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The Use of Cinnamon Essential Oils in Aquaculture: Antibacterial, Anesthetic, Growth-Promoting, and Antioxidant Effects

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Abstract: Cinnamon essential oils (EOs) are widely known for their pharmaceutical properties; however, studies investigating the use of these EOs in aquaculture are scarce. The aims of this study were to evaluate the anesthetic effect of bathing silver catfish (*Rhamdia quelen*) with *Cinnamomum cassia* EO (CCEO) and its nanoemulsion (NCCEO); the growth-promoting and antioxidant effects of dietary supplementation with CCEO in silver catfish; and the in vitro antibacterial effect of CCEO, NCCEO, and *Cinnamomum zeylanicum* EO (CZEO) against bacteria isolated from diseased silver catfish. The two cinnamon EOs showed promising antibacterial activity, which was potentiated by the nanoemulsion. CCEO showed satisfactory anesthetic activity in silver catfish, and its nanoemulsion intensified the sedative activity. Supplementation of 1.0 mL CCEO per kg of diet for 60 days increased weight, length, and weight gain when compared to the control group, evidencing the growth-promoting activity of this EO. Dietary supplementation of CCEO for 30 and 60 days also showed an antioxidant effect, as it decreased levels of thiobarbituric acid reactive species and increased the superoxide dismutase activity in the liver of silver catfish. Therefore, cinnamon EOs have a promising use in aquaculture.

Keywords: *Cinnamomum cassia*; *Cinnamomum zeylanicum*; nanoemulsion; oxidative status; silver catfish; weight gain

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1. Introduction

Several plant essential oils (EOs) and their isolated compounds have been tested in fish, demonstrating their antimicrobial, antioxidant, sedative, and anesthetic activities [1,2]. Thus, these compounds have different uses in the aquaculture industry. True cinnamon (*Cinnamomum zeylanicum*, synonym *C. verum*) and Chinese cinnamon (*Cinnamomum cassia*) are trees of the Lauraceae family, the barks of which are widely used as a spices due to their distinct odor. The EOs extracted from their barks and leaves have been reported to have diverse pharmacological properties [3,4]. According to Ranasinghe et al. [3], the beneficial effects of *C. zeylanicum* on human health are antimicrobial and antiparasitic activity, lowering of blood glucose and pressure, lowering of serum cholesterol, antioxidant and free-radical scavenging properties, inhibition of tau aggregation and filament formation (hallmarks of Alzheimer's disease), inhibitory effects on osteoclastogenesis, antisecretagogue and antigastric ulcer effects, antinociceptive and anti-inflammatory activity, wound healing properties, and hepatoprotective effects. In addition, modern studies have

confirmed that *C. cassia* has a wide range of pharmacological effects, including antitumor, anti-inflammatory and analgesic, antidiabetic and antiobesity, antibacterial and antiviral, cardiovascular protective, cytoprotective, neuroprotective, immunoregulatory, and antityrosinase activity [4]. However, studies related to the use of these two EOs in aquaculture are scarce.

Medicinal herbs have been investigated for use in finfish diets to improve immune response and disease resistance. According to Hoseinifar et al. [5], several herbs present effects on fish resistance to bacterial, viral, and parasitic diseases. Applications of herbal products to combat microbial and parasitic diseases are considered as alternative approaches for sustainable aquaculture. The hydrophobic compounds of EOs can penetrate the bacterial and parasitic cells and cause cell deformities and organelle dysfunctions. Dietary supplementation of EOs also modulate growth, immunity, and infectious disease resistance in aquatic organisms [6].

Nanoemulsion is the mixture between two immiscible liquids in which one of them is in the form of tiny drops dispersed in the other liquid, forming a stable mixture. The use of a nanoemulsion can improve the water solubility of EOs, increasing their bioavailability in the body of fish [1]. Nanotechnology can protect the compounds contained in the EOs against enzymatic hydrolysis and increase their permanence in the bloodstream and sometimes even enable such compounds to be transported through the blood–brain barrier [7].

Studies have reported on the in vitro antibacterial effect of EOs against bacteria isolated from fish, such as *Citrobacter freundii*, *Aeromonas hydrophila*, and *Raoultella ornithinolytica* [8–10]. *A. hydrophila* and *C. freundii* are Gram-negative bacilli that cause gastroenteritis, ulcerations of the skin, and hemorrhagic septicemia in several fish species, such as the silver catfish (*Rhamdia quelen*) [11–13]. *R. ornithinolytica* is a Gram-negative bacillus of the Enterobacteriaceae family that can cause intoxication in humans due to the presence of histamine in contaminated fish [14]. Many EOs and their major compounds can be administered via bathing or as dietary supplements for fish to prevent and/or treat infectious diseases, as several in vitro and in vivo studies have already confirmed their effectiveness [7].

Essential oils (EOs) and their active compounds have shown promising results as fish anesthetics and sedatives when used via a bath [15]. These products may be used in the aquaculture industry to facilitate handling, labeling, transportation, induced spawning, vaccination, blood collection, biopsy, and surgery [1]. According to Hoseini et al. [16], herbal anesthetic use is increasing in aquaculture. To date, several plant essential oils/extracts and some plant bioactive compounds have been studied in fish anesthesia.

Syahidah et al. [17] highlighted the use of Eos as dietary supplements for aquatic organisms, with the purpose of stimulating appetite and promoting growth. Increasing weight gain is desirable in aquaculture, as fish reach their ideal selling weight faster, thus anticipating the fish producer's profits. However, the influence of cinnamon Eos as dietary supplements on the zootechnical parameters of fish remains poorly studied.

Increased production of reactive oxygen species against antioxidant defenses results in oxidative stress, and the fish responses to oxidative stress are related to their life history, strategies, and environmental changes [18]. Several EOs exhibit antioxidant activity when used via a bath or as dietary supplements, thus representing an alternative to prevent or treat oxidative stress in fish [15].

The silver catfish (*Rhamdia quelen*) is an omnivorous freshwater fish from the family Heptapteridae distributed from central Argentina to southern Mexico [19]. This native species is widely used as an animal model in research on substances isolated from plants and is important in fish cultures of southern Brazil [20].

Despite having several pharmacological properties already described, cinnamon EOs have been scarcely studied in terms of the aquaculture industry, mainly in relation to their antibacterial, anesthetic, growth-promoting, and antioxidant effects. Therefore, the aims of this study were to evaluate the anesthetic effect of bathing silver catfish with *Cinnamomum cassia* EO (CCEO) and its nanoemulsion (NCCEO); the growth-promoting and antioxidant effects of dietary supplementation with CCEO in silver catfish; and the in vitro antibacterial

effect of CCEO, NCCEO, and *Cinnamomum zeylanicum* EO (CZEO) against bacteria isolated from diseased silver catfish.

2. Materials and Methods

2.1. Essential Oils

Cinnamomum cassia EO (CCEO) (density 1.05 g/mL) obtained from the steam distillation of leaves, bark, and stalk was purchased from Ferquima™ (Vargem Grande Paulista, Brazil). CZEO (density 1.06 g/mL) obtained from the steam distillation of leaves was purchased from Vimontti™ (Santa Maria, Brazil).

2.2. Quantification of Phytochemicals in EOs and Preparation of CCEO Nanoemulsion

Approximate values of the main compounds of CCEO were provided by the manufacturer, as follows: cinnamaldehyde, 80%; benzaldehyde, 2%; coumarin, 2%; styrene, 2%; and cinnamic alcohol, 2%. Chemical analysis of CZEO was performed according to Garlet et al. [21]. Nanoemulsion-containing CCEO was prepared according to Volpato et al. [22].

2.3. Clinical Isolates

The clinical isolates *A. hydrophila* (Genbank access MF 372510), *C. freundii* (Genbank access MF 565839), and *R. ornithinolytica* (Genbank access MF 372511) were obtained from naturally infected silver catfish and were identified by molecular and biochemical tests according to Bandeira Junior et al. [11].

2.4. In Vitro Antibacterial Activity

The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of CZEO, CCEO, and NCCEO were verified against the three clinical isolates. The guidelines of the Clinical and Laboratory Standards Institute [23] were used to perform the microdilution test. The procedures are described in detail by Bandeira Junior et al. [24].

2.5. Animals and Water Quality Parameters

Silver catfish juveniles with no external lesions were purchased from a fish farm and maintained for one week in 250 L tanks. During the acclimation period, the fish were fed once a day with a commercial feed (Puro Trato™, Santo Augusto, Brazil, 36% crude protein, 4 mm in size). The remains of food and feces were removed daily, and 20% of the water was replaced daily. The water was kept under constant aeration. After the acclimatization period, the fish were transferred to a recirculation system to carry out the diet supplementation experiment. In this system, 20% of the water was renewed each day.

Temperature and oxygen were monitored daily with an oximeter (YSI 50®, Yellow Springs, OH, USA), pH was monitored daily with a portable pH meter (Alfakit AT-315®, Florianópolis, Brazil), and ammonia concentration was monitored daily using a colorimetric kit (LabconTest, Alcon®, Camboriú, Brazil). The water quality parameters remained within the range stipulated as ideal for the species [19], as follows: temperature, 18.5 ± 0.5 °C; dissolved oxygen, 8.55 ± 0.80 mg/L; pH, 7.35 ± 0.25; and total and non-ionized ammonia concentration, lower than 0.50 and 0.018 mg/L mg/L, respectively.

2.6. Induction of Anesthesia and Recovery

Silver catfish (6.62 ± 0.28 g, 8.87 ± 0.22 cm, $n = 8$ per group) were exposed to immersion baths with CCEO and NCCEO (5% EO) at concentrations of 50, 100, 150, and 200 mg/L. The CCEO was previously diluted in 95% ethanol (1:10). The NCCEO was not diluted in ethanol, as the nanoemulsion increases water solubility.

The anesthetic stage was evaluated according to Gomes et al. [25] and divided into four stages, as follows: stage 2 (deep sedation): partial loss of equilibrium and without reaction to external stimuli (hitting the bottom of the aquarium with a glass rod); stage 3a (total loss of equilibrium): fish turns sideways but keeps swimming; stage 3b: loss of ability to swim but still reacts to pressure on the caudal peduncle; stage 4 (anesthesia):

without reaction to pressure on the caudal peduncle. The animals were considered to have recovered when they swam normally and responded to a glass rod hitting the bottom of the aquarium. After anesthesia or a maximum exposure of 30 min, the animals were relocated to aquaria containing pure water for recovery. Anesthesia and recovery times (in seconds) were noted. Animals were observed for mortality over the next 24 h.

2.7. Diet Supplementation with CCEO and Zootechnical Parameters

Silver catfish (n = 240) were divided into five treatments: A, control diet; B, 0.25 mL of CCEO per kg of diet; C, 0.5 mL of CCEO per kg of diet; D, 1 mL of CCEO per kg of diet; and E, 2 mL of CCEO per kg of diet. These EO doses were based on the study of Zeppenfeld et al. [26]. Each treatment contained 48 fish divided into four tanks of 50 L, each containing 12 fish. The initial stock density was approximately 1 kg/m^3. Fish were fed twice a day for 60 days, with a feeding rate of 3% of biomass.

The basal diet and the incorporation of CCEO in the diet were performed according to Zeppenfeld et al. [26]. All ingredients were finely ground, weighed, and kneaded until homogeneous. Different concentrations of CCEO were then added, along with canola oil and, after that, water. The mixtures were dried in a forced-air circulation oven for 24 h (35 °C). Finally, the pellets were broken, sieved, and stored in a freezer until use. The diet formulation is shown in Table 1.

Table 1. Formulation of the experimental diet.

Ingredients	**g/kg**
Soybean meal	300
Meat and bone meal	350
Rice bran	120
Corn	150
Canola oil	30
Salt	10
Vitamin and mineral premix *	30
Phosphate dicalcium	10
Analyzed proximate composition	**g/kg**
Dry matter content	923.6
Protein	461.7
Ether extract	105.4
Crude fiber	29.4
Mineral matter	142.9
Acid detergent fiber	29.1
Neutral detergent fiber	164.1

* Vitamin and mineral mixture (security levels per kilogram of product)—folic acid: 250 mg, pantothenic acid: 5000 mg, antioxidant: 0.60 g, biotin: 125 mg, cobalt: 25 mg, copper: 2000 mg, iron: 820 mg, iodo: 100 mg, manganese: 3750 mg, niacin: 5000 mg, selenium: 75 mg, vitamin A: 1,000,000 UI, vitamin B1: 1250 mg, vitamin B12: 3750 mcg, vitamin B2: 2500 mg, vitamin B6: 2485 mg, vitamin C: 28,000 mg, vitamin D3: 500,000 UI, vitamin E: 20,000 UI, vitamin K: 500 mg, zinc: 17,500 mg.

Biometrics (length and weight) of six animals per tank (four tanks per treatment) were performed at 0, 30, and 60 days of feeding, with randomized sampling to represent each tank, considering each tank as an experimental unit (n = 4) in order to calculate the zootechnical parameters.

Eight fish per treatment (two fish per tank) were randomly selected and euthanized at 30 and 60 days of feeding, and the livers were collected and kept in a freezer at −80 °C until processing for oxidative status marker analysis. Fish were fasted for one day before euthanasia and were then anesthetized for 3 min with 50 mg/L of eugenol and euthanized by spinal cord section. All fish were euthanized at the end of the experimental period.

Mortality was assessed daily throughout the experimental period. The parameters of growth performance (weight gain, relative weight gain, and specific growth rate) were calculated according to Zeppenfeld et al. [26], as follows:

- Weight gain (WG) = final body weight − initial body weight
- Relative weight gain (RWG) = 100 × (final body weight − initial body weight)/initial body weight
- Specific growth rate (SGR) = 100 × (ln final weight − ln initial weight)/days of experiment

2.8. Oxidative Status Markers

The livers were processed as stated by Evelson et al. [27], and the supernatants were utilized to determine the parameters. Superoxide dismutase (SOD) [28], glutathione S-transferase (GST) [29,30], and glutathione peroxidase (GPx) [31] were calculated and expressed as U of enzyme per mg of protein. Thiobarbituric acid reactive species (TBARS) production was determined following Buege and Aust [32] and expressed as nmol of malondialdehyde (MDA) per g of tissue. Protein content in liver homogenates was determined by the spectrophotometric method (595 nm) of Coomassie blue G dye [33], using serum bovine albumin as the standard.

2.9. Statistical Analysis

The homogeneity of variances between groups was determined using the Levene and Kolmogorov–Smirnov tests. When the data were normally distributed, an ANOVA test was used. Alternatively, the Mann–Whitney test was used when the data were not normally distributed. The Mann–Whitney test was used to compare the anesthetic induction time of the CCEO and NCCEO at the same concentrations (median ± interquartile range). Regression analysis was also used to verify the relationship between concentration and anesthesia induction. Recovery data (concentration x recovery time) were not concentration-dependent, so two-way analysis of variance (ANOVA) and Tukey's post hoc test (mean ± standard error) were applied. Anesthesia data were analyzed with GraphPad Prism, version 6.0 (GraphPad Software Inc., San Diego, CA, USA). One-way ANOVA and Tukey's post hoc test were also used for the zootechnical parameters and oxidative status markers, and the results were expressed as the mean ± standard error (Statistica 7.0 StatSoft Inc., Tulsa, OK, USA). The minimum significance level for all tests was set at $p < 0.05$.

3. Results

3.1. Quantification of CZEO Compounds

Seven compounds were identified in CZEO, representing 100% of the total composition. The results indicate that eugenol (91.1%) was the most abundant compound present in CZEO (Table 2).

Table 2. Qualitative and quantitative analyses of *Cinnamomum zeylanicum* EO (CZEO).

RT (min)	Constituent	Relative Percentage (%)	RI Cal	RI Ref
14.8	o-cymene	0.6	1022.9	1022
17.835	Linalool	1.0	1098.5	1096
26.94	Eugenol	91.1	1351.6	1359/1370
29.134	β-caryophyllene	2.8	1418.6	1419
29.902	E-cinnamyl acetate	1.0	1443.0	1446
32.046	Eugenol acetate	1.8	1511.9	1514
39.209	Benzyl benzoate or ascabiol	1.8	1764.1	1762

RT: retention time; RI cal: calculated Kovats retention index; RI ref: reference Kovats retention index [34,35].

3.2. In Vitro Antibacterial Activity

The MIC and MBC results are shown in Table 3. MIC values ranged from 50 to 800 µg/mL for CZEO, 100 to 400 µg/mL for CCEO, and 25 to 200 µg/mL for NCCEO. NCCEO was the most effective compound, reaching very low MIC values and demonstrating the potentiating effect of the nanoemulsion. *A. hydrophila* MF 372510 was the most sensitive strain. MBC values ranged from 100 to 800 µg/mL for CZEO, 400 to 800 µg/mL for CCEO, and 50 to 200 µg/mL for NCCEO. Generally, the MBC values were higher than the MIC values, demonstrating the bacteriostatic effect of the tested substances.

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Cinnamomum zeylanicum* EO (CZEO), *Cinnamomum cassia* EO (CCEO), and its nanoemulsion (NCCEO) against three clinical isolates of fish pathogenic bacteria. The values are expressed in μg/mL.

	***Aeromonas hydrophila* (MF 372510)**		***Citrobacter freundii* (MF 565839)**		***Raoultella ornithinolytica* (MF 372511)**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
CZEO	50	100	400	800	800	800
CCEO	100	400	400	800	200	800
NCCEO	25	50	50	200	50	200

3.3. Induction of Anesthesia and Recovery

CCEO and NCCEO only caused sedation (stages 2 and 3a) at a concentration of 50 mg/L. At higher concentrations (100, 150, and 200 mg/L), both EOs induced anesthesia (stage 4) within a similar timeframe, ranging from 308 to 119 s for CCEO and from 318 to 102 s for NCCEO. NCCEO was more potent in inducing stage 3a (50 mg/L) and stage 3b (100 mg/L) than CCEO (Figure 1). As for recovery, CCEO and NCCEO did not differ in recovery time at the lowest concentration (50 mg/L); however, at the other tested concentrations, the fish anesthetized with NCCEO (172.5–211 s) showed faster recovery than those anesthetized with CCEO (376–488 s) (Figure 2).

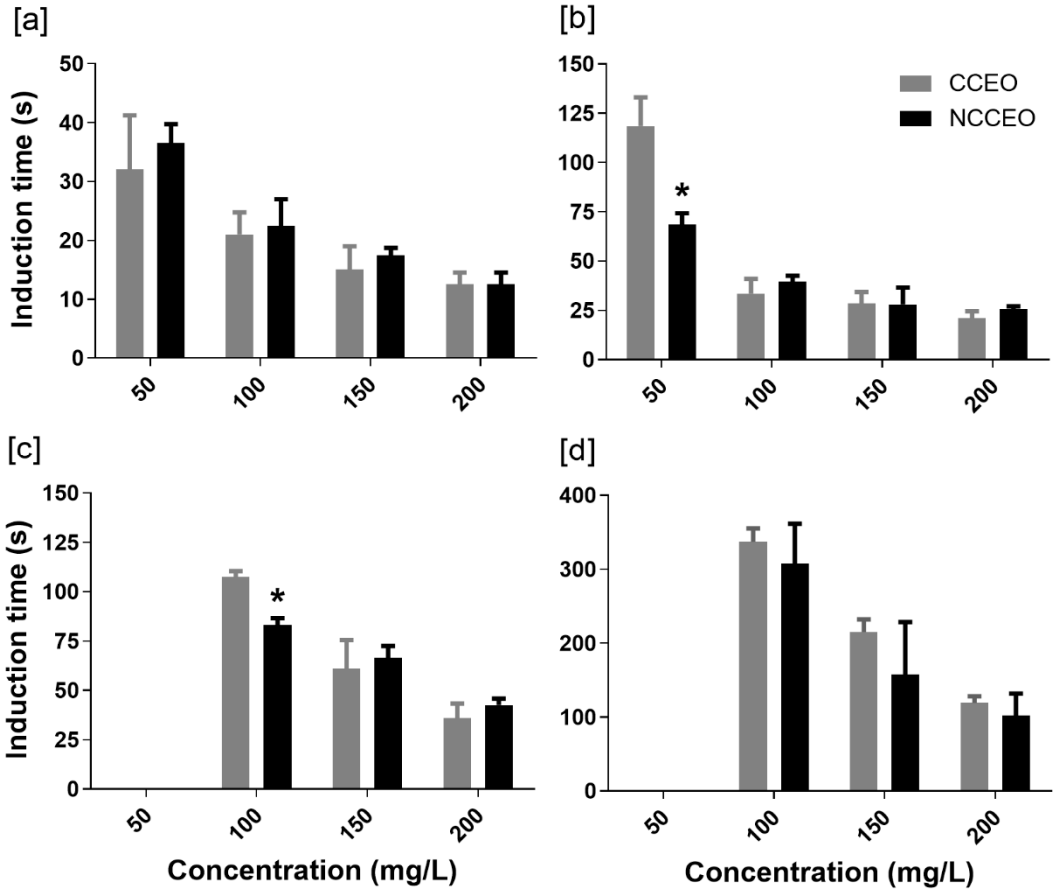

Figure 1. Anesthetic induction time with Chinese cinnamon (*Cinnamomum cassia*) essential oil (CCEO) and its nanoemulsion (NCCEO) in silver catfish (*Rhamdia quelen*) by immersion bath. (**a**) Stage 2; (**b**) stage 3a; (**c**) stage 3b; and (**d**) stage 4. Values are expressed as median ± interquartile range (n = 8). * Asterisk indicates significant difference from CCEO at the same concentration (Mann–Whitney test, $p < 0.05$).

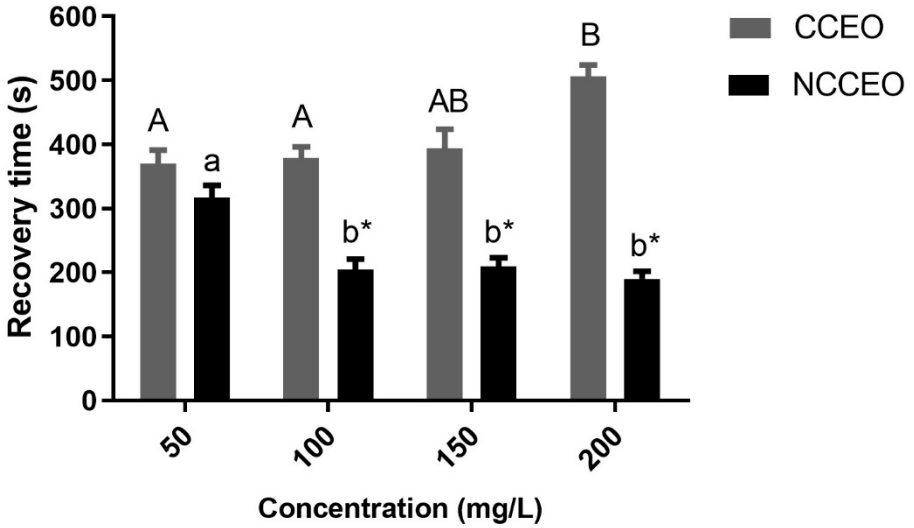

Figure 2. Anesthetic recovery time of Chinese cinnamon (*Cinnamomum cassia*) essential oil (CCEO) and its nanoemulsion (NCCEO) in silver catfish (*Rhamdia quelen*) by immersion bath. Values are expressed as the mean ± SEM (n = 8). Uppercase letters indicate significant difference between CCEO concentrations; lowercase letters indicate significant difference between NCCEO concentrations; * asterisk indicates significant difference from CCEO at the same concentration (two-way ANOVA and Tukey's test, $p < 0.05$).

Regression analysis showed a concentration–response relationship in the induction stages, 3a, 3b, and 4, for CCEO and in all stages for NCCEO (Figure 3). The recovery of CCEO and NCCEO did not show a concentration–response relationship (data not shown). Furthermore, there was no fish mortality within 24 h.

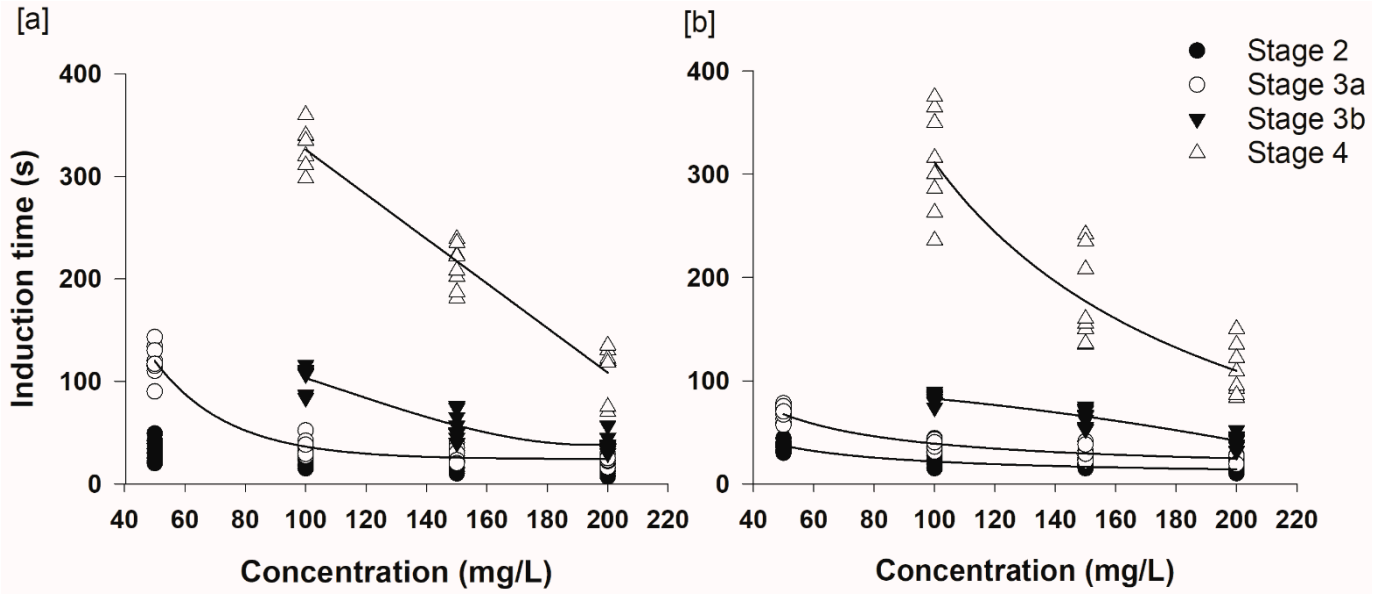

Figure 3. Relationships of concentration x anesthesia induction time in silver catfish (*Rhamdia quelen*) exposed to Chinese cinnamon (*Cinnamomum cassia*) essential oil (CCEO) (**a**) and its nanoemulsion (NCCEO) (**b**). Equations: stage 2: no significant relationship; stage 3a: $y = 23.98 + 746.49^{(-0.0411x)}$, $r^2 = 0.95$; stage 3b: $y = 131.930 + 0.867x - 0.0164x^2 + 4.880^{(-005)} x^3$, $r^2 = 0.86$; stage 4: $y = 543.7035 + (-2.1763x)$, $r^2 = 0.95$ (**a**); and stage 2: $y = 6.27 + 1533.84x$, $r^2 = 0.86$; stage 3a: $y = 10.36 + (2856.92/x)$, $r^2 = 0.90$; stage 3b: $y = 98.25 + (-0.03x) + (-0.0013x^2)$, $r^2 = 0.88$; stage 4: $y = -92.62 + (40{,}425/x)$, $r^2 = 0.83$; recovery (**a**); and (**b**): no significant relationship, data not shown. y = time to reach stage(s) and x = concentration (mg/L).

3.4. Zootechnical Parameters

The weight, length, and weight gain were significantly higher in the group supplemented with 1.0 mL CCEO per kg of diet for 60 days compared to the control group. Fish fed a diet supplemented with 0.5 mL CCEO per kg of diet presented significantly higher weight gain than the control, whereas those fed a diet supplemented with 2.0 mL CCEO per kg of diet showed significantly lower length than control. There was no statistical difference in the other parameters analyzed (Table 4). No deaths occurred during the experimental period.

Table 4. Growth performance of silver catfish (*Rhamdia quelen*) fed with diets containing different concentrations of *Cinnamomum cassia* essential oil (CCEO).

	Diet (mL EO per kg of Diet)				
	0	**0.25**	**0.50**	**1.0**	**2.0**
IW (g)	4.38 ± 0.31	4.03 ± 0.06	4.32 ± 0.55	4.87 ± 0.46	4.45 ± 0.06
ISL (cm)	9.46 ± 0.07	9.08 ± 0.11	9.25 ± 0.38	9.75 ± 0.38	9.33 ± 0.04
30 days					
SL (cm)	9.47 ± 0.08	9.08 ± 0.11	9.25 ± 0.38	9.33 ± 0.04	9.75 ± 0.38
W (g)	5.94 ± 0.32	5.83 ± 0.60	5.78 ± 0.53	6.64 ± 0.23	6.03 ± 0.57
WG (g)	1.55 ± 0.60	1.80 ± 0.55	1.46 ± 1.08	1.77 ± 0.70	1.58 ± 0.52
RWG (%)	37.73 ± 16.32	44.17 ± 12.83	42.68 ± 34.93	39.72 ± 18.04	35.32 ± 11.27
SGR (%/day)	0.51 ± 0.20	0.60 ± 0.14	0.50 ± 0.38	0.53 ± 0.22	0.49 ± 0.14
60 days					
SL (cm)	12.3 ± 0.30 ^{a}	12.44 ± 0.27 ^{a}	13.03 ± 0.10 ^{ab}	13.82 ± 0.38 ^{b}	8.36 ± 0.14 ^{c}
W (g)	8.16 ± 0.58 ^{ab}	7.34 ± 0.35 ^{a}	9.85 ± 0.15 ^{bc}	11.07 ± 0.84 ^{c}	8.35 ± 0.14 ^{ab}
WG (g)	3.78 ± 0.37 ^{a}	3.30 ± 0.30 ^{a}	5.53 ± 0.54 ^{bc}	5.94 ± 0.59 ^{c}	3.91 ± 0.15 ^{ab}
RWG (%)	85.34 ± 5.05	81.37 ± 6.62	138.34 ± 28.77	114.20 ± 7.91	88.09 ± 3.93
SGR (%/day)	1.03 ± 0.05	0.99 ± 0.06	1.40 ± 0.19	1.26 ± 0.08	1.05 ± 0.03

IW: initial weight; ISL: initial standard length; SL: standard length; W: weight; WG: weight gain; RWG: relative weight gain; SGR: specific growth rate. Values are expressed as the mean ± SEM (n = 4 tanks). Different letters in the rows indicate significant difference between groups (one-way ANOVA and Tukey's test, $p < 0.05$).

3.5. Oxidative Status Markers

The TBARS levels in the livers of fish fed a diet supplemented with different doses of CCEO for 30 and 60 days were lower than those in fish fed the control diet. The highest SOD activity was observed in the livers of fish fed a diet supplemented with 2 mL of CCEO per kg of diet for 60 days. There was no statistical difference between groups with respect to the GST and GPx activities in the two analyzed periods (Figures 4 and 5).

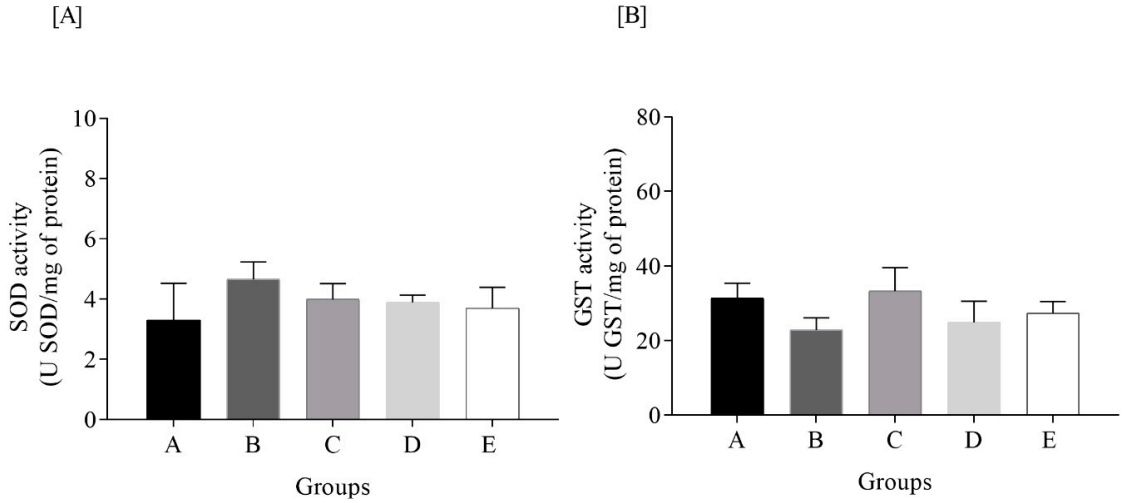

Figure 4. *Cont.*

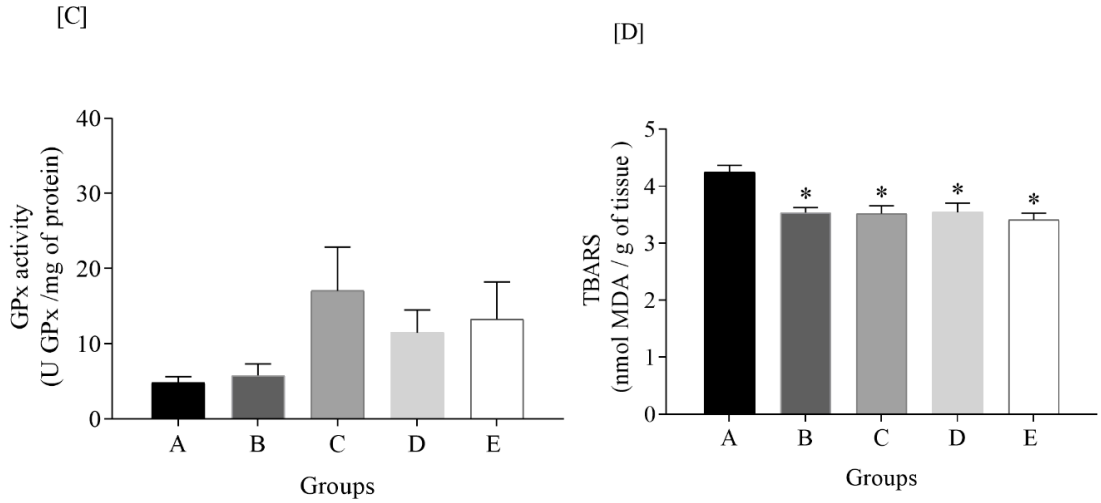

Figure 4. Oxidative status markers in the liver of silver catfish (*Rhamdia quelen*) fed with diets containing different concentrations of *Cinnamomum cassia* essential oil (CCEO) for 30 days (**A**–**D**). Letters of the bars: (A) Control diet; (B) supplementation of 0.25 mL of CCEO per kg of diet; (C) supplementation of 0.5 mL of CCEO per kg of diet; (D) supplementation of 1 mL of CCEO per kg of diet; (E) supplementation of 2 mL of CCEO per kg of diet. SOD: superoxide dismutase; GST: glutathione S-transferase; GPx: glutathione peroxidase; TBARS: thiobarbituric acid reactive species. Values are expressed as the mean ± SEM (n = 8). * Asterisk indicates significant difference from control group (one-way ANOVA and Tukey's test, $p < 0.05$).

4. Discussion

According to Sutili et al. [36], an MIC value lower than 500 µg/mL for plant extractives is considered strong inhibition with regard to the growth of bacteria isolated from fish. Based on this classification, the three substances tested in this study showed excellent MIC results. Treatment with the nanoemulsion potentiated the antibacterial activity of the CCEO, possibly because the nanoemulsion increases the solubility of EOs in water, and the culture medium used for the MIC test was aqueous [1,23].

The antibacterial in vitro effect of CCEO extracted from bark was demonstrated against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*) [37,38], and filamentous fungi and yeasts [39]. In a review, Doyle and Stephens [40] reported the antibacterial activity of cinnamaldehyde (the main constituent of CCEO) and its derivatives against several Gram-positive and Gram-negative bacteria. Therefore, cinnamaldehyde is probably one of the major compounds related to the antibacterial activity of this EO.

Wijesinghe et al. [41] described the antibacterial and antibiofilm effect of the leaf extract of CZEO against *Klebsiella pneumoniae*, *P. aeruginosa*, and *S. aureus*, whereas Gogoi et al. [42] reported good antimicrobial activity of CZEO extracted from bark and leaves against bacteria (*S. aureus* and *Bacillus cereus*) and fungus (*Aspergillus niger*). In addition, Choi et al. [43] reported that CZEO and cinnamaldehyde has a good in vitro antibacterial effect against cariogenic *Streptococcus mutans* and *Streptococcus sobrinus* bacteria strains. The main constituent of CZEO is the phytochemical eugenol, which has been reported to exhibit antibacterial activity against the same strains tested in this study (*C. freundii* MF 565839, *A. hydrophila* MF 372510, and *R. ornithinolytica* MF 372511) [11]. Therefore, eugenol is probably related to the antibacterial activity of this EO. According to Sutili et al. [36], this phytochemical was also efficacious in the in vivo treatment of *A. hydrophila* infection in silver catfish.

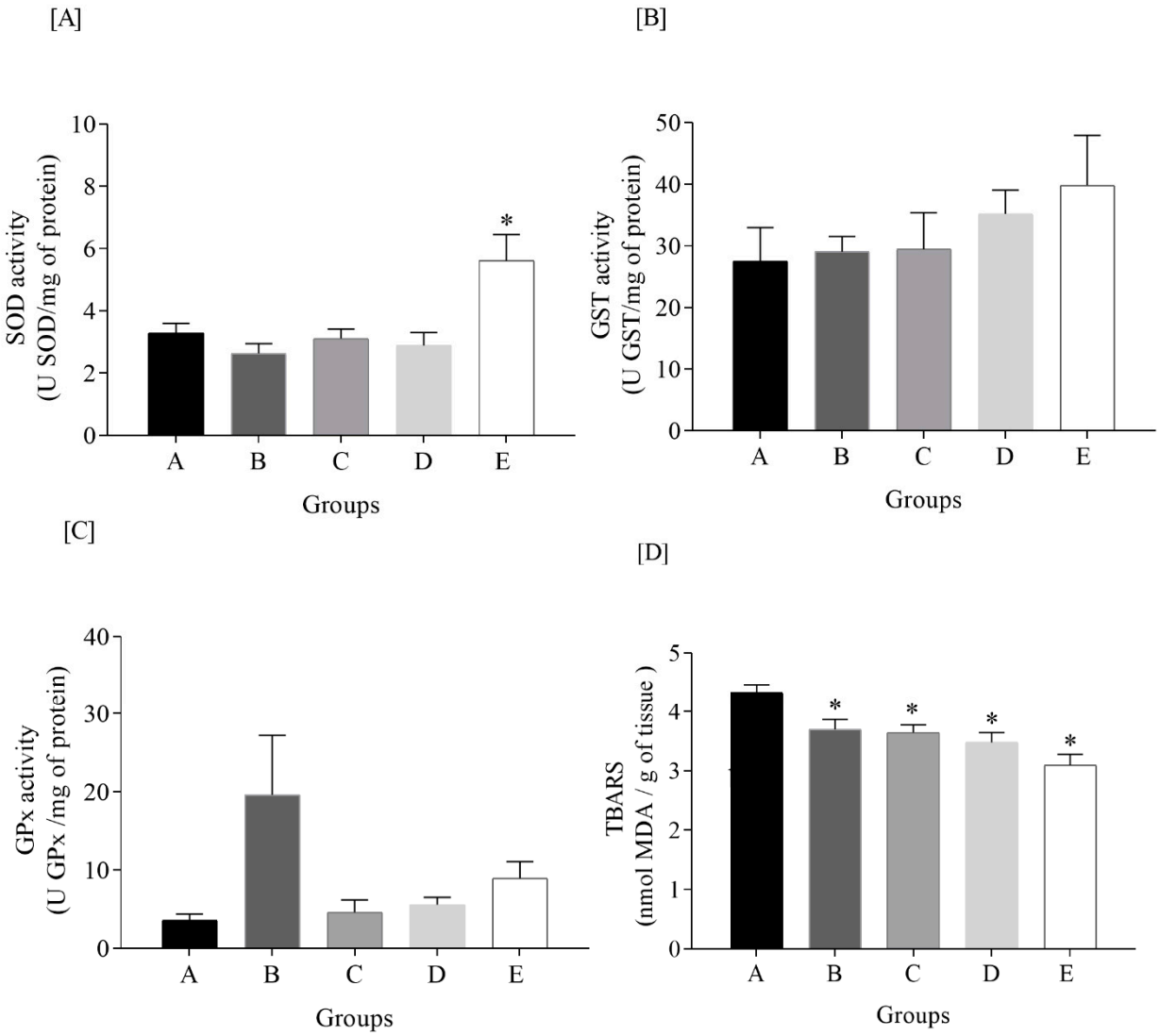

Figure 5. Oxidative status markers in the liver of silver catfish (*Rhamdia quelen*) fed with diets containing different concentrations of *Cinnamomum cassia* essential oil (CCEO) for 60 days (**A**–**D**). Letters of the bars: (A) Control diet; (B) supplementation of 0.25 mL of CCEO per kg of diet; (C) supplementation of 0.5 mL of CCEO per kg of diet; (D) supplementation of 1 mL of CCEO per kg of diet; (E) supplementation of 2 mL of CCEO per kg of diet. SOD: superoxide dismutase; GST: glutathione S-transferase; GPx: glutathione peroxidase; TBARS: thiobarbituric acid reactive species. Values are expressed as the mean ± SEM (n = 8). * Asterisk indicates significant difference from all other groups (one-way ANOVA and Tukey's test, $p < 0.05$).

In this study, CCEO induced sedation in silver catfish at all concentrations tested (50–200 mg/L) and deep anesthesia (stage 4) at concentrations higher than 100 mg/L. Reports on the sedative or anesthetic effects of CCEO on fish are scarce. Power et al. [44] reported the sedative effect of this EO in platy (*Xiphosphorus maculatus*) at a concentration of 0.067 mg/L. The difference in the concentrations required to induce anesthesia in these two species (platy and silver catfish) may be due to the different methodologies used and/or the species' sensibility to CCEO. It is difficult to determine which phytochemicals are responsible for the anesthetic effect of CCEO, as there have been no studies testing the anesthetic effect of cinnamaldehyde in fish; the anesthetic effect may also be the result of the synergistic interaction of the phytochemicals present in CCEO, as described by Benovit et al. [45] for other EOs.

In this study, the nanoemulsion was able to enhance the sedative effect of the CCEO at some concentrations but did not potentiate a state of deep anesthesia (stage 4) as expected. The fast anesthetic induction time (stage 4) achieved by the CCEO (less than 2.5 min) certainly masked any benefit of the nanoemulsion on the bioavailability of the CCEO. Other studies have highlighted the advantages of using the nanoemulsions of other plant EOs as anesthetics for fish, mainly due to the potentiation of the anesthetic effect [46–48], which is associated with factors such as lower volatility and increased light resistance. Another advantage of using the nanoemulsion is its solubility in water, dispensing with the need for pre-solubilization in ethanol.

In this study, the main advantage of nanoemulsion anesthesia was observed in the recovery stage, which occurred in a shorter time with NCCEO. Most lipophilic anesthetics tend to increase recovery time at higher concentration compared to lower concentrations. This occurred with anesthesia by *Aloysia triphylla* EO in Nile tilapia (*Oreochromis niloticus*) [49] and with anesthesia by *Lippia alba* EO in tambacu (*Piaractus mesopotamicus* × *Colossoma macropomum*) [50]. In our study, this effect also occurred with the use of CCEO. The accumulation of large amounts of anesthetic in body fat probably causes a slow release of the substance, increasing the recovery time. However, this did not occur with NCCEO. As it is dispersed in a less lipid-soluble formulation, NCCEO may have shown a lower tendency to accumulate and was consequently more easily eliminated.

We detected growth-promoting activity with CCEO dietary supplementation in silver catfish. This was the first study to evaluate zootechnical parameters in fish that were fed diets supplemented with CCEO. However, there are some studies that have evaluated the growth-promoting activity of cinnamaldehyde in the diet of other fish species [51–55]. El-Hamid et al. [52] reported an increased final body weight (FBW) and improved feed conversion rate (FCR) in Nile tilapia that were fed 100, 200, and 300 mg of cinnamaldehyde nanoemulsion per kg of diet for 12 weeks compared to the control group, whereas the supplementation of 1 mL of cinnamaldehyde per kg of diet in Nile tilapia fingerlings improved the FCR (days 46–60) and increased body weight gain and feed intake (FI) (days 61–75) [51]. Zhou et al. [55] reported that cinnamaldehyde supplementation (36, 72, 108, and 144 mg per kg of diet for 60 days) in grass carp (*Ctenopharyngodon idella*) increased FBW, percent weight gain, SGR, FI, and the feed efficiency ratio when compared to the control diet, with fish fed 72 mg per kg of diet showing the best performance. In addition, the supplementation of 0.1 g of cinnamaldehyde per 100 g of diet in tongue sole (*Cynoglossus semilaevis*) for 60 days revealed significant improvements in the RWG, SGR, FCR, and protein efficiency [54]. Therefore, cinnamaldehyde is probably responsible for the increase in the RWG found in this study; however, additive or synergistic effects with other EO components are possible. The current study and that of Zhou et al. [55] demonstrated that growth response to dietary supplementation with essential oils does not always show a dose–response pattern. Sometimes, higher doses do not improve growth or even impair the fish physiological condition. For example, dietary supplementation with 0.5mL per kg diet of ginger EO (*Zingiber officinale*) improved the growth performance and feed conversion rate of Nile tilapia, but higher doses did not have the same effect and promoted liver disfunction [56]. In addition, 200 mg per kg diet of savory (*Satureja hortensis*) EO increased final weight, weight gain, and specific growth rate of Caspian roach (*Rutilus caspicus*) fry, but those fed 400 mg/kg diet presented the lowest values of these parameters [57].

Supplementation with CCEO in the diet of silver catfish showed an antioxidant effect, as it decreased TBARS levels and increased SOD activity in the liver of these animals. TBARS production is used to measure lipid peroxidation (through the mensuration of a secondary product called MDA), which is related to pathological disorders linked to oxygen toxicity [58]. In addition, SOD is an antioxidant enzyme that prevents oxidative stress by catalyzing the dismutation reaction of the superoxide anion to oxygen and hydrogen peroxide [59].

The antioxidant properties of CCEO have already been reported, especially in terms of its application in food preservation [60]. However, studies testing the antioxidant activity

of this EO in live fish are lacking, although dietary supplementation with cinnamaldehyde significantly reduces MDA formation and increases glutathione reductase (GR) in muscle, as well as catalase (CAT) activity in serum, thus improving the antioxidant protective capacity in fingerlings of Nile tilapia [51]. El-Hamid et al. [52] stated that dietary cinnamaldehyde nanoemulsion supplementation potentiated the expression of genes related to antioxidant enzymes (SOD, CAT, and GPx) in the liver, muscle, and intestine of Nile tilapia. In tongue sole, dietary cinnamaldehyde improved the antioxidant capacity by increasing SOD, CAT, GPx, and total antioxidative capacity (T-AOC) activities and by decreasing the MDA content in the serum, liver, mid kidney, and spleen [54]. In striped catfish (*Pangasianodon hypophthalmus*), the addition of 1 g cinnamaldehyde/kg of diet for 72 days reduced MDA formation and increased SOD activity in liver and meat fillets [53]. In addition, Mousa et al. [61] reported that dietary trans-cinnamaldehyde reduced the oxidative stress in the liver of channel catfish (*Ictalurus punctatus*) infected with *Edwardsiella ictaluri*. These authors reported an increasing in CAT and SOD activities and GSH levels, and a decreasing in MDA and nitric oxide (NO) levels.

Cinnamaldehyde has also been widely studied in fish fillets, as it can increase their shelf life due to its antibacterial and antioxidant properties [62–64]. Therefore, cinnamaldehyde is probably one of the main compounds responsible for the antioxidant activity of CCEO found in this study.

5. Conclusions

The two cinnamon EOs showed promising antibacterial activity, which was potentiated by the nanoemulsion. CCEO showed satisfactory anesthetic activity in silver catfish, and its nanoemulsion intensified the sedative activity and reduced the recovery time. Supplementation of 1.0 mL CCEO per kg of diet for 60 days increased weight, length, and weight gain when compared to the control group, evidencing the growth-promoting activity of this EO. Dietary supplementation of CCEO for 30 and 60 days showed an antioxidant effect, as it decreased TBARS levels and increased SOD activity in the liver of silver catfish. Therefore, cinnamon EOs have a promising use in aquaculture. In future studies, we suggest verifying whether the antibacterial activity of these EOs demonstrated in the in vitro tests remains in in vivo studies. We also suggest that these tests be carried out with CZEO nanoemulsions.

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Data Availability Statement: The data supporting the results obtained in the study are available upon reasonable request to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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