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Prevalence and Characterization of *Aeromonas hydrophila* in Freshwater Fish Farms: A Study in Kafrelsheikh Governorate, Egypt

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ABSTRACT

This study aimed to determine the virulence and prevalence of *Aeromonas hydrophila* in Nile tilapia farms in the Kafrelsheikh governorate. Random samples were collected from 11 fish farms, including water, sediment, and fish. A total of 45 *A. hydrophila* isolates were presumptively identified using morph-chemical conventional tests. The virulence of the retrieved isolates was assessed through the detection and quantitative estimation of lipase activity. A total of 42 *A. hydrophila* isolates proved to be lipase producers. A variation among fish farms in the physicochemical parameters of the water was recorded. In conclusion, the screened lipase activity of the retrieved isolates points to the characteristic ulcerative/ hemorrhagic clinical profile of the retrieved *A. hydrophila* in mortal and moribund Nile tilapias collected from mass mortalities episodes in Kafrelsheikh. It's essential to monitor and control the physicochemical parameters of water to decrease the stressors on fish and avoid the virulence of *A. hydrophila.*

Keywords: Freshwater aquaculture; Nile tilapia; *Aeromonas hydrophila;* Lipase activity; Disease prevalence

INTRODUCTION

Fish are widely recognized as a valuable source of food due to their nutritional composition, high palatability, and digestibility (Colombo et al. 2023). However, diseases significantly impact the fish populations within their ecosystems (Hutson et al. 2023). The prevalence of diseases in aquatic ecosystems is influenced by various environmental factors, including infectious organisms and stressors, which contribute to the susceptibility of fish to diseases (Hutson et al. 2023).

Diseases, particularly those of microbial origin, play a significant role in affecting fish culture (Ina-Salwany et al. 2019). Fish, like other organisms, are exposed to bacteria regularly, but infection usually occurs after prolonged periods of stress. *Aeromonas hydrophila* is among the most common ubiquitous in freshwater habitats worldwide (Akmal et al. 2020). The microorganism exists in diverse environments, including soil, fresh and salt water, chlorinated and non-chlorinated drinking water, and poses a pathogenic risk to both warm and cold-blooded animals (Najeeb et al. 2021).

Consequently, the aquatic environment, along with water and seafood, represents a potential source for the transmission of *A. hydrophila*, posing a risk of human infections. Several potential risk factors, such as physico-chemical parameters of water, have been associated with fish diseases (Mramba and Kahindi 2023). Notably, high thermal stress in fish has been linked to mortality rates as high as 80% due to *Aeromonas* species infections (Sherif et al. 2024). In intensive fish culture, the highest mortality rates resulting from *Aeromonas* species infections were observed during late spring and early summer (Ammar et al. 2023). The Kafrelsheikh governorate is a major contributor to the country's farmed fish production, accounting for 55% of the total yield of 324,479 tonnes. It also produces 44% of farmed tilapia, adding 259,583 tonnes to the overall production, highlighting its significant role in Egypt's aquaculture industry (Kassem et al. 2016).

In light of these considerations, the present study aimed to determine the prevalence of *Aeromonas hydrophila* in Nile tilapia and in the sediment and water of

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various freshwater fish farms in Kafrelsheikh governorate. It also aimed to screen for lipase production, a virulent factor associated with *Aeromonas*'s pathogenicity, and measure the physicochemical parameters of water in fish farms.

MATERIALS AND METHODS

Measurement of physico-chemical parameters of water

Freshwater samples were randomly collected from eleven Nile tilapia fish farms in Kafrelsheikh governorate that experienced mass mortality through the period from July to August 2022. Physicochemical parameters of the water samples were recorded following the protocols described (Trivedy and Goel 1986; APHA 1998). Prior to collecting samples, the bottles were sterilized by autoclaving at 121°C for 1h. Each bottle was labeled with the date and location of collection. Temperature, salinity, total dissolved solids (TDS), electrical conductivity (EC), and resistivity were measured using a HI98192 meter (Hanna, USA), while pH was measured using a pH meter (Hach, United Kingdom). All the above analyses were performed in duplicate.

Samples collection of water, sediment, and fish

Water samples (200mL) were collected at a depth of 50cm from each fish farm. Sediment samples were taken from the water and placed in sterile bags, while fish samples were collected from eight ponds and placed in sterile bags (Eissa 2016)All samples were transferred to the Central Laboratory for Aquatic Organisms Health and Safety Lab (CLAHS), Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, in an icebox provided with crushed ice.

Clinical and postmortem examination

A clinical examination was applied to the fish samples for external signs, and internal organ examination was conducted on freshly deceased fish following the methods described by Eissa (2016).

Bacterial isolation from sediment, water and fish samples

Bacterial isolation was performed according to methods by Eissa (2016) with minor modifications. Sediment and water samples were diluted with sterilized distilled water. Then, 0.1mL of the diluted samples was streaked onto TSA (HiMedia, India) and incubated aerobically at 37°C for 24h. Single colonies grown on TSA were sub-cultured onto *Aeromonas* isolation medium base supplemented with ampicillin selective supplement 5mg/L (FD039) and incubated at 37°C for 24h. For fish samples, a sterile normal saline solution was used to rinse the external surface, followed by sterilization using 70% ethyl alcohol. Under complete aseptic conditions, fish were dissected, and then loopfuls of tissues from the kidney, spleen, liver, and brain were separately streaked onto TSA. Single colonies grown on TSA were further sub-cultured onto *Aeromonas* isolation medium base supplemented with ampicillin selective supplement 5mg/L (FD039) until pure cultures were obtained. Inoculated petri dishes were incubated for 24-48h at 25°C. Suspected colonies showing yellowish, opaque color on TSA and translucent, pale green colonies measuring 0.5-3.0mm in diameter on *Aeromonas* isolation medium base were selected for purification and further characterization.

Morpho-chemical identification of *Aeromonas* spp.

Morphological characterizations, such as shape, size, gram staining, and motility tests, were performed on suspected *Aeromonas* spp. colonies (Koneman et al. 1995). Biochemical tests were conducted for confirmation, including catalase, oxidative-fermentative (OF), acid and gas production from glucose and sucrose, and 0/129 tests (Odds 1981).

Detection of virulence factors of *Aeromonas* spp.

Qualitative screening of lipase-producing isolates

All forty-five bacterial isolates were pre-cultivated in a tryptone soya broth. They were then grown on agar plates with olive oil as substrate and rhodamine B (1mg/mL) as a fluorescent dye. The plates contained 5g peptone, 3g yeast extract, 4g sodium chloride, and 15g agar (pH 7)/L. After autoclaving, 31.25mL olive oil and 10mL of rhodamine B solution (0.001% [wt/vol]) were added and stirred vigorously for 1min before pouring into sterile petri dishes. Lipase-producing isolates were identified by observing the formation of fluorescent halos around colonies after one to two days of incubation under UV light at 350 nm (Katiyar et al. 2017).

Determination of lipase activity

Lipase activity was monitored following the method described by Rehman et al. (2017). Lipase activity was measured using p-nitrophenyl palmitate (p-NPP) as a substrate. A reaction solution of 1000µL was prepared, consisting of 100µL of pNPP, 800µL of solution B (composed of 0.1g of Arabic gum, 0.4mL of Triton, and 100mL of 0.1 M phosphate buffer at pH 7) and 100µL of the cell-free extract. After 10min incubation, the released p-nitrophenol (pNP) was measured at 405 nm using a spectrophotometer (UV-visible spectrophotometer, Nanodroper, EMC-NANO-UV, Germany). Lipase activity (U/mL) is the enzyme required to release 1mol of p-nitrophenol per minute (IU). Lipase activity and specific activities were calculated using the following formula:

$$\text{Lipase activity (U/mL)} = A \times B / (C \times D \times E)$$

Where:

A - µmol of p-Nitrophenol released.
B - Total volume.
C - Volume used in spectrophotometric determination.
D - Volume of enzyme used in the assay.
E - Time of incubation.

Statistical analysis

Data from laboratory were analyzed using analysis of variance (ANOVA) and they are presented in tabular form, showing the Mean ± Standard deviation of duplicates.

RESULTS AND DISCUSSION

Clinical examination

The identification of *Aeromonas* is crucial as it is known to cause mass mortality in Nile tilapia and poses potential risks to human health through various transmission routes, including contaminated water, food, and exposure of wounds. Clinical signs observed in collected fish samples included severe ulceration on the body surface, abdominal distension, and hemorrhages. The postmortem examination revealed congested livers with hemorrhage on their surface,

distended gallbladders, and hemorrhagic spleens (Fig. 1). The clinical and post-mortem findings observed in our collected samples were consistent with previous studies (Yardimci and Aydin 2011), confirming the presence of *Aeromonas* sp. as the causative agent.

Fig. 1: Clinical signs of diseased farmed Nile tilapia. (A) Hemorrhagic skin ulceration; (B) Hemorrhagic enlarged liver.

Physicochemical Parameters of collected water samples

Water quality is a major concern in aquaculture. Changes to physical or chemical factors can harm aquatic organisms, reducing their productivity and causing losses (Banagar et al. 2018). Results in Table 1 show that there is a variation among farms in the physicochemical parameters of the water in the fish farms during January and February 2023. The impact of temperature on aquatic organisms is a critical consideration, as it can significantly affect their digestion, physiology, metabolism, and overall productivity. Understanding and managing temperature conditions is essential for ensuring the optimal health and performance of these organisms (Ibearugbulam et al. 2021). In this study, the lowest recorded temperature was 19.6 °C for farm No. 11, while the highest temperature was 20.6°C for farm No. 1 and 2. A desirable temperature range for the production of Nile tilapia ranging from 20 to 30°C (Makori et al. 2017), that match with the current results. Also, the temperature of fish farms was within WHO and FEPA permissible limit for aquaculture.

The stability of pH is crucial for maintaining life in aquatic environments, and it determines the solubility and bioavailability of nutrients in fish farming (Sanou et al. 2022). An ideal pH range for biological productivity is between 7 and 8.5. A pH ranging from 4 to 6.5 and from 9 to 11 is considered a stress factor leading to death at a pH below 4 or above 11 (Kane et al. 2015). In our study, pH ranges from 8.135 (farm No. 11) to 8.58 (farm No. 5). The recorded pH values fall within the range recommended by WHO and FEPA for breeding *O. niloticus.*

Salinity refers to the amount of salt or dissolved salt present in water. It affects the survival, feeding, and production of aquatic organisms, but the tolerance for salinity varies depending on species. Nile tilapia, for example, can tolerate lower salinity levels (Yue et al. 2024). The lowest value of salinity was observed in the farm No. 4 which was 1.14 ppt, while the highest ppt of salinity was 5.39 in the farm No. 5, these values are within the optimal water salinity for Nile tilapia for better growth performance (El-Leithy et al. 2019).

The measurement of total dissolved solids (TDS) determines the amount of dissolved organic and inorganic substances in a water sample. High TDS values can increase electrical conductivity, hinder light penetration and oxygenation of the aquatic environment, and affect the functions of fish gills and kidneys, which can impact their survival and size (Monira and Mostafa 2023; Olaoluwa 2024).

In our study, it was observed that the lowest value of TDS was 1355mg/L for farm No. 7; while the highest one was 4750mg/L for farm No. 5. The TDS values in the farms that we have studied are higher than the limits recommended by both WHO and FEPA (500mg/L). The TDS present in natural water sources can experience significant fluctuations due to a variety of factors. These may include water usage patterns, precipitation levels, agricultural fertilization practices, or the discharge of industrial waste into water systems (Sanou et al. 2022). Such influences can impact on the overall water balance and can cause fluctuations in TDS levels. In our case, these fish farms are located within agriculture farm and the source of their irrigation coming from this water which are heavily contaminated with fertilizers which increases the TDS in fish farms, in addition to precipitated nutrients from fish feed increase TDS values.

Electrical conductivity (EC) serves as a significant indicator of water freshness, with higher values indicating potential pollution. In the context of fish farming, the nutrient content of fish feed can contribute to elevated EC values in ponds (Kumar 2017). On the other hand, a decrease in EC has been linked to accelerated fish growth (Sanou et al. 2022). Our study has observed the lowest EC value in farm No. 3 (2.550mS/cm) and the highest in farm No. 5 (9.5435mS/cm). It is notable that the EC values of fish ponds in this study exceed both FEPA limits.

In monitoring water ionic purity, either resistivity or conductivity can be used as a cost-effective method. Resistivity, the reciprocal of conductivity, indicates water's ability to resist an electrical current and is directly influenced by the concentration of dissolved salts. If there are ample dissolved salts, water will exhibit lower resistivity (Uiuiu et al. 2020). Our study has observed the lowest resistivity in farm No. 5 (105.5 Ω) and the highest in farm No. 4 (398.5 Ω).

Bacterial isolation and identification

Culture characteristics of suspected *Aeromonas* spp. appeared yellowish opaque on TSA. Growing colonies on TSA were streaked on *Aeromonas* isolation medium base and the colonies were translucent and pale green. Based on these cultural characteristics, 45 isolates from fish, sediment and water samples were suspected as *Aeromonas* spp.

The prevalence of *Aeromonas* sp. in water, sediment and fish samples collected randomly from eleven fish farms at Kafrelsheikh governorate is presented in Table 2. All samples of fish, sediment and water were contaminated with *Aeromonas* spp. In terms of fish sample, the prevalence of *Aeromonas* spp. was the highest in kidney and spleen (100%) followed by liver (62.5%) and brain (25%). The overall prevalence of *Aeromonas* spp. in freshwater fish farms was 100%. This data is matched with a previous study that the presence of bacterial toxins has resulted in acute septicemia, which has caused significant structural damage to both the kidneys and liver (Yardimci and Aydin 2011). Accumulation of yellowish ascitic fluids leads to the common gross lesions that observed in the diseased fish with congestion and enlargement of the kidney and spleen (Ali et al. 2021). The septicemia which disseminated the bacteria to all the internal organs causes the enlargement of the kidney and spleen (Mostafa et al. 2024).

Table 1: Comparison of Physicochemical parameters with FEPA and WHO limits

Farm No.	Temp. (C)	pH	Salinity (ppt)	TDS (mg/L)	EC (mS/Cm)	Resistivity (Ω.m)
1	20.6±0.1	8.375±0.015	3.040±0.02	2836.5±5.5	5.646 ±0.001	178±1
2	20.6±0.2	8.31±0.02	2.050±0.05	1951.5±2.5	3.910±0.002	253±2
3	20.25±0.15	8.245±0.015	1.170±0.02	1280.5±3.5	2.550 ±0.02	389±1
4	20.3±0.1	8.84±0.02	1.140±0.02	1257.5±3.5	2.5065±0.0055	398.5±1.5
5	19.95±0.15	8.58±0.02	5.390±0.03	4750.5±27.5	9.5435±0.0015	105.5±0.5
6	19.9±0.1	8.515±0.015	2.785±0.015	2637.5±5.5	5.2715±0.0035	189.5±0.5
7	20.2±0.1	8.57±0.02	1.260±0.02	1355±1	2.705±0.015	366±2
8	20.25±0.05	8.51±0.02	1.315±0.035	1419.5±01.5	2.835±0.001	352±1
9	20.15±0.05	8.505±0.025	1.840±0.03	1821±2	3.633±0.002	275.5±0.5
10	20.2±0.1	8.155±0.025	1.325±0.035	1363±1	2.7305±0.0025	365±1
11	19.6±0.1	8.135±0.015	1.460±0.03	1490±2	2.970±0.01	336±1
P-value	<0.05	<0.01	<0.01	<0.0001	<0.0001	<0.0001
FEPA[1]	27	6-9	-	500	0.2	-
WHO[2]	<35	6.5-8.5	-	500	-	-
Desirable Range[3]	20-30	6.5-9	0-8 ppt	500mg/L	0.2-1.5	-

Means are significantly different at P<0.05; ppt=parts per thousand, TDS=Total Dissolved Solids, EC=Electrical Conductivity, mS/cm=millisiemens/cm, Ω.m=ohm-meter. The values represent mean±SD of duplicate measurement. [1]Federal Environmental Protection Agency (1991); [2]World Health Organization (1985); [3]Boyd (1990).

Table 2: Prevalence of *Aeromonas* sp. in water, sediment and fish samples collected randomly from eleven fish farms at Kafrelsheikh Governorate

Farm number	Water Sample	Sediment Sample	Fish Sample			
			Kidney	Spleen	Liver	Brain
1	+	+	+	+	+	+
2	+	+	+	+	-	-
3	+	+	+	+	-	-
4	+	+	+	+	+	-
5	+	+	+	+	+	+
6	+	+	+	+	+	-
7	+	+	+	+	+	-
8	+	+	+	+	-	-
9	+	+	*	*	*	*
10	+	+	*	*	*	*
11	+	+	*	*	*	*

(+): Positive, (-): Negative, (*): Not tested.

Characteristics of isolated *Aeromonas* spp.

The phenotypic characteristics and biochemical identification of the isolates (Table 3) showed that the isolates were gram-negative, rod-shaped, motile that exhibited positive catalase reaction, glucose and sucrose fermentation, and resistance to vibriostatic agent 0/129. This data were in line with the established literature, confirming the predominance of *A. hydrophila* in the freshwater aquatic environment of the region (Yardimci and Aydin 2011). In this study, the current phenotypic and biochemical characteristics is matched with original definition of *A. hydrophila* by Schubert (1968) who described the bacterium to be gram-negative, straight rod bacteria, facultative anaerobes, and may either have a polar flagellum for movement or be non-motile. *A. hydrophila* strains are carbohydrate fermenters that produce acid or acid and gas. They are oxidase-positive, reduce nitrates to nitrites, and resist the vibriostatic compound (0/129) (Semwal et al. 2023).

Confirmation of *Aeromonas* sp. by qualitative and quantitative screening for lipase

Qualitative screening for lipase

Out of the 45 isolates, forty-two (93.3 %) were lipase producers when were tested using rhodamine B/olive oil. Fig. 2 displays glowing halos around colonies, indicating lipase production by *A. hydrophila* after exposure to UV light. The screening for lipase production revealed that a significant proportion of the isolates were lipase producers, further implicating the role of lipase as a crucial virulence factor for the invasion and establishment of *Aeromonas* infection in the host. These findings align with previous reports that highlight the contribution of lipase and other virulence genes in the pathogenic nature of *Aeromonas*. The production of lipases in *Aeromonas* spp. is crucial for colonization and pathogenicity, as these enzymes modify the cytoplasmic membrane structure of the host and trigger lysis to sustain the bacterial cells. Moreover, lipase production can interact with leukocytes and affect the immune system's functioning through the free fatty acids generated by the lipolytic activity (Chen and Alonzo III 2019).

Table 3: Morpho-chemical characteristics of *Aeromonas hydrophila*

Characters	Result
Gram stain	-
Motility	+
Shape	Rod Bacilli
Catalase	+
OF test	Fermentative
Acid and gas production from glucose	+
Acid and gas production from sucrose	+
Growth in Vibriostatic agent 0/129	Resistant

(+): Positive, (-): Negative

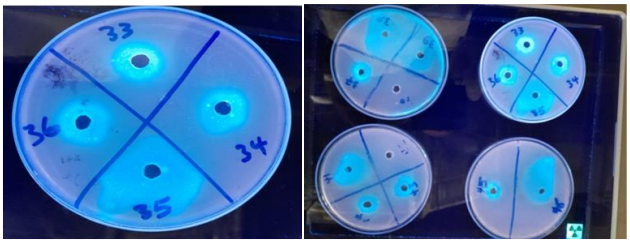

Fig. 2: Lipase production by *Aeromonas hydrophila.*

Quantitative screening for lipase

Four isolates of *A. hydrophila* that were confirmed as higher lipase producers by qualitative screening were used for quantitative test. Table 4 shows the different quantity of lipase as a virulent factor produced by four strains of isolated *A. hydrophila* with different incubation periods.

Fig. 3 shows that lipase activity was highest in the first day of inoculation with isolate No.1 and 2, and then the activity decreased with time. Activity of isolate No.9 increased on the second day of inoculation and reached the highest peak on the third day, while isolate No. 26 showed increasing on the first day then reached the peak on the second day, then decreased.

Table 4: Lipase activity of selected *Aeromonas hydrophila*

Incubation Period	Isolate 1	Isolate 2	Isolate 9	Isolate 26
1st day	73.733	61.4	4.86	32.56
2nd day	13.686	11.033	21.7	57.8
3rd day	12.275	7.625	65.9639	20.76
6th day	8.25	17.917	9.00	24.28

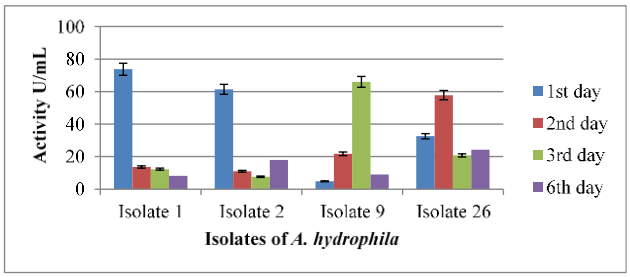

Fig. 3: Lipase activity with rhodamine B of selected *Aeromonas hydrophila*.

While this study provides initial evidence of *Aeromonas* isolation, identification, and characterization in infected Egyptian fish farms, further studies are warranted to explore the organism's molecular and serological characterization. Additionally, future research should focus on assessing Aeromonas strains' molecular epidemiology and virulence profiles better to understand their pathogenicity and potential public health risks.

Conclusion

Egypt is the third largest producer globally after China and Indonesia in tilapia production. Kafrelsheikh governorate is the largest fish producer, which produces about 50% annually of the national fish production. *A. hydrophila* is one of the main bacterial pathogens that cause considerable losses in aquaculture due to high morbidity and mortality rates; therefore, this study investigated the prevalence of *A. hydrophila* in fish farms, considering the physicochemical parameters of these farms. We conclude that *A. hydrophila* is highly prevalent in water, sediment, and fish tissue despite some physico-chemical parameters within the standard limits.

We isolated and identified *Aeromonas* in the freshwater fish farms in the Kafrelsheikh governorate using culture, phenotypic, and biochemical characterization methods, as well as a qualitative and quantitative assay to confirm lipase as a virulent factor as a diagnostic tool. The lipase screening strategy employed in this study provides a new approach to identifying *Aeromonas spp*. This bacterium is a good source of lipase, which could be used in enzymology and biotechnological applications.

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Author contributions: All authors contributed to the study conception and design. AMS collected samples, conducted experiments, and analyzed data. ALAM, AEE, and SOM revised and edited the manuscript draft. All authors revised and approved the final manuscript for publication.

REFERENCES

Akmal M, Rahimi-Midani A, Hafeez-Ur-rehman M, Hussain A and Choi TJ, 2020. Isolation, characterization, and application of a bacteriophage infecting the fish pathogen *Aeromonas hydrophila*. Pathogens 9: 215. https://doi.org/10.3390/pathogens9030215

Ali NJ, Yusoff SM, Tanko PN, Nor NA and Mayowa OO, 2021. Pathological changes associated with *Aeromonas hydrophila* dose-dependent infection and heat stress in Red Hybrid Tilapia. Acta Veterinaria Eurasia 47: 179–187. https://doi.org/10.5152/actavet.2020.20057

Ammar AM, Mohamed BE and Gharib AAEA, 2023. Motile *Aeromonads* as a Nile tilapia bacterial infection: A Review on Prevalence, Molecular Characterization, Effect on Immune Response and Alternatives Control Measures. Zagazig Veterinary Journal 51: 112–128.

APHA AW, 1998. Standard methods for the examination of water. In: Sewage and Industrial Waste. American Public Health Association p. 541.

Banagar G, Riazi B, Rahmani H and Jolodar MN, 2018. Monitoring and assessment of water quality in the Haraz River of Iran using benthic macroinvertebrates indices. Biologia (Bratisl) 73: 965–975. https://doi.org/10.2478/s11756-018-0107-5

Boyd CE, 1990. Water quality in ponds for aquaculture. Alabama Agricultural Experiment Station/Auburn University, Alabama.

Chen X and Alonzo III F, 2019. Bacterial lipolysis of immune-activating ligands promotes evasion of innate defenses. Proceedings of the National Academy of Sciences Proceedings of the National Academy of Sciences 116, 3764–3773.

Colombo SM, Roy K, Mraz J, Wan AHL, Davies SJ, Tibbetts M, Øverland M, Francis DS, Rocker MM, Gasco L, Spencer E, Metian M, Trushenski JT and Turchini GM, 2023. Towards achieving circularity and sustainability in feeds for farmed blue foods. Reviews in Aquaculture 15: 1115–1141. https://doi.org/10.1111/raq.12766

Eissa AE, 2016. Clinical and laboratory manual of fish diseases. Lambert Academic Publishing.

El-Leithy AAA, Hemeda SA, El Naby WSHA, El Nahas AF, Hassan SAH, Awad ST, El-Deeb SI and Helmy ZA , 2019. Optimum salinity for Nile tilapia (*Oreochromis niloticus*) growth and mRNA transcripts of ion-regulation, inflammatory, stress and immune-related genes. Fish Physiology and Biochemistry 45: 1217–1232. https://doi.org/10.1007/s10695-019-00640-7

FEPA, 1991. Guidelines and standards for environmental control in Nigeria. Federal Environmental Protection Agency, Government Press, Lagos p 238.

Hutson KS, Davidson IC, Bennett J, Poulin R and Cahill PL, 2023. Assigning cause for emerging diseases of aquatic organisms. Trends in Microbiology 31: 681–691.

Ibearugbulam HO, Ugwu EI, Ekeleme AC, Njoku CE, Amanamba EC, Ezebuiro V, Ibe OP and Igwegbe E, 2021. A study on physicochemical parameters of fish pond effluents: A case study of Umudibia fish farm. In: Institute of Physics Conference Series: Materials Science and

Engineering. Institute of Physics Publishing p. 012005. https://doi.org/10.1088/1757-899x/1036/1/012005

Ina-Salwany MY, Al-saari N, Mohamad A, Mursidi FA, Mohd-Aris A, Amal MNA, Kasai H, Mino S, Sawabe T and Zamri-Saad M, 2019. Vibriosis in Fish: A Review on Disease Development and Prevention. Journal of Aquatic Animal Health. 31: 3–22. https://doi.org/10.1002/aah.10045

Kane S, Qarri F, Lazo P and Bekteshi L, 2015. The Effect of Physico-Chemical Parameters and Nutrients on Fish Growth in Narta Lagoon , Albania. Journal of Hygiene Engineering and Design10: 62–68.

Kassem MM, Elsbaay AM, AbouZaher SE and Abdelmotaleb A, 2016. Energetic performance assessment of a Thermo-Solar Greenhouse Fish (Nile Tilapia) Hatchery. Misr Journal of Agricultural Engineering 33: 1649–1674. https://doi.org/10.21608/mjae.2016.97625

Katiyar PP, Hare V and Baghel VS, 2017. Isolation, partial purification and characterization of a cold active lipase from *Pseudomonas* spp., isolated from Satopanth Glacier of Western Himalaya, India. International Journal of Scientific Research and Management 5: 6106–6112. https://doi.org/10.18535/ijsrm/v5i7.37

Koneman EW, Allen SD, Janda,WM, Schreckenberger PC, Winn JrWC and Woods GL, 1995. Introduction to diagnostic Microbiology, Archives of Pathology and Laboratory Medicine. JB Lippincott company Philadelphia, Pennsylvania.

Kumar DG, 2017. Study of seasonal water quality assessment and fish pond conservation in Thanjavur, Tamil Nadu, India. Journal of Entomology and Zoology Studies 5: 1232–1238.

Makori AJ, Abuom PO, Kapiyo R, Anyona DN and Dida GO, 2017. Effects of water physico-chemical parameters on tilapia (*Oreochromis niloticus*) growth in earthen pon;ds in Teso North Sub-County, Busia County. Fish and Aquatic Sciences 20: 1–10. https://doi.org/10.1186/s41240-017-0075-7

Monira U and Mostafa MG, 2023. Leather industrial effluent and environmental concerns: a review. Sustainable Water Resources Management 9(6):181.

Mostafa M, Ghaly M and Salama SS, 2024. Prevalence of aerolysin and hemolysin virulence genes and antibacterial resistance profile to control Motile *Aeromonas* Septicemia (MAS) isolated from *Oreochromis niloticus* in the Nile River, Egypt. Bulletin of the Faculty of Science, Zagazig University 2024(2), pp.132-141.

Mramba RP and Kahindi EJ, 2023. Pond water quality and its relation to fish yield and disease occurrence in small-scale aquaculture in arid areas. Heliyon 9(6): e16753. https://doi.org/10.1016/j.heliyon.2023.e16753

Najeeb LM, Hajwal SI, AbdulRazzaq AH and AlJobory MB, 2021. Study the effect of some natural oils to increase some antibiotics activates towards *Aeromonas hydrophila*. Annals of the Romanian Society for Cell Biology 25: 3667–3675.

Odds F, 1981. Biochemical tests for identification of medical bacteria. Journal of Clinical Pathology 34: 572–572. https://doi.org/10.1136/jcp.34.5.572-a

Olaoluwa D, 2024. Assessment of aquaculture wastewater impact on Osun river, Ede, Nigeria. Proceedings of the Nigerian Society of Physical Sciences, pp.79-79.

World Health Organization, 1985. Drinking-water quality control in small-community supplies. In: Drinking-water quality control in small-community supplies, pp: 121-121.

Rehman S, Bhatti HN, Bilal M, Asgher M and Wang P, 2017. Catalytic, kinetic and thermodynamic characteristics of an extracellular lipase from Penicillium notatum. Catalysis Letters 147: 281–291.

Sanou A, Coulibaly S, Guéi AML, Baro M, Tanon EFT, Méité N and Atsé BC, 2022. Assessment of some physico-chemical parameters of the fish farm water in Abengourou, Côte d'Ivoire. Egyptian Journal of Aquatic Biology and Fisheries 26: 319–343. https://doi.org/10.21608/EJABF.2022.261442

Schubert RH, 1968. The taxonomy and nomenclature of the genus *Aeromonas* Kluyver and van Niel 1936: Part III. Suggestions on the definition of the genus *Aeromonas* Kluyver and van Niel 1936. International Journal of Systematic and Evolutionary Microbiology 18(1): 1-7. https://doi.org/10.1099/00207713-18-1-1

Semwal A, Kumar A and Kumar N, 2023. A review on pathogenicity of *Aeromonas hydrophila* and their mitigation through medicinal herbs in aquaculture. Heliyon 9 (3): e14088. doi: 10.1016/j.heliyon.2023.e14088

Sherif AH, Farag EAH and Mahmoud AE, 2024. Temperature fluctuation alters immuno-antioxidant response and enhances the susceptibility of *Oreochromis niloticus* to *Aeromonas hydrophila* challenge. Aquaculture International 32: 2171–2184.

Trivedy RK and Goel PK, 1986. Chemical and biological methods for water pollution studies. Karad Environmental Publications.

Uiuiu P, Cocan D, Constantinescu R, Latiu C, Sava A, Hegedus C, Coroian A, Ihut A, Raducu C and Miresan V, 2020. Water quality parameters which influence rainbow trout (*Oncorhynchas mykiss*) welfare in classic systems. Scientific Papers. Series D. Animal Science 63: 509–515.

Yardimci B and Aydin Y, 2011. Pathological findings of experimental *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus)*. Ankara Üniversitesi Veterinary Fakültesi Derg 58: 47–54.

Yue GH, Ma KY and Xia JH, 2024. Status of conventional and molecular breeding of salinity-tolerant tilapia. Reviews in Aquaculture 16: 271–286