**Iraqi Journal of Veterinary Sciences**

www.vetmedmosul.com

# Efficacy of copper sulfate and potassium permanganate in reducing bacterial infection and mortality in polycultured farmed fish at El-Menoufia, Egypt: A microbiological and environmental analysis

**A.A. Mosleh [1], A.I. El-Bialy[2], D.I. El -Zahaby[3], A.A. El-Saadany[4], A.N. Anter[5], and H.S. Khalefa[6]**

[1]Department of Microbiology, [2]Department of Bacteriology, [3]Department of Food Hygiene, Animal Health Research Institute, Shebin El-Kom, [4]Researcher of Bacteriology, Animal Health Research Institute, Tanta branch, Tanta, [5]Department of Bacteriology, Faculty of Veterinary Medicine, Benha University, Benha, [6]Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

**Article information**

***Article history:***
Received 02 May 2025
Accepted 13 August 2025
Published 22 August 2025

***Keywords***:
*Pseudomonas* spp
*Oreochromis niloticus*
$CuSO_4$
$KMnO_4$

***Correspondence:***
Hanan S. Khalefa
hanansaad04@gmail.com

**Abstract**

Managing fish health effectively requires understanding bacterial coinfections and environmental factors. The effects of copper sulfate and potassium permanganate on bacterial infection in naturally infected farmed fish obtained from private fish farms in Wadi El-Natrun, El Menoufia, Egypt, with high mortality rates have been evaluated. Forty *Mugil capito* and seventy-four *Oreochromis niloticus* were randomly selected from these farms. One pure isolate, including *E. coli* (n=11), *Enterobacter* spp. (n=5), *Klebsiella* spp. (n=5), *Salmonella* spp. (n=3), *Pseudomonas* spp. (n=12), *Aeromonas* spp. (n=2), and *Enterococcus* spp. (n=13), was recovered from the fish samples. *Enterococcus* spp., *E. coli*, and *Pseudomonas* were predominant in both fish species. Compared with other farms, Farms 4 and 5 presented higher frequencies of *Pseudomonas* spp., *Enterobacteriaceae*, and *E. coli* infections in clinically affected fish ($\chi 2$ = 6.02, $p$ = 0.031). Significant differences were observed between farms ($\chi 2$ = 9.37, $p$ = 0.024). These isolates were further identified via *16S rRNA* gene sequencing and phylogenetic analysis. A water analysis revealed that the phosphate, sulfate, hardness, and chloride levels were above permissible limits on farms 4 and 5. During this study, copper sulfate ($CuSO_4$) and potassium permanganate ($KMnO_4$) were applied at 24-hour intervals for seven days, and their effects on bacterial loads, coliform counts, and mortality in morbid Nile tilapia were examined. The pretreatment samples served as a control group. $CuSO_4$ and $KMnO_4$ effectively reduced the bacterial load and mortality rates. Compared with untreated fish, those treated with $CuSO_4$ or $KMnO_4$ had survival rates of 80.0% and 70.2%, respectively.

DOI: 10.33899/ijvs.2025.159766.4272, 

## Introduction

Globally, fish farming has the potential to alleviate nutritional deficiencies and reduce poverty. Egypt and Nigeria are the leading aquaculture countries in Africa because of their comparable production methods (1). Egypt's aquaculture industry produces approximately 1.8 million tons of fish annually (2), with Nile tilapia accounting for approximately 65.15% of the country's fish production (3). Mullets are the second most farmed fish species after Nile tilapia in brackish water (4), where tilapia, carp, and mullets are commonly raised together in polycultured earthen ponds (5). The aquatic environment of Egypt is frequently contaminated by agrochemicals, sewage discharge, and declining water quality standards, all of which contribute to outbreaks of bacterial illnesses among fish species (6,7). Fish

mortality hinders industrial growth and reduces fish production, severely impacting aquaculture (6). Three key factors facilitate disease outbreaks in fish farms: A) fish are raised at higher densities than in the wild, increasing fish-to-fish contact; B) infected fish are not removed as efficiently as they would be by natural predators; and C) farmed fish are more accessible for observation than are wild fish (8). Consequently, pathogens are typically present in small quantities and are nonpathogenic in the wild, which may induce illness in aquaculture systems (9). Significant interconnections exist among hosts, pathogens, and environmental variables. In aquaculture, unfavorable environmental conditions and insufficient abiotic stress mitigation weaken organisms' immune systems, increasing their vulnerability to disease outbreaks (10). Co-infecting pathogens may interact synergistically or antagonistically (5). Abdel-Moneam *et al.* (6) and Khalefa *et al.* (10) noted that synergistic interactions occur when one pathogen suppresses the host's immune response or establishes a point of entry, allowing another pathogen to invade. Such concurrent infections exacerbate disease severity, particularly bacterial infections, and delay diagnosis and treatment, increasing mortality rates (11). Nair *et al.* (12) identified *Escherichia coli*, *Pseudomonas putida*, *Salmonella* sp., *Shigella* sp., *Staphylococcus aureus*, *Enterobacter*, and *Enterococcus faecalis* as the most common bacterial isolates from infected fish skin samples. Several factors contribute to fish mortality, including overcrowding, abrupt temperature fluctuations, poor water quality, and other environmental factors (13). The disease can be attributed primarily to inadequate management and environmental stressors (6). Aquaculture ponds are treated with a variety of disinfectants to reduce disease-causing parasites and pathogenic bacteria (14). However, limited research has demonstrated the effectiveness of $CuSO_4$ and $KMnO_4$ as bactericides for treating bacterial illnesses in fish (15). $KMnO_4$ is frequently used in aquaculture to increase fish health, with its application in baths being a common treatment for aquatic diseases (16,17). According to Darwish *et al.* (18), $CuSO_4$ at 2.1 mg/L (1% total alkalinity) effectively prevented experimental columnaris infections in channel catfish during the early stages of the disease.

The aim of this study was to identify bacterial pathogens responsible for summer mortality syndrome in farmed fish during the summer season in Wadi El-Natrun, El Menoufia, Egypt, via molecular techniques. Additionally, this study aimed to evaluate the efficacy of $KMnO_4$ and $CuSO_4$ treatments in controlling mixed bacterial infections in O. niloticus through a clinical field trial.

## Materials and methods

### Ethics statement

The Research Ethics Committee of Cairo University's Faculty of Veterinary Medicine (Vet CU 18042024939) approved the study protocol following the relevant regulations and guidelines.

### Study area

Wadi El-Natrun is an elongated depression located 23 m below sea level in the western desert of Egypt and is situated between latitudes 30°17′N and 30°19′N and longitudes 30°10′E and 30°25′E. Extending approximately 60 km from north to south. During the summer season of 2023, a field survey was conducted at five fish farms in Wadi El-Natrun, El Menoufia, Egypt, with five fish farms beginning in August and persisting through October (Figure 1).


Figure 1: Satellite map from Google Earth showing various fish farms located in the study area. Wadi El- Natrun, El-Menoufia, Governorate, Egypt (red circles).

### Water quality measurements and sampling:

Three water samples were taken from each investigated farm. The samples were collected in sterile 500-mL glass containers for bacteriological examination and in 2-L bottles for physiochemical analysis. The parameters (temperature, pH, and dissolved oxygen) were analyzed in situ via a precalibrated probe (Yellow Springs Comp., Ohio, model 33, and oximeter model DO200A EcoSense®), whereas the total hardness and concentration of various ions present were measured following the standard protocol from the American Public Health Association (19). EDTA titrimetric, argentometric, phenate, and calorimetric methods were employed to assess the total hardness and chloride (Cl), ammonia ($NH_3$), nitrate ($NO_3$), and nitrite ($NO_2$) levels. The gravimetric method was used to measure the sulfate ($SO_4$) concentration. The stannous chloride method was used to detect phosphorus.

### Microbiological analyses of water samples

The microbiological testing of the water samples was conducted following the guidelines of the APHA (20). The standard presumptive examination employed the multiple-tube fermentation technique with lauryl tryptone broth (Biolife, Italy) to determine the most likely number

(MPN/100 mL water) of total coliforms. Positive presumptive tubes (gas production within 24-48 hours) were transferred to 2% brilliant green bile lactose broth (Difco, Thermo Fisher Scientific) for the MPN confirmation procedure, and gas production was monitored within 48 hours at 35°C. The total bacterial count (TBC) of the water samples was determined via the poured plate method. Following serial dilutions of the water samples, 1 mL was transferred in triplicate to sterile glass Petri dishes. Approximately 15 mL of liquefied nutrient agar medium was added to each plate, mixed, and allowed to solidify. The dishes were incubated at 30°C for 48 hours. The mean value was calculated by counting the number of colonies produced per plate at the same dilution after incubation, and the results are reported as the mean (log10) (21). Further isolation of a representative number of the different colony types detected on the media was achieved by streaking 0.1 mL of each of the last three dilutions of water samples onto selective media. These included Eosin Methylene Blue (EMB; HIMEDIA Laboratories, India), Xylose Lysine Deoxycholate (XLD; Oxoid), and *Aeromonas*-selective agar supplemented with rehydrated ampicillin (HIMEDIA, India) (6). KF *streptococcal* agar media (21), and *Pseudomonas* agar (Oxoid) with *Pseudomonas* CN supplement (Oxoid) (6). These media were used to isolate *E. coli*, *Salmonella* spp., *Aeromonas* spp., *Enterococcus* spp., and *Pseudomonas* spp., respectively. The plates were incubated at 37°C for 24 hours for the detection of bacterial colonies. To obtain pure colonies, the dominant colonies were selected and streaked again on tryptic soy agar (TSA) plates. For further molecular identification, the pure culture was stored at -20°C as a glycerol stock.

**Fish sampling and clinical examination**

A cross-sectional survey approach was used to investigate the prevalence of pathogenic bacteria in five private fish farms in Wadi El-Natrun, El Menoufia, Egypt, and their associations with fish diseases. A total of 114 fish were randomly selected from these farms: 40 *M. capito* (200-220 g) and 74 *O. niloticus* (150-200 g). These farms recorded daily mortality cases (approximately 50-100 fish per day) during the summer of 2023. Diseased fishes displayed patterns of symptoms such as pronounced eye opacity, edemas or hemorrhagic skin irritation. Postmortem (PM) lesions and clinical indicators were analyzed via techniques previously published by Austin and Austin (22). Postmortem examination of the infected fish revealed congestion in the liver and kidney. The fish muscles, internal organs, and tissues were carefully dissected to isolate the microorganisms. The moribund fish were recently housed in tanks with aerators before being transferred to the laboratory.

**Bacteriological examination of fish**

The aseptic culture of liver, skin, and muscle tissue (below the dorsal fin) from the moribund fish samples was followed by subculturing onto TSA and incubating for 24 hours at 30°C. Striking on both general and selective media was useful in purifying a representative number of colony types. The pure colonies were transferred to different media, such as those used for water-based bacterial isolation. The cultures were then incubated at 30°C for 48 hours. Afterward, the bacterial colonies were streaked onto TSA, and all the isolates were identified via biochemical methods. Quinn *et al.* (23) described the phenotypic approaches (motility, Gram staining, and morphology) used to identify the most dominant colonies in the subculture. The pure colonies were stored at -80°C in TSB broth with 20% glycerol for subsequent examinations.

**Serological identification of *E. coli* and Salmonella**

At the serology unit of the Animal Health Research Institute in Egypt, the isolates were serologically identified following the methods of Kok *et al.* (24) via quick diagnostic *E. coli* antisera sets (Serological identification of E. coli was performed by slide agglutination test by using o polyvalent sera and corresponding monovalent one sifin diagnostic gmbh Berlin Germany) for the diagnosis of enteropathogenic types. *Salmonella* species were serologically identified via *Salmonella* Mast Assure antisera (Mast group Ltd., UK) according to ISO6579:2014 part 3 via the Kauffman le minor scheme (25) for the determination of somatic (O) and flagellar (H) antigens.

**Bacterial DNA extraction**

The bacterial DNA was extracted via a QIAamp DNA Micro Kit (Qiagen, Catalog no. 51304, USA) following the manufacturer's instructions. In brief, 200 µL of the sample suspension was incubated with 10 µL of proteinase K and 200 µL of lysate buffer at 56°C for 10 minutes. After incubation, the lysate was rinsed and centrifuged, and 200 µL of 100% ethanol was added. The nucleic acid was then eluted with 100 µL of elution buffer and stored at ~20°C for subsequent use in the PCR steps (26).

***16S rRNA* PCR, sequencing, and phylogenetic analysis**

The universal primers designed by Hall *et al.* (27) were used for analysis: F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and R: 5′-GGTTACCTTGTTACGACTT-3′. A 50 µL reaction mixture containing Maxima Hot Start PCR Master Mix (Thermo Fisher Scientific, Waltham, MA) was used for PCR. The cycling conditions were detailed by Abdelsalam *et al.* (6). A GeneJET PCR Purification Kit (Thermo K0701) was used to purify the amplicons. The amplified *16S rRNA* gene segments of four selected bacterial isolates were analyzed via the ABI 3730xl DNA sequencing machine. After the sequences were obtained, editing and assembly were performed via BioEdit version 7.0 (27). Each isolate's final *16S rRNA* gene sequence was compared to previously published sequences via the National Center for Biotechnology Information (NCBI) BLAST tool.

**Antibiogram testing of the pathogenic bacterial strains**

The in vitro antimicrobial sensitivity of bacteria to six different classes of antibacterial agents was determined via the Kirby-Bauer disc diffusion method, according to Bauer *et al.* (28). The organisms were incubated at 30 ± 2°C for 24 hours. Then, according to the Clinical and Laboratory Standards Institute (CLSI) (29), the strains were categorized as sensitive, moderate, or resistant on the basis of the widths of the inhibitory zones around the discs. The selected antimicrobial discs, obtained from Bioanalyze® (Ankara, Turkey), contained the following: amoxicillin (AML, 25 µg), ciprofloxacin (CIP, 5 µg), norfloxacin (NOR, 10 µg), doxycycline (DO, 30 µg), streptomycin (S, 10 µg), and trimethoprim (Ts, 5 µg).

**Fish treatment trial and experimental design**

Sixty diseased *O. niloticus* (100 g ± 5.0) were randomly selected from the private farms under investigation and used as an experimental model to determine the inhibitory effects of $KMnO_4$ and $CuSO_4$ on the isolated bacteria. The fish were kept in aquarium tanks measuring 70 × 40 × 40 cm. The fish were randomly divided into three treatment groups, with two replicates for each treatment: (i) the untreated control group, (ii) the $KMnO_4$-treated group, and (iii) the $CuSO_4$-treated group, as shown in Figure 2. The dose was administered over seven days, as described by Darwish *et al.* (18), to achieve final concentrations of 2.1 mg/L copper sulfate and 2.5 mg/L $KMnO_4$ in the respective treatment tanks. The experiment was conducted in tanks with adequate aeration, the water temperature was standardized to approximately 27°C, the dissolved oxygen concentration was approximately 7.00 mg/L, and half of the water was exchanged twice a week to maintain the water quality. The fish were examined daily for signs of infection, and those that appeared moribund were removed for necropsy. During the treatment period, the fish were fed twice daily.

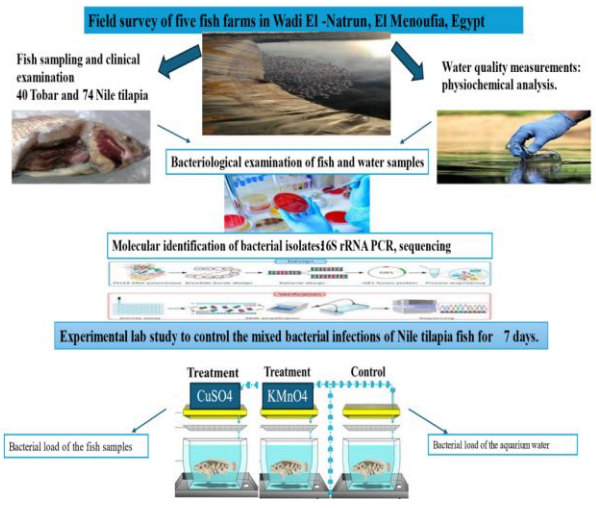


Figure 2: Diagram illustrating the setup for the study.

**Treatment effectiveness assessment**

On days 1, 4, and 7, water and fish samples were collected from each group via a 20-mL sterile glass container and sterile polyethylene bags. The first, second, and third samplings were assigned to these samples in that order. After the last treatment, five fish were randomly selected from each aquaria to assess the impact of the treatment on the general health of the fish. As previously described, the bacteria were reisolated on specific media and counted via the drop-plate method in triplicate to determine the total bacterial count. The mortality rate of the fish was evaluated throughout the treatment period.

**Statistical analysis**

A chi-square test (SAS software) was applied to examine the null hypothesis of multiple farms, with *p* values less than 0.05 indicating a significant difference between the observed parameters. The Shapiro–Wilk test was used to determine whether the data were normally distributed or evenly distributed. One-way ANOVA with Tukey's post hoc test was used to analyze the data for the in vitro experiment in which $KMnO_4$ and $CuSO_4$ were used to control the isolated mixed bacteria. In addition to the Kruskal–Wallis test, which evaluates the density of bacteria in fish muscle tissue and the water quality variables, the Mann–Whitney U test was used to evaluate differences between the groups. SPSS version 18.0 (SPSS Inc., Chicago, IL) was used to analyze all the data, with a statistical threshold set at $p< 0.05$ for all the tests. The findings are expressed as the mean ± SE. Significant differences were determined via the least significant difference test.

**Results**

**Field investigation, clinical examination, and necropsy findings**

During the study, a field assessment revealed substantial fish mortality rates in the fish farms in Wadi El-Natrun, El Menoufia, Egypt. Figure 3 depicts the mortality of the investigated fish farms due to bacterial pathogen coinfection. Clinical examination of *O. niloticus* revealed signs of disease, including rot and patches in the gills. Following necropsy, the postmortem lesions revealed congested gills. Moreover, the abdominal cavity contained a significant volume of red ascitic fluid (Figure 4, A-B). Similarly, most *M. capito* infections (Figure 4C) presented exophthalmia, detached scales, ascites, and mild fin erosion. Additionally, postmortem examination of the infected fish revealed serous fluid accumulation in the stomach cavity and congestion in the gills, liver, and kidney (Figure 4D).

**Water quality**

Table 1 summarizes the quality parameters of the pond water collected from the fish farms during the study period. The water parameters evaluated were all within the

acceptable range except for a decrease in the dissolved oxygen concentration (2.8-3.25 mg/L) and an increase in the ammonia (0.7-1.15 mg/L) and nitrite (7-9 mg/L) concentrations. Farms 4 and 5 also had phosphate, sulfate, hardness, and chloride levels that exceeded the recommended acceptable limits.

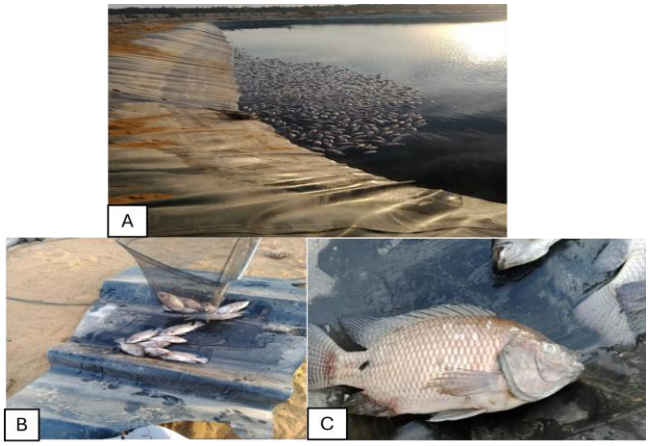


Figure 3: (A-B) Mass mortality in the fish farms under investigation due to coinfection with bacterial pathogens. (C) Diseased *O. niloticus* displayed patterns of symptoms such as pronounced eye opacity, edemas or hemorrhagic skin irritation.

The samples obtained from farms 4 and 5 had the highest bacterial counts, as indicated by the bacterial water analysis in Table 2. In this investigation, Pseudomonas spp. were isolated from farms 2, 4, and 5. The isolation of E. coli and Enterococcus spp. was limited to farms 4 and 5. Salmonella was isolated from only one farm. Farm 5 had the highest total bacterial count (TBC) concentration (7.38 log10 CFU/100 mL) of all the water samples. Total coliforms were detected only on farms 4 and 5, with numbers ranging from 12 to 15 MPN/100 mL. The MPN values of the coliforms in the water samples were within the permissible range for freshwater that can be used for irrigation and fish farming (1000 MPN/100 mL).

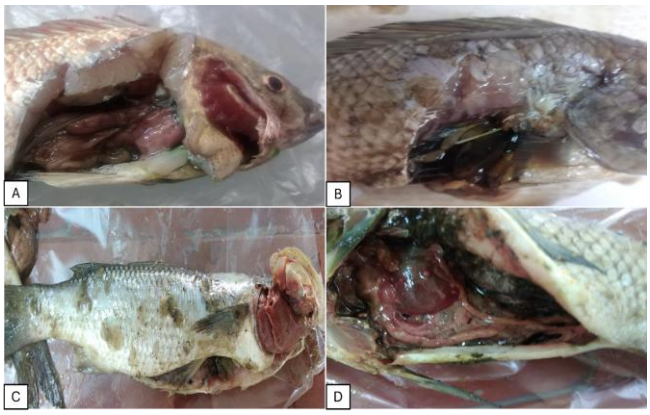


Figure 4. (A-B) Naturally, infected *O. niloticus* presented hemorrhagic and necrotic gills with ulcerative skin lesions. Infected *M. capito* (Figure 4 C) displayed congested gills, ascites, and detached scales (Figure 4 D). Postmortem examination of the infected fish revealed congestion in the liver and kidney.

Table 1: Physical-chemical water analysis (mean ±SE) of five studied fish farms in Wadi El-Natrun, El Menoufia, Egypt, during summer 2023

| | | Location of water samples | | | | |
|---|---|---|---|---|---|---|
| Water parameters | Permissible limits* | Farm 1 | Farm 2 | Farm 3 | Farm 4 | Farm 5 |
| Temp. (◦C) | 20-25 | 29.1 | 29.5 | 31 | 32.1 | 31.7 |
| Dissolved $O_2$ (mg/L) | 5 | 5 | 5.5 | 5.2 | 2.8 | 3.25 |
| pH | 6.5-8.5 | 7.53 | 7.11 | 7.11 | 7.22 | 7.91 |
| Ammonia | 0.05-0.5 mg/l | 0.24 | 0.32 | 0.15 | 1.15 | 0.70 |
| Nitrite (mg/L) | 0.01-0.03 mg/l | 0.02 | 0.01 | 0.02 | 0.02 | 0.025 |
| Nitrate (mg/L) | 2-5 mg/l | 4 | 5 | 9 | 9 | 7 |
| Hardness (mg/L) | >300 mg/l considered hard water | 240 | 275 | 250 | 295 | 310 |
| Chloride (mg/L) | 250 mg/l | 170 | 190 | 200 | 400 | 320 |
| phosphate | 0.5-0.7 mg/l | 0.22 | 0.21 | 0.53 | 0.90 | 1.30 |
| Sulphate | 200 mg/l | 145 | 140 | 120 | 170 | 180 |

*Permissible limits according to Boyd (35).

**Bacteriological examination of fish**

Table 3 displays the total bacterial count (CFU/g) and total coliform count (TCC/g) of the fish muscle samples from the farms under investigation, with samples from muscular tissue from farms 4 and 5 being the highest. Table 4 shows the analysis of the fish samples, with most of the samples being contaminated. One pure isolate was identified from both fish species (*O. niloticus* and *M. capito*) via the

biochemical profile of the recovered isolates. These isolates included 11 *E. coli*, 5 *Enterobacter*, 5 *Klebsiella*, 3 *Salmonella*, 12 *Pseudomonas*, 2 *Aeromonas*, and 13 *Enterococcus* spp.; *E. coli* and *Pseudomonas* were the most common bacteria in both fish species. Farms 4 and 5 had significantly more *Pseudomonas* spp. and *Enterobacteriaceae* infections in fish showing symptoms and *E. coli* ($X^2$ = 6.02, $p$ = 0.031) than did the other fish farms. Significant differences were observed between farms ($X^2$ = 9.37, $p$ = 0.024). The fish samples from the fishponds presented several illnesses or a high degree of contamination.

Table 2: Microbiological analysis of water samples of five studied fish farms in Wadi El-Natrun, El Menoufia, Egypt, during summer 2023

| Bacteriaial isolation | Unit | Site | | | | |
|---|---|---|---|---|---|---|
| | | Farm 1 | Farm 2 | Farm 3 | Farm 4 | Farm 5 |
| Total colony count | log10 CFU[a]/mL | 5.31 | 5.45 | 5.30 | 7.30 | 7.38 |
| Total Coliform | MPN[b]/100 mL | 0.00 | 0.00 | 0.00 | 12 | 15 |
| *E. coli* | log10 CFU/100 mL | 0.00 | 0.00 | 0.00 | 2.53 | 2.87 |
| *Salmonella* spp. | log10 CFU/mL | 0.00 | 0.00 | 0.00 | 0.00 | 2.11 |
| *Aeromonas* spp. | log10 CFU/mL | 0.00 | 0.00 | 0.00 | 2.34 | 0.00 |
| *Pseudomonas* spp. | log10 CFU/mL | 0.00 | 2.23 | 0.00 | 2.34 | 3.23 |
| *Enterococcus* spp. | log10 CFU/mL | 0.00 | 0.00 | 0.00 | 3.23 | 2.76 |

[a] CFU: colony-forming unit. [b]MPN: most probable number.

**Serotyping *E. coli* and *Salmonella* isolates**

Table 5 shows the serotyping of the *E. coli* and *Salmonella* isolates. Serotyping was performed for the *E. coli* isolates, which included O26:K60, O86:K61, O55:59, O103:K-, O114:K90, autoagglutination, O128:K67, O126:K71, O44:K74, and O25:K11. The *Salmonella* isolates were all identified as *Salmonella enteritidis*.

Table 3: Total Bacterial Count TBC (CFU/g) and Total Coliform Count TCC (CFU/g) of fish-muscle samples in the studied fish farms (Mean ± SE) in Wadi El-Natrun, El Menoufia, Egypt, during summer 2023

| Site | TBC | TCC |
|---|---|---|
| Farm 1 | 4.62±1.03 [b] | 3.20±1.04[b] |
| Farm 2 | 4.20±1.02[c] | 3.14±1.02 [b] |
| Farm 3 | 4.77±0.09[b] | 3.60±1.40 [a] |
| Farm 4 | 5.30±1.12 [a] | 3.77±1.15 [a] |
| Farm 5 | 5.34±1.15[a] | 3.69±.08[a] |
| $p$ value | 0.001* | 0.05* |

SE: standard error *The result is significant at $p$ <.05. [a, b, c] Different letters in the same column indicate a significant difference in count between different farms.

**Molecular identification of bacterial pathogens**

BLAST analysis of the *16S rRNA* gene sequences from the four bacterial strains responsible for fish diseases revealed the isolation of *E. coli*, *E. faecium*, *E. faecalis*, and *Pseudomonas* from water samples and fish. The *E. coli* strain exhibited 100% identity with accession number ON126162. The *E. faecium* strains presented 99% identity with MK332163, which was isolated from Qualifiers, and 89% identity with other strains from fermented food and meat in China, including MK332163, MF424510, MF108290, and MZ229662. The *E. faecalis* strain (PP759381), which was isolated from infected fish, showed 100% identity with two *Enterococcus* species (OK271787 and MZ229662) identified from human feces in South Korea and rotten crab meat in China, respectively. Moreover, it exhibited 100% identity with other *E. faecium* strains (MT882700, MG557608, and MW856655) and 99% identity with PQ065633 from fish and water samples in this study. The *E. coli* strain (PP188022), recovered from a fish aquaria, demonstrated 100% similarity with KR148984 from a wetland in China and KC254646 obtained from tap water in India. This strain was 96% similar to ON054416, which was isolated from water in Colombia, and MT177009, which was isolated from Hunan, China. Additionally, it shares 100% similarity with both ON054416 and MT177009.

The neighbor-joining phylogenetic trees constructed for the sequenced *16S rRNA* genes of *E. faecium*, *E. faecalis*, and *E. coli* were grouped with their corresponding sequences, distinguishing them from other related species (Figure 5). The *Pseudomonas* strain (PP187982), recovered from the water of fish aquaria, demonstrated 100% similarity with *Pseudomonas* sp. (MN490878) isolated from water samples from lakes in Austria and JQ308618 obtained from environmental samples in China. The strain showed 99% resemblance to KX714291, which was isolated from soil in Antarctica, and OR841090, which was isolated in China. Furthermore, it shares 100% similarity with MW047419, JQ995152 (sea mud of Yantai), MT177009 (food contact in Pennsylvania), HF548404 (water reservoir in Russia), and KR095629 (tannery effluent in Pakistan) (Figure 6).

Table 4: Occurrence of the different bacteria genera in the studied fish farms in Wadi El-Natrun, El Menoufia, Egypt, during summer 2023

| Bacterial isolates | Farm 1 | Farm 2 | Farm 3 | Farm 4 | Farm 5 | Total | χ2 | P value |
|---|---|---|---|---|---|---|---|---|
| *E. coli* | 0(0) | 0(0) | 1(4.54) | 5(15.6) | 5(21.74) | 11 | 6.02 | 0.031 |
| *Enterobacter* | 1(5.88) | 0(0) | 1(4.54) | 2(6.25) | 1(4.34) | 5 | 0.135 | .987 |
| *Klebsiella* spp. | 0(0) | 1(5) | 0(0) | 2(6.25) | 2(8.69) | 5 | 0.250 | .882 |
| *Salmonella* spp. | 0(0) | 0(0) | 0(0) | 1(3.12) | 2(8.69) | 3 | 0.805 | .369 |
| *Pseudomonas* spp. | 0(0) | 2(10) | 1(4.54) | 2(6.25) | 7(30.43) | 12 | 9.37 | .024 |
| *Aeromonas* spp. | 0(0) | 0(0) | 0(0) | 1(3.12) | 1(4.34) | 2 | 0.057 | .811 |
| *Enterococcus* spp. | 0(0) | 0(0) | 0(0) | 8(25) | 5(21.74) | 13 | 0.197 | .655 |
| Positive | 17 | 20 | 22 | 32 | 23 | | | |
| Total | 1 | 3 | 3 | 21 | 23 | 51 | | |

$X^2$= chi-square. The result is significant at $P$ <0.05.

Table 5: Serotyping of *E. coli* and *Salmonella* isolates obtained from studied fish farms in Wadi El-Natrun, El Menoufia, Egypt, during summer 2023.

| Isolate | Bacterial strain | Source | Biochemical identification | Serotyping | |
|---|---|---|---|---|---|
| | | | | Polyvalent | Monovalent |
| BIE.C1 | *E. coli* | Tilapia fish | √ | I | O26:K60 |
| BIE.C2 | *E. coli* | Tilapia fish | √ | II | O86:K61 |
| BIE.C3 | *E. coli* | Tilapia fish | √ | II | O55:59 |
| BIE.C4 | *E. coli* | Water | √ | III | O103: K- |
| BIE.C5 | *E. coli* | Water | √ | I | O114:K90 |
| BIE.C6 | *E. coli* | Tilapia fish | √ | - | Autoagglutination |
| BIE.C7 | *E. coli* | Tobar fish | √ | II | O128:K67 |
| BIE.C8 | *E. coli* | Tobar fish | √ | II | O126:K71 |
| BIE.C9 | *E. coli* | Tobar fish | √ | I | O44:K74 |
| BIE.C10 | *E. coli* | Water | √ | III | O25:K11 |
| BIE.C11 | *E. coli* | Tilapia fish | √ | I | O44:K74 |
| BIS1 | *Salmonella* | Tobar fish | √ | *Salmonella* Enteritidis | |
| BIS2 | *Salmonella* | Water | √ | *Salmonella* Enteritidis | |
| BIS3 | *Salmonella* | Water | √ | *Salmonella* Enteritidis | |

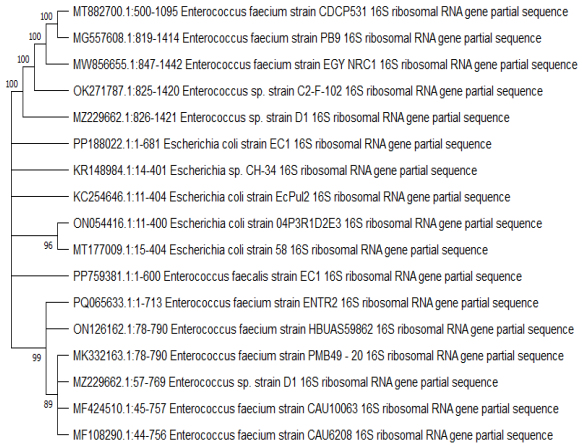


Figure 5: Phylogenetic tree constructed on the basis of the partial *16S rRNA* gene sequences of *E. coli, E. faecalis,* and *E. faecium* and other related partial *16S rRNA* gene sequences isolated from bacterial pathogens.

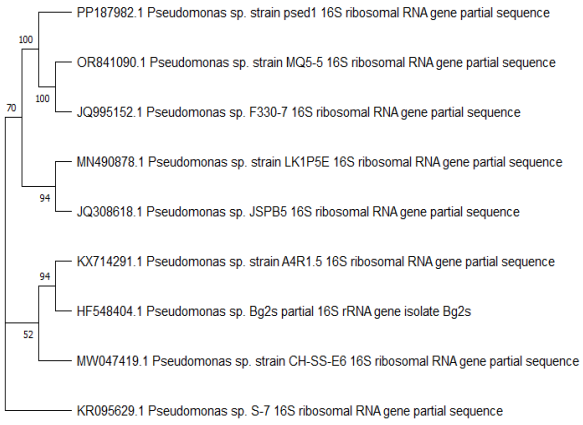


Figure 6. A phylogenetic tree was constructed on the basis of the partial *16S rRNA* gene sequence of the *Pseudomonas* strain (PP187982), which was recovered from the water of fish ponds, and other related partial *16S rRNA* gene sequences.

**Antibiotic susceptibility of the pathogenic bacterial strains**

Each isolate's selected antimicrobial sensitivity test revealed distinct levels of resistance and sensitivity. The 11 *E. coli* isolates presented 100% resistance to tetracycline, 90% resistance to amoxicillin, 90% resistance to trimethoprim, and 54.5% sensitivity to streptomycin. All three *Salmonella* isolates were 100% susceptible to norfloxacin and 100% resistant to amoxicillin, streptomycin, and trimethoprim. The ten *Pseudomonas* isolates were 70% susceptible to ciprofloxacin but exhibited high resistance, with 70% susceptible to trimethoprim, 60% susceptible to tetracycline, and 50% susceptible to amoxicillin. The two *Aeromonas* isolates showed 100% resistance to ciprofloxacin, 50% resistance to trimethoprim, and 100% sensitivity to amoxicillin and doxycycline. The 13 *Enterococcus* isolates displayed high levels of resistance to norfloxacin, doxycycline (30.7%), trimethoprim (15.3%), and ciprofloxacin (61.5%), as shown in Table 6.

Table 6: Antibiogram profile of isolated bacteria obtained from studied fish farms in Wadi El-Natrun, El Menoufia, Egypt, during summer 2023.

| Antimicrobial class | Antibiotic | Conc. | Resistant (%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | *E-coli* | *Salmonella* | *Pseudomonas* | *Enterococcus* | *Aeromonas* |
| Quinolones | Ciprofloxacin | 5 µg | 6 (54.5) | 0 (0) | 1 (10) | 3(23) | 2(100) |
| | Norfloxacin | 10 µg | 5 (45.4) | 0 (0) | 2 (20) | 4(30.7) | 1(50) |
| B-Lactams | Amoxicillin | 25 µg | 10 (90.9) | 3 (100) | 5 (50) | 0 (0) | 0 (0) |
| Macrolides | Streptomycin | 10 µg | 4 (36.3) | 2 (66.6) | 1 (10) | 0 (0) | 0 (0) |
| Tetracycline | Doxycycline | 30 µg | 11(100) | 1 (33.3) | 6 (60) | 4(30.7) | 0 (0) |
| Diaminopyrimidines | Trimethoprim | 5µg | 9 (81.8) | 2 (66.6) | 7 (70) | 2(15.3) | 1(50) |
| Number | | | 11 | 3 | 10 | 13 | 2 |

**Fish treatment trial and experimental design**

Figure 7 shows the bacterial load of the aquarium water at three different time points: pretreatment and days 1, 4, and 7. The quantitative analysis of the TBCs revealed that the number of bacteria in the water decreased from 3.95 ± 0.05 to 3.14 ± 0.30 and 3.27 ± 0.21 before and after the third day of treatment with $KMnO_4$ and $CuSO_4$, respectively. A significant difference ($p < 0.05$) was observed between the untreated and treated groups on days 4 and 7 of treatment.

The total bacteria count revealed that the untreated fish samples had an average of 7.82 ± 0.026 CFUs per gram of skin at the beginning of the trial. When the TBCs of the fish treated with $KMnO_4$ and $CuSO_4$ were compared with those of the untreated control fish, the log count analysis on day 7 revealed a statistically significant decrease. Figure 8 displays the average log counts/g (4.50 ± 0.020 and 3.96 ± 0.124, respectively) in comparison with the control group (8.60 ± 0.33 CFU/g) ($P < 0.05$) at the end of treatment (on day 7).

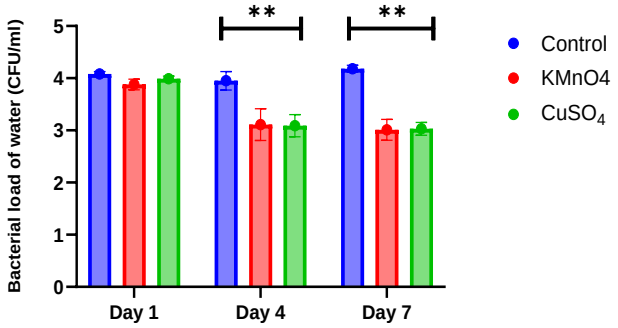


Figure 7: The effects of $Cuso_4$ and $Kmno_4$ on the bacterial load (TBC) of treated aquarium water (mean ± SE) are expressed as CFU/ml at three different time points: day 1 (pretreatment), day 4, and day 7. A significant difference ($p < 0.05$) was observed between the untreated and treated groups on days 4 and 7 of treatment.
*n=3 water samples each time.

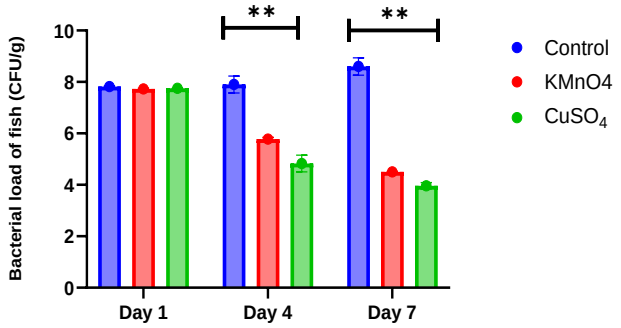


Figure 8: The effects of $Cuso_4$ and $Kmno_4$ on the bacterial load (TBC) are expressed as the mean ± SE of treated fish (CFU/g of skin) at three different time points: day 1, day 4, and day 7. A significant difference ($p < 0.05$) was observed between the untreated and treated groups at the end of treatment (day 7).
*n=5 fish samples each time.

The TBC of the fish treated with $CuSO_4$ and $KMnO_4$ was four times lower than that of the untreated control group. On day seven, the untreated control fish had a mean percent survival of 43% ± 3.5%. The survival rate of the fish treated with CuSO4 was 80.0% ± 2.4%, whereas that of the KMnO4-treated fish was 70.2% ± 4.3% (Figure 9). The survival rate of the fish treated with CuSO4 was 36% greater than that of the untreated control fish. Similarly, the survival rate of the fish treated with $KMnO_4$ was 26% greater than that of the untreated control fish. Survival curve analysis revealed a statistically significant difference in survival rates between treated and untreated control fish ($P < 0.05$).

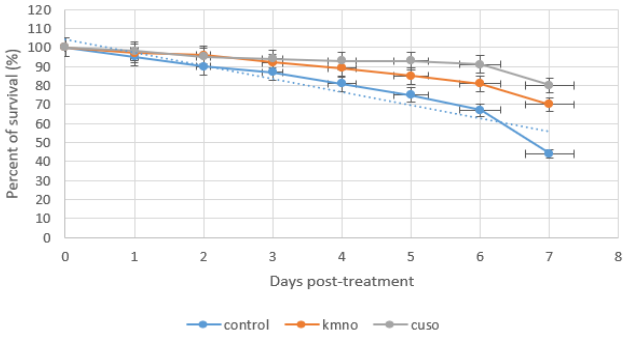


Figure 9: Survival percentage of *Oreochromis niloticus* naturally infected with coinfected bacteria and subjected to one of two treatments: (i) the control, (ii) $KMNO_4$, or (iii) $CUSO_4$ for 7 successive days.

**Discussion**

Field assessment findings indicate that fish farms in Wadi El-Natrun face significant health challenges, especially concerning mortality rates associated with bacterial coinfections. Symptoms such as gill rot, pale patches, and congested gills are consistent with severe infections caused by pathogens such as *Aeromonas* spp., *Vibrio* spp., or *Pseudomonas*, all of which are commonly encountered in aquaculture environments under stressful conditions (6,30,31). There are similar clinical signs in *M. capito*, which suggests widespread bacterial infection, potentially caused by environmental stressors such as low water quality, high stocking densities, or temperature changes (32). Early disease detection, effective water management, and targeted therapeutic interventions are essential for mitigating bacterial infections, enhancing fish health, and minimizing economic losses in aquaculture systems (22).

The overall health and productivity of an aquaculture system may be affected by the water quality parameters of its ponds. Most of the parameters assessed in this study remained within acceptable limits, which is essential for maintaining optimal fish growth and preventing disease outbreaks (33). Nevertheless, the significantly reduced dissolved oxygen (DO) level, reaching 2.8 mg/L, poses a serious concern considering that oxygen is an essential component of fish respiration and metabolism (33). Low DO levels can result in hypoxia, stress, and increased susceptibility to infection, which may explain the high mortality rates observed (6). In addition to elevated levels of ammonia to 1.15 mg/L and nitrite to 9 mg/L, in fish, ammonia is toxic at elevated levels, affecting gill function and causing gill erosion. These compounds increase the susceptibility of farmed fish to bacterial diseases, corroborating findings from previous studies (34). In the bacterial analysis of pond water samples, there were significant differences among farms, especially between farms 4 and 5, which may have implications for fish health and water quality management. The elevated TBC, particularly at farm 5, suggests a high level of microbial contamination, possibly as a result of poor sanitation, an organic load, or an excess of nutrients (6,21). There is evidence that fecal contamination originates from wastewater runoff or insufficient effluent management on farms 4 and 5, posing health risks to fish and humans (34). Although total coliforms were detected only on farms 4 and 5, with counts of 12 to 15 MPN/100 mL, they remain within the permissible range for freshwater irrigation and aquaculture, suggesting that contamination may not be immediately dangerous on the basis of current standards (35). Microbiological analyses of fish muscle samples collected from farms 4 and 5 revealed high levels of contamination, with the highest TBC (CFU/g) and total coliform counts (TCC/g) observed on these farms (Table 3). Pathogenic bacteria can contaminate fish through polluted water, supporting findings by Kim and Lee (36), who reported total bacterial counts ranging from 2.3 to 3.5 log CFU/mL in water and 2.2 to 4.0 log CFU/g in fish. The significantly greater prevalence of *Pseudomonas* spp. and *Enterobacteriaceae* in fish from farms 4 and 5, confirmed by statistical analysis ($\chi 2 = 6.02$, $p = 0.031$; $\chi 2 = 9.37$, $p = 0.024$), indicates a strong association between bacterial contamination and disease manifestation, which is likely exacerbated by environmental stressors. The presence of these bacteria in fish tissues highlights a serious health concern, implying that poor water quality, sanitation, or handling practices contribute to disease outbreaks and contamination within these aquaculture systems (6).

This study confirmed the phenotypic and biochemical identification of bacteria isolated from water and fish through *16S rRNA* sequence analysis. The *16S rRNA* gene sequences of the bacterial isolates in this analysis were more than 98% similar to those of their related bacteria. On the basis of the *16S rRNA* gene sequences of the retrieved isolates, the phylogenetic tree in Figure 5 illustrates the results of the phylogenetic analysis. Among the three isolates identified from water and fish (*E. coli* (PP188022), *E. faecalis* (PP759381), and *E. faecium* (ON126162)) some exhibited 100% identity (in sequence BLAST analysis) with bacterial isolates from the experimental fish, indicating their

potential role in causing infections, which aligns with findings by Abdelsalam *et al.* (37). In our study, *Pseudomonas* was the bacterium most frequently isolated from water and fish on fish farms.

Antimicrobial sensitivity testing indicates diverse resistance patterns among bacterial isolates, emphasizing antibiotic stewardship. *E. coli* showed high resistance to tetracycline, amoxicillin, and trimethoprim, but some sensitivity to streptomycin was observed. *Salmonella* isolates are susceptible to norfloxacin but resistant to amoxicillin, streptomycin, and trimethoprim. There is susceptibility to ciprofloxacin in *Pseudomonas* but resistance to trimethoprim, tetracycline, and amoxicillin in *Pseudomonas*. Despite being resistant to ciprofloxacin, *Aeromonas* is sensitive to amoxicillin and doxycycline. *Enterococcus* shows high resistance to norfloxacin and ciprofloxacin. These findings clearly indicate that continuous monitoring and judicious use of antibiotics are key to preventing multidrug-resistant bacteria. These findings align with prior research showing high resistance to amikacin, streptomycin, trimethoprim, and azithromycin in various genera (38). However, earlier studies reported lower resistance rates: 29.4% for *E. coli*, 46.7% for *Salmonella* spp., and 42.9% for *S. aureus* (38). According to Wang *et al.* (39), antibiotics can alter bacterial abundance and blue-green algae populations in aquaculture systems, indirectly affecting the food web.

Many chemicals have been used in aquaculture for the treatment of diseased animals and, to a lesser extent, to enhance water quality in culture facilities. Chemical applications have the major advantage of quick results (40). $KMnO_4$ and $CuSO_4$ have been shown to significantly reduce bacterial loads in water and on fish skin. This led to improved survival rates in this study. This finding is in line with previous research indicating their antimicrobial efficacy in aquaculture (16-18). In water, the pathogenic microorganism concentration decreased from approximately 3.95 to 3.14-3.27 log CFU/mL. The significant differences on days 4 and 7 ($p < 0.05$) support their efficacy over time. Similar findings were reported by Kumar *et al.* (41), who noted that water treatment primarily reduced the bacterial counts from sources without significant immediate effects. By the end of the experiment on day 7, the bacterial loads in the water of the control group increased, likely because dead fish serve as nutrient-rich substrates for microorganisms. In contrast, the bacterial loads in the treated group decreased rapidly, which is consistent with findings of Faruk *et al.* (42), who investigated the use of chemicals such as salt, lime, formalin, and potassium permanganate for aquarium water disinfection.

The number of bacteria in the skin of the fish decreased to approximately 4.5 log CFU/g. The TBC of the fish treated with $KMnO_4$ and $CuSO_4$ was fourfold lower than that of the untreated fish. This aligns with previous studies indicating that $KMnO_4$ is an effective bactericide that eradicates skin and gill infections through its potent oxidizing properties. Similarly, Jenkins (43) reported a bacterial load of 7.73 ± 0.023 CFU/g in fish skin from the untreated control group, with a significant decrease in the treated groups. Additionally, Moshtaghi *et al.* (44) demonstrated that exposure to copper sulfate and potassium permanganate solutions reduced bacterial loads in the gills, intestines, skin, and surrounding water of sturgeon fries. Interestingly, $CuSO_4$ demonstrated slightly superior results in reducing pathogenic microorganisms, resulting in enhanced fish survival and health. In addition, the significant increase in survival rates-up to 80% with $CuSO_4$-further supports their protective role, although further research on optimal dosing is needed. These findings confirm that $CuSO_4$ and $KMnO_4$ are effective disinfectants and prophylactic agents in aquaculture.

## Conclusion

This study revealed that the elevated mortality rates in polycultured *O. niloticus* and *M. capito* during the summer of 2023 are attributed to substandard water quality and bacterial infections. $CuSO_4$ treatment significantly decreased the bacterial load and mortality rates, whereas $KMnO_4$ improved the clinical signs and survival rates. These findings emphasize the importance of implementing fish health management practices and biosecurity controls to increase productivity and sustainability in aquaculture systems.

## Acknowledgments

Not applicable

## Conflict of interest

The authors declare that there are no potential conflicts of interest that could influence the work reported in this paper.

## References

1. Kaleem O, Bio Singou Sabi AF. Overview of aquaculture systems in Egypt and Nigeria, prospects, potentials, and constraints. Aquac Fish. 2021;6(6):535-547. DOI: 10.1016/j.aaf.2020.07.017
2. Soliman NF. Aquaculture in Egypt under changing climate. Egypt: Alexandria Research Center for Adaptation to Climate Change (ARCA); 2017. DOI: 10.13140/RG.2.2.18235.21284
3. Elsheshtawy A, Yehia N, Elkemary M, Soliman H. Investigation of Nile tilapia summer mortality in Kafr El-Sheikh governorate, Egypt. Genet Aquat Organ. 2019;3(1):17-25. DOI: 10.4194/2459-1831-v3_1_03
4. Plumber A. Fishy business: Assessing Egypt's growing aquaculture sector. Al Noor J Middle East Stud. 2019;1:44-51. DOI: 10.6084/m9.figshare.12501944
5. Mahmoud MA, Attia MM, Abdelsalam M, Abdel-Moneam DA, Zaki Ewiss MA. *Ergasilus extensus* and bacterial co-infection in flathead grey mullet, *Mugil cephalus* (Linnaeus, 1758), are associated with pathological changes and immunological gene expression alterations. Aquac Res. 2021;52(12):6143-6151. DOI: 10.1111/are.15476

6. Abdelsalam M, Ewiss MZ, Khalefa HS, Mahmoud MA, Elgendy MY, Abdel-Moneam DA. Co-infections of *Aeromonas* spp., *Enterococcus faecalis*, and *Vibrio alginolyticus* isolated from farmed Nile tilapia and African catfish in Egypt, with an emphasis on poor water quality. Microb Pathog. 2021;160:105213. DOI: 10.1016/j.micpath.2021.105213
7. Khalefa HS, AbuBakr HO, Aljuaydi SH, Kotp YH, Al-Mokaddem AK, Abdel-Moneam DA. Aquatic assessment of the chelating ability of silica-stabilized magnetite nanocomposite to lead nitrate toxicity with emphasis to their impact on hepatorenal, oxidative stress, genotoxicity, histopathological, and bioaccumulation parameters in *Oreochromis niloticus* and *Clarias gariepinus*. BMC Vet Res. 2024;20(1):262. DOI: 10.1186/s12917-024-04094-9
8. Bray JP, Hewitt CLR, Hulme PE. Bridging aquatic invasive species threats across multiple sectors through One Biosecurity. BioScience. 2024;74(7):440-449. DOI: 10.1093/biosci/biae049
9. Yaseen MM. Hunting of some bacterial species based on isolation and identification from river water and sediments and from tilapia (*Oreochromis niloticus*) in Al-Qadisiyah Province, Iraq. Procedia Eng Med Sci. 2024;9(2):48-57. [available at]
10. Khalefa HS, Attia MM, Abdelsalam M, Mahmoud MA, Zaki Ewiss MA. Immunological status of some edible fishes exposed to parasitic infections in relation to heavy metals pollution. J Parasit Dis. 2022;46(3):653-663. DOI: 10.1007/s12639-022-01479-1
11. Abdel-Latif HM, Dawood MA, Menanteau-Ledouble S, El-Matbouli M. The nature and consequences of co-infections in tilapia: a review. J Fish Dis. 2020;43(6):651-664. DOI: 10.1111/jfd.13164
12. Nair SG, Lipton A, De los Ríos-Escalante P, Ibáñez-Arancibia E. Isolation and characterization of bacterial pathogens, *Pseudomonas aeruginosa* and *Enterobacter cloacae* from the moribund fish, *Etroplus maculatus*. J Mater Environ Sci. 2021;12:1332-1349. [available at]
13. Abdel-Moneam DA, Khalefa HS, Shaalan M, Elshafiee EA, Ahmed ZS. Assessment of the role of wild waterfowl as potential vectors of *Aeromonas hydrophila* and its cross-transmission to Qarun Lake's aquatic environment, considering the altered water quality parameters. Biol Bull. 2025;52(1):1-16. DOI: 10.1134/S1062359024607985
14. Gamal A, Abdel-Moneam DA, Morsi AS, Malak NM, Ali AM, Khalefa HS. In-vitro and in-vivo assessment of the bactericidal potential of peracetic acid and hydrogen peroxide disinfectants against *A. hydrophila* infection in Nile tilapia and their effect on water quality indices and fish stress biomarkers. Sci Rep. 2024;14(1):25715. DOI: 10.1038/s41598-024-76036-2
15. Broom M, Satheesh S, Al-Harbi M, Gabr MH. Effect of disinfectants on sea cucumber juveniles (*Holothuria scabra*) in farming practices. J King Abdulaziz Univ Mar Sci. 2021;31(2). DOI: 10.4197/Mar.31-2.5
16. Rintamäki-Kinnunen P, Rahkonen M, Mannermaa-Keränen AL, Suomalainen LR, Mykrä H, Valtonen ET. Treatment of ichthyophthiriasis after malachite green. I. Concrete tanks at salmonid farms. Dis Aquat Organ. 2005;64(1):69-76. DOI: 10.3354/dao064069
17. Mitchell AJ, Darwish A, Fuller A. Comparison of tank treatments with copper sulfate and potassium permanganate for sunshine bass with ichthyobodosis. J Aquat Anim Health. 2008;20(4):202-206. DOI: 10.1577/H07-048.1
18. Darwish AM, Bebak JA, Schrader KK. Assessment of Aquaflor®, copper sulphate and potassium permanganate for control of *Aeromonas hydrophila* and *Flavobacterium columnare* infection in sunshine bass, *Morone chrysops* × *Morone saxatilis*. J Fish Dis. 2012;35(9):637-647. DOI: 10.1111/j.1365-2761.2012.01393.x
19. American Public Health Association (APHA). Standard methods for the examination of water and wastewater. USA: American Water Works Association, Water Environment Federation; 2005.
20. American Public Health Association (APHA). Standard methods for the examination of water and wastewater. 20th ed. USA: APHA; 1998. DOI: 10.1201/9781315165011
21. Rahman M, Rahman MM, Deb SC, Alam MS, Alam MJ, Islam MT. Molecular identification of multiple antibiotic resistant fish pathogenic *Enterococcus faecalis* and their control by medicinal herbs. Sci Rep. 2017;7(1):3747. DOI: 10.1038/s41598-017-03673-1
22. Austin B, Austin DA. Bacterial fish pathogens: disease of farmed and wild fish. USA: Springer; 2016. DOI: 10.3390/fishes8070357
23. Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, Fitzpatrick E. Veterinary microbiology and microbial disease. USA: John Wiley & Sons; 2011.
24. Kok T, Worswich D, Gowans E. The potential diversity of intestinal *Enterobacteriaceae* in broiler chickens is associated with infectious bursal disease virus infection. Egypt J Vet Sci. 2024;55(4):917-930. DOI: 10.21608/ejvs.2023.246007.1662
25. Grimont PA, Weill FX. Antigenic formulae of the *Salmonella* serovars. WHO collaborating centre for reference and research on *Salmonella*. 2007;9:1-166. [available at]
26. El-Son MA, Nofal MI, Abdel-Latif HM. Co-infection of *Aeromonas hydrophila* and *Vibrio parahaemolyticus* isolated from diseased farmed striped mullet (*Mugil cephalus*) in Manzala, Egypt - a case report. Aquaculture. 2021;530:735738. DOI: 10.1016/j.aquaculture.2020.735738
27. Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95-98. [available at]
28. Bauer KW Jr, Parnell GS, Meyers DA. Response surface methodology as a sensitivity analysis tool in decision analysis. J Multi Criteria Decis Anal. 1999;8(3):162-180. DOI: 10.1002/(SICI)1099-1360(199905)8:3<162::AID-MCDA241>3.0.CO;2-X
29. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. USA: CLSI; 2018.
30. Boyd CE, Tucker CS. Pond aquaculture water quality management. USA: Springer; 2012.
31. Magouz FI, Moustafa EM, Abo-Remela EM, Halawa MR, Barakaat PM, Omar AA. Summer mortality syndrome bacterial pathogens in farmed Nile tilapia (*Oreochromis niloticus*). Open Vet J. 2024;14(1):53. DOI: 10.5455/OVJ.2024.v14.i1.7
32. Abdel-Moneam DA, Ibrahim RA, Nashaat M, Shaalan M. Multifactorial causes of mass mortality in *Oreochromis niloticus* in Kafr El-Sheikh, Egypt. Bull Eur Assoc Fish Pathol. 2021;41(1). [available at]
33. Boyd CE. General relationship between water quality and aquaculture performance in ponds. In: Fish Diseases. UK: Academic Press; 2017. 147-166 p.
34. Mahmoud MA, Abdelsalam M, Mahdy OA, El Miniawy HM, Ahmed ZA, Osman AH, Ewiss MZ. Infectious bacterial pathogens, parasites and pathological correlations of sewage pollution as an important threat to farmed fishes in Egypt. Environ Pollut. 2016;219:939-948. DOI: 10.1016/j.envpol.2016.09.044
35. Al-Taee N, Mohammad M, Abdul-Majeed A. The effect of partial replacement of animal protein with duckweed grown in the treatment unit of the Nineveh pharmaceutical factory water on the growth performance of common carp *Cyprinus carpio* L. Mesopotamia J Agric. 2024;52(3):22-30. DOI: 10.33899/mja.2024.145298.1316
36. Kim JY, Lee JL. Correlation of total bacterial and *Vibrio* spp. populations between fish and water in the aquaculture system. Front Mar Sci. 2017;4:147. DOI: 10.3389/fmars.2017.00147
37. Abdelsalam M, Elgendy MY, Shaalan M, Moustafa M, Fujino M. Rapid identification of pathogenic streptococci isolated from moribund red tilapia (*Oreochromis* spp.). Acta Vet Hung. 2017;65(1):50-59. DOI: 10.1556/004.2017.005
38. Gomes C, Martínez-Puchol S, Palma N, Horna G, Ruiz-Roldán L, Pons MJ, Ruiz J. Macrolide resistance mechanisms in *Enterobacteriaceae*: focus on azithromycin. Crit Rev Microbiol. 2017;43(1):1-30. DOI: 10.3109/1040841X.2015.1136261
39. Wang Z, Han M, Li E, Liu X, Wei H, Yang C, Ning K. Distribution of antibiotic resistance genes in an agriculturally disturbed lake in China: their links with microbial communities, antibiotics, and water quality. J Hazard Mater. 2020;393:122426. DOI: 10.1016/j.jhazmat.2020.122426
40. Haydar H. The effect of adding sweet bean seed powder (*Foeniculum vulgare*) and roselle (*Hibiscus sabdariffa*) seed powder on the growth performance of common carp *Cyprinus carpio* L. Mesopotamia J Agric. 2024;52(2):58-60. DOI: 10.33899/mja.2024.146824.1374

# فعالية كبريتات النحاس وبرمنجنات البوتاسيوم في الحد من العدوى البكتيرية والنفوق في أسماك المزارع متعددة الأنواع في المنوفية، مصر: تحليل ميكروبيولوجي وبيئي

**أماني عبد اللطيف مصلح[١]، أماني إبراهيم البيلي[٢]، دينا إسماعيل الذهبي[٣]، عمرو عبد الخالق عبد الحميد السعدني[٤]، علاء نادي عنتر[٥]، حنان سعد خليفة[٦]**

[١]قسم الأحياء المجهرية، [٢]قسم البكتيريا، [٣]قسم صحة الأغذية، معهد بحوث الصحة الحيوانية، شبين الكوم، [٤]قسم البكتيريا، معهد بحوث الصحة الحيوانية، فرع طنطا، مركز البحوث الزراعية، طنطا، [٥]قسم البكتيريا، كلية الطب البيطري، جامعة بنها، بنها، [٦]قسم صحة البيئة، كلية الطب البيطري، جامعة القاهرة، القاهرة، مصر

**الخلاصة**

تتطلب رعاية الأسماك القدرة على فهم الية العدوى البكتيرية والعوامل البيئية المصاحبة. تم تقييم آثار كبريتات النحاس وبرمنجنات البوتاسيوم على العدوى البكتيرية في الأسماك المستزرعة المصابة طبيعياً والتي تم الحصول عليها من مزارع أسماك خاصة في وادي النطرون، المنوفية، مصر، ذات معدلات نفوق مرتفعة. تم اختيار أربعين من أسماك الطوبار وأربعة وسبعين من أسماك البلطي النيلي عشوائياً من هذه المزارع. تم عزل العديد من البكتريات، تتضمن الإشريكية القولونية (عدد ١١)، والإنتروباكتر (عدد ٥)، والكلبسيلا (عدد ٥)، والسالمونيلا (عدد ٣)، والزائفة الزنجارية (عدد١٢)، والإيروموناس (عدد ٢)، والمعوية (عدد ١٣)، من عينات الأسماك. بالمقارنة مع المزارع الأخرى، أظهرت المزرعتان ٤ و٥ معدلات أعلى للإصابة بأنواع الزائفة الزنجارية، والبكتيريا المعوية، والإشريكية القولونية في الأسماك المصابة سريريًا ($X^2$= 6.02، $P$ = 0.031). ولوحظت فروق ذات دلالة إحصائية بين المزارع ($X^2$ = 9.37، $P$ = 0.024). وتم تحديد هذه المعزولات بشكل أكبر من خلال تسلسل جينات *16S rRNA* والتحليل الوراثي. كما كشف تحليل المياه أن مستويات الفوسفات والكبريتات وعسر المياه والكلوريد كانت أعلى من الحدود المسموح بها في المزرعتين ٤ و٥. خلال هذه الدراسة، استخدمت كبريتات النحاس وبرمنجنات البوتاسيوم على فترات ٢٤ ساعة لمدة سبعة أيام، ودرست آثارها على الأحمال البكتيرية، وعدد القولونيات، ومعدلات الوفيات في أسماك البلطي النيلي المصابة. استخدمت عينات المعالجة المسبقة كمجموعة ضابطة. وقد خفض كل من كبريتات النحاس وبرمنجنات البوتاسيوم الحمل البكتيري ومعدلات الوفيات بشكل فعال. وبالمقارنة مع الأسماك غير المعالجة، بلغت معدلات بقاء الأسماك المعالجة بكبريتات النحاس أو برمنجنات البوتاسيوم ٨٠ % و٧٠,٢ % على التوالي.

41. Kumar P, Prasad Y, Patra AK, Ranjan R, Swarup D, Patra RC, Pal S. Ascorbic acid, garlic extract and taurine alleviate cadmium-induced oxidative stress in freshwater catfish (*Clarias batrachus*). Sci Total Environ. 2009;407(18):5024-5030. DOI: 10.1016/j.scitotenv.2009.05.030
42. Faruk MAR, Ali MM, Patwary ZP. Evaluation of the status of use of chemicals and antibiotics in freshwater aquaculture activities with special emphasis to fish health management. J Bangladesh Agric Univ. 2008;6(2):381-390. DOI: 10.3329/jbau.v6i2.4838
43. Jenkins JA, Bart HL Jr, Bowker JD, Bowser PR, MacMillan JR, Nickum JG, Warkentine BE. Guidelines for the use of fishes in research. Bethesda (MD): American Fisheries Society; 2014. [available at]
44. Moshtaghi B, Khara H, Pazhan Z, Shenavar A. Histopathological and bacterial study of Persian sturgeon fry, *Acipenser persicus* (Borodin, 1897) exposed to copper sulfate and potassium permanganate. J Parasitol Dis. 2016;40:779-784. DOI: 10.1007/s12639-014-0578-4