Received: 24 March 2022 | Revised: 3 May 2022 | Accepted: 10 May 2022

DOI: 10.1111/are.15910

Aquaculture Research WILEY

ORIGINAL ARTICLE

Anthelmintic efficacy of *Copaifera reticulata* oleoresin in the control of monogeneans and haematological and histopathological effects on *Colossoma macropomum*

Dayna Filocreão Malheiros[1] | Marcela Nunes Videira[2] | Irlon Maciel Ferreira[3] | Marcos Tavares-Dias[1,4]

[1]Programa de Pós-Graduação em Biodiversidade Tropical (PPGBIO), Universidade Federal do Amapá (UNIFAP), Macapá, Brazil

[2]Universidade do Estado do Amapá (UEAP), Macapá, Brazil

[3]Laboratório de Biocatálise e Síntese Orgânica Aplicada, Universidade Federal do Amapá, Macapá, Brazil

[4]Embrapa Amapá, Macapá, Brazil

Correspondence
Marcos Tavares-Dias, Embrapa Amapá, Rodovia Josmar Pinto Chaves, km 5, 2600, 68903-419 - Macapá, AP, Brazil.
Email: marcos.tavares@embrapa.br

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 303013/2015-0

Abstract

This study investigated for the first time the anthelmintic efficacy of therapeutic baths with *Copaifera reticulata* oleoresin and nanoemulsion with this oleoresin against monogeneans of *Colossoma macropomum*, as well as the haematological and histological effects in this fish. In the therapeutic baths of three consecutives were used 100 mg/L of *C. reticulata* oleoresin or 250 mg/L of nanoemulsion containing *C. reticulata* oleoresin. Therapeutic baths with 100 mg/L of *C. reticulata* oleoresin had anthelmintic an efficacy of 48.5% against monogeneans (*Anacanthorus spathulatus*, *Notozothecium janauachensis* and *Mymarothecium boegeri*), while baths with nanoemulsion of *C. reticulata* oleoresin had not anthelmintic efficacy, which was discussed. Baths for 1 h with 100 mg/L of *C. reticulata* oleoresin or 2 h with 250 mg/L of *C. reticulata* nanoemulsion increased levels of plasma total protein and glucose, mean corpuscular volume (MCV) and neutrophils number in *C. macropomum* and decreased in the number of total leucocytes and lymphocytes. In the gills of fish exposed and controls occurred detachment of the epithelium, hyperplasia and hypertrophy, resulting in moderate fusion of the secondary lamellae. Therefore, therapeutic baths with *C. reticulata* oleoresin and nanoemulsion of *C. reticulata* oleoresin have a low toxicity to *C. macropomum*, as there were few changes to the blood parameters.

KEYWORDS
aquaculture, blood, freshwater fish, parasite, toxicity, treatment

1 | INTRODUCTION

In the last decades, global aquaculture industry has succeeded and continues to increase while achieving the critical goals of environmental, economic and societal sustainability. Aquaculture is, therefore, the fastest growing agricultural activity, but it needs technological innovations to achieve improvements in the health management of farmed fish and thus avoid parasitic outbreaks and economic losses, which are the main obstacles to the development of this activity on an industrial scale (Luz et al., 2021; Soler-Jiménez et al., 2017; Tavares-Dias, 2018). In this scenario, the nanotechnology can reduce the economic losses caused by mass mortality in fish farming, as it has emerged as a new science and technology platform aimed at the development and transformation of agrifood systems, producing more qualified and disease-free products (Luis et al., 2019; Malheiros et al., 2020; Pimentel-Acosta et al., 2019; Shah & Mraz, 2020).

Currently, there is a growing search for environmentally friendly antiparasitic treatments, such as the use of oils derived from medicinal plants (Costa et al., 2017; Gonzales et al., 2020; Ling et al., 2015; Luz et al., 2021; Morales-Serna et al., 2019; Soares et al., 2016; Tavares-Dias, 2018). However, essential oils and oleoresins may

have the desired efficacy compromised, due to low water solubility, which is the major disadvantages for the use in fish farms (Costa et al., 2017; Malheiros et al., 2020; Tavares-Dias, 2018). This can be solved with the use of nanotechnology because nanostructured tools can remove barriers that hinder the success of the fishing industry but its application is still recent (Khosravi-Katuli et al., 2017; Luis et al., 2019; Shah & Mraz, 2020).

Aquaculture industry can be revolutionized by nanotechnology with new tools for rapid disease detection and treatments. Recently, we demonstrated that nanoemulsions with oleoresin of *Copaifera reticulata* Duckei improved in vitro anthelmintic efficacy in the control of monogeneans *Anacanthorus spathulatus*, *Notozothecium janauachensis* and *Mymarothecium boegeri* of *Colossoma macropomum*, when compared to oleoresin without nanoformulation. In addition, tolerance assays with *C. reticulata* oleoresin and showed that *C. macropomum* tolerated only 100 mg/L, while 250 mg/L of the nanoemulsions of *C. reticulata* oleoresin has been tolerated during 2 h of exposure (Malheiros et al., 2020). Therefore, we showed that the loading of bioactive constituents such as oleoresin in a nanoemulsion system has been a significant method for increasing potential activity of medicinal plants, due to decreased toxicity, reduced volatility and increased stability of active components in parasites. However, nanoemulsions with oleoresin of *C. reticulata* and oleoresin without nanoformulation has been not used to evaluate in vivo anthelmintic efficacy in the control of monogeneans in fish.

Copaifera reticulata is large tree of the Leguminosae family found in the Amazon Rainforest, and it has a resin with a non-volatile portion consisting mainly of acid diterpenes and sesquiterpenes, including γ-macrocarpene, α-bergamotene, β-selinene and β-caryophyllene. These majority compounds of *C. reticulata* oleoresin have shown in vitro efficacy against monogeneas of *C. macropomum* (Malheiros et al., 2020) and therefore exists a growing interest in the use of oils derivate of medicinal plants and their bioactive compounds for controlling and treating fish infected by monogeneans (Luis et al., 2019; Malheiros et al., 2020; Morales-Serna et al., 2019; Tavares-Dias, 2018), including oleoresin in nanoemulsion. Thus, this study compared the anthelmintic efficacy of therapeutic baths with *C. reticulata* oleoresin and nanoemulsions prepared with this oleoresin in the control of monogeneans on the gills of *C. macropomum* and studied the effects of the treatments in haematology and histopathology of this fish exposed.

2 | MATERIALS AND METHODS

2.1 | Fish and acclimation

Fingerlings of *Colossoma macropomum* (±15 cm) were purchased of a commercial fish farm from Macapá (AP) and kept in the Aquaculture and Fisheries Laboratory of Embrapa, Macapá, Amapá state (Brazil). The fish were acclimatized in 500 L tanks with constant aeration and continuous water renewal (1.1 L/min), fed twice a day with a commercial ration containing 32% crude protein (Guabi®, Brazil). These fish naturally infested by monogeneans were used in all trials.

The following water quality parameters were monitored daily: temperature (29.7 ± 0.1°C), dissolved oxygen (5.5 ± 0.2 mg/L), pH (5.8 ± 0.2), ammonia (0.4 ± 0.2 mg/L), alkalinity (10.0 ± 0.001 mg/L) and hardness (10.0 ± 0 mg/L), with the aid of a multiparameter probe (YSI, USA). The tank was siphoned weekly to remove organic matter accumulated at the bottom.

2.2 | Therapeutic baths with oleorresina of *C. reticulata* and nanoemulsion with this oleoresin against monogeneans of *C. macropomum*

A total of 180 fingerlings of *C. macropomum* (59.9 ± 29.7 g and 17.6 ± 9.8 cm), naturally parasitized, were distributed in 100 L tanks and acclimatized during 7 days in a static water system and with constant aeration. The experimental design consisted of baths with 100 mg/L of *C. reticulata* oleoresin and with 250 mg/L of *C. reticulata* oleoresin nanoemulsion. Each treatment was performed with three replicates, containing 10 fish per replicate (30 fish per treatment). In the baths with *C. reticulata* oleoresin, two control groups were used: one with water from the culture tank and the other with water from the culture tank + DMSO. In the baths with *C. reticulata* nanoemulsion two control groups were used: one with water from the culture tank and the other with water from the culture tank + Tween 20. Baths with 100 mg/L of *C. reticulata* oleoresin were performed during 1 h per day, while baths with 250 mg/L of *C. reticulata* oleoresin nanoemulsion were performed during 2 h per day. All baths were performed on three consecutive days and the tanks were maintained with constant aeration and without water renewal. During the baths, the fish remained fasting, and every 15 min they were observed to record changes in behaviour such as erratic swimming, agitation, jumping, convulsion, accelerated opercular beating, tipping over, lethargy and death (Malheiros et al., 2020).

After the last therapeutic bath, to evaluate the efficacy of the treatments, the fish were euthanized by medullar section. Ten fish from each of the three replicates (30 fish per treatment) were necropsied and the gills were collected and fixed in 5% formalin for later quantification of monogeneans. Subsequently, the prevalence and mean abundance of monogeneans (Bush et al., 1997) and the efficacy of therapeutic baths (Wang et al., 2008) were determined.

2.3 | Blood parameters of *C. macropomum* exposed to oleoresin of *C. reticulata* and nanoemulsion with this oleoresin

After the therapeutic baths, five fish were collected from each of three replicates (15 and fish per treatment) for evaluation of blood parameters. Blood sample was collected by puncture of the caudal vessel using syringes containing EDTA (10%), and it was divided into two aliquots. The first aliquot of blood was used for the following

determinations: haematocrit (Hct) using the microhematocrit method, total erythrocyte count (RBC) using the Neubauer chamber and haemoglobin concentration [Hb] using the cyanomethemoglobin method. Hematimetric indices such as mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the values of Hct, RBC and [Hb]. Blood smears were made and panchromatically stained with a combination of May Grünwald-Giemsa-Wright for differential leucocyte counts in up to 200 cells of interest. These blood smears were also used to determine the number of total leucocytes and total thrombocytes (Ranzani-Paiva et al., 2013).

The second aliquot of blood was centrifuged to obtain plasma for the determination of glucose levels using the enzymatic-colorimetric glucose oxidase method and total proteins using the biuret method, using kits (Doles, GO, Brazil) and UV/Visible spectrophotometer readings.

2.4 | Histopathology of gills from *C. macropomum* exposed to oleoresin of *C. reticulata* and nanoemulsion with this oleoresin

At the end of the therapeutic baths with 100 mg/L of *C. reticulata* oleoresin and 250 mg/L of *C. reticulata* oleoresin nanoemulsion, three fish from each of the three replicates (nine fish per treatment) were collected and euthanized by medullar section, and the first branchial arch on both sides were removed and fixed in buffered formalin (10%). The gill arches were dehydrated in a series of ethanol solutions, diaphanized in xylene and later embedded in paraffin, following routine techniques. Histological sections were performed using a microtome (Easypath EP 31–20,093, Brazil). After confection of slides (in duplicates), they were stained with haematoxylin and eosin (HE) for morphological analysis of the gills (Behmer et al., 1994). The images were captured in a common optical microscope, coupled to a photographic camera (Leica DM 1000, EUA) and computer containing the image capture software (Leica Application Suite 1.6.0). The histopathological analyzes performed were semi-quantitative using the mean assessment values (MAV) (Schwaiger et al., 1997) and histopathological alteration index (IAH) (Poleksić & Mitrović-Tutundžić, 1994).

2.5 | Statistical analysis

All histopathological, parasitic and blood data were previously evaluated under normality and homoscedasticity using Shapiro–Wilk and Bartlett, respectively. As the data did not follow a normal distribution, the Kruskal–Wallis test was used, followed using the Dunn test, to compare medians at $p < 0.05$ (Zar, 2010).

3 | RESULTS

3.1 | Therapeutic baths with oleoresin of *C. reticulata* and nanoemulsion with this oleoresin against monogeneans of *C. macropomum*

During the therapeutic baths, there was no fish mortality in any of the treatments and the behaviour of the fish was similar to that described by Malheiros et al. (2020) during tolerance trials.

All *C. macropomum* used in therapeutic baths had their gills naturally parasitized by monogeneans (*A. spathulatus*, *M. boegeri* and *N. janauachensis*). There was a reduction in the parasite load in fish exposed to 100 mg/L of *C. reticulata* oleoresin, with an efficacy of 48.5%. In the therapeutic baths with 250 mg/L of nanoemulsion containing *C. reticulata*, it was not possible to observe the efficacy of the treatments (Table 1).

3.2 | Blood parameters of *C. macropomum* exposed to oleoresin of *C. reticulata* and nanoemulsion with this oleoresin

In fish exposed to 100 mg/L of *C. reticulata* oleoresin the plasma levels of glucose and total protein, and MCV increased ($p < 0.05$) when compared to controls with culture tank water and culture water +

TABLE 1 Prevalence (P), mean abundance (MA) and efficacy (E) of monogeneans in gills of *Colossoma macropomum* exposed to the oleoresin and nanoemulsion of *Copaifera reticulata*

Treatment	P (%)	MA	Minimum–maximum	*E* (%)
Oleoresin				
Water	96.7	7.4 ± 4.6^{a}	1–17	–
Water + DMSO	100	81.0 ± 34.9^{b}	11–138	–
100 mg/L	100	41.7 ± 30.0^{c}	11–129	48.5
Nanoemulsion				
Water	67.85	3.7 ± 5.0^{a}	1–17	–
Water + Tween 20	96.7	6.0 ± 7.0^{ab}	1–38	–
250 mg/L	96.7	7.6 ± 6.5^{b}	1–31	0

Note: Different letters in the same column indicate differences significant between treatments ($p < 0.05$).

DMSO. The number of total thrombocytes was higher ($p < 0.05$) in fish exposed to culture tank water + DMSO when compared to fish exposed only to culture tank water. The number of total leucocytes, lymphocytes and eosinophils decreased ($p < 0.05$) in fish exposed to 100 mg/L of *C. reticulata* oleoresin when compared to control fish exposed to culture water + DMSO. The number of monocytes and neutrophils increased ($p < 0.05$) in fish exposed to 100 mg/L of *C. reticulata* oleoresin and in those exposed to culture water + DMSO when compared to the control exposed only to culture tank water (Table 2).

In fish exposed to nanoemulsion containing 250 mg/L of *C. reticulata* oleoresin, plasma glucose, plasma total protein, MVC and neutrophils had increased values ($p < 0.05$) when compared to control group with tank water. The number of total thrombocytes decreased ($p < 0.05$) when compared to the control with water from the culture tank + Tween 20. The number of total leucocytes increased ($p < 0.05$) in fish exposed to water + Tween 20 and decreased ($p < 0.05$) in fish exposed to nanoemulsion with 250 mg/L of C. reticulata oleoresin (Table 2).

3.3 | Histopathology of gills from *C. macropomum* exposed to oleoresin of *C. reticulata* and nanoemulsion with this oleoresin

Histological analyzes revealed changes in the gills of all treatments, including the control groups. The main alterations found in the gills of exposed fish were detachment of the epithelium, hyperplasia and

TABLE 2 Blood parameters of *Colossoma macropomum* exposed to oleoresin and nanoemulsion of *Copaifera reticulata*

Oleoresin			
Parameters	**Water**	**Water + DMSO**	**100 mg/L**
Glucose (mg/dl)	86.8 ± 25.2^{a}	74.1 ± 17.8^{a}	154.8 ± 25.9^{b}
Total protein (g/dl)	2.9 ± 0.9a^{b}	2.8 ± 0.4^{a}	3.5 ± 0.9^{b}
Haematocrit (%)	23.6 ± 3.1^{a}	22.8 ± 2.2^{a}	23.6 ± 3.1^{a}
Haemoglobin (g/dl)	6.3 ± 0.78^{a}	6.2 ± 1.1^{a}	5.7 ± 1.2^{a}
Erythrocytes (×10^{6}/μl)	1.52 ± 0.23^{a}	1.77 ± 0.41^{a}	1.44 ± 0.46^{a}
MVC (fL)	157.3 ± 20.6^{a}	133.2 ± 23.0^{a}	204.2 ± 139.4^{b}
MCHC (g/dl)	27.0 ± 3.9^{a}	27.1 ± 4.2^{a}	24.5 ± 5.6^{a}
Thrombocytes (μl)	34.595 ± 17.482^{a}	46.665 ± 22.381^{b}	46.618 ± 12.888^{ab}
Leucocytes (μl)	31.953 ± 10.258^{ab}	36.378 ± 16.856^{a}	28.390 ± 8291^{b}
Lymphocytes (μl)	24.371 ± 7533^{a}	22.856 ± 9993^{a}	12.364 ± 4249^{b}
Monocytes (μl)	3727 ± 2373^{a}	7309 ± 4264^{b}	6924 ± 3207^{b}
Neutrophils (μl)	3364 ± 1883^{a}	5640 ± 4130^{b}	8810 ± 4020^{b}
Eosinophils (μl)	0^{a}	330 ± 512^{a}	152 ± 384^{b}
PAS-GL (μl)	491 ± 561^{a}	242 ± 280^{a}	141 ± 191^{a}
Nanoemulsion			
Parameters	**Water**	**Water + Tween 20**	**250 mg/L**
Glucose (mg/dl)	75.1 ± 22.6^{b}	99.2 ± 26.6^{a}	97.5 ± 23.0^{a}
Total protein (g/dl)	2.9 ± 0.5^{b}	3.6 ± 0.5^{a}	3.3 ± 1.3^{a}
Haematocrit (%)	25.2 ± 3.1^{ab}	26.6 ± 3.3^{a}	21.1 ± 5.6^{b}
Haemoglobin (g/dl)	8.4 ± 1.5^{ab}	8.9 ± 1.2^{a}	7.2 ± 2.1^{b}
Erythrocytes (×10^{6}/μl)	2.28 ± 0.56^{a}	2.53 ± 0.89^{a}	1.52 ± 0.53^{b}
MVC (fl)	116.0 ± 25.5^{a}	114.3 ± 29.3^{a}	146.8 ± 27.1^{b}
MCHC (g/dl)	33.3 ± 5.1^{a}	33.5 ± 4.0^{a}	34.1 ± 4.1^{a}
Thrombocytes (μl)	78.809 ± 26.213^{ab}	106.166 ± 47.164^{a}	65.526 ± 18.140^{b}
Leucocytes (μl)	46.026 ± 19.055^{a}	53.786 ± 31.369^{b}	32.638 ± 8410^{b}
Lymphocytes (μl)	34.342 ± 15.245^{a}	33.386 ± 22.259^{a}	12.849 ± 5883^{b}
Monocytes (μl)	6478 ± 3206^{a}	9656 ± 6337^{a}	9167 ± 3563^{a}
Neutrophils (μl)	4880 ± 3782^{a}	6577 ± 4908^{a}	10.484 ± 2514^{b}
Eosinophils (μl)	11 ± 43^{a}	349 ± 774^{a}	25 ± 95^{a}
PAS-GL (μl)	314 ± 280^{a}	232 ± 384^{a}	112 ± 136^{a}

Note: Data express mean ± deviation standard. Different letter, in the same line, indicate different by the Dunn test ($p < 0.05$).

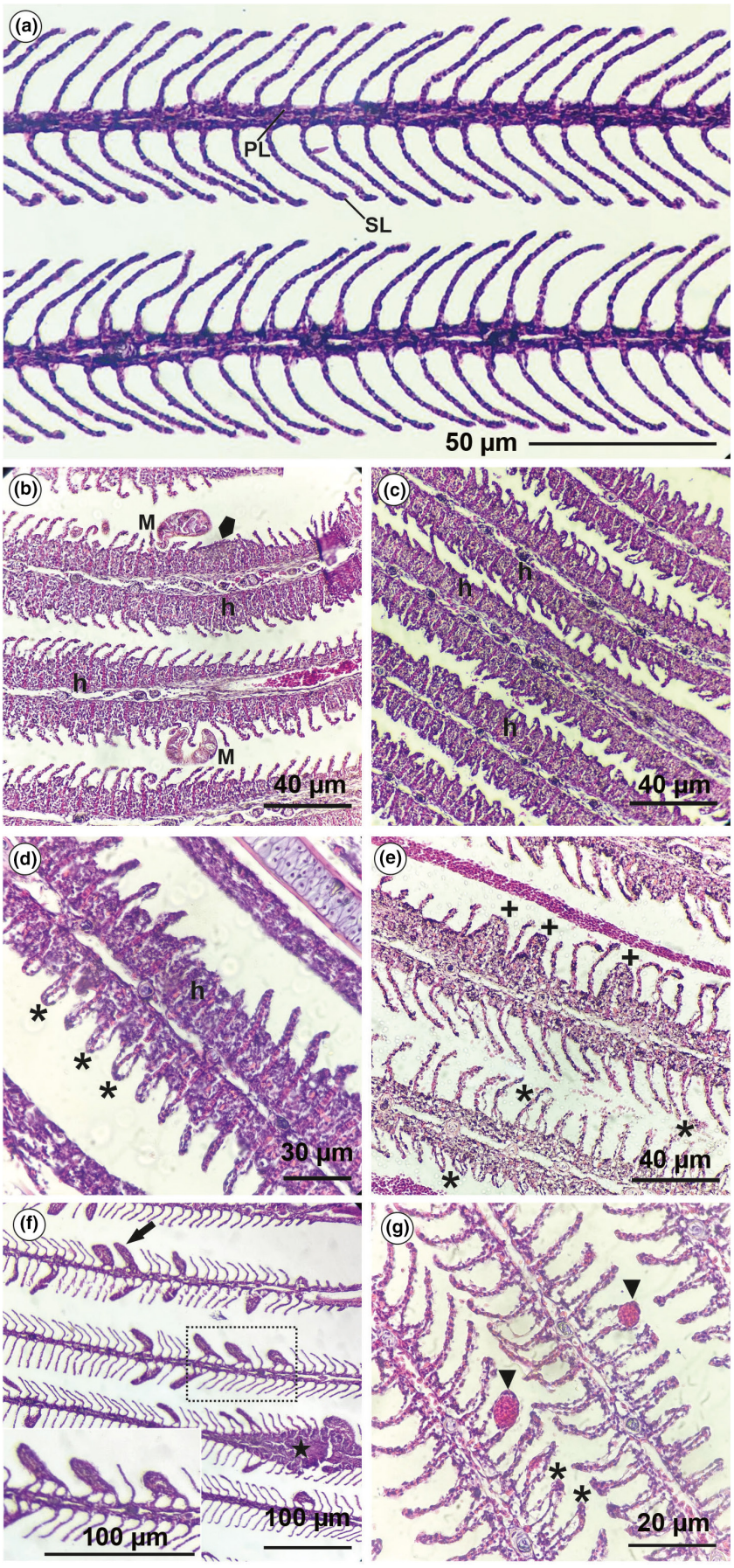

FIGURE 1 Histopathology of gills from *Colossoma macropomum* exposed to 100 mg/L of oleoresin of *Copaifera reticulata* and 250 mg/L of nanoemulsion with this oleoresin. (a) Gills of fish exposed to culture tank water (control) showing primary (PL) and secondary (SL) lamellae. (b) Gills filament with monogenean (M) and hyperplasia (h) in fish of control group exposed to DMSO. (c) Hyperplasia of gill epithelium (h) of fish exposed to DMSO. (d) Detachment of the lamellar epithelium (asterisk) in the gills of fish exposed to 100 mg/L of *Copaifera reticulata* oleoresin. (e) Hyperplasia with fusion of secondary lamellae (+) in the gills of fish exposed to tween 20 (control). (f) Lamellar hyperplasia with partial (dotted rectangle) and total (star) fusion in the gills of fish exposed to culture tank water (control). (g) Lamellar aneurysm (triangle) in the gills of fish exposed to 250 mg/L of nanoemulsion. Stained with haematoxylin and eosin (HE).

TABLE 3 Values of histopathological alteration index (HAI) and mean assessment values (MAV) for gills of *Colossoma macropomum* exposed to the oleoresin and nanoemulsion of the *Copaifera reticulata*

Treatments	N	MAV	HAI	Severity of the lesions according to the HAI
Oleoresin				
Water	9	8.7 ± 3.3^{ab}	3.4 ± 3.3^{a}	Normal functioning of the organ
Water + DMSO	9	12.2 ± 3.9^{b}	14 ± 8.0^{b}	Mild to moderate of the organ damage
100 mg/L	9	7.6 ± 2.2^{a}	8.9 ± 5.2^{ab}	Normal functioning of the organ
Nanoemulsion				
Water	9	7.3 ± 3.1^{a}	9.4 ± 4.3^{a}	Normal functioning of the organ
Water + Tween 20	9	7.0 ± 2.5^{a}	9.4 ± 4.6^{a}	Normal functioning of the organ
250 mg/L	9	8.1 ± 2.3^{a}	12.4 ± 5.3^{a}	Mild to moderate of the organ damage

Note: Data express mean ± deviation standard. Different letter, in the same column, indicate different by the Dunn test ($p < 0.05$).

hypertrophy, resulting in moderate fusion of the secondary lamellae (Figure 1). In fish exposed to 250 mg/L of nanoemulsion with *C. reticulata* oleoresin there were no differences ($p > 0.05$) in histopathological alteration index (HAI) and mean assessment values (MAV) when compared to controls, although lesions with moderate damage have been observed. The mean assessment values (MAV) were higher ($p < 0.05$) in fish exposed to culture tank water + DMSO when compared to fish exposed to 100 mg/L of *C. reticulata* oleoresin. The histopathological alteration index (HAI) of fish exposed to culture tank water + DMSO was higher ($p < 0.05$) than in fish exposed only to culture water (Table 3).

4 | DISCUSSION

Oleoresin of *Copaifera* species generally have a complex chemical composition, and they vary between species regarding the presence and abundance of each chemical compound. In general, oleoresins of *Copaifera* species are composed mostly by sesquiterpenes such as caryophyllene, copaene and humulene, and they interact with different target proteins on parasites (Arruda et al., 2019; Malheiros et al., 2020). The major compounds present in the oleoresin of *C. reticulata* of this study were the γ-macrocarpene (14.2%), α-bergamotene (13.6%), β-selinene (13.4%) and β-caryophyllene (11.7%), which were effectives in the in vitro activity against monogeneans of *C. macropomum* (Malheiros et al., 2020), as well as in vivo antiparasitic activity was here investigated.

With the growing demand by food fish in recent years, the aquaculture needs innovative biotechnological interventions to overcome the challenges in terms of development of suitable technologies for diseases management in culture systems and methods for controlling disease outbreaks in the fish farms. In the last decades, nanotechnology had diverse new developments in almost every field of science and technology, including the biology, because the nanotechnology presents a great opportunity to develop effective products for controlling pathogenic agents, for example the use in control of monogeneans of fish (Malheiros et al., 2020; Valentim, Duarte, Oliveira, Cruz, Carvalho, Conceição et al., 2018; Valentim, Duarte, Oliveira, Cruz, Carvalho, Solans et al., 2018). Therapeutic baths with 250 mg/L of nanoemulsion containing *C. reticulata* oleoresin were not effective against *C. macropomum* monogeneans, since there was a low parasite abundance in all treatments, thus compromising the expected efficacy. This unexpected problem was caused by the good quality of water in the culture tanks and the low stocking density of *C. macropomum*. This was the first study on the therapeutic baths with nanoemulsion for controlling and treating parasites of fish.

Therapeutic baths with 100 mg/L of *C. reticulata* oleoresin for three consecutive days had a low efficacy against monogeneans of *C. macropomum* gills (48.5%). However, results in vitro with *C. officinalis* oleoresin (Valentim, Duarte, Oliveira, Cruz, Carvalho, Solans et al., 2018) and *C. reticulata* oleoresin (Malheiros et al., 2020) demonstrated a high efficacy against monogeneans of *C. macropomum*. In vitro assays serve primarily for the initial recognition of the existence of antiparasitic properties of therapeutic substances that are being investigated (Tavares-Dias, 2018).

Baths for 1 h with 100 mg/L of *C. reticulata* oleoresin or 2 h with 250 mg/L of *C. reticulata* nanoemulsion increased levels of plasma total protein and glucose and MVC in *C. macropomum*. Gonzales et al. (2020) reported also an increase in plasma glucose levels in *C. macropomum* exposed to 60 mg/L of *Cymbopogon citratus* essential oil. In contrast, therapeutic baths of *C. macropomum* with 300 mg/L of *Alpinia zerumbet* essential oil decreased the levels of plasma glucose and total protein (Luz et al., 2021). This hyperglycaemia is indicative of stress in fish submitted to therapeutic baths with these phytotherapics. Total protein level depends on intracellular mechanisms and specific proteins that can be affected by stress fish (Luz et al., 2021). Furthermore, *C. macropomum* exposed to 100 mg/L of *C. reticulata* oleoresin or 250 mg/L of *C. reticulata* nanoemulsion showed a decrease in the total number of leucocytes and lymphocytes, with an increase in neutrophils number. These leucocyte

alterations may be related to stress and/or damages to the branchial epithelium caused by exposure to oleoresin from *C. reticulata* and nanoformulation with this oleoresin.

In the present study, exposure to 100 mg/L of *C. reticulata* oleoresin or 250 mg/L of *C. reticulata* nanoemulsion caused structural changes in the gills of *C. macropomum*. However, lesions capable of impairing gill function were observed only in fish from the control group with water from the culture tank + DMSO and 250 mg/L of *C. reticulata* nanoemulsion. Structural alterations in the gills of *C. macropomum* caused by both the toxicity of *Lippia alba* essential oil and the diluent used (ethylic alcohol) were also described by Soares et al. (2016). The main gill changes found in all treatments of this study with *C. macropomum* were detachment of the epithelium, aneurysm, hyperplasia and hypertrophy resulting in moderate fusion of the secondary lamellae. Winkaler et al. (2007) reported that gill alterations such as epithelial detachment and hyperplasia are adaptive strategies to increase the distance between the external environment and the blood and thus make contact with the stressor agent more difficult.

In conclusion, therapeutic baths with 100 mg/L of *C. reticulata* oleoresin for three consecutive days had low efficacy against monogeneans and few physiological changes in *C. macropomum*. However, an increase in this exposure time, to 6–7 days, may increase this efficacy. Since the treatment with oleoresin nanoemulsion of *C. reticulata* was inconclusive, further studies are need. Lastly, as the fish exposed to nanoemulsion with *C. reticulata* oleoresin showed lesions capable of compromising their gill function, these results indicate that there must be parsimony in the use of these nanostructured products in aquaculture. Furthermore, the results reinforce the need for a detailed evaluation of the possible factors responsible for the toxicity of these products, because it is important to have a safe therapeutic application.

AUTHOR CONTRIBUTIONS

Dayna Filocreão Malheiros, execution in vivo experiments and redaction of paper; Marcela Nunes Videira, histopathological analyses; Irlon Maciel Ferreira, preparation of nanoemulsions; Marcos Tavares-Dias, project financing, project coordination and redaction of paper.

ACKNOWLEDGEMENTS

M. Tavares Dias thanks the National Council for Scientific and Technological Development (CNPq) for the research fellowship (Grant No. 303013/2015-0). The authors would like to thank Kamukaia III project of Embrapa by the supply of copaiba oil used in this study.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

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

ETHICAL APPROVAL

This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA) and with authorization from the Ethics Committee in the Use of Animals of Embrapa Amapá (Protocol No. 013-CEUA/CPAFAP).

ORCID

Marcos Tavares-Dias https://orcid.org/0000-0002-8376-1846

REFERENCES

Arruda, C., Aldana-Mejía, J. A., Ribeiro, V. P., Borges, C. H. G., Martins, C. H. G., Veneziani, R. C. S., Ambrosio, S. R., & Bastos, J. K. (2019). Occurrence, chemical composition, biological activities and analytical methods on *Copaifera* genus. A review. *Biomedicine & Pharmacotherapy*, *109*, 1–20. https://doi.org/10.1016/j.biopha.2018.10.030

Behmer, A., Castro Tolosa, E. M., & Neto, A. G. F. (1994). *Manual de técnicas para histologia normal e patológica*. Faculdade de Medicina da Universidade de São Paulo.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al revisited. *Journal of Parasitology*, *83*(4), 575–583. https://doi.org/10.2307/3284227

Costa, J. C., Valladão, G. M. R., Pala, G., Gallani, S. U., Kotzent, S., Crotti, A. E. M., Fracarolli, L., Silva, J. M., & Pilarski, P. (2017). *Copaifera duckei* oleoresin as a novel alternative for treatment of monogenean infections in pacu *Piaractus mesopotamicus*. *Aquaculture*, *471*, 72–79. https://doi.org/10.1016/j.aquaculture.2016.11.041

Gonzales, A. P. P. F., Yoshioka, E. T. O., Mathews, P. D., Mertins, O., Chaves, F. C. M., Videira, M. N., & Tavares-Dias, M. (2020). Anthelminthic efficacy of *Cymbopogon citratus* essential oil (Poaceae) against monogenean parasites of *Colossoma macropomum* (Serrasalmidae), and blood and histopathological effects. *Aquaculture*, *528*, 735500. https://doi.org/10.1016/j.aquaculture.2020.735500

Khosravi-Katuli, K., Prato, E., Lofrano, G., Guida, M., Vale, G., & Libralato, G. (2017). Effects of nanoparticles in species of aquaculture interest. *Environmental Science and Pollution Research*, *24*, 17326–17346.

Ling, F., Jiang, C., Liu, G., Li, M., & Wang, G. (2015). Anthelmintic efficacy of cinnamaldehyde and cinnamic acid from cortex cinnamon essential oil against *Dactylogyrus intermedius*. *Parasitology*, *142*, 1744–1750. https://doi.org/10.1017/S0031182015001031

Luis, A. I. S., Campos, E. V. R., Oliveira, J. L., & Fraceto, L. F. (2019). Trends in aquaculture sciences: From now to use of nanotechnology for disease control. *Reviews in Aquaculture*, *11*, 119–132. https://doi.org/10.1111/raq.12229

Luz, J. G. R., Nogueira, J. N., Alves, C. M. G., Videira, M. N., Canuto, K. M., Castro, K. N. C., & Tavares-Dias, M. (2021). Essential oil of *Alpinia zerumbet* (Zingiberaceae) has anthelmintic efficacy against monogenean of *Colossoma macropomum* (Characiformes: Serrasalmidae). *Aquaculture Research*, *52*, 5340–5349. https://doi.org/10.1111/are.15404

Malheiros, D. F., Sarquis, I. R., Ferreira, I. M., Mathews, P. D., Mertis, O., & Tavares-Dias, M. (2020). Nanoemulsions with oleoresin of *Copaifera reticulata* (Leguminosae) improve anthelmintic efficacy in the control of monogenean parasites when compared to oleoresin without nanoformulation. *Journal of Fish Diseases*, *43*, 687–695. https://doi.org/10.1111/jfd.13168

Morales-Serna, F. N., Caña-Bozada, V. H., López-Moreno, D. G., Medina-Guerrero, R. M., Morales-Serna, J. A., & Fajer-Ávila, E. J. (2019). *In vitro* efficacy of two terpenes against ancyrocephalid monogeneans from Nile tilapia. *Journal of Parasitic Diseases*, *43*, 739–742. https://doi.org/10.1007/s12639-019-01150-2

Pimentel-Acosta, C. A., Morales-Serna, F. N., Chávez-Sánchez, M. C., Lara, H. H., Pestryakov, A., Bogdanchikova, N., & Fajer-Ávila, E. J. (2019). Efficacy of silver nanoparticles against the adults and eggs of monogenean parasites of fish. *Parasitology Research*, *118*, 1741–1749. https://doi.org/10.1007/s00436-019-06315-9

Poleksić, V., & Mitrović-Tutundžić, V. (1994). Fish gills as a monitor of sublethal and chronic effects of pollution. In R. Muller, & R. Lloyd (Eds.), *Sublethal and chronic effects of pollutants on freshwater fish* (pp. 339–352). Fishing News Books.

Ranzani-Paiva, M. J. T., Pádua, S. B., Tavares-Dias, M., & Egami, M. I. (2013). *Métodos para análises hematológicas em peixes* (1a ed.). Eduem.

Schwaiger, J., Wanke, R., Adam, S., Pawert, M., Honnen, W., & Triebskorn, R. (1997). The use of histopathological indicators to evaluate contaminant- related stress in fish. *Journal of Aquatic Ecosystem Stress and Recovery*, *6*, 75–86. https://doi.org/10.1023/A:1008212000208

Shah, B. R., & Mraz, J. (2020). Advances in nanotechnology for sustainable aquaculture and fisheries. *Reviews in Aquaculture*, *12*, 925–942. https://doi.org/10.1111/raq.12356

Soares, B. V., Neves, L. R., Oliveira, M. S. B., Chaves, F. C. M., Dias, M. K. R., Chagas, E. C., & Tavares-Dias, M. (2016). Antiparasitic activity of the essential oil of *Lippia alba* on ectoparasites of *Colossoma macropomum* (tambaqui) and its physiological and histopathological effects. *Aquaculture*, *452*, 107–114. https://doi.org/10.1016/j.aquaculture.2015.10.029

Soler-Jiménez, L. C., Paredes-Trujillo, A. I., & Vidal-Martínez, V. M. (2017). Helminth parasites of finfish commercial aquaculture in Latin America. *Journal of Helminthology*, *91*, 110–136. https://doi.org/10.1017/s0022149x16000833

Tavares-Dias, M. (2018). Current knowledge on use of essential oils as alternative treatment against fish parasites. *Aquatic Living Resources*, *31*, 1–11. https://doi.org/10.1051/alr/2018001

Valentim, D. S. S., Duarte, J. L., Oliveira, A. E. M. F. M., Cruz, R. A. S., Carvalho, J. C. T., Solans, C., Fernandes, C. P., & Tavares-Dias, M. (2018). Effects of a nanoemulsion with *Copaifera officinalis* oleoresin against monogenean parasites of *Colossoma macropomum*: A neotropical Serrasalmidae. *Journal of Fish Diseases*, *41*, 1041–1048. https://doi.org/10.1111/jfd.12793

Valentim, D. S. S., Duarte, J. L., Oliveira, A. E. M. F. M., Cruz, R. A. S., Carvalho, J. C. T., Conceição, E. C., Fernandes, C. P., & Tavares-Dias, M. (2018). Nanoemulsion from essential oil of *Pterodon emarginatus* (Fabaceae) shows in vitro efficacy against monogeneans of *Colossoma macropomum* (Pisces: Serrasalmidae). *Journal of Fish Diseases*, *41*, 443–449. https://doi.org/10.1111/jfd.12739

Wang, G., Zhou, Z., Cheng, C., Yao, J., & Yang, Z. (2008). Osthol and isopimpinellin from *fructus cnidii* for the control of *Dactylogyrus intermedius in Carassius auratus*. *Veterinary Parasitology*, *158*, 144–151. https://doi.org/10.1016/j.vetpar2008.07.034

Winkaler, E. U., Santos, T. R. M., Machado-Neto, J. G., & Martinez, C. B. R. (2007). Acute the lethal and sublethal effects of neem leaf extract on the neotropical freshwater fish *Prochilodus lineatus*. *Comparative Biochemistry and Physiology Parte C: Toxicology & Pharmacology*, *145*, 236–244. https://doi.org/10.1016/j.cbpc.20.06.12.009

Zar, J. H. (2010). *Biostatistical analysis* (5th ed.). Prentice-Hall.

How to cite this article: Malheiros, D. F., Videira, M. N., Ferreira, I. M., & Tavares-Dias, M. (2022). Anthelmintic efficacy of *Copaifera reticulata* oleoresin in the control of monogeneans and haematological and histopathological effects on *Colossoma macropomum*. *Aquaculture Research*, *53*, 4087–4094. https://doi.org/10.1111/are.15910