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Systematic Review

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Effects of Zinc Oxide Nanoparticles on the Growth Performance and Immune Response of Nile Tilapia (*Oreochromis niloticus*): A Systematic Review

Ayesha Ali[1], Fariha Ibrahim[2*], Iffat Imran[1] and Bushra Anzar[1]

[1]Department of Biomedical Engineering, Ziauddin University, Karachi, Pakistan
[2]Department of Biotechnology, Bahria University, Karachi, Pakistan

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***Corresponding Author:**
Fariha Ibrahim
Department of Biotechnology, Bahria University, Karachi, Pakistan
farihaibrahim.buhsck@bahria.edu.pk

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ABSTRACT

Nile Tilapia, Oreochromis niloticus, is a globally important aquaculture species. Production of this species is confronted with challenges such as disease outbreaks and environmental stress. However, the application of nanoparticles in aquaculture, especially zinc oxide nanoparticles (ZnO NPs), has been reported with remarkable positive results due to high bioavailability, but there is limited information about optimum doses and their potential toxicity. **Objective:** To determine the effects of zinc oxide nanoparticles on the growth performance and immune system in Nile tilapia. **Methods:** In this study, 20 research articles published from 2020 to 2025 were reviewed. These articles were specifically selected for their focus on the impacts of ZnO NPs on Nile tilapia. **Results:** A total of twenty studies were considered in the present review. The use of ZnO NPs was found to be effective in enhancing the weight gain, antioxidant activity, IgM content, and survival rate of Nile tilapia. The best concentration of ZnO NPs was found to be in the range of 10-60 mg/kg for the diet and 0.5 mg/L for water. However, high concentrations of ZnO NPs reported a negative impact on fish health. ZnO NPs could improve growth and immunity of Nile tilapia at the optimum dosage. **Conclusions:** This review shows that optimized doses of ZnO NPs improved the growth and immunity of Nile tilapia. However, green-synthesized nanoparticles are comparatively safer. Doses above recommended limits can be toxic for Nile tilapia.

INTRODUCTION

The aquaculture industry continues to grow into a vital source of high-quality protein for food security and economic benefits [1, 2]. Known as the "aquatic chicken," the Nile tilapia (*Oreochromis niloticus*) is cultured in more than 120 countries because of its fast growth, environmental tolerance, and rich nutritive profile [3-5]. Despite its importance in commercial tilapia farming, there are several issues associated with tilapia culture, such as diseases, environmental factors, and inefficient feed use [6]. Recent advances in nanotechnology have paved the way for new ways of improving aquaculture efficiency. Being extremely small (1-100 nm), nanoparticles have a large surface-area-to-volume ratio that allows them to penetrate biological cells [7, 8]. Among nanoparticles, zinc oxide nanoparticles (ZnO NPs) have attracted attention because zinc plays an important role in catalysis of enzymatic reactions, protein synthesis, and immune responses [9]. Compared to common zinc additives, ZnO NPs possess much higher biological availability, with studies demonstrating 15-20 times greater intestinal absorption [10]. Supplementation with ZnO NPs at 10-60 mg/kg consistently increased final body weight, specific growth rate (SGR), and feed conversion ratio (FCR), and improved survival under pathogen challenge [11]. On the

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other hand, dose-dependent toxicity is still a key factor. ZnO NPs overdose causes oxidative stress, lipid peroxidation, DNA damage, and permanent histological changes in the liver and gills [12]. Synthesis techniques, particle size, surface charge, and route of administration also determine the effectiveness and safety of ZnO NPs [13].

Although the ideal amount of ZnO NPs in Nile tilapia is yet to be established, their effectiveness cannot be underestimated. Whereas many researchers have studied the use of ZnO NPs in aquaculture, only a few have studied the overall impact of these nanoparticles on the growth performance and immunity of the Nile tilapia (*Oreochromis niloticus*). Moreover, results from previous studies have not been consistent, and there has been no critical evaluation of all existing knowledge in this particular field. Thus, it is important that a systematic review is performed in order to consolidate information regarding the impacts of ZnO NPs on Nile tilapia. Therefore, this systematic review aimed to determine the effects of green-synthesized ZnO NPs on growth and immune function of Nile Tilapia.

METHODS

A thorough literature search was conducted using electronic databases such as ScienceDirect, PubMed, and Google Scholar. to collect relevant articles, literature search was conducted using keywords such as "zinc oxide nanoparticles", "ZnO nanoparticles", "nano zinc oxide", "Nile tilapia", "*Oreochromis niloticus*", "growth performance", "immune response", "oxidative stress", and "aquaculture", combined by using Boolean operators "AND" and "OR'. In PubMed, appropriate MeSH terms were also applied to improve search precision. The related articles were imported to reference management software, Endnote. First, the duplicates were removed, followed by title and abstract screening. After this, full-text articles were obtained via different channels, and they were then examined carefully for inclusion criteria. All articles were carefully examined by all authors, and any differences were sorted through discussion or through adjudication by an independent third senior reviewer. This paper followed the PICO structure: P (Population): Nile tilapia (O. niloticus) at all life stages, I (Intervention): Feeding/dissolving of ZnO NPs via diet through different methods of production, C (Comparison): Effects of ZnO NPs containing diet or control without supplementation with ZnO NPs. O (Outcomes): Growth parameters (weight gain, SGR, FCR), immune response, antioxidant activity, survival, and toxicity. This article includes studies in which experimental research was conducted evaluating zinc oxide nanoparticles administered either through feed or water to Nile tilapia. Furthermore, only the studies with clear results of effect and characterization of nanoparticles with well-defined methodologies were included. Additionally, only the peer-reviewed research articles in the English language were included, excluding review papers, conference proceedings, non-English articles, and studies lacking sufficient methodological details.

Data extraction was conducted independently by two reviewers using a customized data extraction form that had been previously tested on a pilot sample for this systematic review. The form included the following information: author and year of publication; study design; sample size; methodology for synthesizing ZnO NPs; particle size; dose/concentration; exposure duration; mode of administration; and all quantitative results (growth parameters, immunological parameters, antioxidant enzyme activity, mortality, and toxicity results. Due to the diversity of the nanoparticles used, sizes, dosages, and duration of the experiment, some of the data were analysed qualitatively rather than through statistical means. By comparing the results of the treated groups with those of the respective controls, general trends regarding the effects of zinc oxide nanoparticles on Nile tilapia were identified. Ethical compliance of the selected primary studies was judged based on the ARRIVE 2.0 guidelines. The systematic search resulted in a total of 323 records, while an additional 27 studies were found using citation chaining. After deduplication, 350 distinct papers were screened. The title and abstract screening process excluded 312 records based on their lack of relevance to Nile tilapia, non-experimental study type, and failure to characterize ZnO NPs. Out of 38 full-text papers, 18 were rejected from the study for having unsatisfactory methodology (n=7); testing with other fish species than Nile tilapia or other varieties of tilapia (n=7); and unspecified nanoparticle characteristics (n=4). The remaining 20 studies were finally selected for qualitative analysis.

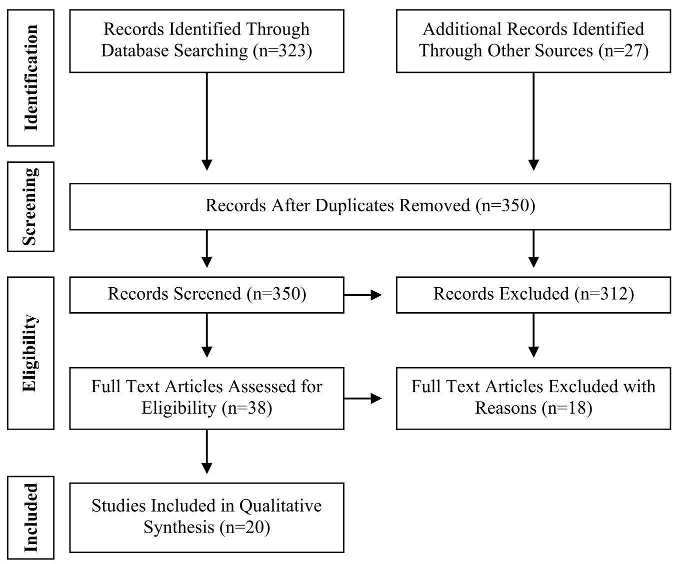

Figure 1: PRISMA Flow Diagram Illustrating the Procedure Adopted in Selecting the Relevant Articles

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RESULTS

The potential bias in the included articles was assessed using an adapted version of the Cochrane Risk of Bias 2 (RoB 2) assessment tool, focusing on in vivo animal exposure trials, including randomized controlled, quasi-experimental, and dose-response trials. The assessments in each study were made in relation to the five main RoB 2 criteria: (1) the randomization procedure; (2) any deviations from the interventions planned; (3) missing outcome data; (4) the measurement of the outcome; and (5) the reporting of the outcome. Any differences among the two independent assessors were addressed through consensus or involving a third reviewer where necessary. The assessments were shown using traffic lights, with green signifying low risk, yellow signifying some concerns, and red signifying high risk. All included studies indicated some concern or a high-risk bias in Domains 1, 2, 4, and 5, except for Domain 3 (missing outcome data) (Figure 2).

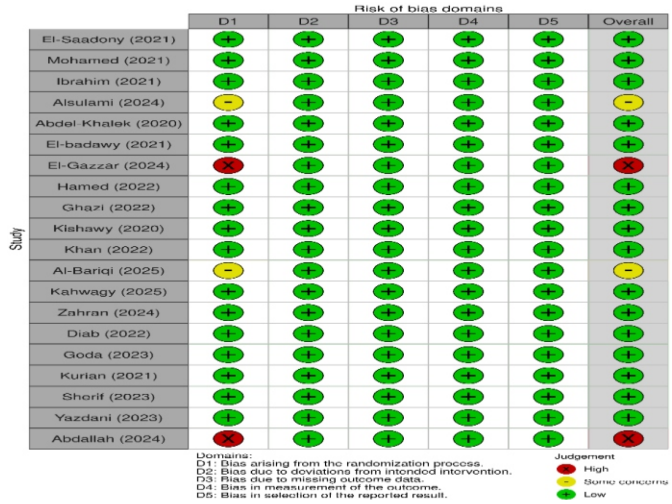

Figure 2: A traffic Light Plot for the Cochrane Risk of Bias (RoB 2) Tool's Risk of Bias Assessment. Red, Yellow, and Green

The main features of the 20 selected studies are comprehensively documented. It should be highlighted that different types of nanoparticles and experimental conditions in terms of synthesis procedure, nanoparticle size (17 to 77 nm), dose calculation (mg/kg feed or mg/L water), duration of exposure (between 48 h and 12 weeks), and obtained results differ significantly among the studies presented.

Table 1: Study Characteristics

References	Objectives	Synthesis Method	Particle size	Duration	Dose	Key Findings
[10]	Assess the antibacterial activity of biological Zn Nps against pathogenic bacteria and their influence on performance and immunity in Nile tilapia.	Biological (Aspergillus niger) & Chemical (Zn nitrate + NaOH)	45 (Bio); 75 (Chem.)	10 weeks	0.5–1 mg/L	Biological Zn NPs had a positive effect on growth, feed efficiency, and immunity. 1 mg/L group had the lowest cortisol level and highest growth hormone level. 0.5 mg/L group had an enhancement effect on liver function and immune values.
[11]	Compare the influence of inorganic ZnO, organic Zn-methionine, and nano-zinc oxide on growth, antioxidant status, etc.	Chemical (Commercial ZnONPs)	<65	60 days	20–40 mg/kg	Zn NPs at 40 mg/kg significantly improved growth, haemoglobin, white blood cells, up-regulated antioxidant genes such as SOD, GPx, and immune genes.
[12]	Evaluate and compare the genotoxicity in DNA damage potential of ZnNPs and bulk Zn.	Chemical	<50	7–28 days	90 mg/L	ZnNPs exhibited higher genotoxicity in micronuclei and nuclear abnormalities than bulk Zn. DNA damage was tissue-specific: gills > liver > muscles.
[14]	Assessed the toxic effects of ZnNPs compared to bulk Zn on the haematology and histopathology of tilapia.	Chemical	Not Reported	96 h	0.02 mg/L	ZnNPs induced a significant increase in WBCs and platelets but reduced RBCs and Hb levels. Histopathological changes were noted in the gills, liver, and intestines.
[15]	Assess the effect of algal ZnNPs on growth, digestion, intestinal integrity, and immune response.	Biological (Pediastrum boryanum)	29.35	8 weeks	30–60 mg/kg	Significant increase in weight gain and SGR at 60 mg/kg dose level. Increased intestinal amylase activity, villus height, and IgM and antioxidant levels.
[16]	Assess the effectiveness of green synthesized ZnO NPs as an anti-fungal drug against Candida albicans in Nile tilapia.	Biological (Ulva fasciata extract)	~30	8 weeks	40–60 mg/kg	40 mg/kg dosage significantly increased growth, survival, and immune-related genes. Effectively managed candidiasis and increased digestive enzymes.
[17]	Evaluate the biochemical and immune response to ZnO Nps challenge and the protective role of dietary parsley.	Chemical	25–35	6 weeks	8 mg/L	8 mg/L of ZnO NPs raised the levels of stress markers like cortisol and glucose, and lowered the levels of IgG antibodies. Parsley nanoparticles (4 g/kg) showed protective action against the toxicity of ZnO Nps.

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[18]	Assess the health effects of dietary nano-zinc prepared by chemical and green synthesis method with respect to bioaccumulation, antioxidant status, and DNA damage.	Green (Leucas aspera) & Chemical	29–35	60 days	100–400 mg/kg	Green synthesis ZnO NPs at 400 mg/kg enhanced antioxidant status as well as immunity without any damage to health, whereas chemical synthesis ZnO Nps induced liver necrosis and DNA damage
[19]	Investigate immunomodulatory activity of ZnO NPs and their efficacy against infection by A. hydrophila.	Chemical (Precipitation)	45	8 weeks	30–60 μg/g	ZnO NPs increased phagocytic activity/ lysozyme activity, and immune genes were upregulated. Survival rate increased after infection.
[20]	Assess the effect of curcumin-loaded ZnO NPs on Nile tilapia's growth, antioxidant activity, immune response, and resistance against Aeromonas hydrophila infection.	Green (Curcumin-mediated reduction)	29–43	56 days	5–10 mg/kg	Significant improvement reported in growth, haematology, antioxidant capacity, and immunity at a 10 mg/kg dose. However, it decreased MDA, cholesterol, and mortality against A. hydrophila.
[21]	Control S. agalactiae infection in Nile tilapia using ZnO NPs and plant extracts.	Chemical (Commercial)	<35	72 h	21–42 mg/well	ZnO NPs reported the dose of 42.5 mg/ well was effective against the viable count within 72h through ROS formation.
[22]	Assess the protective effect of vitamins E+C on ZnO Nps accumulation and changes in chemical composition.	Physical (Ultrasonication)	Not Reported	7–28 days	0.7–2.8 mg/L	ZnO NPs had higher accumulation in the gills than muscles. Vitamin E+C at 500 mg/kg reduced Zn accumulation and changed composition.
[23]	Evaluate the efficacy of bulk and ZnNPs on performance, intestinal morphology, and enzymes.	Not specified	59	84 days	30–60 mg/kg	Significantly improved the growth and digestive enzyme activity at the 60 mg/ kg dose (amylase and lipase), and haematological values with the 30 mg/ kg dose. Antioxidants were increased, and MDA levels were decreased.
[24]	Assessed the antiparasitic potential of bacterial ZnNPs against T. heterodentata and their effects on growth and immunity.	Biological (Bacillus subtilis)	45	8 weeks	100–500 mg/kg	Infection was significantly mitigated, survival was enhanced (88%), and weight gain was improved along with immunological parameters like SLA and IL-6 at a dose of 500 mg/kg
[25]	To investigate the effect of protein level and Zn in different forms (bulk and nano) on growth and immunity.	Not specified	59	84 days	60 mg/kg	Fish at 35% protein with ZnNPs showed the best growth response. ZnNPs enabled reduction of protein level from 35% to 25% without compromising growth response. Increased activity of SOD, CAT, and GSH.
[26]	Evaluate the antifungal activities as well as antibiofilm potential of ZnNPs and ZnNCs against C. albicans.	Biological (Aspergillus fumigatus)	45.8	48 h	10–100 μg/mL	ZnNCs at a concentration of 10 mu g/mL exhibited 84.4% biofilm inhibition. ZnNCs demonstrated higher antifungal potential than ZnNPs
[27]	Evaluate the impact of Moringa oleifera nanoparticles (MO-NPs) on the alterations in growth/ health resulting from ZnNPs toxicity.	Chemical	Not Reported	2 months	8 mg/L	ZnNPs toxicity in 8 mg/L aqueous solution induced stress and growth retardation. MO-NPs in the diet at 2.5 g/kg alleviated ZnNPs toxicity.
[28]	Evaluate the synergistic effects of Se-NPs and ZnNPs on growth, immunity, and oxidative stress responses.	Not specified	Not Reported	60 days	10 mg/kg	The synergistic supplement has been found to enhance the final body weight, SGR, and FCR. It has been found to increase the activity of SOD, CAT, and lysozyme.
[29]	Elucidate the apoptotic effects of microplastics in Nile Tilapia and the remedial effect of photocatalytic ZnNPs.	Photocatalytic ZnNPs	Not Reported	Not Reported	Effluent treatment	ZnNPs treatment significantly reduced the apoptotic alterations in Nile Tilapia due to the degradation of organic pollutants.
[30]	Investigated the interaction of ZnNPs and malathion pesticide in liver damage, antioxidant enzymes, and immune genes.	Chemical	<100	30 days	2 mg/L	ZnNPs increased antioxidant enzymes and reduced lipid peroxidation in malathion-treated fish. Significant reductions in pro-inflammatory cytokines.

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Biological ZnO NPs are safer than their chemical counterparts. In Nile tilapia, 0.5 mg/L of biological ZnO NPs led to a 100% survival rate as opposed to the 90% survival rate of chemical nanoparticles. Green ZnO NPs can be used in large concentrations without compromising safety since the highest amount that can be consumed is 500 mg/kg compared to chemical nanoparticles. ZnO NPs provide more protection against stress and pathogens as compared to bulk zinc. Addition of minerals such as FeN and plants such as Moringa increases the survival rate. Dosage for biological ZnO NPs ranges between 10 and 60 mg/kg, whereas for the latter, higher doses of 300 to 450 mg/kg will lead to a decrease in antioxidants (Figure 3).

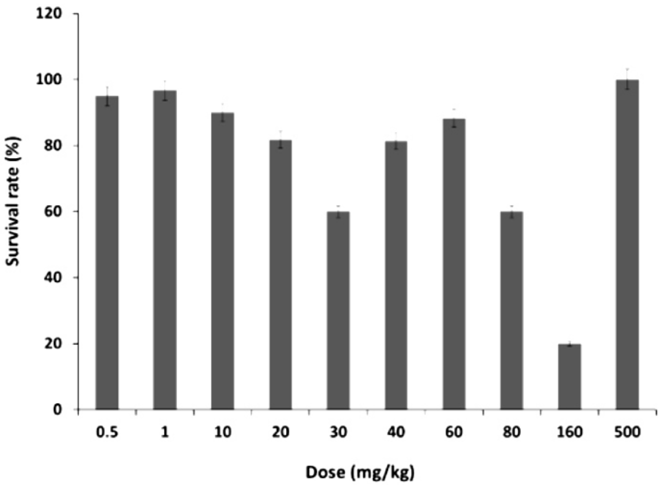

Figure 3: Survival Rate of Nile Tilapia on Different Concentrations of ZnO NP

The green ZnO NPs are seen to be more effective than chemical zinc oxide, as shown by more weight gain and survivability of Nile Tilapia, due to natural capping agents like fungi or plants, which also makes it less toxic, as shown by an LC50 of 25.5 mg/L for green zinc oxide and 3.1 mg/L for chemical zinc oxide. Nano oxide zinc is seen to be 15-20 times more efficiently absorbed than bulk zinc, showing more weight gain. For example, 60 mg/kg of ZnO NPs showed 49.77 g of weight gain. Compared with 35.49 g for bulk ZnO NPs with 60 mg/kg. The combination of ZnO NPs with other elements like selenium and plant nanoparticles like Moringa is effective in enhancing weight gain and reducing toxic stress. The weight gain increases from 57.62% to 77.61%. The optimal dose of ZnO NPs for weight gain is found to be in the range of 10-60 mg/kg of feed and 0.5 mg/L of water. Excessive use of ZnO NPs, i.e., 300-450 mg/kg of feed and 8 mg/L of b water, leads to reduced weight gain and toxicity (Figure 4).

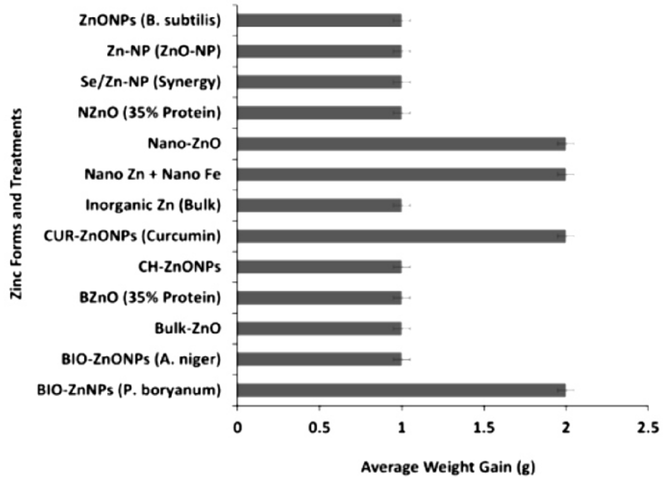

Figure 4: Comparative Analysis of the Effect of Zinc Oxide Sources and Nanoparticles on Weight Gain in Nile Tilapia

The results obtained through testing also validate the above findings since there was a notable dose-dependent and form-dependent elevation in the antioxidant and immune functions. The supplementation with ZnO NPs was more efficacious compared to bulk Zn and controls, and this was particularly evident when the 60 mg/kg dosage was employed. There was a marked increase in the activity of antioxidant enzymes (SOD, CAT, and GPx), which clearly shows improved ability to counteract the oxidative effects. Furthermore, glutathione indicators, such as GSH, GST, and GSR, increased in the ZnO NPs treated fish, thus providing evidence of a strengthened cellular protective system against diseases. Moreover, MDA content was notably lower among the ZnO NPs treated fish, which again indicates lesser damage caused by lipid peroxidation. Lastly, lysozyme activity attained peak values when the 60 mg/kg dosage of ZnO NPs was used (Table 2).

Table 2: Effect of ZnO NPs on Immunoglobulin M (IgM) and Lysozyme Activity in Nile Tilapia

Parameter (Unit)	0 mg/kg (Control)	30 mg/kg (Bulk-Zn)	60 mg/kg (Bulk-Zn)	30 mg/kg (Nano-ZnO)	60 mg/kg (Nano-ZnO)
LZM (ng mL^{-1})	0.59 ± 0.03	0.72 ± 0.03	0.68 ± 0.03	0.76 ± 0.03	0.90 ± 0.03
CAT (ng mL^{-1})	54.00 ± 2.67	58.50 ± 2.67	57.00 ± 2.67	63.50 ± 2.67	73.50 ± 2.67
SOD (U mL^{-1})	31.25 ± 1.96	32.50 ± 1.96	35.25 ± 1.96	35.00 ± 1.96	44.25 ± 1.96
GPX (ng mL^{-1})	27.75 ± 1.26	28.00 ± 1.26	34.00 ± 1.26	31.00 ± 1.26	38.88 ± 1.26
GSH (ng mL^{-1})	07.50 ± 1.06	08.75 ± 1.06	08.95 ± 1.06	10.50 ± 1.06	11.13 ± 1.06
GST (ng mL^{-1})	00.35 ± 0.03	00.54 ± 0.03	00.35 ± 0.03	00.38 ± 0.03	00.39 ± 0.03
GSR (mu mL^{-1})	02.71 ± 0.19	03.49 ± 0.19	03.69 ± 0.19	03.06 ± 0.19	03.80 ± 0.19
MDA (nmol mL^{-1})	73.50 ± 5.35	59.00 ± 5.35	59.00 ± 5.35	49.00 ± 5.35	39.00 ± 5.35

The values are represented as the mean ± standard deviation

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In statistical analysis, two-way ANOVA was used to determine the interaction between the two variables, ZnO NPs and their dose level. Results revealed significant effects of ZnO NPs, their dose level, and interaction on several immune biomarkers in Nile Tilapia ($p<0.005$). The results showed that supplementation with 60 mg/kg of ZnO NPs has significantly improved antioxidant enzyme activities and immune responses, while reducing lipid peroxidation compared with bulk zinc treatments and the control group (Table 3).

Table 3: Statistical Interpretations of the Immunological Effects Using Two-Way ANOVA

Parameters	ZnO Nps (p-value)	Dose Effect (p-value)	Interaction ZnO Nps × Dose (p-value)
LZM	<0.001	0.020	<0.001
CAT	<0.001	0.024	0.005
SOD	<0.001	<0.001	0.020
GPX	0.063	<0.001	0.232
GSH	0.012	0.516	0.734
GST	0.008	<0.001	0.004
GSR	0.182	0.002	0.039
MDA	<0.001	0.1442	0.144

p-value <0.005 is considered significant

Moreover, according to statistical analysis represented zinc oxide nanoparticles exhibited predominantly significant beneficial effects in Nile tilapia, and 65% of studies have reported improvements in immunity, growth, disease resistance, and antioxidant activity. Whereas 25 % of the studies reported adverse effects, primarily at higher concentrations (Table 4).

Table 4: Immunity, Growth, Disease Resistance, and Antioxidant Activity

Parameters	Statistical Summary
Total studies reviewed	20
Studies reporting dose	19 (1 Study Did Not Report Dose)
Median dose	42.5
Q1 (25th percentile)	8
Q3 (75th percentile)	60
Interquartile Range (IQR)	52
Minimum reported dose	0.02
Maximum reported dose	500
Positive outcomes (growth, immunity, antioxidant status, disease resistance, antimicrobial activity, environmental remediation)	13 Studies (65%)
Negative outcomes (genotoxicity, histopathological damage, oxidative stress, growth retardation, bioaccumulation)	5 Studies (25%)
Mixed/Protective outcomes (toxicity observed but mitigated by protective agents or green synthesis)	2 Studies (10%)
Chemical synthesis studies	10 (43.5%)
Biological/Green synthesis studies	7 (30.4%)
Physical synthesis studies	1 (4.3%)
Most common beneficial dose range (IQR)	8-60

DISCUSSION

This systematic review examines the effects of ZnO NPs on the growth, health, and immunity of Nile tilapia [10]. Nano zinc oxide demonstrates higher bioavailability and activity compared to other zinc sources [11], and its efficiency depends on particle size, surface area, and synthesis method [12]. The small size of ZnO NPs allows better intestinal absorption and greater reactivity than bulk zinc [23]. These results correlate with previous reviews on aquaculture nano supplements, which have found improved nutrient absorption and immunomodulation when administered at small concentrations [31, 32]. Unlike other reviews, which aggregated various nanoparticles into one category, this study focuses solely on ZnO NPs to distinguish their safety levels based on synthesis and dosing trends. Compared to other nanoparticles, ZnO NPs are easier and more eco-friendly to produce, often using biological resources. For example, CuO nanoparticles can be synthesized chemically or biologically, though green synthesis may lead to aggregation due to extract viscosity [13, 31]. Selenium nanoparticles often use microalgae as reducing agents but may require additional steps like UV irradiation or physical methods [31, 32]. Silver nanoparticles are mostly chemically synthesized, forming well-structured particles but with higher toxicity [33], while SiO_2 nanoparticles require complex chemical reactions such as the Stöber method [34, 35]. Species sensitivity and dosage are critical. Tilapia tolerate dietary ZnO NPs at 10–60 mg/kg [23] and waterborne biological ZnO NPs at 0.5 mg/L [10], achieving high survival, while chemically synthesized forms have lower LC50 (5.6–80.9 mg/L). Other species, such as Asian seabass and stinging catfish, are far more sensitive [36], and prolonged exposure in Rare Minnow reduces growth and gene expression [37]. Unique effects include delayed zebrafish hatching due to ZHE1 inhibition [38, 39] and disturbances in ionic balance in Blue Parrotfish [38]. Overall, the synthesized data suggest that ZnO NPs have the potential to increase growth, antioxidant activity, and immunity in small amounts [23, 25]. However, these results are merely descriptive and not statistically combined. Considering the differences in experimental approaches and standards for data reporting, it is important to consider the optimum ranges as preliminary guidelines. ZnO NPs show strong potential as a dietary supplement for Nile tilapia, improving growth, antioxidant capacity, and immunity when applied at moderate levels [23, 25].

However, there are some shortcomings associated with this review. First, differences in terms of nanoparticle size, synthesis process, dosing, and endpoints affected the

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ability to compare results. Additionally, there were no appropriate characterizations for the nanoparticles used in most of the reviewed articles. Therefore, further research needs to focus on standardizing the reporting of the characteristics of nanoparticles, conducting longer duration experiments, and comparing green-synthesized ZnO NPs and chemically synthesized nanoparticles under similar conditions.

CONCLUSION

Based on the current data, the administration of ZnO NPs in doses of 8–60 mg/kg (in diet) and 0.5 mg/L (in water) may improve growth, antioxidants, and immunity in Nile tilapia. It also seems that green-synthesized nanoparticles pose less risk than their chemical analogy. Nonetheless, doses above 300 mg/kg and 8 mg/L were always followed by oxidative stress and toxic effects. This information comes from a descriptive synthesis and experiments of moderate quality. The findings should not be considered definitive values for clinical application.

Authors' Contribution

Conceptualization: AA, FI
Methodology: AA, FI
Formal analysis: AA, II, BA
Writing and Drafting: AA, FI, BA
Review and Editing: AA, FI, II, BA

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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