Toxic and Growth Effects of Ag-TiO_2 Nanoparticles on *Etroplus suratensis* Fingerlings with Statistical Application

Kumar Pandion [a], Aarrthy M. Arunachalam [b], Archana Sharma [c], Kantha Deivi Arunachalam [c,*]

[a] *Center for Environmental Nuclear Research, Directorate of Research, SRM Institute of Science and Technology, Kattankulathur, 603203, Chennai, TN, India*

[b] *Kaplan Teaching Centre, Washington D.C., 20036, USA*

[c] *Faculty of Sciences, Marwadi University, Rajkot, Gujarat, 360 003, India*

Editor's note: Improper disposal of nanoparticles poses significant risks to environmental health. The extremely small size of silver-titanium dioxide (Ag-TiO_2) nanoparticles results in a high surface area-to-weight ratio, which enhances their reactivity and allows toxic substances to adhere to their surfaces. Pandion et al. assessed the impact of dietary exposure to Ag-TiO_2 nanoparticles on the growth performance of *Etroplus suratensis* fingerlings. The findings demonstrate that Ag-TiO_2 nanoparticles exert concentration-dependent effects on these fish, with extended exposure to elevated concentrations adversely affecting growth and survival rates.

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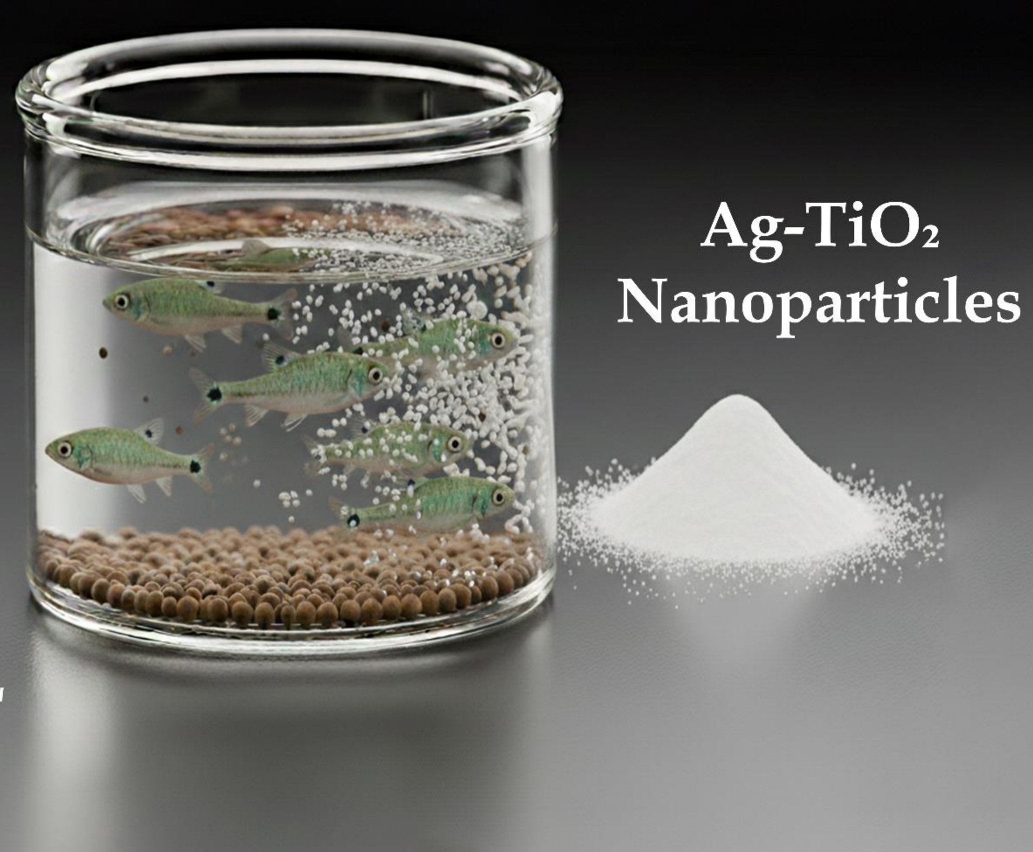

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Original Research

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[b] *Kaplan Teaching Centre, Washington D.C., 20036, USA*

[c] *Faculty of Sciences, Marwadi University, Rajkot, Gujarat, 360 003, India*

Abstract

Silver-doped titanium dioxide nanoparticles (Ag-TiO_2 NPs) are widely used and increasingly released into aquatic environments, raising concerns about their ecological impacts. This study evaluated the effects of dietary Ag-TiO_2 nanoparticle exposure on the growth performance of *Etroplus suratensis* fingerlings. Fish were fed diets supplemented with Ag-TiO_2 nanoparticles at concentrations of 1, 10, 100, and 1000 mg kg^{-1} for 12 weeks under controlled water quality conditions. Acute toxicity (LC_{50}) tests revealed concentration-dependent mortality, with the highest mortality (24% within 96 h) observed at 1000 mg kg^{-1}. Growth performance, assessed through length and weight measurements, exhibited a clear concentration and time-dependent response. The 10 mg kg^{-1} treatment group showed the highest percentage weight gain (134.24%) and final biomass (34.01 g), indicating an optimal growth response. One-way ANOVA confirmed significant differences among treatments ($p < 0.05$). Pearson correlation analysis demonstrated a strong positive relationship between weight gain and biomass, while mortality was negatively correlated. Principal component analysis identified weight gain and biomass as major contributors to PC1, whereas mortality dominated PC2. Cluster analysis further highlighted the adverse effects of higher Ag-TiO_2 concentrations. Overall, the findings demonstrate that Ag-TiO_2 nanoparticles exert concentration-dependent effects on *Etroplus suratensis*, with prolonged exposure to higher concentrations impairing growth and survival, indicating potential ecological risks in nanoparticle-contaminated aquatic ecosystems.

Keywords: Ag-TiO_2 nanoparticles; Fish; Growth; Statistical application.

* Corresponding author.
Email address: kanthadevi.arunachalam@marwadieducation.edu.in (K.D. Arunachalam)

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1. Introduction

Silver-doped titanium dioxide nanoparticles (Ag-TiO_2) are widely used engineered materials incorporated into manufacturing products such as dyes, textiles, cosmetics, individual care products, and even nutrition, owing to their great anti-corrosion capacity, chemical solidity, and strong photo-catalytic concentration [1]. Advances in nanotechnology have enhanced nanoparticle synthesis techniques, improved their physical and chemical properties, and led to their extensive use in diverse industries, including cosmetics, textiles, and electronics.

However, careless disposal of nanoparticles can result in environmental toxicity. Due to their extremely small size, Ag-TiO_2 has a greater surface area-to-weight relation, which increases their surface reactivity and allows toxic substances to adsorb onto their surface [2]. These nanoparticles can cross physiological barriers such as the blood-brain and blood-eye barriers, potentially causing xenobiotic effects [3, 4]. For example, silver nanoparticles have been shown to accumulate in the head, yolk, plasma, and fish embryos, leading to severe tissue destruction [5].

Once discharged into aquatic environments, Ag-TiO_2 nanoparticles undergo various physical, chemical, and biological transformations that alter their physicochemical properties and toxicity. Interactions such as deposition, aggregation, and stabilization (DLVO theory) determine their transport and persistence in natural and artificial waters [6]. In the incidence of natural organic material (NOM), Ag-TiO_2 nanoparticles tend to form aggregates ranging from nanometers to micrometers and can adsorb other ligands, increasing their toxicity [7]. Furthermore, interaction with biological macromolecules forms a bio-corona layer, conferring a "biological identity" that influences their environmental and toxicological behavior [8].

Toxicity tests, such as the 96-hour LC_{50}, measure the concentration of a substance that is lethal to 50% of a test population within 96 hours [9]. Acute toxicity refers to the short-term entry of toxicants into organisms, causing physiological disruption or organ damage. Standard criteria for acute toxicity in fish include mortality, immobility, loss of equilibrium, and impaired growth. Accumulation of nanoparticles in low-flow aquatic systems can increase their local concentration, intensifying toxic effects. Small fish species can play a critical role in trophic dynamics because of their mid-level position in food webs [10].

Most existing studies have examined waterborne exposure of Ag-TiO_2, whereas dietary exposure data, especially regarding effects on fish growth indices, are scarce. *Etroplus suratensis* (E. *suratensis*), a commercially important brackish water species in South Asia, was chosen for the present study due to its fast growth, excessive yield, and market demand. Previous research has reported toxic impacts of Ag-TiO_2 on fish, such as gill pathology and reduced Na^+/K^+ ATP concentration in rainbow trout [11] and concentration- and time-dependent growth inhibition in fish [12].

Numerous investigations have documented Ag-TiO_2 toxicity in aquatic systems and humans [13-15]. Reported harmful impacts include gastrointestinal, pulmonary, and dermal toxicity in mammals [16] and genotypic changes such as ribosomal function alterations in aquatic organisms [17]. However, most studies have been short-term, and few have tested unmodified Ag-TiO_2 in aquatic species under long-term exposure [18-21].

This study investigates the effects of dietary Ag-TiO_2 exposure over 12 weeks on the growth performance of E. *suratensis* fingerlings under controlled brackish water conditions. In addition to growth indices, physicochemical water parameters and LC_{50} concentrations were monitored to better understand long-term nanoparticle toxicity in aquaculture-relevant conditions.

2. Experimental

2.1. Animal and acclimatization

Etroplus suratensis fingerlings, with a standard initial mass of 10 ± 2.5 g and length of 4 ± 2.8 cm, were procured from the fish seed hatchery at CIBA, Chennai. Healthy, uniformly sized individuals were chosen and given a potassium permanganate ($KMnO_4$) bath to eliminate any organisms. Earlier to experimental revelation, animals were adjusted for two weeks in 100 L concrete units under a 12 h brightness:12 h darkness cycle and fed a basal diet two times daily.

2.2. Nutrition formulation

The nutrition (30% crude protein; Table 1) and nanomaterials of silver titanium dioxide (anatase form,

< 25 nm) were procured from local vendors (Sigma company, USA). Trial diets were prepared by incorporating each nanoparticle type at concentrations of 1, 10, 100, and 1000 mg kg^{-1} into the feed, following the trial protocol. Blackstrap molasses (7%) was treated as a binder to combine the powdered ingredients into a semisolid paste, which was then processed through a grinding machine to produce feed of approximately 4 mm width. The feed was sliced into constant pellets, dried out, and stored in sealed jars until consumed [22].

Table 1. Diet formulation

Crude Protein	min. 30%
Crude Fat	min. 5.8%
Crude Fiber	max. 6.7%
Moisture	max. 10%
Ash	max. 8.5%

2.3. Experimental design

Adjusted animals were independently weighed and determined before being randomly allocated into fifteen chambers (59 × 34 × 40 cm) at a density of 50 fish per tank, with three replicate tanks per treatment. Therapies were defined by the absorption of silver-doped titanium dioxide nanoparticles incorporated into the feedstuff: T0 (control), T1 (1 mg kg^{-1}), T2 (10 mg kg^{-1}), T3 (100 mg kg^{-1}), and T4 (1000 mg kg^{-1}). The animals were kept alive in a flow-through system (current rate: 5.1–6.0 L h^{-1}) under controlled water quality conditions: hotness 29.4–30.1 °C, acid or alkali in a substance 7.4–7.6, and amount of oxygen in water 11.1–11.5 mg L^{-1}, monitored with a HANNA multipara meter probe (HI 9829). Animals were fed the respective test diets twice a day (08:00 and 20:00 h) for 30 min to ensure complete consumption. Control fish received the basal diet to satiation following the same schedule. Not consumed feed and fecal matter were drained off after feeding. The trial lasted for 12 weeks, during which mortality was recorded daily, and dead fish were removed promptly [23].

2.4. Physicochemical parameters of the water

Water samples were gathered in bottles and saved in a refrigerator (4 °C) until transported to the test site. Temperature, pH, saltiness, dissolved oxygen, and thick with suspended matter were determined on-site using an involving more than one parameter probe. The tests were then carried to the test site and saved in a refrigerator at 4 °C until further analysis. Prior to chemical analysis, tests were removed through Whatman No. 42 clean paper and acidulated with concentrated nitric acid (3 mL L^{-1}) and chloroform (0.5 mL L^{-1}) to prevent microbial growth. They were subsequently passed through a 0.45 μm Millipore removed nutrients, including essential elements, were determined using the usual analytical procedures. A pigment absorption was assessed following pigment removal with 90% acetone, and absorbance was recorded utilizing a UV-visible light (Shimadzu UV series).

2.5. Acute-lethal toxicity test (LC_{50})

Adjusted animals were independently weighed and determined, then randomly assigned to fifteen tanks (59 × 34 × 40 cm) at a mass of 50 fish per chamber, with three duplicate tanks per treatment. Treatments corresponded to silver-doped titanium dioxide nanoparticle concentrations incorporated into the feedstuff: T0 (control), T1 (1 mg kg^{-1}), T2 (10 mg kg^{-1}), T3 (100 mg kg^{-1}), and T4 (1000 mg kg^{-1}). A static toxicity exposure system was used, with the test solution remaining unchanged throughout the 96-h trial. Water condition factors were determined before and after the experiment. Mortality and abnormal behavioral responses of E. *suratensis* were monitored continuously. Fish were considered dead when showing complete immobility and no abdominal flexion upon forced extension.

2.6. Fish growth

Five animals from every tank (50 per tank) were tested at the start of the experiment (day 0) and subsequently at fortnightly intervals. Fish were euthanized by immersion in a water solution of 3-aminobenzoic acid ethyl ester (MS-222; Sigma-Aldrich Chemical, St. Louis, MO, USA) at a concentration of 1.0 g L^{-1}, until opercular activity ceased. The juvenile fish were rinsed in water, blotted dry with towels, weighed, and measured for length. Growing performance factors were determined as follows: Exact growing rate (SGR, % day^{-1}) = [(ln Final weight - ln Initial weight)/Experimental period days] × 100.

2.7. Statistical analysis

Every analytical information was processed utilizing Python software. Statistical studies involved a one-way study of modification (ANOVA), Pearson association, principal module study (PCA), and a classified cluster study (HCA) to assess the relationships between nanoparticle exposure and growth performance parameters (weight gain and biomass) of *Etroplus suratensis*. Consequence was determined at $p < 0.05$.

3. Results and discussion

3.1. Physicochemical properties of water

Silver-doped titanium dioxide nanoparticles can enter fish either directly via the water route or indirectly through trophic transfer. Under both exposure routes, Ag-TiO_2 nanoparticles have been shown to accumulate in fish tissues [24], though maximum investigates have used waterborne exposure, particularly in zebrafish [25, 26]. Water temperature, a key factor influencing biological and chemical processes [27], ranged from 28.0 ± 1.0 °C during the study (Table 2). Surface temperatures were slightly higher than deeper waters due to sunlight, photoperiod length, and wind mixing [28]. The pH remained alkaline, ranging from 7.5 ± 0.5, consistent with seawater's high buffering capacity [29].

Salinity ranged from 20 ± 7.0 PSU, like other Indian coastal waters [30]. Dissolved oxygen (DO) levels were 5.5 ± 1.0 mg L^{-1}, reflecting the combined influence of photosynthesis, organic matter decomposition, and atmospheric exchange [31]. Turbidity averaged 2.5 ± 0.5 NTU, primarily influenced by sediment suspension, runoff, and phytoplankton biomass [32]. Silicate, an essential nutrient for marine primary producers, ranged from 11.0 ± 1.5 µmol L^{-1}, influenced by riverine input, sediment adsorption, and biogeochemical interactions [33]. Ammonia concentrations ranged from 9.0 ± 2.5 µmol L^{-1}, arising from organic matter decay, nitrification, and excretion [34]. Phosphate ranged from 30 ± 1.5 µmol L^{-1}, driven by river inflow, biological uptake, and benthic-pelagic coupling [35]. Nitrate concentrations were 4.0 ± 1.0 µmol L^{-1} [36].

Total nitrogen (TN) was 40 ± 2.0 µmol L^{-1}, with high levels potentially promoting eutrophication and harmful algal blooms [37]. Total phosphorus (TP) was 1.5 ± 0.5 µmol L^{-1}, mostly in particulate form [38]. Chlorophyll-a was an indicator of autotrophic biomass. Although Ag-TiO_2 tended to settle at the tank's lowest due to variability in water, a portion remained suspended in the water route throughout the experiment [39].

Table 2. Physicochemical properties of water

Sample No.	Parameters	Values
1	Temperature	28±1.0 °C
2	pH	7.5±0.5 pH
3	Salinity	20±7.0 PSU
4	Dissolved oxygen	5.5±1.0 mg L^{-1}
5	Turbidity	2.5±0.5 NTU
6	Nitrate	4.0±1.0 µmol L^{-1}
7	Ammonia	9.0±2.5 µmol L^{-1}
8	Silicate	11.0±1.5 µmol L^{-1}
9	Phosphate	30±1.5 µmol L^{-1}
10	TN	40±2.0 µmol L^{-1}
11	TP	1.5±0.5 µmol L^{-1}
12	Chlorophyll a	1.5±0.5 mg m^{-3}

3.2. Exposure and mortality of *Etroplus suratensis* juveniles to TiO_2 nanoparticles

This research represents the first information on the impacts of Ag-doped TiO_2 nanoparticles (Ag-TiO_2) on water animals. The 96-h LC_{50} of Ag-TiO_2 was determined to be 112 mg L^{-1}, whereas bulk Ag-TiO_2 showed no toxicity up to 300 mg L^{-1}. These results align with earlier studies: Xiong et al. [40] reported no mortality in fish at concentrations up to 50 mg L^{-1} for the majority of Ag-TiO_2 and 300 mg L^{-1} for Ag-TiO_2 with a recorded LC_{50} of 124.5 mg L^{-1} for Ag-TiO_2. Similarly, Diniz et al. [41] assessed the 96-h LC_{50} for Ag-TiO_2 in fish at 100 mg L^{-1}.

In the present experiment, *E. suratensis* juveniles were exposed to Ag-TiO_2 via dietary administration in five treatment groups: T0 (control), T1, T2, T3, and T4, over 96 hours. Exposure induced noticeable changes in opercular rate and visible physical damage, particularly on the gill surfaces. Mortality patterns varied across treatments (Table 3). The highest concentrations of T3 (100 mg kg^{-1}) and T4 (1000 mg kg^{-1}) showed early signs of toxicity within the first 8 h, leading to the most rapid onset of mortality. In contrast, the T2 group (10 mg kg^{-1}) exhibited first signs of mortality only after ~50 h of exposure.

These results indicate a clear concentration-dependent toxicity, with higher Ag-TiO_2 levels causing faster and more severe effects. Comparable findings have been reported for fish where 40%, 50%, and 80% mortality occurred at 150, 175, and 200 mg L^{-1}, respectively, within 96 h, and 100% mortality occurred at 225 mg L^{-1}, particularly in 24 h [42]. Similarly, Chen et

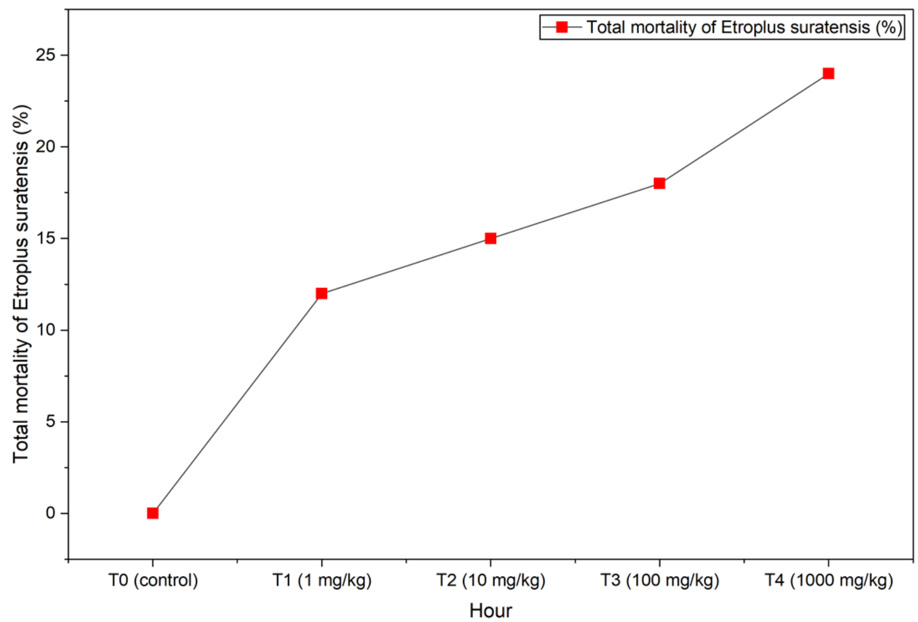

Figure 1. Exposure and Mortality of *Etroplus suratensis* Juveniles to TiO_2 Nanoparticles.

al. [43] observed elevated mortality in animals exposed to 1–7 mg L^{-1} Ag-TiO_2, with the highest mortality (16.7%) at 7 mg L^{-1}. In the current study, significant mortality occurred primarily within the first 8 h, suggesting acute toxicity effects were most pronounced early on. Thereafter, mortality rates stabilized, possibly due to reduced nanoparticle bioavailability or physiological adaptation by the fish. As shown in Figure 1, the T4 group recorded the highest mortality rate (24%), whereas T2 had the lowest (5%).

3.3. Growth performance of Etroplus suratensis juveniles exposed to Ag-TiO_2 nanoparticles

Etroplus suratensis is a commercially valuable species widely cultured in China's coastal region due to its tasty white meat, rapid growth, disease resistance, and great

Table 3. Exposure and Mortality of *Etroplus suratensis* Juveniles to Ag-TiO_2 Nanoparticles

Hour	T0 (control)	T1 (1 mg kg^{-1})	T2 (10 mg kg^{-1})	T3 (100 mg kg^{-1})	T4 (1000 mg kg^{-1})
1	0	0	0	0	0
2	0	0	0	0	0
4	0	0	0	0	0
8	0	0	0	0	3
12	0	0	0	2	4
16	0	0	0	1	2
24	0	0	0	3	1
36	0	1	0	4	1
48	0	1	0	2	4
50	0	0	3	0	3
62	0	4	1	0	2
72	0	3	1	3	3
96	0	3	0	2	2
Total mortality of *Etroplus suratensis* (%)	0	12	05	18	24

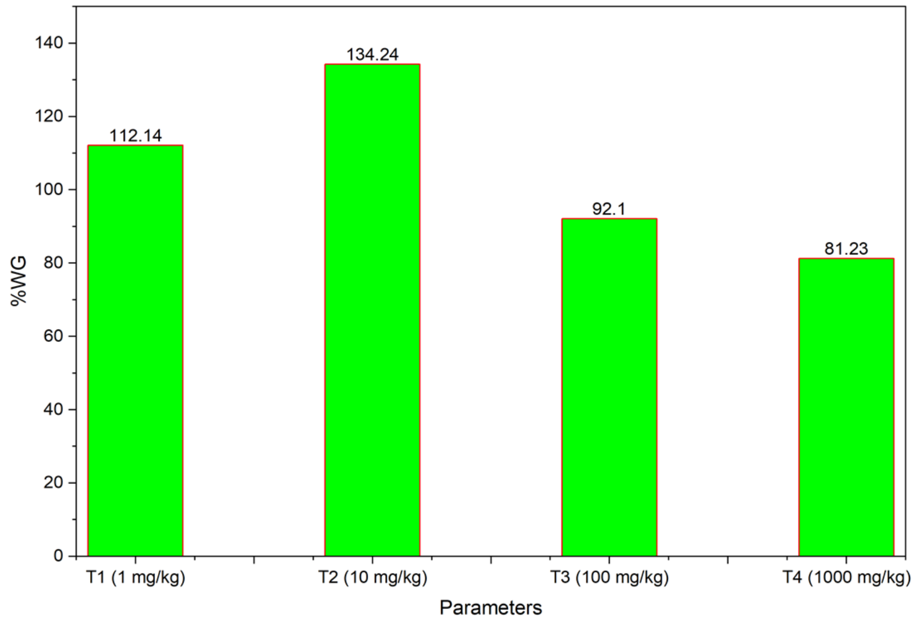

Figure 2. The highest percentage weight gain (%WG).

commercial value [44-46]. Sheethal et al. [47] reported acute toxicological effects of TiO_2 exposure in pearl spot, with muscular tissue being particularly sensitive even at very low concentrations. Similar neurotoxic effects of metals in aquatic animals have been recognized in previous reports [48-52]. In the present research, no mortality was noted in the control group (T0; basal diet), while minimal mortality occurred in T1 (1 mg kg^{-1}) and T2 (10 mg kg^{-1}). In contrast, higher concentrations, T3 (100 mg kg^{-1}) and T4 (1000 mg kg^{-1}), produced significant mortality after 12 weeks of dietary exposure (Figure 1).

The enhanced growth observed at low dietary Ag-TiO_2 concentrations, particularly in the T2 group, reflects a dose-dependent adaptive response that supports physiological homeostasis. At optimal levels, Ag-TiO_2 nanoparticles may improve metabolic efficiency and nutrient utilization, thereby promoting somatic growth without inducing stress. Similar low-dose growth stimulation has been reported in fish exposed to TiO_2 nanoparticles [11, 12]. In contrast, higher concentrations likely exceed the tolerance threshold, leading to oxidative stress and metabolic disruption. Consequently, growth inhibition at elevated doses is consistent with earlier findings in fish [53].

Growth performance differed significantly among treatments. Collections T1 and T2 confirmed the highest percentage weight gain (%WG) at 112.14% and 134.24%, respectively (Figure 2), outperforming even the control group. During the first four weeks, all groups gained weight at a similar rate (up to ~1 g). From week 6 onwards, growth declined sharply in T3 and T4. This trend aligns with Chen et al. 2011, who documented growth and involving impairments in fish under prolonged exposure to high Ag-TiO_2 nanoparticle concentrations. The highest weight gain (WG) and individual growing rate (SGR) were maintained by T1 and T2 throughout the 12-week trial (Figures 2 and 3), while T3 and T4 showed marked reductions by the experiment's end. This suggests a bi-modal effect of Ag-TiO_2 nanoparticles, low concentrations stimulated growth, whereas high concentrations were detrimental (Table 4). Similar growth-enhancing effects at optimal supplementation levels have been noted in other species, such as hybrid grouper fed with inositol [54].

One-way ANOVA (Tables 5 and 6; Figures 4 and 5) explained major differences ($p < 0.05$) in %WG, final biomass, and mortality among treatments, supported by high F-values. Both %WG and final biomass peaked in T1 and T2, whereas T4, and to a lesser extent T0 and T3, had lower values. Mortality followed a dose–response pattern: lowest in T0 and highest in T4. Pearson correlation analysis (Figure 5; Table 6) further revealed:

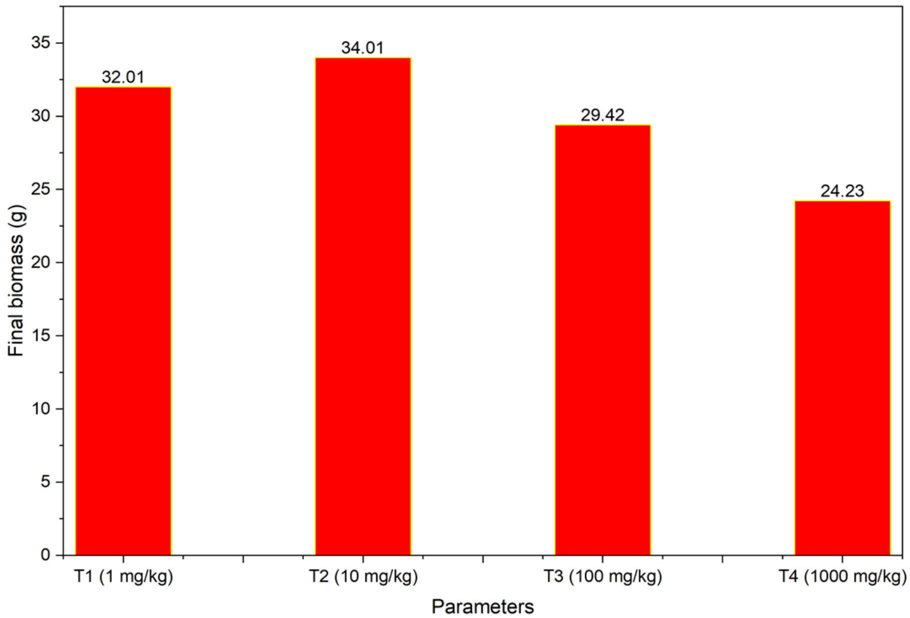

Figure 3. The highest percentage of biomass.

%WG vs. Final Biomass: Strong positive correlation (r = 0.90), increases in %WG were mirrored by biomass gains from T0 to T2, both declining in T3 and T4. %WG vs. Mortality: Moderate negative correlation (r ≈ –0.70), higher mortality corresponded to reduced %WG. Final Biomass vs. Mortality: Moderate-to-strong negative biomass correlation (r ≈ –0.70) decreased as mortality increased. These findings confirm that intermediate Ag-TiO_2 nanoparticle doses (T1, T2) optimize growth and biomass accumulation while minimizing mortality, whereas high doses (T4) severely impair growth and survival. The lowest losses recorded in the T2 treatment (10 mg kg^{-1}) likely reflect a hormetic response, in which exposure to an optimal nanoparticle concentration

Table 4. The bi-modal effect of Ag-TiO_2 nanoparticles at low concentrations stimulated growth

Parameters	Treatment	Initial weight and weight gains in the nth week						
		1st	2nd	4th	6th	8th	10th	12th
WG (g)	T0 (control)	10 ± 2.4	10.5 ± 2.0	11.1 ± 1.4	11 ± 1.5	11.2 ± 1.8	12 .1± 0.4	12 ± 0.5
	T1 (1 mg kg^{-1})	10 ± 2.5	10 .3± 2.2	11.3 ± 1.2	11.3 ± 1.2	11.3 ± 1.7	12 .3± 0.2	12.1 ± 0.4
	T2 (10 mg kg^{-1})	10 ± 2.4	10 .4 ± 2.1	11 ± 1.5	11 ± 1.5	11.2 ± 1.9	12 .2± 0.3	12 ± 1.5
	T3 (100 mg kg^{-1})	10 ± 2.5	10.3 ± 2.3	11.1 ± 1.4	11.5 ± 1.0	11.1 ± 1.9	12 .3± 0.2	12.3 ± 0.2
	T4 (1000 mg kg^{-1})	10 ± 2.5	10.4 ± 2.1	11 ± 1.5	11.7 ± 1.8	11.8 ± 1.2	12 .4± 0.1	12 ± 0.5
SGR (g)	T0 (control)	0.0 ± 0.05	0.54 ± 0.05	1.1 ± 0.05	1.0 ± 0.05	1.2 ± 0.05	2.1 ± 0.05	2.0 ± 0.05
	T1 (1 mg kg^{-1})	0.0 ± 0.05	0.30 ± 0.05	1.0 ± 0.05	1.3 ± 0.05	1.3 ± 0.05	2.3 ± 0.05	2.1 ± 0.05
	T2 (10 mg kg^{-1})	0.0 ± 0.05	0.4 ± 0.05	1.0 ± 0.05	1.0 ± 0.05	1.2 ± 0.05	2.2 ± 0.05	2.0 ± 0.05
	T3 (100 mg kg^{-1})	0.0 ± 0.05	0.3 ± 0.05	0.9 ± 0.05	1.0 ± 0.05	1.1 ± 0.05	2.3 ± 0.05	2.3 ± 0.05
	T4 (1000 mg kg^{-1})	0.0 ± 0.05	0.4 ± 0.05	1.0 ± 0.05	1.2 ± 0.05	1.8 ± 0.05	2.4 ± 0.05	2.5 ± 0.05
length (cm)	T0 (control)	4 ± 2.6	4.4 ± 0.4	5.4 ± 0.2	5.4 ± 0.5	5.8 ± 0.3	6.4 ± 0.2	6.5 ± 0.3
	T1 (1 mg kg^{-1})	4 ± 2.8	4.3 ± 0.5	5.4 ± 0.4	5.6 ± 0.2	5.8 ± 0.3	6.3 ± 0.4	6.6 ± 0.2
	T2 (10 mg kg^{-1})	4 ± 2.8	4.2 ± 0.6	5.3 ± 0.5	5.2 ± 0.6	5.8 ± 0.3	6.4 ± 0.2	6.7 ± 0.2
	T3 (100 mg kg^{-1})	4 ± 2.7	4.1 ± 0.7	5.2 ± 0.6	5.6 ± 0.2	5.8 ± 0.3	6.3 ± 0.2	6.4 ± 0.4
	T4 (1000 mg kg^{-1})	4 ± 2.8	4.0 ± 0.7	5.1 ± 0.4	5.4 ± 0.4	5.8 ± 0.3	6.0 ± 0.5	6.4 ± 0.4

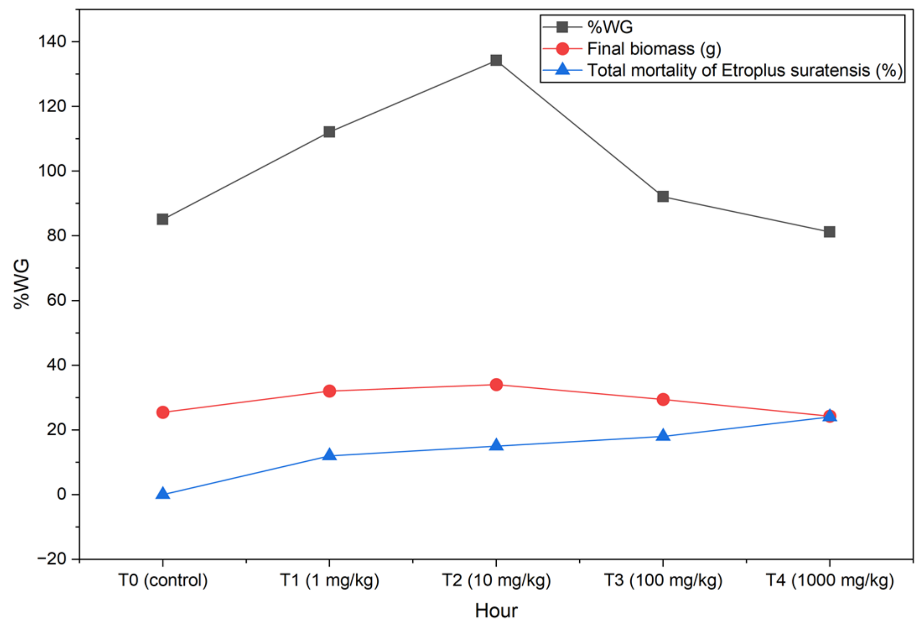

Figure 4. Boxplots illustrating patterns.

promotes physiological homeostasis and efficient metabolic functioning. At this intermediate dose, titanium oxide nanoparticles may enhance nutrient utilization, antioxidant capacity, and stress adaptation without inducing cellular damage. In contrast, the lower concentration (T1) may be insufficient to elicit such beneficial adaptive responses, while higher concentrations (T3 and T4) likely exceed the tolerance threshold of *Etroplus suratensis*, leading to oxidative stress, cellular disruption, and impaired physiological functions, thereby increasing mortality. This growth suppression at high concentrations may be linked to Ag-TiO_2 -induced redox inequality and improved reactive oxygen species (ROS) production, disrupting antioxidant defenses [53, 55].

3.4. ANOVA analysis

ANOVA was performed to compare weight gain percentage (%WG), final biomass, and mortality among treatments (T0–T4). Every variable indicated statistically significant differences ($p < 0.05$), with high F-values confirming strong treatment effects. Weight gains were highest in intermediate doses (T1 and T2), while the highest dose (T4) and, to a lesser extent, T0 and T3, resulted in reduced gains. Final biomass followed a similar pattern, peaking at T1 and T2 and decreasing at T0 and T4. Mortality exhibited a clear dose–response trend, being lowest in T0 and highest in T4.

Boxplots (Figure 4) illustrate these patterns: %WG: Higher medians in T1 and T2; lowest in T4. Final biomass: Peak at T2, declines in T3 and T4. Mortality: Near-zero in T0, moderate in T1–T3, maximum in T4. These results indicate that intermediate nanoparticle doses promote optimal growth and low mortality, whereas the highest dose (T4) markedly impairs growth and increases mortality (Table 5).

3.5. Qualitative and quantitative Pearson Correlation study

A Pearson correlation study was used to assess relationships among %WG, final biomass, and mortality (Figure 5; Table 6). %WG vs Final Biomass: Both variables increased from T0 (85 %, 25 g) to T2 (134 %, 34

Table 5. ANOVA study

Variable	F-value	p-value	Interpretation
%WG	124.27	7.95×10^{-14}	Significant differences between treatments
Final Biomass	23.70	2.39×10^{-7}	Significant differences between treatments
Mortality	41.53	2.04×10^{-9}	Significant differences between treatments

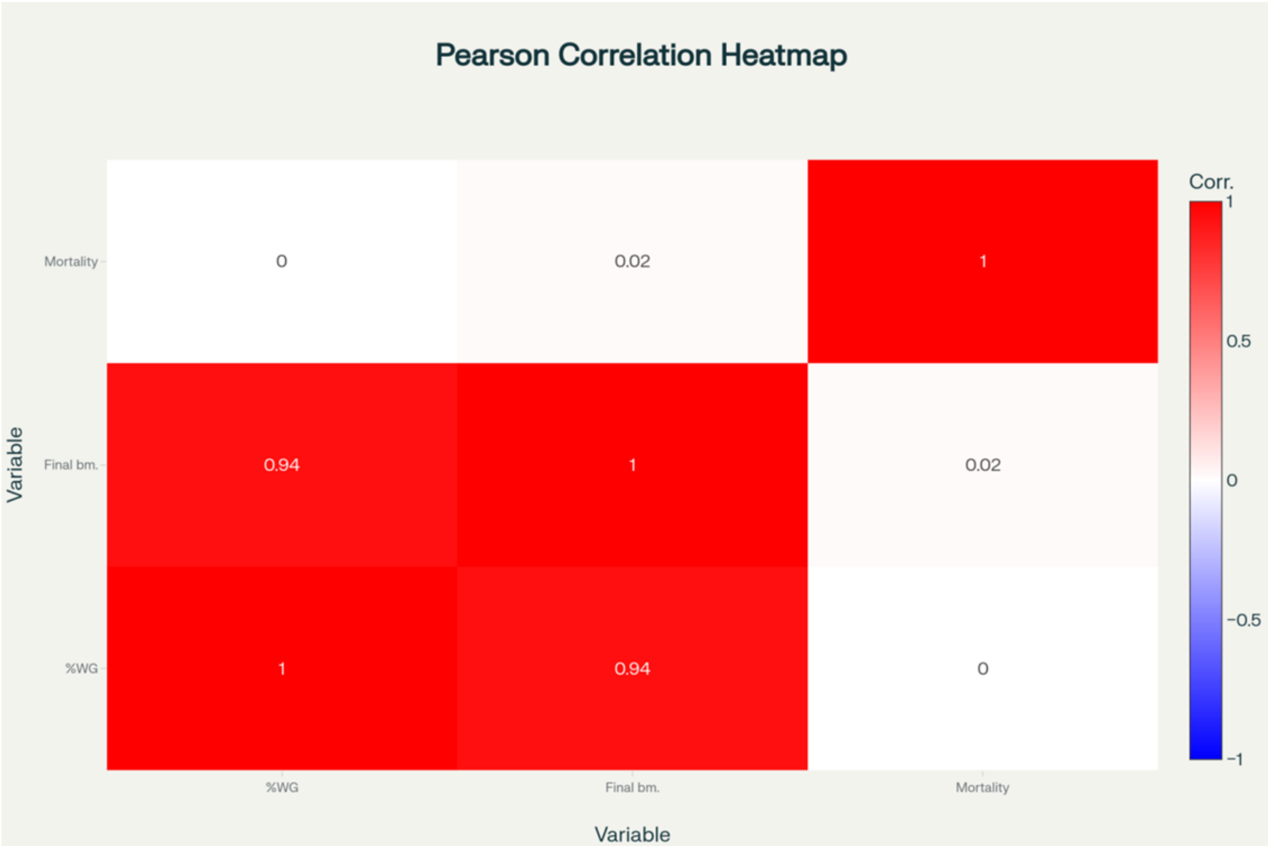

Figure 5. Qualitative and quantitative Pearson correlation analyses.

g), then declined in T3 and T4. A strong positive correlation (r = 0.90) confirms that higher weight gain translates directly into greater final biomass. %WG vs Mortality: As mortality rose from 0 % (T0) to 24 % (T4), %WG generally declined (85 % to 81 %), yielding a moderate negative correlation (r ≈ –0.70).

Final Biomass vs Mortality: Similarly, biomass decreased as mortality increased, with a moderate-to-strong negative correlation (r ≈ –0.70). In summary, %WG and final biomass are strongly and positively correlated, whereas both are negatively correlated with mortality. These findings indicate that optimal growth and biomass occur under low mortality conditions, typically at intermediate nanoparticle exposure levels.

3.6. Principal Component Analysis

Principal Module Analysis (PCA) was applied to %WG, final biomass, and mortality to reduce dimensionality and explore multivariate relationships among treatments (T0–T4). The unique two principal sections described 53.65% and 44.41% of the modification; correspondingly, the method of accounting accounted for almost 100 % of the entire variation (Figure 6). The PCA biplot showed clear treatment separation. T1 and T2 clustered together, reflecting similar performance marked by high %WG and biomass with low mortality. T4 was positioned furthest from these groups, driven by high mortality and poor growth. T0 and T3 occupied intermediate positions. Variable loadings indicated that %WG and final biomass loaded strongly and positively on PC1, whereas

Table 6. Pearson correlation analysis

Pair	Expected Pearson r	Relationship
%WG vs Final biomass	+0.95	Strong positive
%WG vs Mortality	-0.7	Moderate negative
Biomass vs Mortality	-0.7	Moderate negative

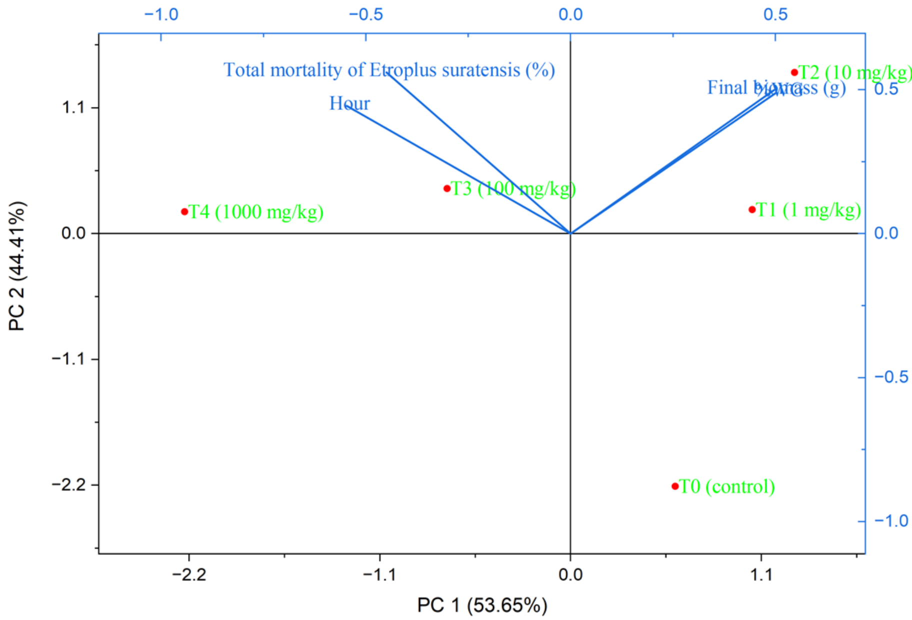

Figure 6. The principal component analysis (PCA) study.

mortality loaded in the opposite direction, mainly along PC2. This multivariate view highlights distinct grouping patterns and trade-offs between growth and survival, providing insights beyond those from individual pairwise correlations.

3.7. Hierarchical Clustering Dendrogram analysis

Hierarchical clustering using Euclidean distance was applied to %WG, final biomass, and mortality to group treatments (T0–T4) (Figure 7). T1 and T2 clustered at the smallest Euclidean distance, reflecting their close

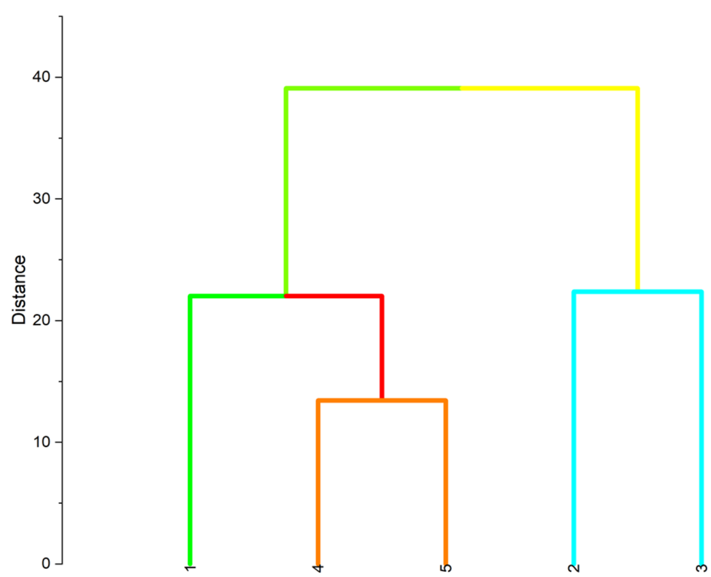

Figure 7. The hierarchical clustering studies. 1.T0 (control), 2. T1 (1 mg/kg), 3.T2 (10 mg/kg), 4. T3 (100 mg/kg), and 5. T4 (1000 mg/kg).

similarity in performance, characterized by high %WG, high biomass, and low mortality. T3 and T4 formed a separate subcluster, both showing reduced growth and elevated mortality, with T4 emerging as an outlier due to its extreme mortality and minimal growth. The control (T0) joined the T1–T2 cluster at a higher merge height, indicating moderate similarity to these high-performing treatments and marked dissimilarity from T3 and T4.

The largest vertical separation occurred when the T1–T2–T0 cluster merged with the T3–T4 cluster, indicating two primary performance groups: High growth / low-to-moderate mortality: T1, T2, T0 and Low growth / high mortality: T3, T4 Optimal outcomes for *Etroplus suratensis* occurred at low (T1, T2) or zero (T0) Ag-TiO_2 nanoparticle doses. In contrast, high-dose treatments (T3, T4) were linked to poor growth and increased mortality, with T4 (1000 mg/kg) showing the most severe negative effects. The dendrogram thus clearly separates effective from harmful concentrations, underscoring a dose-dependent trade-off between growth and survival.

4. Conclusions

The present study demonstrates that long-term dietary exposure to Ag-TiO_2 nanoparticles induces clear dose-dependent effects on the growth performance and survival of *Etroplus suratensis* fingerlings. Intermediate dietary concentrations (1 and 10 mg kg^{-1}) significantly enhanced growth indices, including percentage weight gain and final biomass, while maintaining low mortality, indicating a stimulatory or hormetic response at low exposure levels. In contrast, higher concentrations (100 and 1000 mg kg^{-1}) markedly impaired growth and increased mortality, with the highest dose producing the most pronounced adverse effects.

Statistical and multivariate analyses (ANOVA, Pearson correlation, PCA, and hierarchical clustering) consistently distinguished treatments into two major response groups: low-dose treatments associated with optimal growth and survival, and high-dose treatments associated with reduced growth performance and elevated mortality. The strong positive correlation between weight gain and biomass, coupled with their negative association with mortality, highlights the trade-off between growth and survival under increasing nanoparticle stress.

Overall, the findings indicate that E. *suratensis* is highly sensitive to dietary Ag-TiO_2 nanoparticle exposure and can serve as an effective bioindicator for nanoparticle-induced toxicity in aquatic ecosystems. The observed growth inhibition and increased mortality at elevated concentrations raise concerns regarding the ecological risks of uncontrolled nanoparticle discharge into aquatic environments. These results emphasize the need for regulatory guidelines and sustainable management strategies to minimize nanoparticle contamination and protect aquatic biodiversity.

Conflict of Interest

The authors declare no conflict of interest.

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