

Effect of zinc supplementation from *Moringa oleifera* in feed and environment on growth performance and nutritional content of Nile tilapia

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Abstract. Zinc is an essential element that is crucial in various biological processes. Common sources of zinc for fish are typically in inorganic forms. The use of inorganic zinc can potentially lead to metal accumulation, posing risks of negative impacts on fish and the environment. One alternative that can be offered is the use of natural zinc sources from plants that have high zinc content, such as *Moringa oleifera*. This study aims to evaluate the effects of zinc supplementation from *Moringa oleifera* on growth performance and nutritional content in Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)). The research consists of three experimental forms: supplementation in feed (F), supplementation in the environment (E), and a combination of feed + environment (FE). The feed experiment involved adding moringa leaf extract to the feed, while the environmental supplementation included adding moringa extract to the rearing medium. The combination experiment (feed + environment) was conducted by adding moringa extract to both the feed and the rearing medium. The results indicate that the overall zinc supplementation in tilapia significantly enhances growth performance, protein content, and zinc levels in the body. This study concludes that the utilization of *Moringa oleifera* as a natural zinc source has been proven to significantly improve growth performance, protein content, and mineral zinc levels in *O. niloticus*.

Key Words: aquaculture, *Moringa oleifera* leaf, natural mineral source, *Oreochromis niloticus*.

Introduction. Zinc (Zn) is an essential element that plays a crucial role in various biological processes in animals (Rahman et al 2022). In aquaculture, this mineral functions as a specific cofactor in many metabolic pathways, such as antioxidant enzyme activation (e.g., superoxide dismutase), as well as supporting growth, bone mineralization, and physiological functions in fish (Mohammady et al 2021; Sansuwan et al 2023). Zinc deficiency in fish can cause a range of health issues, reduced growth, increased mortality rates, and may lower egg production and hatchability (Thangapandiyam & Monika 2020; Lall & Kaushik 2021).

The zinc requirements for various fish species have been established, including Nile tilapia. This species is among the most popular freshwater fish cultivated in aquaculture across many countries. In 2022, *O. niloticus* ranked as the fourth most-produced aquaculture commodity, with a production volume reaching 5.3 million tonnes (FAO 2024). The optimal dietary zinc intake for Nile tilapia growth is reported to be in the range of 20–50 mg kg⁻¹ (Suprayudi et al 2024).

Although zinc is an essential element, it can become toxic to fish when administered in excessive amounts (Rajkumar et al 2022). Common sources of zinc used for fish typically come in inorganic forms, such as zinc sulfate or zinc oxide (Aziz et al 2020; Rohaidi et al 2022). The use of inorganic zinc can potentially lead to heavy metal accumulation, posing risks of negative impacts on fish and the environment (Garai et al 2021).

The use of natural zinc sources from plants has not yet been extensively explored. One plant with a high zinc content is moringa (*Moringa oleifera*). The leaves of *M. oleifera* contain zinc ranging from 1.0 to 1.6 mg/100 g (Valdez-Solana et al 2015). This plant has been widely utilized in feed formulations, which can enhance the growth performance of

fish (Abd El-Gawad et al 2020). The use of *M. oleifera* as a zinc source in fish feed represents an innovative approach to reducing dependency on inorganic mineral supplements, while also offering a more environmentally friendly alternative. Therefore, this study aims to evaluate the effects of zinc supplementation from *M. oleifera* in feed and environment on the growth performance and nutritional content of *O. niloticus*.

Material and Method

Preparation of containers and test animals. The research containers are aquariums with a water volume of 20 liters per aquarium. Each aquarium is equipped with an aerator as an oxygen source. The test animals used are juvenile *O. niloticus* with an initial weight of 10 grams and a length of 5 cm. The tilapia are maintained at a stocking density of 2 fish per liter. Thus, each aquarium contains a total of 40 juvenile *O. niloticus*.

Extraction of *Moringa oleifera* leaf. The extraction was carried out following the methodology established by Syahputra et al (2021), with minor modifications to optimize the process. Only fresh *M. oleifera* leaves, ideally neither too mature nor too young, were selected for extraction. Mature leaves tend to be dry, leading to a reduced concentration of essential chemical compounds, while younger leaves contain lower levels of these bioactive substances. The extraction process began by thoroughly washing the selected fresh leaves with clean water to remove any contaminants. The leaves were then subjected to a controlled drying process indoors at a temperature ranging from 25–30°C for 3 days, shielded from direct sunlight to preserve their chemical integrity. Once fully dried, the leaves were weighed to determine their dry mass, approximately 1 kg. The dried *M. oleifera* leaves were placed in a glass jar, and 96% ethanol was added at a ratio of 3 × 3000 mL. This mixture was left to stand for 24 hours to ensure optimal extraction. Afterward, the filtrate was carefully separated using a rotary evaporator set at 50°C and 200 mbar for 42 minutes, ensuring complete removal of the solvent. The resulting filtrate was then prepared for further analyses and observations.

Experimental design. This research comprises three experimental approaches: feed addition (F), environmental addition (E), and a combination of feed and environmental addition (FE). In the feed experiment, *M. oleifera* leaf extract is incorporated into the feed using the repleting method. The details of the feed experiment are presented in Table 1. The environmental addition involves the introduction of *M. oleifera* leaf extract into the rearing medium, with specific details outlined in Table 2. The combination treatment of feed and environment entails adding *M. oleifera* leaf extract to both the feed and the rearing medium, as detailed in Table 3. The dosages used in both feed and environmental treatments were determined based on preliminary trials. The experimental design employed is a completely randomized design, where each of the three experiments is tested separately. Each experiment includes three replicates. The maintenance of *O. niloticus* is carried out over three months. During this maintenance period, feeding occurs twice daily at 08:00 and 18:00 (UTC +8), with a feeding frequency of 5% of the total body weight of the fish (Muin et al 2017). The feed provided contains 35% protein and has a particle size of 1-2 mm.

Table 1

Details of the feed experiment

<i>Treatment</i>	<i>Description</i>
FC	Control treatment (Feed without <i>M. oleifera</i> leaf extract addition)
F50	Feed with the addition of 50 mg kg ⁻¹ <i>M. oleifera</i> leaf extract
F100	Feed with the addition of 100 mg kg ⁻¹ <i>M. oleifera</i> leaf extract
F150	Feed with the addition of 150 mg kg ⁻¹ <i>M. oleifera</i> leaf extract

Table 2

Details of the environmental experiment

<i>Treatment</i>	<i>Description</i>
EC	Control Treatment (Without addition of <i>M. oleifera</i> leaf to the rearing medium)
E50	Addition of 50 mg/container of <i>M. oleifera</i> leaf extract to the rearing medium
E100	Addition of 100 mg/container of <i>M. oleifera</i> leaf extract to the rearing medium
E150	Addition of 150 mg/container of <i>M. oleifera</i> leaf a extract to the rearing medium

Table 3

Details of the feed + environment combination experiment

<i>Treatment</i>	<i>Description</i>
FEC	Control Treatment: No addition of <i>M. oleifera</i> leaf extract to the feed and rearing medium.
FE50	Combination of 50 mg kg ⁻¹ feed + 150 mg/container <i>M. oleifera</i> leaf extract
FE100	Combination of 100 mg kg ⁻¹ feed + 100 mg/container <i>M. oleifera</i> leaf extract
FE150	Combination of 150 mg kg ⁻¹ feed + 50 mg/container <i>M. oleifera</i> leaf extract

Observation parameters. The parameters observed include growth performance indicators such as absolute weight, absolute length, Food Conversion Ratio (FCR), Feed Efficiency, and Survival Rate (Panase & Mengumphan 2015; Thaiso et al 2024). Additionally, the proximate composition of the body is assessed, including protein, carbohydrates, fat, ash content, crude fiber, and moisture content (Karapanagiotidis et al 2019). The mineral content of zinc in the fish body is also measured (Yu et al 2021). Water quality parameters, including temperature, pH, Dissolved Oxygen (DO), nitrate, nitrite, ammonia, and phosphate, are also monitored in this study.

Statistical analysis. Proximate composition and water quality data are analyzed descriptively. Meanwhile, growth performance data and zinc mineral content in the fish body are subjected to analysis of variance (ANOVA) using SPSS 20. If the ANOVA results indicate significant differences, further analysis will be performed using the Least Significant Difference (LSD) test.

Results. The results of the growth performance observation of *Oreochromis niloticus* (tilapia) fed with moringa leaf extract through feed are presented in Table 4. The experiment involving the addition of moringa leaf extract through feed showed a significant effect on growth performance, including final weight, weight growth, final length, length growth, FCR, and feed efficiency ($p < 0.05$). However, the data on survival rate (SR) did not show a significant effect ($p > 0.05$). The results of the growth performance of tilapia given moringa leaf extract through the rearing medium are shown in Table 5. This experiment showed significant effects on growth performance parameters, including final weight, weight growth, final length, length growth, FCR, feed efficiency, and SR ($p < 0.05$). The results of the growth performance observation of tilapia given moringa leaf extract through a combination of feed and environment are presented in Table 6. This experiment also showed a significant impact on growth performance indicators such as final weight, weight growth, final length, length growth, FCR, feed efficiency, and SR ($p < 0.05$). Overall, the administration of moringa leaf extract, whether through feed, the rearing medium, or a combination of both, produced significantly better results compared to the control.

Table 4

Growth performance of Nile tilapia fed with moringa leaf extract through feed

	<i>Treatment</i>			
	F50	F100	F150	FC
Initial weight (g)	13.00±0.00 ^a	13.00±0.00 ^a	13.00±0.00 ^a	13.00±0.00 ^a
Final weight (g)	13.75±14.28 ^b	14.29±14.62 ^b	17.95±18.23 ^c	10.88±10.91 ^a
Weight growth (g)	3.75±4.28 ^b	4.29±4.62 ^b	7.95±8.23 ^c	0.88±0.91 ^a
Initial length (cm)	5.00±0.00 ^a	5.00±0.00 ^a	5.00±0.00 ^a	5.00±0.00 ^a
Final length (cm)	7.68±8.06 ^b	8.07±8.30 ^b	10.68±10.88 ^c	5.63±5.65 ^a
Length growth (cm)	2.68±3.06 ^b	3.07±3.30 ^b	5.68±5.88 ^c	0.63±0.65 ^a
FCR	0.77±0.82 ^b	0.74±0.77 ^b	1.16±1.30 ^a	0.68±0.71 ^b
Feed efficiency (%)	74.71±76.20 ^b	77.39±79.86 ^b	89.73±92.30 ^c	66.20±70.65 ^a
SR (%)	88.33±95.00 ^a	80.00±90.00 ^a	68.33±80.00 ^a	68.33±80.00 ^a

Data (Mean ± SD) in the same row with different letters are significantly different ($p < 0.05$). The absence of letters indicates no significant difference between treatments.

Table 5

Growth performance of Nile tilapia treated with moringa leaf extract through the rearing medium

	<i>Treatment</i>			
	E50	E100	E150	EC
Initial weight (g)	13.00±0.00 ^a	13.00±0.00 ^a	13.00±0.00 ^a	13.00±0.00 ^a
Final weight (g)	13.30±13.70 ^c	11.45±11.57 ^b	11.35±11.48 ^b	10.84±10.94 ^c
Weight growth (g)	3.30±3.70 ^c	1.45±1.57 ^b	1.35±1.48 ^b	0.84±0.94 ^a
Initial length (cm)	5.00±0.00 ^a	5.00±0.00 ^a	5.00±0.00 ^a	5.00±0.00 ^a
Final length (cm)	7.35±7.64 ^c	6.04±6.12 ^b	5.97±6.06 ^b	5.60±5.67 ^a
Length growth (cm)	2.35±2.64 ^c	1.04±1.12 ^b	0.97±1.06 ^b	0.60±0.67 ^a
FCR	1.06±1.30 ^a	0.86±0.91 ^{ab}	0.72±0.75 ^b	0.64±0.69 ^b
Feed Efficiency (%)	85.00±86.34 ^c	74.88±76.89 ^b	71.21±73.21 ^b	61.35±62.78 ^a
SR (%)	61.67±70.00 ^b	68.33±75.00 ^b	91.67±95.00 ^c	43.33±55.00 ^a

Data (Mean ± SD) in the same row with different letters are significantly different ($p < 0.05$). The absence of letters indicates no significant difference between treatments.

Table 6

Growth performance of Nile tilapia treated with moringa leaf extract through the combination of feed and rearing environment

	<i>Treatment</i>			
	FEC	FE50	FE100	FEC
Initial weight (g)	13.00±0.00 ^a	13.00±0.00 ^a	13.00±0.00 ^a	13.00±0.00 ^a
Final weight (g)	15.77±16.30 ^a	16.83±18.32 ^{ab}	17.80±18.04 ^b	14.75±15.08 ^a
Weight growth (g)	5.77±6.30 ^a	6.83±8.32 ^{ab}	7.80±8.04 ^b	4.75±5.08 ^a
Initial length (cm)	5.00±0.00 ^a	5.00±0.00 ^a	5.00±0.00 ^a	5.00±0.00 ^a
Final length (cm)	9.12±9.50 ^a	9.88±10.94 ^{ab}	10.57±10.74 ^b	8.39±8.63 ^a
Length growth (cm)	4.12±4.50 ^a	4.88±5.94 ^{ab}	5.57±5.74 ^b	3.39±3.63 ^a
FCR	0.78±0.80 ^b	0.83±0.89 ^b	1.18±1.33 ^a	0.67±0.71 ^b
Feed Efficiency (%)	72.31±74.68 ^a	70.42±72.60 ^a	85.88±88.74 ^b	66.10±71.32 ^a
SR (%)	63.33±70.00 ^b	46.67±60.00 ^a	81.67±85.00 ^c	60.00±65.00 ^{ab}

Data (Mean ± SD) in the same row with different letters are significantly different ($p < 0.05$). The absence of letters indicates no significant difference between treatments.

Table 7 presents the results of the body proximate composition analysis. The observations indicate that the administration of moringa leaf extract through feed, rearing medium, or a combination of both resulted in higher protein, ash, crude fiber, and moisture content in the body of Nile tilapia (*O. niloticus*) compared to the control. Meanwhile, carbohydrate and fat content remained relatively stable.

Table 7

Proximate composition of Nile tilapia treated with *Moringa oleifera* leaf extract through feed, rearing medium, and a combination of both

<i>Treatment</i>	<i>Protein</i>	<i>Carbohydrates</i>	<i>Fat</i>	<i>Ash content</i>	<i>Crude fiber</i>	<i>Moisture content</i>
<i>Administration of M. oleifera</i> leaf extract through feed						
F50	11.66	0.12	0.62	3.37	0.71	78.01
F100	11.23	0.09	0.56	3.15	0.58	76.81
F150	11.92	0.16	0.47	3.52	0.79	78.45
FC	10.87	0.16	1.54	3.05	0.63	75.63
<i>Administration of M. oleifera</i> leaf extract through the rearing medium						
E50	29.15	0.36	1.86	10.11	2.13	78.01
E100	28.08	0.27	1.68	9.45	1.74	76.81
E150	29.80	0.48	1.41	10.56	2.37	78.45
EC	27.18	0.48	4.62	9.15	1.89	75.63
<i>Administration of M. oleifera</i> leaf extract through a combination of feed and rearing environment						
FE50	13.83	0.08	1.40	3.90	0.79	81.02
FE100	12.41	0.12	0.89	3.57	0.88	81.46
FE150	11.83	0.04	0.97	3.51	0.96	80.76
FEC	11.27	0.08	0.70	3.49	1.04	79.31

The results of water quality observations are presented in Table 8. The observations show that the temperature was 26°C, pH 7, dissolved oxygen (DO) ranged from 5 to 7 mg L⁻¹, and ammonia was 1.5 mg L⁻¹. The temperature was below the optimal range, while pH and DO were within optimal conditions. However, ammonia levels exceeded the optimal condition.

Table 8

Water quality during rearing with the administration of *Moringa oleifera* leaf extract through feed (F), rearing medium (E), and a combination of both (FE)

<i>Parameter</i>	<i>E</i>	<i>F</i>	<i>FE</i>	<i>Optimum</i>
Temperature (°C)	26.3-26.5	26.3-26.4	26.3-26.6	29-31 ^a
pH	7.0-7.3	7.0-7.5	7.6-7.9	6-9 ^a
DO (mg L ⁻¹)	5.9-7.1	4.8-6.8	7.6-7.9	>4 ^b
Ammonia (mg L ⁻¹)	1.5	1.5	1.5	<0.1 ^a

^aAbd El-Hack et al 2022; ^bGodoy et al 2021.

The results of the mineral content observations are presented in Figure 1. The administration of Moringa leaf extract, whether through feed, the rearing medium, or a combination of both, resulted in higher protein content, ash content, crude fiber, and moisture content in the body of Nile tilapia compared to the control. Meanwhile, carbohydrate and fat content remained relatively stable.

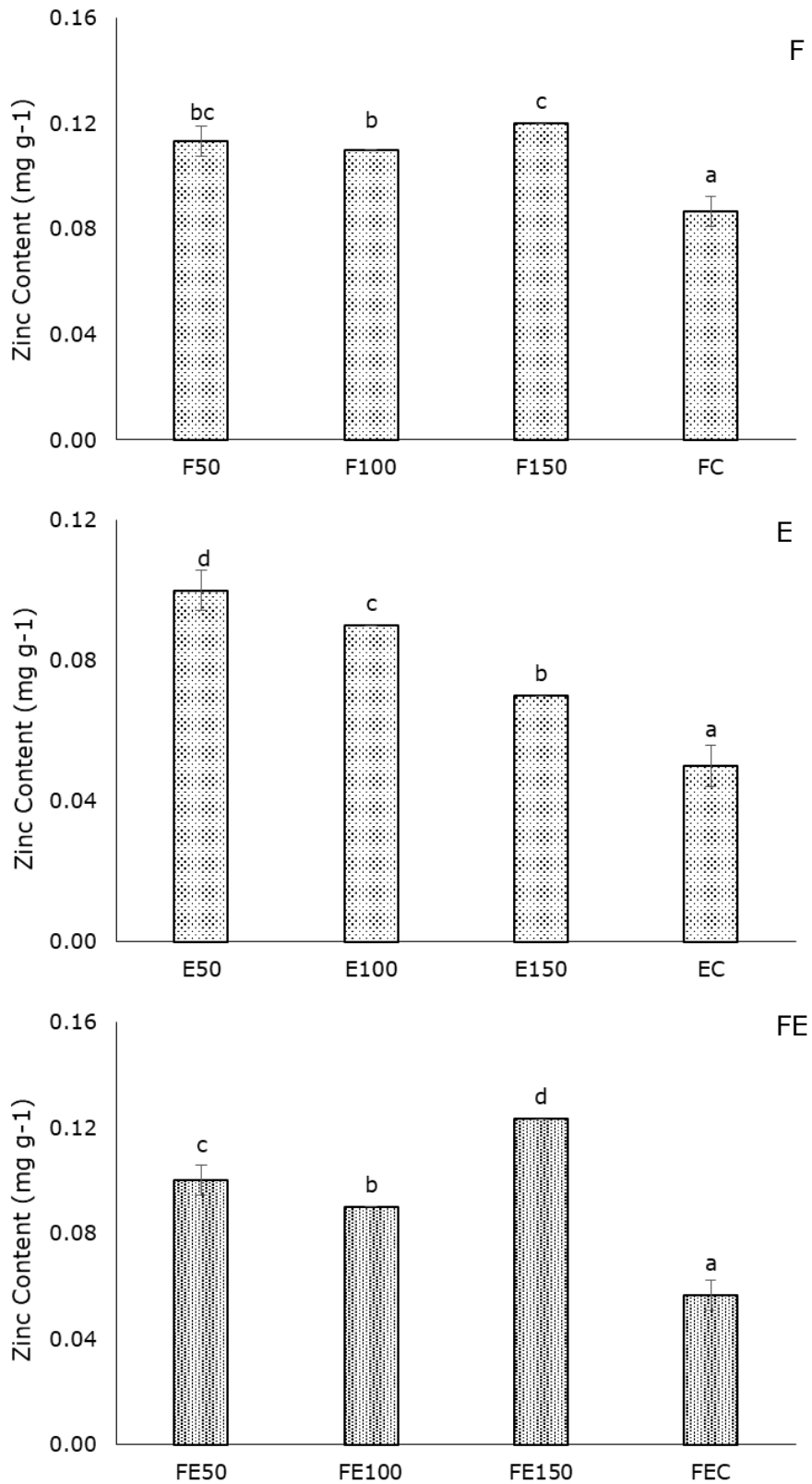


Figure 1. Zinc mineral content in the body of Nile tilapia was administered with *Moringa oleifera* leaf extract through feed (F), rearing medium (E), and a combination of both (FE).

Discussion. Zinc is an essential element that plays a crucial role in various biological processes in aquaculture commodities, serving as a specific cofactor in many metabolic pathways, growth, bone mineralization, and physiological functions of fish (Mohammady et al 2021). Zinc deficiency in fish can lead to various health issues, reduced growth, increased mortality rates, and decreased egg production and hatchability (Thangapandiyan & Monika 2020; Lall & Kaushik 2021). However, zinc can also become toxic to fish when provided in excessive amounts (Rajkumar et al 2022). The common sources of zinc for fish are usually in inorganic forms, such as zinc sulfate or zinc oxide (Aziz et al 2020; Rohaidi et al 2022). The use of inorganic zinc may result in the accumulation of heavy metals, potentially posing negative impacts on both fish and the environment (Garai et al 2021).

The use of natural zinc sources from plants has not yet been extensively explored. One plant that is known to have a high zinc content is *Moringa oleifera*. The utilization of Moringa leaves in this study revealed that it could significantly enhance the growth of *O. niloticus*, showing a notable difference compared to the control treatment without Moringa. This finding is consistent with Abd El-Gawad et al (2020) on Nile tilapia fry. Moringa leaves can enhance growth due to their high mineral content, including zinc, ranging from 1.0 to 1.6 mg/100 g (Valdez-Solana et al 2015). Zinc plays a vital role in growth by supporting the release of growth hormone (GH) and increasing the synthesis of insulin-like growth factor (IGF-I), both of which are key regulators of cell proliferation and protein synthesis. IGF-I, in particular, stimulates the growth of muscle and bone tissues, contributing to overall body growth (Estefan et al 1998). Moreover, zinc positively influences the composition of gut microbiota by promoting the proliferation of beneficial probiotic strains, thereby improving nutrient absorption efficiency and digestion (Diao et al 2021). This enhanced digestion enables more effective nutrient absorption, supporting the body's energy needs and contributing to improved growth and development (De Marco et al 2023), which can ultimately enhance the growth of *O. niloticus*.

This study also found an increase in protein and zinc mineral content in the bodies of fish subjected to treatments with Moringa leaf extract. This is suspected to be due to one of the functions of zinc, which can enhance protein synthesis in the body, including in fish muscle tissues (Suganya et al 2020). Additionally, zinc influences myogenesis, which is the formation of new muscle cells, and the regeneration of skeletal muscle through the activation, proliferation, and differentiation of muscle cells. With zinc supplementation, these processes can increase muscle mass and protein content in fish bodies, potentially enhancing the zinc levels in fish flesh or muscles. This occurs because zinc accumulates in body tissues alongside the increased synthesis of proteins and the formation of new cellular structures (Hernández-Camacho et al 2020). Thus, the study shows an increase in protein and zinc minerals in *O. niloticus*.

Water quality can also impact growth performance and other biological conditions. Observations showed that the temperature was at 26°C, pH was 7, dissolved oxygen (DO) ranged from 5 to 7 mg L⁻¹, and ammonia was at 1.5 mg L⁻¹. The temperature was below the optimal range of 29–31°C; however, Nile tilapia can still grow at temperatures as low as 11°C (Abd El-Hack et al 2022). The pH and DO levels were within optimal conditions, which can support the growth of Nile tilapia (Godoy et al 2021; Abd El-Hack et al 2022). Meanwhile, ammonia levels exceeded the optimal condition of <0.1 mg L⁻¹; although it reached 1.5 mg L⁻¹, this concentration was still not toxic to Nile tilapia, which is above 7 mg L⁻¹ (Abd El-Hack et al 2022). Therefore, water quality can support the growth of *O. niloticus* in this study.

Conclusions. The utilization of *M. oleifera* as a natural source of zinc has been shown to significantly enhance the growth performance, protein content, and zinc mineral levels in the bodies of Nile tilapia. This study highlights the potential benefits of incorporating *M. oleifera* leaf extract into aquaculture practices, as it not only contributes to improved growth rates but also boosts the nutritional quality of the fish. The increased availability of zinc from this natural source supports vital biological processes, such as protein synthesis and muscle development, ultimately leading to healthier and more robust fish. Therefore, integrating *M. oleifera* into fish diets presents a promising strategy for enhancing aquaculture productivity while simultaneously promoting sustainable practices.

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Conflict of interest. The authors declare that they have no conflict of interest.

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