Nalidixic acid	NA	30	-	-	100
Colistin	CT	10	-	-	100
Cefepime	FEP	30	-	-	100
Gentamicin	CN	10	33.3	_-	66.6

TABLE 4. Effect of Se-NPs supplementation on hematological variables of O. niloticus challenged with *A. hydrophila*

Group / Parameters	Group1(Control)	Group 2 (Se-NPs)	Group 3 (Infected control)	Group 4
RBCs X 10^6/cmm	2.78 ^{b} ± 0.10	3.36 ^{a} ± 0.15	1.72 ^{d} ± 0.14	2.14 ^{c} ± 0.09
Hb g/dl	6.28 ^{b} ± 0.21	7.43 ^{a} ± 0.24	5.02 ^{c} ± 0.16	5.84 ^{b} ± 0.19
Hct %	26.46 ^{b} ± 0.55	29.80 ^{a}± 0.44	22.64 ^{c} ± 0.38	24.56 ^{b} ± 0.62
MCV Fl	95.18 ^{c} ± 2.88	88.70 ^{c}± 2.63	131.62 ^{a}± 4.62	114.77 ^{b} ± 3.11
MCH Pg	22.59 ^{b}± 0.57	22.11 ^{b}± 0.45	29.18 ^{a} ± 0.59	27.28 ^{a} ± 0.61
MCHC g/dl	23.73^{a} ± 0.60	24.93 ^{a}± 0.63	22.17 ^{a} ± 0.57	23.77 ^{a} ± 0.65

* Values represent means ± standard error (n = 5). Means with distinct small letters in the same row show significant differences (P < 0.05).

TABLE 5. Effect of Se-NPs supplementation on leukogram of *O. niloticus* challenged with *A. hydrophila*

Group / Parameters	Group 1 Control	Group 2 Se-NPs	Group 3 Infected control	Group 4
WBCs X10^3/cmm	12.83 ^{c} ± 0.41	14.45 ^{b}± 0.36	16.88 ^{a} ± 0.42	14.92 ^{b} ± 0.40
Neutrophils X10^3/cmm	3.43 ^{d} ± 0.11	3.95 ^{c} ± 0.19	7.96 ^{a} ± 0.32	5.49 ^{b} ± 0.24
Lymphocytes %X10^3/cmm	6.82 ^{b} ± 0.22	7.66 ^{a} ± 0.26	5.47 ^{d} ± 0.21	6.24 ^{c} ± 0.16
Monocytes X10^3/cmm	1.63 ^{c} ± 0.04	1.85 ^{b} ± 0.05	2.36 ^{a} ± 0.07	2.15 ^{a} ± 0.08
Eosinophils X10^3/cmm	0.69 ^{a}± 0.03	0.71 ^{a} ± 0.04	0.79 ^{a} ± 0.07	0.73 ^{a}± 0.05
Basophils X10^3/cmm	0.26 ^{a} ± 0.03	0.28 ^{a} ± 0.02	0.30 ^{a} ± 0.03	0.31 ^{a} ± 0.02

* Values represent means ± standard error (n = 5). Means with distinct small letters in the same row show significant differences (P < 0.05).

TABLE 6. Effect of Se-NPs supplementation on biochemical parameters of *O. niloticus* challenged with *A. hydrophila*

Group / Parameters	Group 1 Control	Group 2 Se-NPs	Group 3 Infected control	Group 4 Aeromonas+ nano
ALT U/L	9.21^{b} ± 0.16	9.51^{b} ± 0.37	18.20 ^{a} ±. 2.534	14.77 ^{a} ± 0.99
AST U/L	101.91^{c} ± 1.22	102.96 ^{c} ± 0 .90	152.10 ^{a} ± 1.154	116.23 ^{b} ± 1.58
ALK U/L	11.10 ^{c} ± 0.53	12.20 ^{c}± 0.57	19.20 ^{a} ± 0.55	16.26 ^{b} ± 0.56
GGT U/L	1.17 ^{c} ± 0.117	1.16 ^{c}± .031	2.93 ^{a} ± 0.199	1.59 ^{b} ± 0.005
total protein g/dl	3.42 ^{a} ± 0.15	3.48 ^{a} ± 0.06	2.23 ^{c} ± 0.060	2.92 ^{b} ± 0.035
Albumin g/dl	1.92 ^{a} ± 0.14	1.95 ^{a}± .036	1.28 ^{c} ± 0.044	1.60 ^{b} ± 0.057
Globulin g/dl	1.50 ^{a} ± 0.048	1.53 ^{a} ± 0.046	0.95 ^{c} ± 0.018	1.32 ^{b} ± 0.014
Urea Mg/dl	0.88 ^{c} ± 0.020	0.84 ^{c} ± 0.026	1.72 ^{a} ± 0.133	1.20 ^{b} ± 0.057
Creatnine Mg/dl	0.28 ^{c} ± 0.014	0.26 ^{c} ± 0.015	0.57 ^{a} ± 0.018	0.40 ^{b} ± 0.005
Uric Acid Mg/dl	0.71 ^{c} ±.026	0.70 ^{c} ± 0.024	0.99 ^{a} ± 0.017	0.82 ^{b} ± 0.029

* Values represent means ± standard error (n = 5). Means with distinct small letters in the same row show significant differences (P < 0.05).

TABLE 7. Effect of Se-NPs supplementation on oxidative stress responses, phagocytic % and phagocytic index of *O. niloticus* challenged with *A. hydrophila*

Group / Parameters	Group 1 Control	Group Se-NPs	Group 3 Infected control	Group 4
SOD (U/mg protein)	51.29^{b}±0.55	88.6^{a}± 0.32	38.6^{d}± 0.32	47.63^{c}±1.06
Cat (U/mg protein)	2.62^{b}±.14518	5.49 ^{a}±0.44	1.04^{d}± 0.41	2.09 ^{c}±. 2.81
Gpx (U/mg protein)	61.10^{b}± 0.52	99.61^{a}±1.57	45.76^{d}± 1.2	55.26^{c}±2.59
Phagocytic %	49.68 ^{c} ± 0.82	54.82 ^{b}± 0.69	61.14 ^{a}± 0.73	60.25 ^{a} ± 0.98
Phagocytic index	1.83 ^{c} ± 0.13	2.51 ^{b} ± 0.16	3.94 ^{a} ± 0.14	3.76 ^{a} ± 0.18

* Values represent means ± standard error (n = 5). Means with distinct small letters in the same row show significant differences (P < 0.05).

Fig.1. *A. hydrophila* on Aeromonas Base medium: Opaque green colonies with a darker center

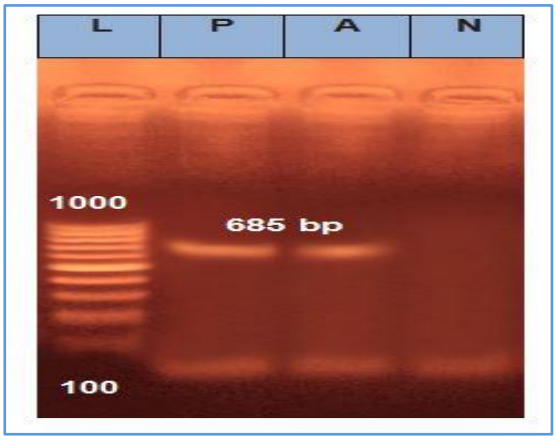

Fig.2. uniplex: 16s rRNA gene specific for *A. hydrophila* (685 bp)

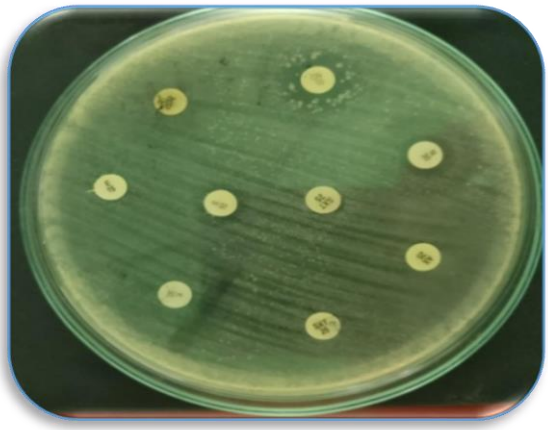

Fig. 3_a. *A. hydrophila* showing multidrug resistance via disc diffusion sensitivity test

Fig. 3. disc diffusion technique and zone of inhibition after exposure of *A. hydrophila* to Se NPs showing inhibition zones ranging from 11mm: 28mm.

References

1. Food and Agriculture Organization of the United Nations (FAO). The state of world fisheries and aquaculture. Rome: FAO. (2024). doi:10.4060/cc9930en.
2. El-Sayed, A. F. M**.** Tilapia culture. Academic Press. (2019). doi:10.1016/C2017-0-03902-4
3. Austin, B. and Austin, D. A. Bacterial fish pathogens: Disease of farmed and wild fish (6th ed.). *Springer*. (2016). doi:10.1007/978-3-319-32674-0
4. Li, M. H., Robinson, E. H., Tucker, C. S., Manning, B. B. and Khoo, L. Oxidative stress and fish diseases: The role of antioxidants. *Fish Physiology and Biochemistry*, **37**(1), 1–12 (2011). .doi:10.1007/s10695-010-9426-2
5. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G. and Hoekstra, W. G. Selenium: Biochemical role as a component of glutathione peroxidase. *Science*, **179**(4073), 588–590 (1973) doi:10.1126/science.179.4073.588
6. Huang, Q., Xu, W. and Zhang, Y. Effect of dietary selenium on growth, antioxidant status, and disease resistance of fish: *A review. Fish & Shellfish Immunology*, **33**(2), 324–331 (2012). doi:10.1016/j.fsi.2012.05.003
7. Wang, C., Lovell, R. T. and Payne, R. L. Bioavailability and safety of different selenium sources in aquaculture. *Aquaculture Research*, **51** (5), 1831–1844. (2020). doi:10.1111/are.14515
8. Zhou, X., Wang, Y. and Gu, Q. Effects of different dietary selenium sources (selenium nanoparticle and selenomethionine) on growth performance, muscle composition, and glutathione peroxidase enzyme activity in crucian carp (*Carassius auratus gibelio*). *Aquaculture*, **291**(1–2), 78–81. (2009). doi:10.1016/j.aquaculture. 2009.03.002
9. Salah, A. S., Mahmoud, S., El-Sayed, R. and Abdeen, A. Dietary nano-selenium alleviates oxidative stress and enhances disease resistance in fish. *Aquaculture*, **547**, 737468 (2022). doi:10.1016/j.aquaculture. 2021.737468
10. Guo, L., Li, J., Yang, X., Wang, X. and Xu, Z. Nano-selenium enhances antioxidant capacity and immune function in fish. *Aquaculture Reports*, **21**, 100900. (2021). doi:10.1016/j.aqrep.2021.100900
11. Abdelghany, A. M., Ayaad, D. M. and Mahmoud, S. M. *Antibacterial and energy gap correlation of PVA/SA biofilms doped with selenium nanoparticles*. *Biointerface Res. Appl. Chem***, 10**(5), (2020). doi:10.33263/BRIAC105.62366244
12. ElSheikh, S. K., Eid, E. S. G., Abdelghany, A. M. and Abdelaziz, D. Physical/mechanical and antibacterial properties of composite resin modified with selenium nanoparticles. *BMC Oral Health***, 24** (1), 1245. (2024).doi:10.1186/s12903-024-04965-5
13. Abdelghany, A. M., Soliman, H. A. and Khatab, T. K. Biosynthesized Selenium nanoparticles as a new catalyst in the synthesis of quinazoline derivatives in pentacyclic system with docking validation as (TRPV1) inhibitor. *Journal of Organometallic Chemistry* **944,** 121847 (2021). Doi:10.1016/j.jorganchem.2021.121847
14. Mohamed, A. A., Zaghloul, R. A., Abdelghany, A. M. and El Gayar, A. M. Selenium nanoparticles and quercetin suppress thioacetamide-induced hepatocellular carcinoma in rats: Attenuation of inflammation involvement. *Journal of Biochemical and Molecular Toxicology*, **36** (4), e22989 (2022). doi:10.1002/jbt.22989
15. Waly, A. L., Abdelghany, A. M., and Tarabiah, A. E. Study the structure of selenium modified polyethylene oxide/polyvinyl alcohol (PEO/PVA) polymer blend. *Journal of Materials Research and Technology*, **14,** 2962-2969. (2021) doi:10.1016/j.jmrt.2021.08.078.
16. Saad, S., Abdelghany, A. M., Abou-ElWafa, G. S., Aldesuquy, H. S., and Eltanahy, E. Bioactivity of selenium nanoparticles biosynthesized by crude phycocyanin extract of Leptolyngbya sp. SSI24 cultivated on recycled filter cake wastes from sugar-industry. *Microbial Cell Factories*, **23** (1), 211. (2024). doi:10.1186/s12934-024-02482-2
17. Zaghloul, R. A., Abdelghany, A. M., and Samra, Y. A. Rutin and selenium nanoparticles protected against

STZ-induced diabetic nephropathy in rats through downregulating Jak-2/Stat3 pathway and upregulating Nrf-2/HO-1 pathway. *European Journal of Pharmacology*, **933,** 175289. (2022). Doi10.1016/j.ejphar.2022.175289

18. Rathore, H.S. Murthy, S.K. Girisha, M.S. Nithin, S. Nasren, M.A.A. Mamun, T.G. Puneeth K. Rakesh, B.T.N and Kumar, M. Pai. Supplementation of nano-selenium in fish diet: Impact on selenium assimilation and immune-regulated selenoproteome expression in monosex Nile tilapia (Oreochromis niloticus) *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **240,** 108907 (2021). doi: 10.1016/j.cbpc.2020.108907

19. AlYahya, S.A, Fuad, A., Khalidah, S. Al-Niaeem, Bashar, A. Al-Sa'adi, Sarfaraz Hadi and Ashraf, A. Mostafa. Histopathological studies of experimental Aeromonas hydrophila infection in blue tilapia, *Oreochromis aureus. Saudi Journal of Biological Sciences*, 182–185 (2018). Doi:10.1016/j.sjbs.2017.10.019

20. Handfield, M., P. Simard, and R. Letarte Differential media for quantitative recovery of waterborne Aeromonas hydrophila. *Applied Environmental Microbiology* **62,** 3544-3547 (1996).

21. Nicky, B.J.C. Bacteria from fish and other aquatic animals. a *practical identification manual*, **106,** 83-116 (2004).

22. Gordon, L., Giraud, E., Ganière, G.P., Armand, F., Bouju-Albert, A., de la Cotte, N., Mangion, C. and Le Bri, H. Antimicrobial resistance survey in a river receiving effluents from freshwater fish farms. *Journal of Applied Microbiology*, **102**, 1167–1176. (2007).

23. Alghuthaymi, M. A. Antibacterial action of insect chitosan/gum Arabic nanocomposites encapsulating eugenol and selenium nanoparticles. *J. King Saud Univ. Sci.*, **34,** 102219 (2022). Doi:10.1016/j.jksus.2022.102219

24. Liu, C., Wang, L., Xu, H., Wang, S., Gao, S., Ji, X., Xu, Q. and Lan, W. "One pot" green synthesis and the antibacterial activity of g-C3N4/Ag nanocomposites. *Materials Letters*, **164**, 567-570, (2016)./doi:/10.1016/j.matlet.2015.11.072.

25. Humphries, R., Bobenchik, A.M., Hindler, J.A. and Schuetz, A.N.J.J. Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, *M100*., **59,** 10.1128/jcm 00213-00221 (2021). doi:10.1128/JCM.00213-21

26. Coyle, M,B. Manual of Antimicrobial Susceptibility Testing. Washington, D.C. *American Society for Microbiology*, (2004).

27. Brown, L.J. A new instrument for the simultaneous measurement of total hemoglobin percentage oxyhemo globin percentage carboxyhemoglobin percentage methemoglobin, and oxygen content in whole blood. *IEEE Trans. Biomed. Eng.*, **27,** 132–138 (1980).

28. Jain, N.C. *Schalm's veterinary hematology*, 4th ed., Lea & Febiger. (1986)

29. Coles, E. *Veterinary Clinical Pathology*. 4th Ed.W.B. Saunders Comp. Philadelphia, London and Toront (1986).

30. Lie, Ø., Evensen, Ø., and Sørensen, A. Study on lysozyme activity in some fish species. *Diseases of Aquatic Organisms* **6**(1), 1–5. (1989). doi.org/10.3354/dao006001

31. Young, D.S. Effects of disease on Clinical Lab. Tests, 4th ed. AACC (2001).

32. Persijn, J. P., van der Slik, W., and van Kamp, G. J. Serum creatinine and its measurement in clinical chemistry. *Journal of Clinical Chemistry and Clinical Biochemistry*, **9**(14), 421–427 (1976). Doi:10.1515/cclm.1976.9.14.421

33. Wenger, C., Kaplan, A., Pesce, A.J. and Kazmierczak, S.C. Alkaline phosphatase. In: Kaplan A, Pesce AJ, Kazmierczak SC, editors. *Clinical Chemistry: Theory, Analysis, and Correlation*. St. Louis (MO): The C.V. Mosby Company, p1094–1098 (1984).

34. Burtis, C.A., Ashwood, E.R., editors. *Tietz Textbook of Clinical Chemistry*. 3rd ed. Washington (DC): AACC Press, (1999).

35. Busher, J. T. Serum albumin and globulin. In H. K. Walker, W. D. Hall, & J. W. Hurst (Ed.), *Clinical Methods: The History, Physical, and Laboratory Examinations* (3rd ed., pp. 497-499). Boston: Butterworths. (1990).

36. Murray, R. L. Creatinine. Kaplan, A. et al. *Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton*, 1261-1266 and 418(1984).

37. Kaplan, A. Urea. In: Kaplan A, Pesce AJ, Kazmierczak SC, editors. *Clinical Chemistry: Theory, Analysis, and Correlation.* St. Louis (MO): The C.V. Mosby Company, pp. 1257–1260, 418, 437. (1984).

38. Fossati, P. and Prencipe, L. Serum Triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*, **26**(2)**,** 227–231. (1980). doi.org/10.1093/clinchem/26.2.227

39. Paoletti, F. and Mocali, A. Determination of superoxide dismutase activity by purely chemical system based on NAD (P)H oxidation. *Methods in Enzymology*, **186,** 209–220 (1990). doi.10.1016/0076-6879(90)86110

40. Paglia, D. E. and Valentine, W. N**.** Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of Laboratory and Clinical Medicine*, **70**(1), 158–169**.** PMID: 6066618**.** (1967).

41. Xu, J., Yuan, X., Lang, P., Dong, P. and Zhang, B. Determination of catalase activity and its application in food and biology. *Progress in Biochemistry and Biophysics*, **24,** 199–202 (1997).

42. Jensch-Junior, B., Pressinotti, L., Borges, J. and Silva, J. Characterization of macrophage phagocytosis of the tropical fish *Prochilodus scrofa* (Steindachner, 1881). *Aquaculture.* **251,** 509–515. **(**2006).

43. Cleveland, J. and Montgomery, K. Gut characteristics and assimilation efficiencies in two species of

herbivorous dam shellfishes. *Mar. Biol.* **142** (1), 35–44 (2008).

44. Yucel, N, Aslim, B and Beyatli, Y. Prevalence and resistance to antibiotics for *Aeromonas* spp isolated from retail fish in Turkey. *Int. J. Food Qual.*, **28** (4), 313**–**321 **(**2005).

45. Radu, S., Ahmad, N., Ling, F.H. and Reezal, A. Prevalence and resistance to antibiotics for Aeromonas spp from retail fish in Malaysia. *Int. J. Food Microbiol*., **81** (3), 261–266 (2003).

46. Hafez, A. E., Darwish, W. S., Elbayomi, R. M., Hussein, M.A.M. and El Nahal, S. M. Antibiogram and molecular characterization of aeromonas hydrophila isolated from frozen fish marketed in Egypt *Slov. Vet. Res.,* **20,** 445–54. (2018). DOI 10.26873/SVR-671-2018.

47. Sherif, A.H. and Kassab, A. S**.**: Multidrug-resistant Aeromonas bacteria prevalence in Nile tilapia broodstock., *BMC Microbiology*, **23,** 80 (2023). doi.10.1186/s12866-023-02827-8

48. Dash, J. P., Mani, L. and Kumar, S. Nayak: Antibacterial activity of Blumea axillaris synthesized selenium nanoparticles against multidrug resistant pathogens of aquatic origin, *Egyptian Journal of Basic and Applied Sciences*, **9,** 65-76. (2022). DOI: 10.1080/2314808X.2021.2019949

49. Khalaf, N. H., Hassane, A. M. A., El-Deeb, B. A. and Abo-Dahab N. F.: Antimicrobial Efficacy Mediated by Mycogenic and Characterized Selenium Nanoparticles *Sohag J. Sci.*, **9**(3), 255-260. (2024). doi.10.21608/sjsci.2024.254285.1161

50. Cittrarasu, V.: Green synthesis of selenium nanoparticles mediated from Ceropegia bulbosa Roxb extract and its cytotoxicity, antimicrobial, mosquitocidal and photocatalytic activities. *Sci. Rep.*, **11,** 1032. (2021). Doi:10.1038/s41598-020-80327-9

51. Nassar, A.A., Eid, A. M., Atta, H. M., El Naghy, W. S. and Fouda, A.: Exploring the antimicrobial, antioxidant, anticancer, biocompatibility, and larvicidal activities of selenium nanoparticles fabricated by endophytic fungal strain Penicillium verhagenii. *Scientifc Reports*, **13,** 9054 (2023). doi:10.1038/s41598-023-35360-9

52. Saffari, S., Keyvanshokooh, S., Zakeri, M., Johari, S.A., Pasha-Zanoosi, H. and Mansour, M.T. Effects of dietary organic, inorganic, and nanoparticulate selenium sources on growth, haemato-immunolog ical, and serum biochaemical parameters of common carp (*Cyprinus carpio*). *Fish Physiol, Biochaem.*, **44,** 1087–1097. (2018). doi:10.1007/s10695-018-0496-y

53. Hao, X., Q. Ling and F. Hong. Effects of dietary selenium on the pathological changes and oxidative stress in loach (Paramisgurnus dabryanus). *Fish Physiol. Biochem.*, **40** (5), 1313-1323 (2014). Doi:10.1007/s10695-014-9926-7

54. Ibrahim, D., Neamat-Allah, A. N., Ibrahim, S. M., Eissa, H. M., Fawzey, M., Mostafa, D. I., Abd El-Kader, S. A., Khater, S. and Khater, S. I. Dual effect of selenium loaded chitosan nanoparticles on growth, antioxidant, immune related genes expression, transcriptomics modulation of caspase 1, cytochrome P450 and heat shock protein and Aeromonas hydrophila resistance of Nile Tilapia (Oreochromis niloticus). *Fish Shellfish Immunol*, **110,** 91-99. (2021). doi:10.1016/j.fsi.2021.02.027

55. Parrino, V., Cappello, T., Costa, G., Cannavà, C., Sanfilippo, M., Fazio, F. and Fasulo, S.. Comparative study of haematology of two teleost fish (Mugil cephalus and Carassius auratus) from different environments and feeding habits. *Eur. Zool. J*., **85**(1), 193-199 (2018). doi:10.1080/24750263.2018.1460694

56. Khan, K. U., Zuberi, A., Nazir, S., Fernandes, J. B. K., Jamil, Z. and Sarwar, H. Effects of dietary selenium nanoparticles on physiological and biochaemical aspects of juvenile Tor putitora. *Turk. J. Zool.*, **40,** 704–712**.** (2016). doi:10.3906/zoo-1510-5 47.

57. Ashouri, S., Keyvanshokooh, S., Salati, A.P., Johari, S.A. and Pasha-Zanoosi, H. Effects of different levels of dietary selenium nanoparti cles on growth performance, muscle composition, blood biochaemical profiles and antioxidant status of common carp (Cyprinus carpio). *Aquaculture*, **446,** 25–29. (2015). doi.10. 1016/j.aquaculture.2015.04.021

58. Hardi, E.H., Kusuma, I.W. and Suwinarti W. Antibacterial activities of some Borneo plant extracts against pathogenic bacteria of Aeromonas hydrophila and Pseudomonas sp. Aquaculture, *Aquarium, Conservation and Legislation International Journal of the Bioflux Society*, **9**(3), 638–64 (2016).

59. Ayotunde, E.O., Offem, B.O. and Bekeh, A.F. Toxicity of Carica papaya lim: Haematological and piscicidal effect on Adult catfish (*Clarias gariepinus*). *Journal of Fisheries and Aquatic Science*, **6**(3), 291-308. (2011). doi:10.3923/jfas.2011.291.308

60. Omoregie, E. Changes in the haematology of the Nile Tilapia Orochronis niloticus under the effect of crude oil. *Hydrobiol*., **40**(4), 287-292 (1995)

61. Amphan S., Unajak S., Printrakoon C. and Areechon N. Feeding regimen of β-glucan to enhance innate immunity and disease resistance of Nile tilapia, Oreochromis niloticus Linn, against Aeromonas hydrophila and Flavobacterium columnare. *Fish & Shellfish Immunology*, **87,** 120- 128**.** (2019). doi:10.1016/j.fsi.2019.02.020

62. Biller-Takahashi, J. D., Takahashi, L. S., Mingatto, F. E. and Urbinati, E. C. The immune system is limited by oxidative stress: dietary selenium promotes optimal antioxidative status and greatest immune defense in pacu Piaractus mesopotamicus. *Fish Shellfish Immunol.,* **47,** 360–367**.** (2015). doi: 10.1016/j.fsi.2015.09.022

63. Takahashi, L. S., Biller-Takahashi, J. D., Mansano, C. F. M., Urbinati, E. C., Gimbo, R. Y. and Saita, M. V. Long-term organic selenium supple mentation overcomes the trade-off between immune and antioxidant systems in pacu (Piaractus mesopotamicus). *Fish Shellfish Immunol.*, **60,** 311–317. (2017). doi.org/10.1016/j.fsi.2016.11.060

64. Aluta, U.P., Aderolu, A.Z., Lawal, M.O. and Olutola, A.A. Inclusion effect of onion peel powder in the diet of African catfish, Clarias gariepinus: Growth, blood chemistry, hepatic antioxidant enzymes activities, and

SOD mRNA responses. *Sci African*, **12,** e00780. (2021) doi:10.1016/j.sciaf.2021.e00780

65. Kurniawan, R., Putri, M.N., Riswan, M., Wahyuni, S., and Mursawal, A.: Immunostimulant Effect of Chaetomorpha sp in Tilapia Infected with Aeromonas hydrophila. *Aceh Journal of Animal Science*, **10** (2), 64-69 (2025).

66. Talpur, A. D. and Ikhwanuddin, M. Azadirachta indica (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, Lates calcarifer challenged with Vibrio harveyi. *Fish Shellfish Immunol.*, **34**, 254–264. (2013). doi:10.1016/j.fsi. 2012.11.003

67. Harris, J. and Bird, D.J. Modulation of the fish immune system by hormones. *Vet Immunol. Immunopathol*, **77,** 163-176 (2000). doi:10.1016/s0165-2427(00)00235-x

68. Buchmann, K. Neutrophils and aquatic pathogens. *Parasite Immunology*, **44**(6). **(**2022). doi:10.1111/pim.12915

69. Haugland, G.T., Jordal, A.E.O. and Wergeland, H.I. Characterization of small, mononuclear blood cells from salmon having high phagocytic capacity and ability to differentiate into dendritic like cells. *Plos One*, **7**(11) (2012). Doi:10.1371/journal.pone.0049260

70. Birkert, A. The fish immune armaments in response to pathogen invasion-a tour inside the macrophages *Elsevier eBooks*, pp. 49-62 (2023).

71. Speirs, Z.C., Loynes, C.A., Mathiessen, H., Elks, P.M., Renshaw, A. and Jørgensen, L. What can we learn about fish neutrophil and macrophage responses to immune challenge from studies in zebrafish. *Fish & Shellfish Immunology*, **146,** 109490 (2024). doi:10.1016/j.fsi.2024.109490

72. Sarkar, B., Bhattacharjee, S., Daware, A., Tribedi, P., Krishnani, K. K. and Minhas, P. S. Selenium nanoparticles for stress-resilient fish and livestock. *Nanoscale Research Letters*, **10**(1), 1-1 (2015).

73. Abdel-Daim, M.M., Abdeen, A., Taha, R., Aleya, L., Bungău, S.G. and Najda, A. Protective role of dietary antioxidants against bacterial diseases in fish. *Fish Shellfish Immunol*. **98**, 158–166 (2020). doi:10.1016/j.fsi.2020.01.023

74. Harikrishnan, R., Balasundaram, C. and Heo, M.S. Immunomodulatory effect of herbal products against bacterial infection in fish. *Aquaculture* **317**(1–4), 110–116. (2011). doi:10.1016/j.aquaculture.2011.04.009

75. Wang, C., Lovell, R.T., Wang, Y., Li, X., Zhang, Y., Liu, Z., Zhao, P. and Chen, L. Nano-selenium supplementation improves hepatic health in fish under bacterial challenge. *Fish & Shellfish Immunology*. **102**, 233–241 (2020) doi:10.1016/j.fsi.2020.03.013.

76. Huang, Y., Zhou, J., Wang, Y., Wu, J., Zhang, C. and Li, Q. Selenium supplementation improves immune responses in fish. *Fish Physiology and Biochemistry*. **38**(3), 763–772 (2012). doi:10.1007/s10695-011-9564-7.

77. Mohamed, R.A. Renal pathology in fish due to bacterial infection. *Veterinary Pathology*,**46**(1), 82–89. (2009). doi:10.1354/vp.46-1-82.

78. Shi, .L, Xun, W., Yue, W., Zhang, C., Ren, Y., Shi, L. and Wang, Q. Selenium in aquaculture: Growth, immunity, and antioxidant capacity. *Reviews in Aquaculture*. **10**(4), 843–857 (2018). doi:10.1111/raq.12205.

79. Betancor, M. B., Caballero, M. J., Terova, G., Saleh, R., Atalah, E., Benítez-Santana, T., Hernández-Cruz, C. M., Izquierdo, M. S., and Montero, D. Selenium inclusion decreases oxidative stress indicators and muscle injuries in sea bass larvae fed high-DHA microdiets. *British Journal of Nutrition*, **108**(12), 2115–2128. (2012). doi:10.1017/S0007114512000376

80. Durigon, E.G., Kunz, D.F., Peixoto, N.C., Uczay, J. and Lazzari, R. Diet selenium improves the antioxidant defense system of juvenile Nile tilapia (*Oreochromis niloticus* L.). *Brazilian Journal of Biology*, **79**(3), 527–532. (2019). doi:10.1590/1519-6984.188597.

81. Kohrle, J., Brigelius-Flohe, R., Bock, A., Gartner, R., Meyer, O. and Flohe, L. Selenium in biology: facts and medical perspectives. *Biol. Chaem.*, **381**, 849–864. (2000) doi:10.1515/BC.2000.107

82. Watanabe, T., Kiron, V. and Satoh, S. Trace minerals in fish nutrition. *Aquaculture*, **151**(1–4), 85–207 (1997). doi:10.1016/S0044-8486(96)01503-7.

83. Hamed, G. M. S. and Selim, M. A. Protective effect of nano-selenium against experimentally induced toxicity by aflatoxin B1 (AFB1) on the gingiva and periodontal ligament of albino rats: histological and immunohistochemical study. *Egyptian Dental Journal*. **67**(1), 357–366. (2021). doi:10.21608/edj.2021.52706

84. Abdel-Tawwab, M., Samir, F., Abd El-Naby, A. S., and Monier, M.N. Antioxidative and immunostimulatory effect of dietary cinnamon nanoparticles on the performance of Nile tilapia (*Oreochromis niloticus* L.) and its susceptibility to hypoxia stress and Aeromonas hydrophila infection. *Fish & Shellfish Immunology*, **74**, 19–25 (2018). Doi: 10.1016/j.fsi.2017.12.033

85. Neamat-Allah, A. N., Mahmoud, E. A., and Abd El Hakim, Y. E. Efficacy of dietary nano-selenium on growth, immune response, antioxidant, transcriptomic profile, and resistance of Nile tilapia (Oreochromis niloticus) against Streptococcus iniae infection. *Fish & Shellfish Immunology*, **94**, 282–291 (2019). doi.10.1016/j.fsi.2019.09.019

86. Sunyer, J. O. Evolutionary and functional relationships of B cells from fish and mammals: insights into their novel roles in phagocytosis and presentation of particulate antigen. *Infectious Disorders Drug Targets*, **12**, 200–212 (2012). Doi: 10.2174/187152612800564403

87. Mansour, A.T.E., Goda, A.A., Omar, E.A., Khalil, H.S. and Esteban MÁ Dietary supplementation of organic selenium improves growth, survival, antioxidant and immune status of meagre, Argy rosomus regius, juveniles. *Fish Shellfish Immunol*, **68**, 516–524. (2017). doi:10.1016/j.fsi.2017.07.060

88. Murray, P.J., and Wynn, T.A. Protective and pathogenic functions of macrophage subsets. *Nature Reviews Immunology*, **11**, 723–733 (2011). Doi:10.1038/nri3073

89. Dawood, M. A. O. Evaluating the possible feeding strategies of selenium nanoparticles on the growth rate and wellbeing of European seabass (*Dicentrarchus labrax*). *Aquaculture Reports*, **18**, 100539 (2020). Doi10.1016/j.aqrep.2020.100539

90. Abu-Elala, N. M., Shaalan, M., Ali, S. E. and Younis, N. A. Immune responses and protective efficacy of diet supplementation with selenium nanoparticles against cadmium toxicity in *Oreochromis niloticus*. *Aquaculture Research*, **52**, 3677–3686 (2021). Doi:10.1111/are.15212

91. Abd El-Kader, M.F., Fath El-Bab, A. F., Abd-Elghany, M. F., Abdel-Warith, A.-W. A., Younis, E. M., and Dawood, M. A. O. Selenium nanoparticles act potentially on the growth performance, hemato-biochemical indices, antioxidative, and immune-related genes of European seabass (Dicentrarchus labrax). *Biological Trace Element Research*, **199**, 3126–3134 (2021). Doi:10.1007/s12011-020-02431-1

92. Pereira, C., Duarte, J., Costa, P., Braz, M., and Almeida, A. Bacteriophages in the control of Aeromonas sp. in aquaculture systems: an integrative view. *Antibiotics*, **11**(2), 163 (2022). Doi:10.3390/antibiotics11020163

دراسة تأثير جزيئات السيلينيوم النانوية على مقاومة الايروموناس في أسماك البلطي النيلي

صفاء المسلمي[1]، رندا خلاف[2]، عزة رفاعي[3]، سحر عبد الحميد[4] وأمنية العويسي[3]

[1] قسم الكيمياء الحيوية والسموم ونقص الأعلاف، معهد بحوث صحة الحيوان، مركز بحوث الزراعة، فرع الزقازيق، مصر.
[2] قسم الكيمياء الحيوية والسموم ونقص الأعلاف، معهد بحوث صحة الحيوان، مركز بحوث الزراعة، الدقي، الجيزة، مصر.
[3] قسم الجراثيم، معهد بحوث صحة الحيوان، مركز بحوث الزراعة، فرع الزقازيق، مصر.
[4] قسم علم الأمراض والباثولوجيا الإكلينيكية، معهد بحوث صحة الحيوان، مركز بحوث الزراعة، فرع الزقازيق، مصر.

الملخص

أحد أهم المسببات المرضية التي تصيب أسماك المياه الدافئة، *Aeromonas hydrophila* تعد بكتيريا وخاصة أسماك البلطي النيلي، حيث تؤدي إلى ظهور العديد من الأمراض البكتيرية. هدفت هذه الدراسة إلى *hydrophila* بحث القيود المرتبطة باستخدام المضادات الحيوية في علاج العدوى الناجمة عن) كخيار علاجي بديل. أجريت الدراسة في (Se-NPs بالإضافة إلى تقييم فعالية جسيمات السيلينيوم النانوية محافظة الشرقية بجمهورية مصر العربية، حيث أظهرت النتائج انتشاراً مرتفعاً لبكتيريا بنسبة 92% من العزلات البكتيرية، وجميعها أبدت مقاومة متعددة للمضادات الحيوية شملت 11 من أصل 13 مضاداً حيوياً تم اختبارها. تم تنفيذ تجربة تغذية استمرت 35 يوماً على 120 من أسماك البلطى النيلي، بجزيئات قُسمت إلى أربع مجموعات: مجموعة ضابطة سالبة، مجموعة تغذت على علائق أساسية مدعمة ومجموعة مصابه تغذت علي جزيئات*A. hydrophila* النانو سلينيوم ومجموعة ضابطة موجبة مصابه ب النانو سلينيوم. واظهرت النتائج ان العدوي أحدثت تغيرات في المؤشرات الدموية والبيوكيميائية، تمثلت في ارتفاع مستويات كل من اليوريا والكرياتينين وحمض (ALT, AST, ALP, GGT) إنزيمات الكبد البوليك، إلى جانب انخفاض البروتين الكلي، الألبومين، الجلوبيولين، وإنزيمات مضادات الأكسدة. وعلى النقيض، فإن إضافة جزيئات النانو سلينيوم إلى العليقة حسّنت بصورة ملحوظة هذه المعايير، من خلال تعزيز النشاط المضاد للأكسدة وتنشيط الاستجابة المناعية في الأسماك. علاوة على ذلك، أسهمت جزيئات النانو سلينيوم في التخفيف من التأثيرات المرضية الناتجة عن العدوى، مما يشير إلى إمكان استخدامها كخيار علاجي واعد للسيطرة على الأمراض البكتيرية في أنظمة الاستزراع السمكي.

الكلمات الدالة: سمك البلطي النيلي، جسيمات السيلينيوم النانوية، بكتيريا إيروموناس هيدروفيلا، أمراض الدم. مضادات الأكسدة، نسبة البلعمة.