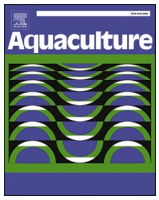
Aquaculture 574 (2023) 739695

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Prophylactic and therapeutic effects of a nanocomposite (silver nanoparticle plus *Terminalia catappa*) against *Saprolegnia parasitica* in tambaqui

Juliana Oliveira Meneses [a], Joel Artur Rodrigues Dias [b], Fernanda dos Santos Cunha [a], Hugo Leandro dos Santos [c], Thays Brito Reis Santos [a], Cindy Caroline Moura Santos [a], Ricardo Marques Nogueira Filho [a], Peterson Emmanuel Guimarães Paixão [a], Natalino da Costa Sousa [b], Márcia Valéria Silva do Couto [b], Higo Andrade Abe [b], Francisco José dos Santos [d], Silvia Patrícia Carraschi de Oliveira [e], Alexandre Nízio Maria [d], Juliana Cordeiro Cardoso [a,f], Luiz Pereira da Costa [g], Rodrigo Yudi Fujimoto [d,*]

[a] *Post Graduation Program in Health and Environment, Tiradentes University, 49032-490, Brazil*
[b] *Post Graduation Program in Animal Science, Federal University of Pará, 660758-110, Brazil*
[c] *Graduation in Fishery Engineering, Federal University of Sergipe, 49100-000, Brazil*
[d] *Brazilian Agricultural Research Corporation, 49025-040, Brazil*
[e] *University of Araraquara, 14801-320, Brazil*
[f] *Technology and Research Institute (ITP), 49032-490, Brazil*
[g] *Institute of Exact Science and Technology (ICET), Amazon Federal University, Amazonas, Brazil*

A R T I C L E I N F O

Keywords:
Saprolegnia parasitica
Mycelium
Zoospore
Colossoma macropomum
Prophylaxis

A B S T R A C T

Infectious diseases have been the main limiting factor for international fish farming, especially saprolegniosis, which causes large economic losses. Therefore, this study evaluated the prophylactic and therapeutic effect of a nanocomposite (Silver nanoparticle plus extract of *Terminalia catappa*) to control *Saprolegnia parasitica* in tambaqui *Colossoma macropomum*. Two tests were carried out: 1) a therapeutic assay including long and short-term baths, containing two concentrations of nanocomposite each, on infected tambaqui and 2) a prophylactic assay where the zoospore and nanocomposite (four different concentrations) were added at the same time into water for 72 h. Mortality, prevalence index, hematology, and infected areas with *S. parasitica* on the fish body were evaluated in both tests. In the therapeutic test, all fish from the control group (without nanocomposite) had increased infected area, as well as lethargy and hemorrhage, resulting in 100% mortality. They also had reduced red blood cells, lymphocytes, neutrophils, monocytes, and thrombocytes. In group exposed to nanocomposite, higher concentrations affected mycelial growth, especially in the highest concentration (4.33 mg L^{-1}), reducing infected areas on the fish body by 98.70% and achieving 100% survival. The treatment with long-term bath at the lowest concentration (0.54 mg L^{-1}) had increased values of neutrophils. In the prophylactic test, fish groups without handling stress and the methylene blue had no clinical signs or mortality. However, the fish group submitted to handling stress presented the highest prevalence and infected areas resulting in 100% mortality. However, the increasing of nanocomposite concentration promoted less oomycete prevalence, pathology intensity, and mortality. The most effective concentration in the prophylactic assay was 0.87 mg L^{-1}, preventing the infection without blood alterations. Then, the nanocomposite as prophylactic measured at concentration of 0.87 mg L^{-1} is the best strategy to prevent *S. parasitica* infection in fish.

* Corresponding author.
E-mail address: ryfujim@hotmail.com (R.Y. Fujimoto).

https://doi.org/10.1016/j.aquaculture.2023.739695
Received 4 September 2022; Received in revised form 10 May 2023; Accepted 15 May 2023
Available online 16 May 2023

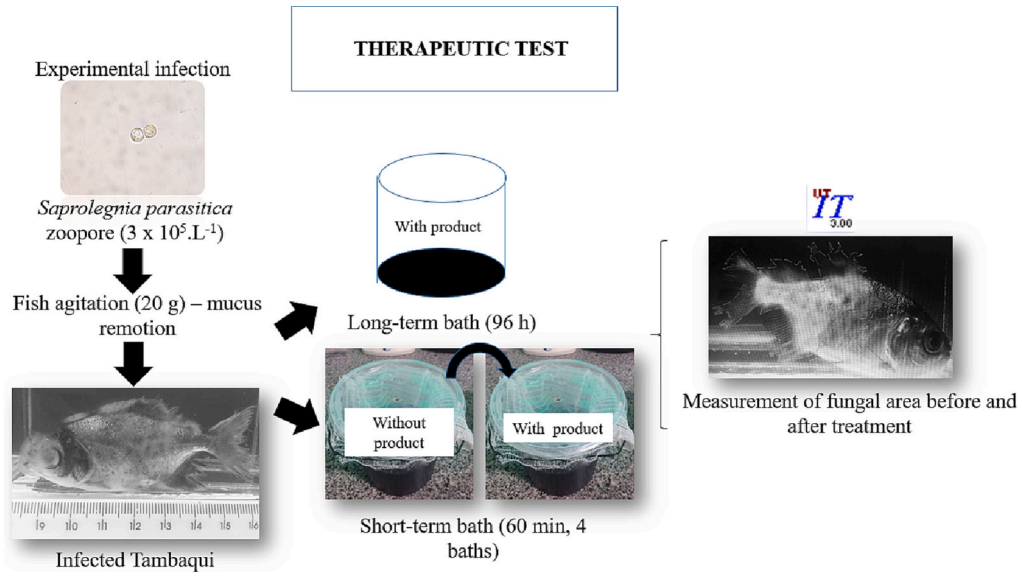


Fig. 1. Experimental design of therapeutic test with nanocomposite against *Saprolegnia parasitica* in infected juvenile *C. macropomum*.

1. Introduction

Oomycetes present filamentous mycelial growth and were initially classified as Eumycota (fungi), but have motile biflagellate zoospores and are currently classified as Stramenopiles or Chromista groups (fungus-like) (Beakes et al., 2012). Oomycetes are one of the main problems for fish farming around the world especially *Saprolegnia parasitica*. This is a fungus-like from the Saprolegniaceae family characterized as an opportunistic parasite quickly infesting fish or eggs, causing saprolegniosis diseases (Zaki and Fawazi, 2015; Sarowar et al., 2019; Magray et al., 2021).

This fish pathogen has been responsible for worldwide outbreaks in rainbow trout *Oncorhynchus mykiss*, Nile tilapia *Oreochromis niloticus*, common carp *Cyprinus carpio*, American catfish *Ictalurus punctatus,* and Salmon coho *Oncorhynchus kisutch* (Hatai and Hoshiai, 1992, 1993; Bly et al., 1994; Singh et al., 2012; Van Den Berg et al., 2013; Lone and Manohar, 2018; Ali et al., 2019; Alwash et al., 2021). Reports about the American catfish estimated a mass mortality of approximately 50% total production, generating an economic loss in the United States of 40 million dollars (USD) (Bly et al., 1994; Van West, 2006; Bruno et al., 2011). In Japan, mortality reached above 50% of reared *Salmon coho* (Hatai and Hoshiai, 1992).

The *Colossoma macropomum* is one of the most produced fish species in South America, totaling over 100 thousand tons in 2020 (FAO, 2022). Brazil is the largest producer of this species, surpassed only by tilapia production. This fish presents resistance to hypoxia, easy acceptance for industrial feed and high market value (Valenti et al., 2021), however is sensitive to saprolegniosis after handling procedures.

Silver nanoparticles have emerged as an alternative to control *S. parasitica* due to their fungicide and fungistatic effect. However, when only nanoparticles are used, higher concentrations are necessary (1800 mg L^{-1}) to reach the fungicide effect, as observed by Johari et al. (2015) to control infection of rainbow trout eggs. Despite effectively controlling the disease, high concentration of silver nanoparticles can cause toxic effects on the fish and be an environmental problem (Khan et al., 2015).

To minimize the toxic effects, an alternative to control this pathogen could be the use of nanocomposites (silver nanoparticles plus aqueous extract of *T. catappa*). Thus, the present study evaluated the therapeutic and prophylactic effects of nanocomposite (aqueous extract of *T. catappa* plus silver nanoparticles) for controlling *S. parasitica* in tambaqui *C. macropomum*.

2. Material and methods

2.1. Characterization of the nanocomposite

Nanocomposite was prepared and characterized according to Meneses et al. (2021) with the following features: silver nanoparticle size 12.97 ± 10.90 nm and zeta potential −15.20 ± 1.93 mV. The *T. catappa* aqueous extract was produced with 25 g of dried leaves powder included into 500 mL of water at temperature 60 °C during one hour. Afterwards, its content was filtered and then replaced to another 500 mL of water at the same temperature (60 °C/1 h), totalizing two hours of extracting procedure. This aqueous extract presented the following chemical profile: gallic acid (7.36 μg mg^{-1}), ellagic acid (9.54 μg mg^{-1}), α-punicalagin (13.32 μg mg^{-1}), and β-punicalagin (22.87 μg mg^{-1}) (Meneses et al., 2020). The nanocomposite stocking solution contained silver nanoparticle (AgNPs) and *T. catappa* extract at concentrations of 1830 and 2500 mg L^{-1}, respectively.

2.2. Zoospores of Saprolegnia parasitica

The *Saprolegnia. parasitica* was acquired from the laboratory of aquaculture in the Embrapa Tabuleiros Costeiros. This pathogen was previously isolated from naturally infected tambaqui and molecularly identified (gen bank access KY418035) (https://www.ncbi.nlm.nih.gov/nuccore/KY418035).

The oomycete grown up in Potato Dextrose Agar (PDA) throughout the 96 h at room temperature (25 °C). After the complete growth, 1-cm^2 fragment were placed into a new plate containing only PDA. In addition, sterile sesame seeds were distributed around the fragment and grown at room temperature for 24 h. All seeds were removed after 24 h and placed into specific solution (SS) for an additional 48 h at 25 °C. An air supply was used to aid the zoospore production in the solution. At the end, seeds were discarded and then the zoospores were counted using Neubauer Chamber (number of zoospores of 8.85 ± 5.38 × 10^4 mL^{-1}).

The specific solution (SS) contained one liter of distillated water, 0.5 mL of solution 1 (8.7 g K_2HPO_4, 6.8 g KH_2PO_4, 6.6 g $(NH_4)_2HPO_4$, and 50 mL of distilled water), and 0.1 mL of solution 2 (5.08 g $MgCl_2.6H_2O$, 3.67 g $CaCl_2.2H_2O$, and 50 mL of distilled water).

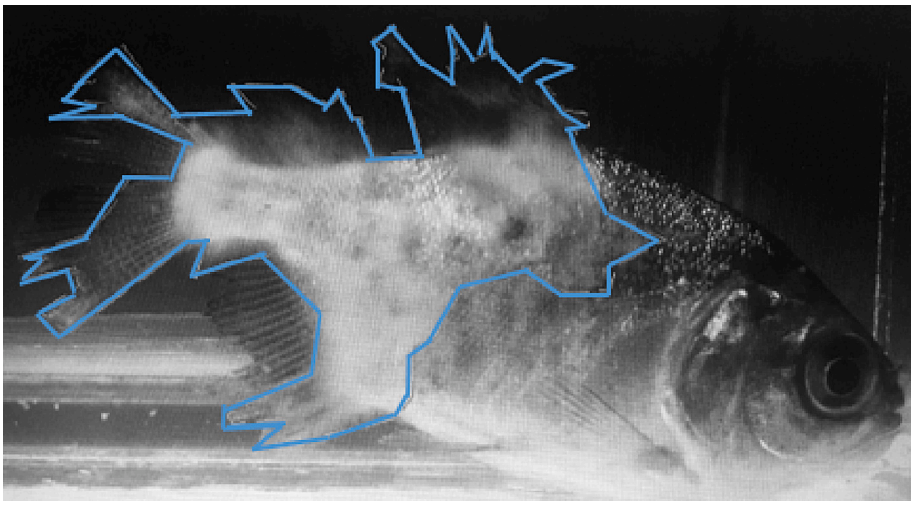


Fig. 2. Measuring infected areas on the fish body using the software *image tool*.

2.3. Nanocomposite therapeutic test in juvenile Colossoma macropomum infected with mycelium of Saprolegnia parasitica

This experiment was approved by the ethics committee for animal use from Tiradentes University (03031R/2018). Initially, for the infection process 60 juvenile fish (10–12 g) were shaken in a small fishing net for 20 s to cause stress (handling stress) and mucus remotion. Afterwards, the animals were placed into 20 polyethylene recipients at stocking density of three fish per unit with final volume of 1 L previously added with 3×10^{5} zoospore L^{-1} (Firouzbakhsh et al., 2012). The procedure to promote stress, the stocking density, the inoculation, and zoospore concentration was adapted from Firouzbakhsh et al. (2012).

The complete infection on fish were observed 48 h after addition of zoospores. Afterwards, 50 infected fish were distributed separately into recipients containing only water for 24 h to ensure the fungal infection on the fish body. The initial water quality parameters were: temperature 23.35 ± 0.26 °C, dissolved oxygen 6.99 ± 0.55 mg L^{-1}, pH 7.60 ± 0.15, electric conductivity of 155.71 ± 26.37 μS cm^{-1}, and toxic ammonia of 0.00 ± 0.00 mg L^{-1}

After 24 h, the experiment occurred with different concentrations of nanocomposite applied in either a long-term bath (infected fish exposed to **T1-LB**: 0.54 and **T2-LB**: 1.08 mg L^{-1} for 96 h) and a short-term bath (infected fish submitted to **T3-SB**: 2.16 and **T4-SB**: 4.33 mg L^{-1} for 60 min once a day for 4 days) and a negative control (infected fish not exposed to nanocomposite). Each fish was considered a replicate totaling ten replicates per treatment (control and 4 treatments × 10 replicates = 50 fishes). This test lasted 96 h and the fish received no food or water exchange to avoid cross contamination (Fig. 1).

Infected fish subjected to a short-term bath were kept in a fishing net inside the tanks without nanocomposite. Afterwards, the net was removed from the original recipient and relocated in another tank containing the nanocomposite concentrations (according to the experimental design) and then exposure for 60 min. After the exposuring time, the fish returned to the original recipient and the process was repeated daily for 4 days. The maintenance of the fish inside an internal fishnet was intended to facilitate the handling of the application of the short-term bath (Fig. 1).

Mortality was recorded, and infected areas on the fish body were evaluated after 96 h with the aid of digital photos analyzed using the software *image tool*. The fish were evaluated before and after the test to determine the infected area (cm^2) for both sides on the body (left and right) (Fig. 2). The water quality parameters evaluated daily throughout the experimental time included temperature, dissolved oxygen (YSI® 55-12FT), pH (AKROM® KR20), and electric conductivity (YSI® 30-10FT). Toxic ammonia was evaluated before and after the test (Hanna® professional plus).

2.4. Nanocomposite prophylactic test against zoospores of Saprolegnia parasitica in juvenile Colossoma macropomum

This experiment was approved by the ethics committee for animal use from Tiradentes University (03031R/2018). In the prophylactic test, 63 fish (10–12 g) were used. Initially, 54 fish were submitted to the handling stress procedures (shaking before infection with pathogen according to Firouzbakhsh et al., 2012), and nine fish was not shaking or exposed to nanocomposite.

The fish were distributed into tanks (1 L) at stocking density of 3fish/recipient with air supply and the animals were not fed throughout the experimental time. All fish were exposed to zoospores of *Saprolegnia parasitica* at a concentration of 3×10^{5} L^{-1}.

Afterwards, they were immediately submitted to four different concentrations of nanocomposite (P0.1, P0.2, P0.4, and P0.8 mg L^{-1}) and three control groups, with three fish per replicate, in a completely randomized arrangement in triplicate (7 treatments × 3 fishes × 3 replicates). The control groups were: CS group (CS: Control Shaking) consisted of fish submitted to handling stress (shaking stress) procedure according to Firouzbakhsh et al. (2012) but not exposed to nanocomposite; CWS group (CWS: control without shaking or nanocomposite, **negative control**), the fish were introduced into recipients without handling stress procedure as previously mentioned; and CMB group where the fish in this group undergone to shaking stress and then exposed to methylene blue at concentration of 2 mg L^{-1} (Rahman et al., 2017). The methylene blue is an effective antifungal commonly used in the aquaculture to *Saprolegnia* control and for that was considered as a **positive control**.

The experiment lasted 72 h, daily the percentage of fish that demonstrated fungal infection (prevalence) and mortality were determined. The intensity of infection was measured through the images taken of the fish body and analyzed in computer with the aid of a software image tool (evaluating digital photos). Water quality parameters were also measured daily with the aid of a multiparameter *professional plus YSI* determining temperature (°C), dissolved oxygen (mg L^{-1}), pH, electric conductivity (μS cm^{-1}), and toxic ammonia (mg L^{-1}).

2.5. Hematological parameters analysis

The blood samples were taken by caudal puncture vessel using syringes containing EDTA 10% from dying fish (demonstrating erratic swimming, no reaction to stimuli, lethargic and with reduced opercular beating) and all other fish at the end of experiments, which underwent

Table 1
Mortality of infected tambaqui with *Saprolegnia parasitica* and exposure to different concentrations of nanocomposite applied as long-term or short-term baths.

Treatments	Mortality (% and number of dead fish)	Mortality time (hours)
C	100% or 10 dead fish	54.14
$T_{1\text{-LB}}$	20% or 2 dead fish	48
$T_{2\text{-LB}}$	20% or 2 dead fish	48
$T_{3\text{-SB}}$	0	–
$T_{4\text{-SB}}$	0	–

C: control group, long-term bath strategy T_1: 0.54 mg L^{-1} and T_2: 1.08 mg L^{-1}, short-term bath strategy T_3: 2.16 mg L^{-1} and T_4: 4.33 mg L^{-1}.

an anesthesia procedure (60 mg L^{-1}). Blood smears were stained using *Panotic Newprov Kit* for differential counting of leukocytes and total thrombocytes (Tavares-Dias and Moraes, 2004; Ranzani-Paiva et al., 2013). The total counting of erythrocytes (cell × 10^6 μL^{-1} according to Garcia-Navarro, 2005), percentage of hematocrit (Goldenfarb et al., 1971), hemoglobin concentration (g dL^{-1} using the biochemical analyzer LAB-TP6000 PLUS), glucose concentration (mg dL^{-1} using the Accu Chek Active), and total plasma protein (g dL^{-1} using RHC-200/AT) were also evaluated. Based on these data, hematimetric indexes were calculated according to Vallada (1999).

2.6. Statistical analysis

Data of the therapeutic test were submitted to *t*-test for paired samples by comparing before and after the infected areas on the fish body. The difference among treatments in infection areas were submitted to normality and homoscedasticity tests (Shapiro Wilk and Bartlett respectively) following Analysis of Variance (ANOVA) with post hoc Tukey test ($\alpha = 0.05$) for mean comparisons. Data of prophylactic test (prevalence, infected areas and mortality) and blood values were conducted to premises tests following ANOVA with post hoc Tukey test ($\alpha = 0.05$).

3. Results

3.1. Therapeutic assay

Different nanocomposite concentrations did not cause statistically changes in water quality parameters among the treatments throughout experimental time. The water quality parameters were temperature of 23.83 ± 0.41 °C, dissolved oxygen of 5.40 ± 1.33 mg L^{-1}, pH of 7.95 ± 0.26, electric conductivity of 170 ± 30 μS cm^{-1}, and toxic ammonia of 0.02 ± 0.01 mg L^{-1}.

In the therapeutic assay, fish infected with *Saprolegnia parasitica* in the control group showed lethargic, petechial hemorrhages and large areas of fungal cotton-like infection. The control group also suffered the highest mortality (100% or 10 dead fish), a significant difference compared to long-term bath treatments, which presented 20% (or 2 dead fish) of mortality. However, the fish that received short-term bath did not suffer any mortality by the end of the experiment (Table 1).

At the beginning of the experiment, all fish had similar infected area on their bodies (**C**: 3.41 ± 1.71, **T1-LB**: 1.63 ± 1.20, **T2-LB**: 2.19 ± 0.96, **T3-SB**: 2.60 ± 1.69, and **T4-SB**: 3.19 ± 2.16 cm^2, $p = 0.23$). An increase of infected area (230.5%) was observed in the control group. The nanocomposite treatments reduced the infected area by 50.30%, 52.05%, 56.15%, and 97.80% for T1-**LB**, T2-**LB**, T3-**SB**, and T4-**SB**, respectively (Fig. 3 and 4A, B, C, D e E).

At the end of the experimental time, the infected areas were not statistically different among the nanocomposite treatments, only the control group showed the largest infected areas compared to all treatments (Fig. 5).

Fish from the control group had lower glucose values than the nanocomposite treatments (T2-LB, T3-SB, and T4-SB), as well as reduced erythrocytes number, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and total plasmatic protein. For defense cells, the control group presented monocytosis and thrombocytopenia (Table 2) compared to nanocomposite treatments.

T1-LB and T2-LB presented no difference in glucose, total plasmatic protein, hematocrit, hemoglobin, erythrocytes, hematimetric indexes, total leukocytes, lymphocytes, monocytes, and thrombocytes, however, T1-LB had higher neutrophil value than T2-LB (Table 2).

The nanocomposite treatment, independent of strategy (long-term or short bath), achieved lower values of monocytes number (2.90 ± 1.97a; 2.28 ± 1.39a; 2.11 ± 0.77a and 4.29 ± 1.74a), but high values for thrombocytes number (39.90 ± 2.75a; 29.05 ± 2.95a; 31.35 ± 3.11a and 35.23 ± 10.72a) compared to the control group. The fish from T2-LB presented the lowest values for neutrophils (Table 2).

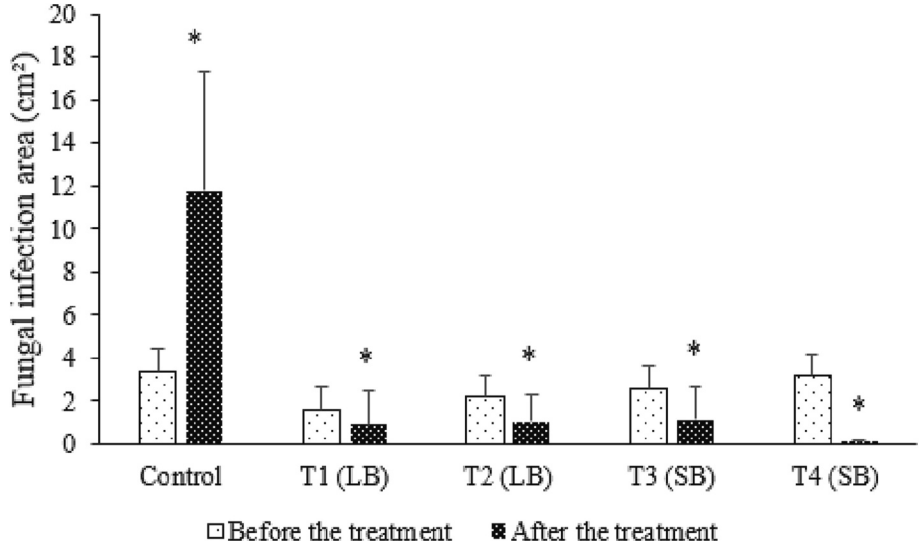


Fig. 3. Fungal infection area after nanocomposite treatments. *: Statistical difference before and after bath strategy to control the fungal infection using *t-test* for paired samples, **LB**: Long-term bath (96 h) **SB**: Short-term bath (one bath a day for 60 min, for four consecutive days). C: control group, long-term bath strategy T1-LB: 0.54 mg L^{-1} and T2-LB: 1.08 mg L^{-1}, short-term bath strategy T3-SB: 2.16 mg L^{-1} and T4-SB: 4.33 mg L^{-1}.

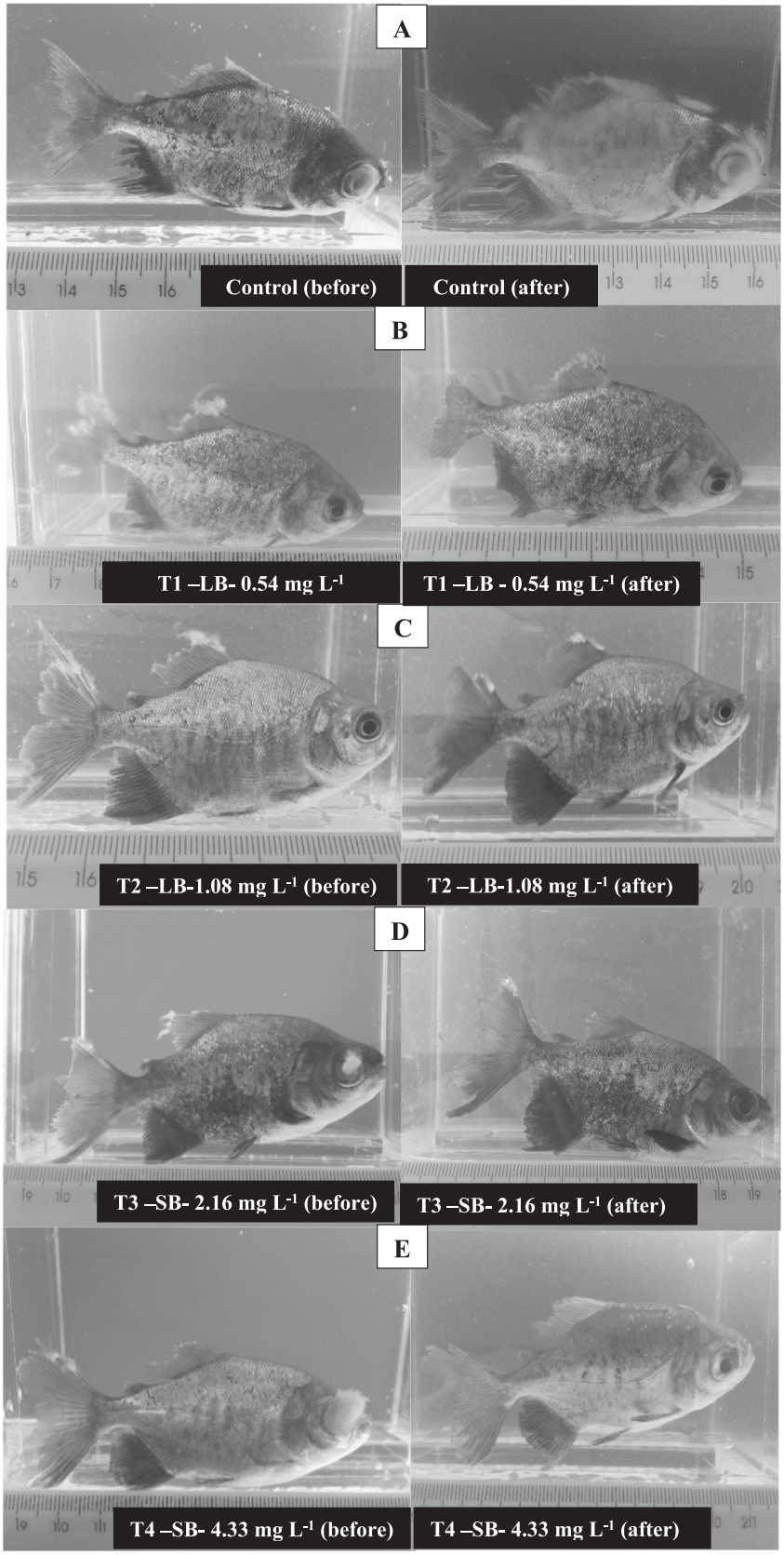


Fig. 4. Fish infected before and after the therapeutic test with nanocomposite. **LB**: Long-term bath (96 h) **SB**: Short-term bath (one bath a day for 60 min, for four consecutive days). C: control group, long-term bath strategy T1-LB: 0.54 mg L^{-1} and T2-LB: 1.08 mg L^{-1}, short-term bath strategy T3-SB: 2.16 mg L^{-1} and T4-SB: 4.33 mg L^{-1}.

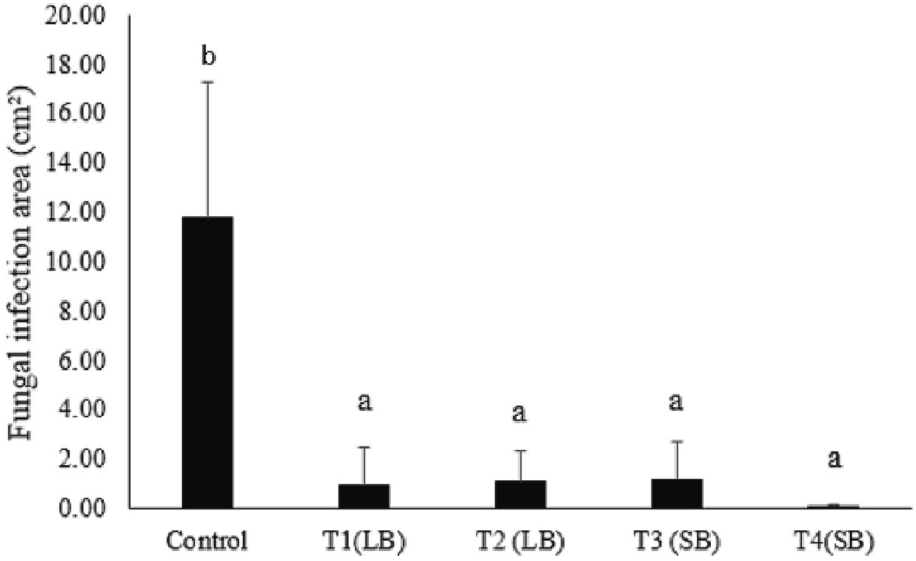


Fig. 5. Infected areas of short-term and long-term baths with nanocomposite concentrations in the final of experiment. **LB**: Long-term bath (96 h) **SB**: Short-term bath (one bath a day for 60 min, for four consecutive days). C: control group, long-term bath strategy T1-LB: 0.54 mg $L^{-1}$and T2-LB: 1.08 mg L^{-1}, short-term bath strategy T3-SB: 2.16 mg L^{-1} and T4-SB: 4.33 mg L^{-1}.

Table 2
Blood parameters of tambaqui *C. macropomum* submitted to nanocomposite therapeutic baths (long and short-term baths).

Parameters	C	T1-LB	T2-LB	T3-SB	T4-SB
Glu	21.67 ± 3.79b	41.00 ± 11.95ab	43.75 ± 8.46a	53.40 ± 7.20a	55.14 ± 9.0.8a
TPP	2.86 ± 0.40b	3.84 ± 0.54a	3.48 ± 0.41ab	3.53 ± 0.60ab	3.55 ± 0.49ab
Hg	2.25 ± 0.94b	8.40 ± 3.67a	6.51 ± 2.17a	7.67 ± 2.74a	7.26 ± 2.58a
Ht	11.10 ± 5.20b	25.24 ± 6.75a	28.79 ± 6.53a	25.37 ± 5.30a	25.39 ± 5.09a
Er	0.54 ± 0.15b	1.28 ± 0.19a	1.10 ± 0.08a	1.28 ± 0.14a	1.32 ± 0.32a
MCV	175.85 ± 48.97b	204.59 ± 23.73ab	292.44 ± 62.06a	214.39 ± 51.11ab	243.33 ± 69.00ab
MCHC	17.38 ± 6.34a	23.39 ± 5.29a	26.76 ± 9.69a	28.87 ± 10.48a	28.53 ± 14.03a
Leu	42.37 ± 9.32a	39.71 ± 6.00a	34.84 ± 4.30a	39.19 ± 5.28a	38.15 ± 11.47a
Lym	27.05 ± 5.12a	29.82 ± 2.60a	28.67 ± 2.80a	31.72 ± 4.42a	31.25 ± 8.52a
Mon	7.87 ± 2.23b	2.90 ± 1.97a	2.28 ± 1.39a	2.11 ± 0.77a	4.29 ± 1.74a
Neu	6.54 ± 1.55b	5.99 ± 1.59ab	3.71 ± 1.61a	4.45 ± 0.97ab	4.25 ± 1. 48ab
Thro	3.11 ± 0.60b	39.90 ± 2.75a	29.05 ± 2.95a	31.35 ± 3.11a	35.23 ± 10.72a

Glu: glucose mg dL^{-1}, TPP: total plasma protein g dL^{-1}, Hg: hemoglobin g dL^{-1}, Ht: hematocrit (%), Er: erythrocyte cell × 10^6 μL^{-1}, MCV: mean corpuscular volume (fL), and MCHC: mean corpuscular hemoglobin concentration (g dL^{-1}). Different letters in the row means statistical difference by Tukey test ($p < 0.05$), **LB**: Long-term bath (96 h) **SB**: Short-term bath (one bath a day for 60 min, for four consecutive days). C: control group, long-term bath strategy T1-LB: 0.54 mg L^{-1} and T2-LB: 1.08 mg L^{-1}, short-term bath strategy T3-SB: 2.16 mg L^{-1} and T4-SB: 4.33 mg L^{-1}.

3.2. Prophylactic test

In this test, no statistical difference occurred in the water parameters, discarding any possible interference of the nanocomposite or methylene blue among the treatments. The water quality parameters were temperature (23.47 ± 0.39 °C), dissolved oxygen (5.15 ± 0.57 mg L^{-1}), pH (7.52 ± 0.24), electric conductivity (230 ± 50 μS cm^{-1}), and toxic ammonia (0.01 ± 0.05 mg L^{-1}).

Fish from the CWS (without handling stress) and methylene blue treatment did not show saprolegniosis infection throughout the experimental time. However, fish in the CS group (submitted to handling stress) presented darkened skin, petechial hemorrhage (Figs. 6 and 7), lethargy, reduced opercular beating, higher fungal prevalence (Fig. 8A), and large infected area (Fig. 8B), provoking 100% mortality by 72 h (Fig. 8C).

A dose-response relationship was observed, where increases in nanocomposite concentration reduced the infection prevalence, pathology intensity and mortality (Fig. 8A, B and C). Similar results of prevalence, fungal infection area and mortality among the highest concentrations (P0.8 mg L^{-1}) and the groups CWS and methylene blue were found (Fig. 8).

The higher concentration (P0.8 mg L^{-1}) achieved better results of prevalence (Fig. 8A), infected area (Fig. 8B) and mortality (Fig. 8C) compared to CS in 72 h. In particular, the highest treatment (P0.8 mg L^{-1}) obtained 100% fish survival without infection signs, similar result to the methylene blue treatment (positive control) (Fig. 8C).

At the lowest concentration (P0.1 mg L^{-1}), greater infection occurred at 24 h compared to the control with shaking procedure (CS) (Fig. 8B). However, the fungal area on the fish body reduced over time, unlike the control (CS) in which fish suffered an increased in infection area (Fig. 8B). In the next concentration (P0.2 mg L^{-1}), in the first 24 h, an intermediate infection occurred, but it was reduced between 24 and 48 h (Fig. 8B).

The blood parameters, except glucose, showed changes in all nanocomposite treatments as well as groups CS and methylene blue. The CS group that presented higher values of fungal infection, displayed a reduction of erythrocytes, hematocrit, hemoglobin, total plasma protein, MCV, and MCHC (Table 3).

Increasing the nanocomposite concentration, the blood parameters remained similar to methylene blue (positive control) and CWS groups (negative control). Fish in the treatment P0.4 mg L^{-1} had the highest values of total plasma protein, hemoglobin, and hematocrit. At the lower concentrations (P0.1 and P.0.2 mg L^{-1}), the fish presented similar red blood cells count and biochemical values to the methylene blue group (intermediate values) (Table 3).

Among the nanocomposite treatments, the P0.8 mg L^{-1} stands out as the most effective prophylactic concentration and presented intermediate blood values compared to the CWS and methylene blue treatment

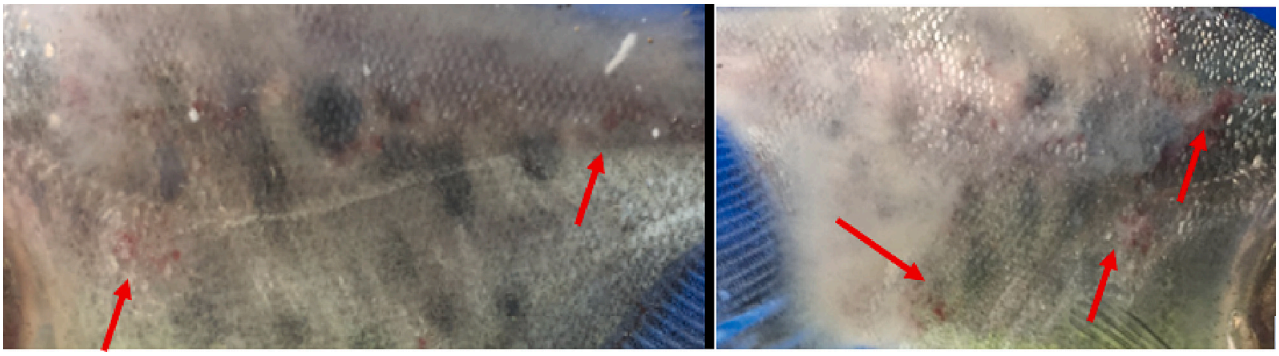


Fig. 6. Petechial hemorrhage on the skin of *C. macropomum* caused by Saprolegniosis infection in the control group with shaking procedure (CS).

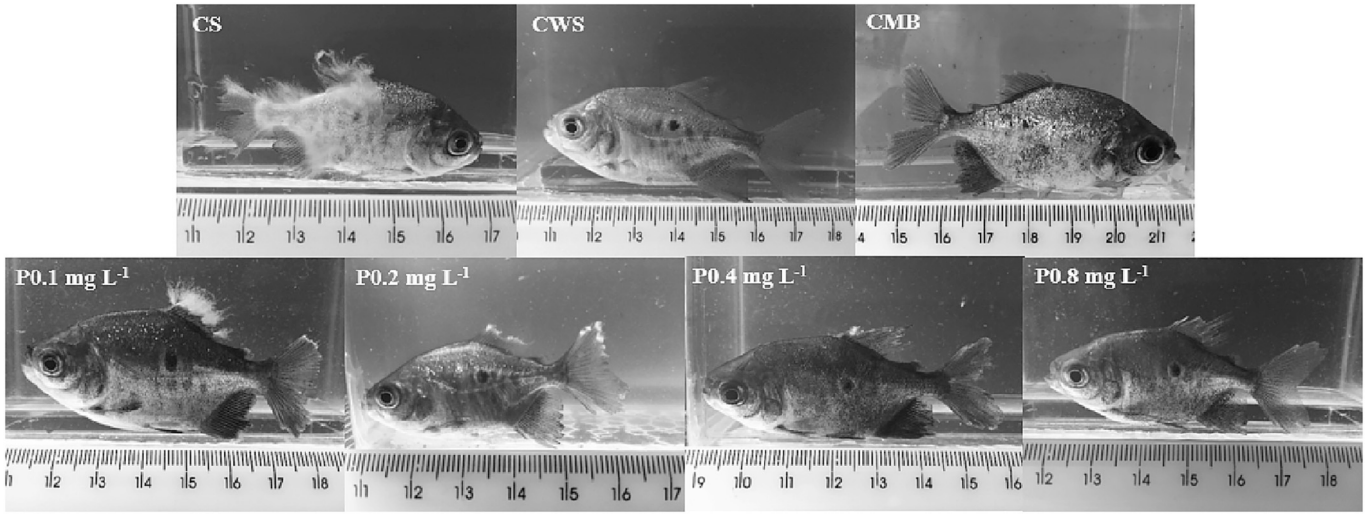


Fig. 7. Fungal infection area of tambaqui in the prophylactic test in different experimental groups. Note the intense mycelial growth in CS group.

(Table 3).

Comparing the CWS and methylene blue treatment, the fish exposed to methylene blue had higher values of leukocytes, lymphocytes, and total thrombocytes. Nanocomposite treatments (P0.4 and P0.8 mg L^{-1}) also altered leukocytes similar to the methylene blue treatment (Table 3).

There was uncontrolled monocytosis with methylene blue, unlike observed in the other treatments (Table 3). Neutrophilia was observed in the CWS and neutropenia in the lowest nanocomposite concentration (P0.2 mg L^{-1}). For thrombocytes, the highest values were observed in the methylene blue, P0.2 mg L^{-1}, and P0.4 mg L^{-1} treatments, while a reduction of this cell count occurred in the CWS group (Table 3).

4. Discussion

Infected fish in both assays demonstrated lethargic behavior, hemorrhage, and hemodilution, which are clinical signs also observed in other fish infected by *Saprolegnia parasitica* such as common carp *Cyprinus carpio* (Singh et al., 2012; Alwash et al., 2021), rainbow trout *Oncorhynchus mykiss* (Singh et al., 2012), and tenca *Tinca tinca* (Shah, 2010).

The hyphae of the oomycete penetrate the epithelial tissue and cause damage (within 3 to 5 days), provoking hemorrhage and osmotic imbalance, which result in hemodilution and consequently mortality (Shah, 2010). Similar physiological issues probably occurred in this study explaining the mortality of *C. macropomum*.

Reduced thrombocyte values in infected fish in both tests (Therapeutic and prophylactic) could indicate the presence of a hemorrhage process. Thrombocytes play an important role in cell defense (Tavares-Dias et al., 2008) and blood coagulation (Rahkonen and Pasternack, 1998).

Different fish can demonstrate different alterations in leukocytes when infected by *S parasitica*. In *C. carpio* (Salih and Mustafa, 2017), a monocytosis and neutrophilia as well as reduced lymphocytes number were observed. *Salmo trout* have similar blood alterations (increased neutrophil number and reduced lymphocytes number) after saprolegniosis (Jamalzadeh et al., 2009). For African catfish *C. gariepinus* (Chauhan et al., 2014), monocytes and neutrophil increased throughout oomycete infection. For tilapia *O. niloticus* infected with *Saprolegnia* sp., they also have reduced values of lymphocytes as result of infection (Elgendy et al., 2022).

All these leukocytes alterations allied to alterations in red blood parameters are common during stressing moments and have been cited in oomycete infection. The infected tambaqui from control group of therapeutic assays presented similar response to saprolegnia infection as observed in other fish species as increased glucose, reduction of erythrocytes, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, total plasmatic protein and thrombocytes (Barde et al., 2020). These factors can allow the rapid development of infection and clinical signs (cotton-like areas on the fish body) resulting in outbreaks (Phillips et al., 2008, Lone and Manohar, 2018).

To avoid this problem, the therapeutic strategy evaluated in the present study has demonstrated a promising reduction of fungal infection without blood issues and consequently reduction of mortality, with a 97.8% reduction of the infected area using the highest concentration in a short-term bath (T4-SB).

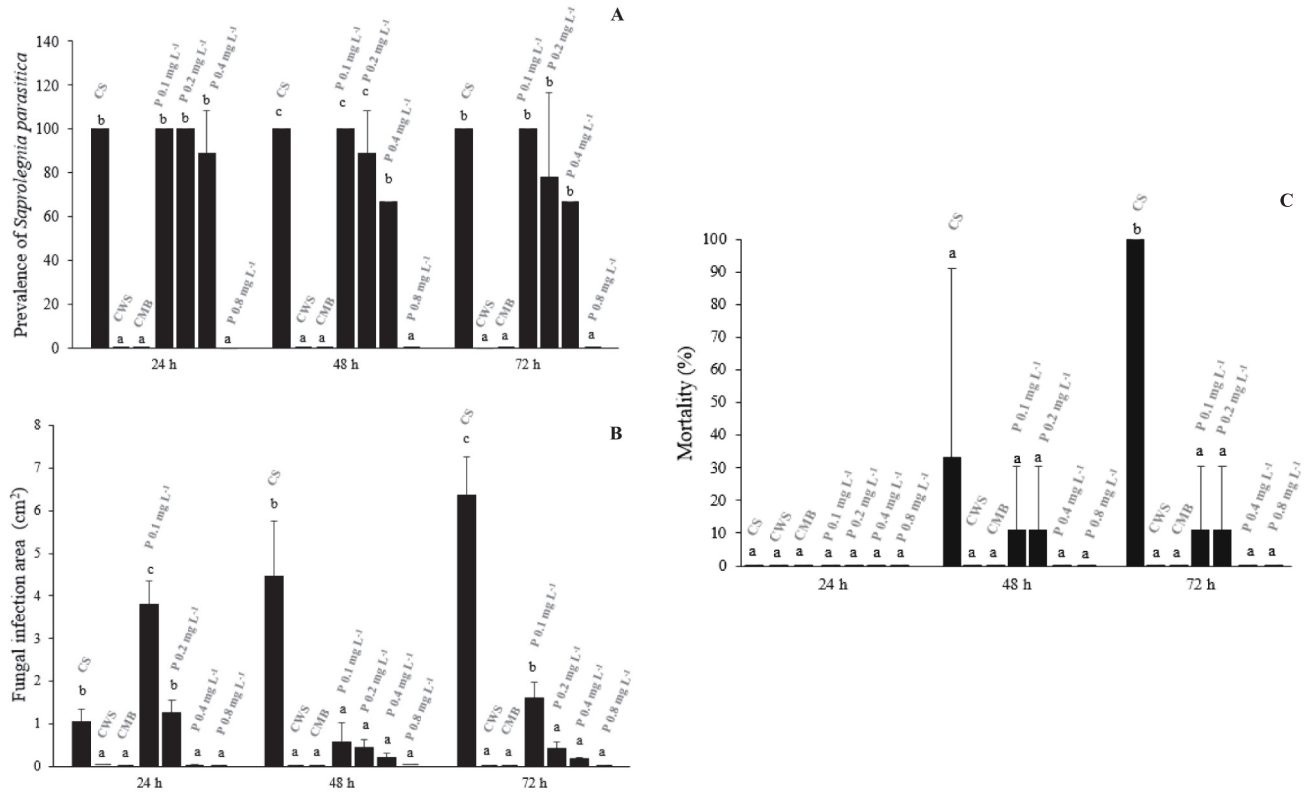


Fig. 8. Prevalence (A), fungal infected area (B) and mortality (C) of juvenile *C. macropomum* infected with *Saprolegnia parasitica* after nanocomposite treatments in a prophylactic test over time. Different letters in the columns (24, 48, and 72 h) indicate statistical difference by Tukey test ($p < 0.05$). CS: shaking control, CWS: negative control, CMB, methylene blue treatment (positive control), P0.1, P0.2, P0.4, and P0.8 mg L^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Tambaqui *C. macropomum* hematologic parameters after exposed to nanocomposite in the prophylactic assay.

Hematologic parameters	CS	CWS	MB	P0.1 mg L^{-1}	P0.2 mg L^{-1}	P0.4 mg L^{-1}	P0.8 mg L^{-1}
Glucose (mg dL^{-1})	40.83 ± 16.10**a**	39.00 ± 9.66**a**	52.75 ± 13.82**a**	55.50 ± 16.38**a**	51.00 ± 19.08**a**	48.33 ± 18.03**a**	48.50 ± 12.24**a**
Total plasma protein (g dL^{-1})	3.10 ± 0.72**b**	4.02 ± 0.39**a**	3.45 ± 0.35**ab**	3.81 ± 0.66**ab**	3.55 ± 0.22**ab**	4.03 ± 0.23**a**	3.76 ± 0.49**ab**
Hemoglobin (g dL^{-1})	3.34 ± 0.95**c**	3.65 ± 2.13**bc**	6.73 ± 0.95**ab**	5.86 ± 2.20**abc**	5.63 ± 0.95**abc**	7.29 ± 1.31**a**	4.46 ± 2.17**bc**
Hematocrit (%)	13.59 ± 3.43**b**	26.65 ± 4.00**a**	15.10 ± 5.13**b**	19.52 ± 5.75**ab**	19.92 ± 5.13**ab**	25.62 ± 3.42**a**	23.96 ± 6.52**ab**
Erythrocyte (x10^6 mL^{-1})	0.63 ± 0.19**bc**	0.60 ± 0.15**c**	1.14 ± 0.31**a**	0.54 ± 0.15**c**	0.66 ± 0.20**bc**	0.97 ± 0.27**ab**	0.81 ± 0.21**abc**
MCV (fL)	277.44 ± 61.66**b**	403.68 ± 118.05**b**	134.03 ± 31.42**a**	359.62 ± 56.58**b**	283.11 ± 62.11**b**	263.38 ± 25.91**b**	258.99 ± 100.75**ab**
MCHC (g dL^{-1})	27.68 ± 4.62**b**	28.75 ± 8.47**b**	53.03 ± 4.04**a**	30.44 ± 11.67**b**	29.51 ± 7.54**b**	28.26 ± 3.07**b**	26.15 ± 12.89**b**
Total Leukocytes (10^3 mL^{-1})	35.13 ± 5.62**ab**	34.13 ± 5.33**ab**	53.46 ± 11.07**a**	29.28 ± 7.80**b**	38.06 ± 8.75**ab**	42.88 ± 7.42**ab**	37.06 ± 8.78**ab**
Lymphocyte (10^3 mL^{-1})	23.97 ± 0.74**bc**	22.55 ± 2.72**c**	42.04 ± 9.88**a**	28.70 ± 1.65**bc**	23.44 ± 1.25**c**	35.20 ± 4.39**ab**	30.95 ± 6.53**abc**
Monocyte (10^3 mL^{-1})	1.24 ± 0.30**b**	1.49 ± 0.75**b**	6.02 ± 1.79**a**	0.57 ± 0.22**b**	1.71 ± 0.70**b**	1.53 ± 0.52**b**	1.44 ± 0.15**b**
Neutrophil (10^3 mL^{-1})	5.66 ± 0.65**ab**	7.07 ± 1.22**a**	4.34 ± 1.47**ab**	2.90 ± 1.05**b**	6.30 ± 2.01**ab**	6.87 ± 3.25**ab**	5.28 ± 1.14**ab**
Thrombocyte (10^3 mL^{-1})	3.91 ± 1.85**b**	4.90 ± 3.06**b**	14.78 ± 2.25**a**	12.22 ± 4.50**ab**	17.54 ± 4.72**a**	17.57 ± 5.55**a**	12.21 ± 0.60**ab**

MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration. Different letters in the row means statistical difference by Tukey test ($p < 0.05$). CS: shaking control, CWS: negative control, CMB, methylene blue treatment (positive control), P 0.1, P 0.2, P 0.4, and P 0.8 mg L^{-1}.

The chemical profile of nanocomposite (silver nanoparticles plus aqueous extract of *Terminalia catappa*) would explain this beneficial effect. *T. catappa* has tannins that inhibit the enzymes and proteins on the fungal membrane (Cowan, 1999) and phenolic compounds (gallic acid, ellagic acid, α and β punicalagin) which can cause damage on the fungal membrane affecting its metabolism (Hili et al., 1997; Meneses et al., 2021). The silver nanoparticle, in particular, can increase the permeability of fungal membrane affecting osmoregulation, losing the ability to replicate DNA (Feng et al., 2000; Yamanaka et al., 2005; Song et al., 2006).

In the present study, the nanocomposite used in long or short-term baths were efficient to control *Saprolegnia parasitica* showing survival above 90% compared to control group without nanocomposite. Other chemical products, such as potassium permanganate 100 mg L^{-1} and hydrogen peroxide 420 mg L^{-1}, demonstrated 70 and 63% of survival, respectively (Sherif and Abdel-Hakim, 2016). Another study using copper sulfate at the concentration of 1 mg L^{-1} (Sun et al., 2014) achieved a reduced infection rate as well as 56.67% survival. Despite the positive result in different fish species, these survival rates reported were lower compared to the present study, which achieve 90% survival with lower concentration of nanocomposite in long-term bath.

Furthermore, the short-term baths with 4.33 mg L^{-1} promoted a reduction of 97.8% of infected area. No more comparison were possible due to lack of scientific data on the reduction of infected areas in the fish body, making this the first report using this method of evaluating infection.

The therapeutic measure is focused on the control of the mycelia present in the tegument of the fish, however, the scientific literature

reports that different concentrations of chemical products can affect different stages of life of *S parasitica* (zoospore and mycelium). The infective phase (zoospore) is more sensitive than the mycelium, making it important to determine which strategy to control this pathogen is most appropriate (Xue-Gang et al., 2013; Sun et al., 2014). This difference was verified in prophylactic test of present study, where the concentration of 0.8 mg L^{-1} demonstrated greater effectiveness on zoospores, preventing the development of mycelia.

For the blood parameters in a prophylactic test, methylene blue caused physiological alterations such as increased young red cells in circulated blood (cells with reduced MCV). Methylene blue also promoted high values for monocytes, demonstrating an improved cell immune response. For nanocomposite concentrations, all blood parameters were similar to the methylene blue treatment indicating no toxic effect. In addition, the lower glucose values verify that the nanocomposite and methylene blue do not cause stress for tambaqui (Silbergeld, 1974).

Methylene blue has been widely used to control *Saprolegnia parasitica* providing a survival rate similar than our study. According to Rahman et al. (2017), methylene blue at concentration of 3 mg L^{-1} in short-term baths (four baths a day for 30 min each bath) provided survival of 94.33 ± 4.73% for *Labea bata* larvae infected with *S. parasitica*. Another study also using methylene blue at the lowest concentration (0.2 mg L^{-1}) provided 50% survival of infected Asian Catfish *Heteropneustes fossilis* (Neowajh et al., 2017). Despite the benefits of methylene blue, the concentration used in prophylactic assay is higher than nanocomposite concentrations to promote similar efficacy.

For these reasons, nanocomposite used as a therapeutic or prophylactic measure stands out as a promising alternative to control *S. parasitica*, being an alternative to methylene blue. However, some factors must be considered such as its lethal concentration, time of rearing, the concentration for therapeutic short-term baths, and the opportunistic behavior of *S. parasitica* after stress.

Thus, the nanocomposite used as the prophylactic procedure would be a better alternative than the therapeutic treatment considering the rearing time, complexity of aquatic environments and the use of lower concentrations.

The absence of infection and mortalities at the higher concentration in the prophylactic test corroborate this recommendation to prophylactic use. The prophylactic use of nanocomposite in fish farming to avoid any infection and to reduce concentrations of therapeutic products are important points to mitigate environmental impact of chemicals in water.

An effective product such as nanocomposite plays an important role to protect the fish at stressing moments, such as transporting and biometric procedures, which cause tissue damage allowing infection. For this reason, the use of nanocomposite at stressing moments could avoid the infection without blood alterations as well as reduce the use of other chemicals, which would be used at high concentrations.

5. Conclusion

The nanocomposite is recommended as a therapeutic treatment at concentration of 4.33 mg L^{-1} in short-term bath and as a prophylactic procedure using 0.8 mg L^{-1} without stress effects or blood changes. It can be used for common procedures during rearing, such as biometric or transport, since these activities can generate an opening for *S. parasitica* infection.

Funding source

This study has no specific funding to declare.

CRediT authorship contribution statement

Juliana Oliveira Meneses: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization. **Joel Artur Rodrigues Dias:** Methodology, Investigation. **Fernanda dos Santos Cunha:** Methodology, Investigation. **Hugo Leandro dos Santos:** Methodology, Investigation. **Thays Brito Reis Santos:** Methodology, Investigation. **Cindy Caroline Moura Santos:** Methodology, Investigation. **Ricardo Marques Nogueira Filho:** Methodology, Investigation. **Peterson Emmanuel Guimarães Paixão:** Methodology, Investigation. **Natalino da Costa Sousa:** Methodology, Investigation. **Márcia Valéria Silva do Couto:** Methodology, Investigation. **Higo Andrade Abe:** Methodology, Investigation. **Francisco José dos Santos:** Methodology, Investigation. **Silvia Patrícia Carraschi de Oliveira:** Methodology, Investigation. **Alexandre Nízio Maria:** Methodology, Investigation. **Juliana Cordeiro Cardoso:** Conceptualization, Methodology, Resources, Writing – review & editing. **Luiz Pereira da Costa:** Conceptualization, Methodology, Resources, Writing – review & editing. **Rodrigo Yudi Fujimoto:** Conceptualization, Methodology, Validation, Formal analysis, Data curation, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors report no conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgment

The authors would like to thank the National Council for Scientific and Technological Development for the financial support to Rodrigo Yudi Fujimoto (304533/2019-0) and Luiz Pereira da Costa (311002/2020-0), the Coordination for the Improvement of Higher Education Personnel of Brazil (CAPES) – Financing Code 001, the Center Integrated Fisheries Resources and Aquaculture of Itiúba-Al (Codevasf) for donating the fish to carry out the experiment, and BRS Aqua (BNDES/EMBRAPA/SAP/CNPQ). Luiz Pereira da Costa would also like to thank the Amazonas State Research Support Foundation (FAPEAM), for the POSGRAD financial assistance.

References

Ali, S.E., Garril, A.A., Skaar, I., Evensen, Ø., Charo-Karisa, H., 2019. Efficacy and safety of boric acid as a preventive treatment against *Saprolegnia* infection in Nile tilapia (*Oreochromis niloticus*). Sci. Rep. 9 (1), 1–9. https://doi.org/10.1038/s41598-019-54534-y.

Alwash, S.W., Alfaragi, J.K., Alsaadi, T.M., 2021. Assessment of the mint-copper nanoparticles treatment to control the infection of Saprolegniasis in *Cyprinus carpio* L. Ann. Roman. Soc. Cell Biol. 25 (4), 16010–16019.

Barde, R.D., Deshpande, M., Nagthane, N., Darak, O., Baig, M.M.V., 2020. A review of Saprolegnia infection in freshwater fishes and control of the saprolegniosis. Sustain. Humanosph. 16 (1), 702–711.

Beakes, G.W., Glockling, S.L., Sekimoto, S., 2012. The evolutionary phylogeny of the oomycete "fungi". Protoplasma. 249 (1), 3–19. https://doi.org/10.1007/s00709-011-0269-2.

Bly, J.E., Lawson, L.A., Abdel-aziz, E.S., Clem, L.W., 1994. Channel catfish, *Ictalurus punctatus*, immunity to *Saprolegnia* sp. J. Appl. Aquac. 3 (1–2), 35–50. https://doi.org/10.1300/J028v03n01_04.

Bruno, D.W., Van West, P., Beakes, G., 2011. *Saprolegnia* and other Oomycetes. In:. Woo P.T.K., BRUNO D.W. Fish Diseases and Disorder, Viral, Bacterial, and Fungal Infections (Eds.). Wallingford: CABI 1 (1), 669–720.

Chauhan, R., Faroq, A., Lone, S., Ganaie, S., 2014. Hematological and histological investigations on healthy and *Saprolegnia* sp. infected *Clarias gariepinus* (Burchell, 1822). Int. J. Exp. Pharmacol. 4 (2), 97–100.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12 (4), 56–64. https://doi.org/10.1128/CMR.12.4.564.

Elgendy, M.Y., Ali, S.E., Abdelsalam, M., El-Aziz, A., Tamer, H., Abo-Aziza, F., Abbas, W. T., 2022. Onion (*Allium cepa*) improves Nile tilapia (*Oreochromis niloticus*) resistance to saprolegniasis (*Saprolegnia parasitica*) and reduces immunosuppressive effects of cadmium. Aquac. Int. 254 (2022), 1–25. https://doi.org/10.1007/s10499-022-01035-x.

FAO, 2022. Global Aquaculture Production Quantity (1950–2020). accessed by. https://www.fao.org/fishery/statistics-query/en/aquaculture/aquaculture_quantity.

Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N., Kim, J.O., 2000. A mechanistic study of the antibacterial effect of silver ions on *E. coli* and *Staphylococcus aureus*. J. Biomed. Mater. Res. 52 (4), 662–668. https://doi.org/10.1002/1097-4636(20001215)52:4<662::AID-JBM10>3.0.CO;2-3.

Firouzbakhsh, F., Mehrabi, Z., Heydari, M., Khalesi, M.K., Tajick, M.A., 2012. Protective effects of a synbiotic against experimental *Saprolegnia parasitica* infection in rainbow trout (*Oncorhynchus mykiss*). Aquac. Res. 45 (4), 609–618. https://doi.org/10.1111/j.1365-2109.2012.03261.x.

Garcia-Navarro, C.E.K., 2005. Manual de Hematologia Veterinária, primeira ed. Varela, São Paulo.

Goldenfarb, P.B., Bowyer, F.P., Hall, E., Brosius, E., 1971. Reproductibility in the hematology laboratory: the microhematocrit determination. Am. J. Clin. Pathol. 56 (1), 35–39. https://doi.org/10.1093/ajcp/56.1.35.

Hatai, K., Hoshiai, G., 1992. Mass mortality in cultured coho salmon (*Oncorhynchus kisutch*) due to *Saprolegnia parasitica* Coker. J. Wildl. Dis. 284 (4), 532–536. https://doi.org/10.7589/0090-3558-28.4.532.

Hatai, K., Hoshiai, G.-I., 1993. Characteristics of two Saprolegnia species from coho salmon with saprolegniosis. J. Aquat. Anim. Health 5 (2), 115–118. https://doi.org/10.1577/1548-8667(1993)005<0115:COTSSI>2.3.CO;2.

Hili, P., Evans, C.S., Veness, R.G., 1997. Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. Lett. Appl. Microbiol. 24 (4), 269–275. https://doi.org/10.1046/j.1472-765X.1997.00073.x.

Jamalzadeh, H.R., Keyvan, A., Ghomi, M.R., Gherardi, F., 2009. Comparison of blood indices in healthy and fungal infected Caspian salmon (*Salmo trutta caspius*). Afr. J. Biotechnol. 8 (2), 319–322.

Johari, S.A., Kalbassi, M.R., Soltani, M., Yu, I.J., 2015. Study of fungicidal properties of colloidal silver nanoparticles (AgNPs) on trout egg pathogen, *Saprolegnia* sp. Int. J. Aquat. Biol. 3 (3), 191–198.

Khan, M.S., Jabeen, F., Qureshi, N.A., Asghar, M.S., Shakeel, M., Noureen, A., 2015. Toxicity of silver nanoparticles in fish: a critical review. J. Biodiv. Env. Sci. 6 (5), 211–227.

Lone, S.A., Manohar, S., 2018. *Saprolegnia parasitica*, a lethal oomycete pathogen: demands to be controlled. J. Mol. Biol. 6 (1), 36–44. https://doi.org/10.17582/journal.jimb/2018/6.2.36.44.

Magray, A.R., Lone, S.A., Ganai, B.A., Ahmad, F., Hafeez, S., 2021. The first detection and in vivo pathogenicity characterization of *Saprolegnia delica* from Kashmir Himalayas. Aquaculture. 542 (1), 736876 https://doi.org/10.1016/j.aquaculture.2021.736876.

Meneses, J.O., Cunha, F.S., Dias, J.A.R., Cunha, A.F.S., Santos, F.J., Sousa, N.C., Couto, M.V.S., Paixão, P.E.G., Abe, H.A., Lima, B.S., Neto, A.G.C., Araújo, A.A.S., Costa, L.P., Cardoso, J.C., Fujimoto, R.Y., 2020. Acute toxicity of hot aqueous extract from leaves of the *Terminalia catappa* in juvenile fish *Colossoma macropomum*. Aquac. Int. 28 (6), 2379–2396. https://doi.org/10.1007/s10499-020-00596-z.

Meneses, J.O., Silva, I.C.A., Cunha, A.F.S., Souza, J.C.N., Nascimento, V.R.S., Santos, C.C. M., Cunha, F.S., Paixão, P.E.G., Dias, J.A.R., Sousa, N.C., Couto, M.V.S., Abe, H.A., Filho, R.M.N., Santos, T.B.R., Santos, F.J., Costa, L.P., Cardoso, J.C., Fujimot, R.Y., 2021. Efficacy of Terminalia catappa-AgNP nanocomposite towards *Saprolegnia parasitica* infection in angelfish (*Pterophyllum scalare*) eggs. Aquaculture. 543 (1), 736–914. https://doi.org/10.1016/j.aquaculture.2021.736914.

Neowajh, M.S., Rashid, M.M., Al-Asif, A., Zafar, M.A., Hossain, A., 2017. Effects of chemotherapeutics against experimentally injured stinging catfish *Heteropneustes fossilis*. Asian J. Med. Biol. Res. 3 (4), 476–487. https://doi.org/10.3329/ajmbr.v3i4.35338.

Phillips, A.J., Anderson, V.L., Robertson, E.J., Secombes, C.J., Van West, P., 2008. New insights into animal pathogenic oomycetes. Trends Microbiol. 16 (1), 13–19. https://doi.org/10.1016/j.tim.2007.10.013.

Rahkonen, R., Pasternack, M., 1998. Effect of experimental *Diphyllobotrium dentriticum* infection on the blood leucocytes pattern of brown trout at two temperature levels. Boreal Environ. Res. Helsinki. 3 (1), 381–386.

Rahman, M.A., Rahman, M.H., Yeasmin, S.M., Asif, A.A., Mridha, D., 2017. Identification of causative agent for fungal infection and effect of disinfectants on hatching and survival rate of Bata (*Labeo. Bata*) larvae. Adv. Plants Agric. Res. 7 (1), 00264. https://doi.org/10.15406/apar.2017.07.00264.

Ranzani-Paiva, M.J.T.R., de Pádua, S.B., Tavares-Dias, M., Egami, M.I., 2013. Métodos para análise hematológica em peixes primeira ed. Editora da Universidade Estadual de Maringá, Paraná.

Salih, S.T., Mustafa, S.A., 2017. Efficiency of dietary synbiotic on hematological, histopathological changes and resistance against *Saprolegnia* spp. in common carp, *Cyprinus carpio* L. JEZS. 5 (6), 2092–2098.

Sarowar, M.N., Hossain, M.J., Nasrin, T., Naznin, T., Hossain, Z., Rahman, M.M., 2019. Molecular identification of oomycete species affecting aquaculture in Bangladesh. Aquac. Fish. 4 (3), 105–113. https://doi.org/10.1016/j.aaf.2018.12.003.

Shah, S.L., 2010. Impairment in the haematological parameters of tench (*Tinca tinca*) infected by *Saprolegnia* spp. Turk. J. Vet. Anim. Sci. 34 (4), 313–318. https://doi.org/10.3906/vet-0706-4.

Sherif, A.H., Abdel-Hakim, S., 2016. Treatment trails of Saprolegniosis in *Oreochromis Niloticus*. Alexandria J. Veterin. Sci. 49 (2), 99–105. https://doi.org/10.5455/ajvs.226020.

Silbergeld, E.K., 1974. Blood glucose: a sensitive indicator of environmental stress in fish. Bull. Environ. Contam. Toxicol. 11 (1), 20–25. https://doi.org/10.1007/BF01685023.

Singh, R., Gupta, M., Pandey, N.N., 2012. Hematological changes in common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*), infected with *Saprolegnia* spp. Israeli J. Aquac. Bamidgeh. 65 (1), 20–670.

Song, H.Y., Ko, K.K., Oh, L.H., Lee, B.T., 2006. Fabrication of silver nanoparticles and their antimicrobial mechanisms. Europ. Cells Mater. 11 (1), 58.

Sun, Q., Hu, K., Yang, X.L., 2014. The efficacy of copper sulfate in controlling infection of *Saprolegnia parasitica*. J. World Aquacult. Soc. 45 (2), 220–225. https://doi.org/10.1111/jwas.12113.

Tavares-Dias, M., Moraes, R.F., 2004. Hematologia de Peixes Teleósteos, primeira ed. Lillimpress Complexo Gráfico, Ribeirão Preto.

Tavares-Dias, M., Moraes, F.R., Martins, M.L., 2008. Hematological assessment in four Brazilian teleost fish with parasitic infections, collected in feefishing from Franca, São Paulo. Brazil. Boletim do Instituto de Pesca. 34 (2), 189–196.

Valenti, W.C., Barros, H.P., Moraes-Valenti, P., Bueno, G.W., Cavalli, R.O., 2021. Aquaculture in Brazil: past, present and future. Aquaculture Reports 19, 100611.

Vallada, E.P., 1999. Manual de técnicas hematológicas, primeira ed. Atheneu, São Paulo.

Van Den Berg, A.H., Mclaggan, D., Diéguez-Uribeondo, J., Van West, P., 2013. The impact of the water moulds *Saprolegnia diclina* and *Saprolegnia parasitica* on natural ecosystems and the aquaculture industry. Fung. Biol. Rev. 27 (2), 33–42. https://doi.org/10.1016/j.fbr.2013.05.001.

Van West, P., 2006. *Saprolegnia parasitica*, an oomycete pathogen with a fishy appetite: new challenges for an old problem. Mycologist. 20 (3), 99–104. https://doi.org/10.1016/j.mycol.2006.06.004.

Xue-Gang, H., Lei, L., Cheng, C., Kun, H., Xian-Le, H., Gao-Xue, W., 2013. In vitro screening of Chinese medicinal plants for antifungal activity against *Saprolegnia* sp. and *Achlya klebsiana*. N. Am. J. Aquac. 75 (4), 468–473. https://doi.org/10.1080/15222055.2013.808298.

Yamanaka, M., Hara, K., Kudo, J., 2005. Bactericidal action of a silver ion solution on Escherichia coli, studied by energy-filtering transmission electron microscopy and proteomic analysis. Appl. Environ. Microbiol. 71 (11), 7589–7593. https://doi.org/10.1128/AEM.71.11.7589-7593.2005.

Zaki, M.S., Fawazi, O.M., 2015. *Saprolegnia parasitica* in fish. Life Sci. J. 12 (2), 156–157.