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Essential oils of *Ocimum gratissimum*, *Lippia grata* and *Lippia origanoides* are effective in the control of the acanthocephalan *Neoechinorhynchus buttnerae* in *Colossoma macropomum*

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ABSTRACT

This study investigated the *in vivo* efficacy of the essential oils (EOs) of *Lippia grata* (EOLG), *Lippia origanoides* (EOLO) and *Ocimum gratissimum* (EOOG) in the control of the acanthocephalan *Neoechinorhynchus buttnerae* in *Colossoma macropomum* (tambaqui). In addition, the parasitic and growth indices, and hematological, biochemical, enzymatic and histopathological parameters were evaluated in the tambaqui. After 30 days of feeding with diets containing 1.52 g EOLO kg^{-1}, the length, mean weight and mean weight gain decreased. There was 100% survival of fish fed with the EOs, and a decrease in the rates of parasitism in fish fed with diets containing 0.86 g EOLG kg^{-1}, 0.76 g EOLO kg^{-1}, 1.03 and 2.06 g EOOG kg^{-1}. For these concentrations, the anthelmintic efficacy of the EOLG was 62.1%, EOLO was 61.8% and EOOG was 58.7% and 59.8%, respectively. An increase in plasma levels of total protein and alkaline phosphatase was found in the fish fed with diets containing highest concentrations of EOLG and EOLO. Maintenance levels of alanine aminotransferase in plasma and aspartate aminotransferase, together with the higher frequency of mild to moderate damages in liver tissue and presence of focal point necrosis, suggest the influence of a high abundance of parasites on the biochemical and enzymatic processes of the host fish. Histomorphological and physiological indicators and a decrease in the rates of parasites with diets containing 0.86 g EOLG kg^{-1}, 0.76 g EOLO kg^{-1} and 1.03 and 2.06 g EOOG kg^{-1} indicate that this is a promising therapeutic alternative in the control of acanthocephalosis in tambaqui.

1. Introduction

In fish farming, the spread and establishment of parasitosis caused by helminth species is harmful to the health of the fish, and this situation can be aggravated as a result of the lack of use of good management practices and of lack of biosecurity management plans (Soler-Jiménez et al., 2017; Souza et al., 2019, 2022; Kyule-Muendo et al., 2022). Epizootic outbreaks caused by the acanthocephalans *Neoechinorhynchus buttnerae* have already been reported for *Colossoma macropomum* (tambaqui), which is the main species of native fish cultivated in northern Brazil and other regions of the Amazon (Chagas et al., 2019; Valladão et al., 2020; Valenti et al., 2021; Maciel-Honda et al., 2022). Obstruction of the intestinal lumen, impairment of the villi in the mucous layer, thickening and necrosis of the muscle layer and leukocyte infiltration, as

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well as reduction in the growth of fish, have been related as a result of high rates of infection by *N. buttnerae* in tambaqui (Jerônimo et al., 2017; Matos et al., 2017; Aguiar et al., 2018). These problems involving acanthocephalosis have led to economic losses in the production of tambaqui (Silva-Gomes et al., 2017) and to decreases the productivity.

The control of gastrointestinal helminths is a challenge in fish farming due to difficulty potential therapeutic agents have in accessing the lumen of the intestine, which is the most common location of endohelminths, such as *N. buttnerae* in tambaqui (Soler-Jiménez et al., 2017). Hence, the oral administration of EOs when added to fish feed and associated with procedures and techniques that ensure therapeutic efficacy has been an important alternative in the control of endoparasites in infected fish (Tavares-Dias, 2018; Costa et al., 2020; Oliveira et al., 2021). Some studies have shown the EOs of *Lippia grata* (EOLG), *Lippia origanoides* (EOLO) and *Ocimum gratissimum* (EOOG) to be promising against helminths monogeneans found in tambaqui gills (Boijink et al., 2016; Soares et al., 2017; Barriga et al., 2020). However, so far, few studies have addressed the control of *N. buttnerae* using EOs in diet of farmed fish.

For the validation of the therapeutic efficacy of EOs and their bioactive compounds, the potential effect on the growth performance and general condition of fish health should be evaluated. This can be done by analyzing the growth, physiological and histopathological responses (Costa et al., 2020; Almeida et al., 2021; Monteiro et al., 2021). Enzymatic studies are also essential for a better understanding of the physiology of the digestion and metabolism of nutrients, and enable more precise adjustments in the elaboration of diets for farmed fish (Panserat et al., 2019). In addition, tissue alterations in the liver are considered important indicators for understanding the nutritional status of fish fed with feed that is supplemented with EOs, as well as the effects of these as food additives (Karataş et al., 2020; Acar et al., 2021; Chung et al., 2021).

The aim of this study was to investigate the anthelmintic efficacy of EOLG, EOLO and EOOG in the diet of tambaqui for the control of *N. buttnerae*, as well as evaluate parasitic infection indices and growth, physiological and histopathological parameters for this host.

2. Materials and methods

2.1. Animals

Juvenile tambaqui ($n = 252$, 173.5 ± 4.5 g) that were naturally infected by *N. buttnerae* were acquired from a commercial fish farm in the city of Manaus, state of Amazonas (Brazil). Fish were acclimated in the fish farming sector of Embrapa Amazônia Occidental, in Manaus, for two weeks in 1000 L tanks with a water heating system and constant aeration. During this period, the fish were fed twice a day with commercial feed containing 32% crude protein until apparent satiety.

This study was developed after the approval of its protocol by the Ethics Commitee for the Use of Animals (CEUA) at Embrapa Amazônia Occidental (protocol N^{o} 03/2018).

2.2. Preparation of experimental diets

For the preparation of 1 kg of experimental feed with EOs, a solution was prepared with 300 mL of 70% commercial ethyl alcohol, 7.5 g of ethyl cellulose polymer and the respective concentrations of EOLG, EOLO and EOOG, which was based on results of *in vitro* assays carried out by Oliveira et al. (2021). For the control diets, only 70% ethyl alcohol and ethylcellulose were added. After the addition of each solution, which was sprinkled on the feed pellets while under constant stirring for uniform distribution of the product, drying was carried out with the aid of a diffuser and the pellets were then stored in plastic bags in a dark environment and under refrigeration. One day before feeding, the required amount of each feed, estimated according to the biomass of the fish in each experimental unit, was weighed and stored (4 °C) until the moment of supply.

The EOLG, EOLO and EOOG, incorporated into the diets, were previously obtained by hydrodistillation process in a Clevenger apparatus and their chemical composition was determined by gas chromatography and mass spectrometry. All procedures that involved the collection of plants were approved by the Ministry of the Environment (MMA) and the Genetic Heritage Management Council (CGEN) (process N^{o} A643761 and AB1F0FA).

2.3. Experimental design

After the period of acclimation, the fish were distributed in 1000 L tanks, in an entirely randomized experimental design with seven treatments, one control group without the addition of EO (0 g kg^{-1}) and the rest with two concentrations of each EO evaluated: EOLG (0.86 g kg^{-1} and 1.72 g kg^{-1}), EOLO (0.76 g kg^{-1} and 1.52 g kg^{-1}) and EOOG (1.03 g kg^{-1} and 2.06 g kg^{-1}) (Oliveira et al., 2021), with three replicates with 12 fish each (36 fish per treatment). Feeding was provided twice a day, in the amount estimated according to the biomass of the fish (3%) in each experimental unit, for 30 days.

During the experiments, water quality parameters were monitored and did not differ between treatments: dissolved oxygen (5.2 ± 0.04 mg L^{-1}) and temperature (28.1 ± 0.04 °C), measured with a digital oximeter (YSI Pro20, YSI Inc., USA), pH (5.8 ± 0.23) using digital pH meter (YSI F-1100, YSI Inc., USA), alkalinity (4.9 ± 0.3 mg L^{-1}) and hardness (8.6 ± 0.5 mg L^{-1}) using titrimetry, and total ammonia (0.7 ± 0.03 mg L^{-1}) *via* the indophenol method, according to APHA (American Public Health Association) (1998).

2.4. Growth performance parameters of tambaqui

With the weight and length data obtained from biometrics of the fish at the beginning and at the end of 30 days of fish feeding, the following zootechnical performance indices were calculated: weight gain (final weight-initial weight), specific growth rate (SGR: 100 × [(ln final weight-ln initial weight)/t], feed conversion rate (FCR: (final weight – initial weight)/initial weight), in addition to the survival percentage rate and relative condition factor (Kn).

2.5. Determination of blood parameters of tambaqui

After 30 days of feeding with the experimental diets, six fish of each one of the three replicates (18 fish per treatment) of the control group without the addition of EO (0 g kg^{-1}) and of the experimental groups with EOLG (0.86 g kg^{-1} and 1.72 g kg^{-1}), EOLO (0.76 g kg^{-1} and 1.52 g kg^{-1}) and EOOG (1.03 g kg^{-1} and 2.06 g kg^{-1}) were anesthetized with benzocaine (100 mg L^{-1}) for blood sampling by puncture of the caudal vessel using syringes containing EDTA (10%), and the samples were divided into two aliquots. One blood aliquot was used to obtain the plasma after centrifugation (75 G) and then used for the determination of plasma glucose by the glucose oxidase method and absorbance reading at 520 nm; total protein using the colorimetric method of biuret reaction and absorbance reading at 545 nm; alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in kinetic mode with absorbance reading at 340 nm and 405 nm, respectively, and alkaline phosphatase (ALP) using the colorimetric method and absorbance reading at 590 nm. In all analyses, commercial kits (LabTest®, GO, Brazil) were used and all absorbance readings were performed on a spectrophotometer (Genesys 10s UV–Vis, Thermo Scientific, USA).

The second blood aliquot was used to determine the total erythrocyte number; hematocrit (Hct) using the method of microhematocrit with centrifugation at 80 G for 10 min in a microhematocrit centrifuge (207 N, Fanem, Brazil) and concentration of hemoglobin (Hb), which was determined according to the cyanmethemoglobin method. These data were used for determining the hematimetric indices such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) (Ranzani-Paiva et al., 2013).

2.6. Parasitological indices of tambaqui

An initial survey to confirm the presence of *N. buttnerae* in the intestine of tambaqui ($n = 20$) resulted in a mean intensity of 772.9 ± 147.5 parasites per fish, thus proving the high rate of infection.

To evaluate the effectiveness of control group (0 g kg^{-1}), EOLG (0.86 g kg^{-1} and 1.72 g kg^{-1}), EOLO (0.76 g kg^{-1} and 1.52 g kg^{-1}) and EOOG (1.03 g kg^{-1} and 2.06 g kg^{-1}), after 30 days of feeding, ten fish of each one of three replicates (30 fish per treatment) were subjected to deep anesthesia with benzocaine (250 mg L^{-1}), followed by euthanasia by medullar section (Conselho Nacional de Controle de Experimentação Animal (CONCEA), 2018) for necropsy and the removal of the intestines, which were fixed in formalin (5%). Subsequently, the acanthocephalans were quantified under a stereomicroscope (Leica EZ4, Brazil). With these results, the parasitic indices, such as prevalence, mean intensity and mean abundance, were calculated according to Bush et al. (1997). The effectiveness of the treatments with the EOs was calculated according to the following formula: $E = ANPC\text{-}ANPT \times 100/NMPC$ (E: efficacy, ANPC: average number of parasites in the control group, ANPT: average number of parasites in the treatment groups) (Dotta et al., 2015).

2.7. Histopathological analysis of tambaqui liver

Liver fragments of four fish of each one of the three replicates (12 fish per treatment) of the control (0 g kg^{-1}), EOLG (0.86 g kg^{-1} and 1.72 g kg^{-1}), EOLO (0.76 g kg^{-1} and 1.52 g kg^{-1}) and EOOG (1.03 g kg^{-1} and 2.06 g kg^{-1}) treatments were collected and fixed in buffered 5% formalin, where they remained for 24 h. Afterwards, the samples were submitted to the following stages of histological processing: dehydration in increasing series of alcohol, diaphanization in xylol, and impregnation in liquid paraffin at 60 °C. Subsequently, the samples were embedded in solidified paraffin blocks to obtain histological sections of 7 μm using a microtome (2 sections per fish; 36 and 246 in total). Histological damage was semi-quantitatively evaluated using the histopathological alteration index (HAI) and the mean alteration value (MAV), which were adapted from the methodologies described by Schwaiger et al. (1997) and Poleksić and Mitrović-Tutundžić (1994).

2.8. Statistical analysis

The data are presented as mean ± standard deviation. All data were previously evaluated for normality and homoscedasticity using the Shapiro–Wilk and Bartlett tests, respectively. The mean values of the fish's zootechnical performance, and their hematological, biochemical, enzymatic and histopathological parameters were evaluated using ANOVA (one-way), followed by the Tukey test, or Kruskal-Wallis followed by the Dunn test, when the normality of the data was not observed. The difference in the mean values of parasitic intensity after the treatments in relation to the control was verified using the Kruskal-Wallis test followed by the Dunn test. Differences were considered significant when $p < 0.05$ (Zar, 2010).

3. Results

3.1. Parasitological indices of tambaqui

The parasitological analysis revealed that the prevalence was of 100% in all treatments after 30 days of feeding. Significantly lower values ($p < 0.05$) of mean intensity for *N. buttnerae* in the intestine of fish were found in the treatments with the lower concentration of EOLG and EOLO, as well as in two concentrations of EOOG, when compared to the control group. The treatments with 0.76 g EOLO kg^{-1} and 2.06 g EOOG kg^{-1} presented a lower range of variation in the values of infection intensity. Regarding the anthelmintic effect of the EOs against *N. buttnerae*, the efficacy ranged of 43.8 to 62.1%, though there was no significant difference ($p > 0.05$) among the treatments with EOLG, EOLO and EOOG. However, the higher efficacy against *N. buttnerae* was observed in the diets supplemented with 0.86 g EOLG kg^{-1} (Table 1).

3.2. Zootechnical performance parameters of tambaqui

Regarding the parameters of the zootechnical performance of the fish, after 30 days of feeding, the mean values of standard length, final weight and weight gain were significantly lower ($p < 0.05$) in fish fed with diets containing 1.52 g EOLO kg^{-1}, when compared to the control group. The fish condition factor, specific growth rate (SGR) and feed conversion rate (FCR) in all the treatments with EOs varied; however there was no difference ($p > 0.05$) among treatments. The fish condition factor ranged from 0.99 to 1.03, and fish survival was of 100% in all treatments (Table 2).

3.3. Hematological and biochemical parameters of tambaqui

After 30 days of feeding with diets containing the three EOs, no significant differences were observed ($p > 0.05$) for the Hct and hemoglobin concentrations. For Hct, the variation averaged from 31.8% to 35.1% and, for Hb, from 8.7 and 9.8 g dL^{-1}. For RBCs, significantly lower values ($p < 0.05$) were observed in fish fed with the diets containing 1.03 g EOOG kg^{-1} when compared to treatment with 1.52 g EOLO kg^{-1}, and this reduction was negatively correlated with the values of MCV ($r = -0.825$; $p < 0.0001$). Regarding the values of the corpuscular constants MCV and MCHC, no significant statistical difference was observed ($p > 0.05$) among the treatments with the three EOs (Table 3).

Regarding biochemical parameters, plasma glucose levels showed no difference ($p > 0.05$) among the treatments with the EOs and the control group. Comparing the treatments with the EOs from the same plant, it was noted that only for EOLO were the glucose levels significantly higher ($p < 0.05$) in the treatment with the highest concentration of EO added to the feed (Fig. 1A). Plasma protein levels were significantly higher ($p < 0.05$) in the treatment with 1.52 g EOLO kg^{-1}, when compared to the control and the treatment with the EO species of this same plant at the lowest concentration and the treatment with EOOG in both the evaluated concentrations (Fig. 1B). Plasma albumin and triglycerides levels were significantly higher ($p < 0.05$) in fish fed with the highest concentrations of EOLG and EOLO, when compared to the

Table 1
Parasitological indices of *Neoechinorhynchus buttnerae* in *Colossoma macropomum*, after 30 days of feeding with diets containing essential oils from *Lippia grata* (EOLG), *Lippia origanoides* (EOLO) and *Ocimum gratissimum* (EOOG).

Treatments (g kg diet^{-1})	Prevalence (%)	Ranges	Mean intensity	Mean abundance	Efficacy (%)
Control	100	174.0–947.7	634.1 ± 129.7	634.1 ± 129.7	–
0.86 EOLG	100	156.3–371.7	240.3 ± 41.3*	240.3 ± 41.3*	62.1 ± 2.44
1.72 EOLG	100	79.3–434.3	312.8 ± 61.4	312.8 ± 61.4	50.7 ± 7.26
0.76 EOLO	100	175.7–326.3	242.3 ± 25.1*	242.3 ± 25.1*	61.8 ± 5.48
1.52 EOLO	100	52.0–539.3	355.9 ± 71.2	355.9 ± 71.2	43.8 ± 8.21
1.03 EOOG	100	75.7–451.0	261.8 ± 58.5*	261.8 ± 58.5*	58.7 ± 9.84
2.06 EOOG	100	177.3–378.0	254.4 ± 32.2*	254.4 ± 32.2*	59.8 ± 7.53
P (Kruskal-Wallis)			0.047	0.047	0.438

[*] Indicates a significant difference with respect to control by the Dunn test ($p < 0.05$).

Table 2

Growth performance parameters of *Colossoma macropomum*, after 30 days of feeding with diets containing essential oils from *Lippia grata* (EOLG), *Lippia origanoides* (EOLO) and *Ocimum gratissimum* (EOOG).

Treatments (g kg diet^{-1})	Final length (cm)	Final weight (g)	Weight gain (g)	SGR (%)	FCR	Kn	Survival (%)
Control	21.3 ± 0.34^{a}	349.7 ± 18.78^{a}	163.9 ± 9.45^{a}	2.75 ± 0.24	2.06 ± 0.18	1.02 ± 0.02	100
0.86 EOLG	19.7 ± 0.14^{ab}	272.7 ± 8.17^{a}	115.5 ± 12.48^{ab}	2.27 ± 0.15	2.59 ± 0.24	0.99 ± 0.06	100
1.72 EOLG	20.4 ± 0.35^{ab}	307.6 ± 16.03^{a}	120.2 ± 21.41^{ab}	2.54 ± 0.36	2.85 ± 0.52	1.03 ± 0.02	100
0.76 EOLO	20.9 ± 0.23^{a}	332.3 ± 14.61^{a}	165.2 ± 13.44^{a}	2.79 ± 0.05	1.99 ± 0.32	0.99 ± 0.02	100
1.52 EOLO	18.8 ± 0.35^{b}	257.0 ± 16.39^{b}	104.2 ± 16.03^{b}	2.28 ± 0.14	2.77 ± 0.33	1.01 ± 0.05	100
1.03 EOOG	20.6 ± 0.34^{ab}	305.5 ± 20.78^{a}	130.4 ± 9.35^{ab}	2.14 ± 0.48	2.48 ± 0.21	1.00 ± 0.02	100
2.06 EOOG	19.8 ± 0.67^{ab}	296.8 ± 15.46^{a}	112.4 ± 11.74^{ab}	2.24 ± 0.05	2.86 ± 0.29	0.99 ± 0.01	100
p (ANOVA)	0.006	0.017	0.037	0.446	0.312	1	

Different letters in same column indicate a significant difference between treatments according to the Tukey test ($p > 0.05$). SGR = Specific growth rate, FCR = Feed conversion rate, Kn = Relative condition factor.

Table 3

Red blood cell parameters of *Colossoma macropomum* infected with *Neoechinorhynchus buttnerae*, after 30 days of feeding with diets containing essential oils from *Lippia grata* (EOLG), *Lippia origanoides* (EOLO) and *Ocimum gratissimum* (EOOG).

Treatments (g kg diet^{-1})	Hct (%)	Hb (g dL^{-1})	RBC (x 10^{6} µL^{-1})	MCV (fL^{-1})	MCHC (g dL^{-1})
Control	34.5 ± 0.9	9.1 ± 0.3	1.89 ± 0.06^{ab}	185.6 ± 7.8	26.6 ± 1.1
0.86 EOLG	33.8 ± 1.1	9.3 ± 0.3	1.88 ± 0.06^{ab}	183.9 ± 8.7	27.3 ± 0.7
1.72 EOLG	35.0 ± 1.4	8.7 ± 0.2	1.81 ± 0.06^{ab}	195.4 ± 8.4	25.3 ± 0.8
0.76 EOLO	31.9 ± 1.3	8.5 ± 0.3	1.79 ± 0.05^{ab}	178.7 ± 6.7	27.0 ± 0.9
1.52 EOLO	33.9 ± 0.4	9.8 ± 0.5	2.05 ± 0.08^{a}	170.1 ± 7.2	28.9 ± 1.4
1.03 EOOG	33.9 ± 1.1	9.2 ± 0.3	1.71 ± 0.08^{b}	207.0 ± 12.6	27.4 ± 0.8
2.06 EOOG	34.4 ± 0.8	9.2 ± 0.3	1.88 ± 0.07^{ab}	187.2 ± 8.6	26.8 ± 0.7
P (ANOVA)	0.699	0.207	0.032	0.117	0.193

Different letters in the same column indicate a significant difference according to the Tukey test ($p > 0.05$). Hct = hematocrit, Hb: hemoglobin, RBC = number of erythrocytes, MCV = mean corpuscular volume, MCHC = mean corpuscular hemoglobin concentration.

control and with the treatment with the lowest concentration of EOLO. Plasma albumin and triglyceride levels in these treatments did not differ from each other ($p > 0.05$), but the treatment with the highest concentration of EOLO showed significantly higher values ($p < 0.05$) than the levels of these two parameters observed in the treatment with 1.03 g EOOG kg^{-1} (Fig. 1C and 1 D).

3.4. Plasma enzymatic parameters of tambaqui

The activity values of the AST and ALT of fish fed with EOs were similar ($p > 0.05$) to those of control group (Fig. 2). For ALP, higher plasma levels ($p < 0.05$) were observed in the treatments with EOLG and EOLO at the concentrations of 0.86 g kg^{-1} and 1.52 g kg^{-1}, respectively, when compared to the values of activity of this enzyme in the control group. In comparison between the treatments with EOs, fish fed with feed containing *O. gratissimum* EO, in the two tested concentrations, had significantly lower ALP values ($p < 0.05$) than the fish fed with feed containing treatments with EOLG and EOLO (Fig. 2).

3.5. Histopathological evaluation of tambaqui liver

In the liver of tambaqui, the hepatic parenchyma was observed consisting of rounded to polyhedral hepatocytes with large, central basophilic nucleus, evident nucleolus and cytoplasm with a granular or vesicular appearance, which were arranged in linear cords on the periphery of the sinusoid capillaries of narrow lumen, and which converge into the central vein (Fig. 3A). After 30 days of treatment with diets containing EOLG, EOLO and EOOG, liver damage including cellular and vascular damage was observed. For all the treatments with the EOs similar alterations were observed, the most frequently damage was classified as Level I and II damage, with 57.12% and 40.62% of occurrence, respectively (Table 4).

Among the Level I damage, the most common was hypertrophy, vacuolization of hepatocytes, dilatation and congestion of sinusoid capillaries (Fig. 3B); and nuclear pyknosis, vascular congestion and cell disruption were the most observed at Level II damage (Fig. 3C). Only 2% of the total damage corresponded to Level III damage; in this case, the damage observed was the dissolution of small groups of hepatocytes and the loss of the normal architecture of the hepatic parenchyma, without association with infiltration of leukocytes and corresponding to focal necrosis (Fig. 3D).

Considering the frequency of occurrence of individual HAI values, it is observed that in most treatments they were between 21 and 100 (mild and severe damage) on the damage scale, but these alterations are reversable. The exception was in the treatment with 1.52 g EOLO kg^{-1}, for which values were above 100 HAI, an indication of severe and irreversible damage to the liver tissue, and these values were more frequent (Fig. 4). In this treatment with EOLG and EOLO, the mean values for HAI were significantly higher ($p < 0.05$) than the average recorded for the control group and for the treatments with EOOG (Fig. 5A). For the mean alteration values (MAV), there was no difference ($p > 0.05$) among the treatments with EOLG, EOLO and EOOG, with mean values between 1.03 and 1.35, which are indicative of the occurrence of lesions in the liver of the fish (Fig. 5B).

4. Discussion

The main compounds of the EOLG (42.2% of carvacrol, 11.2% of *p*-cymene and 10.7% of γ-terpinene), EOLO (37.8% of *p*-cymene, 14.0% of carvacrol and 11.6% of γ-terpinene) and EOOG (39.5% of 1.8-cineole, 14.7% of eugenol and 12.6% of β-selinene) were previously reported by Oliveira et al. (2021). Similarly, in EOLG and EOLO, the monoterpenes carvacrol and *p*-cymene were found to be the compounds with the highest percentage, while in EOOG, cineole and eugenol were the majority compounds. Terpenes and terpenoids are generally the primary components of EOs obtained from medicinal plants and are exploited as anthelminthics. Furthermore, many terpenes are safe and approved for use in the food, cosmetics and pharmaceutical industry (Mirza et al., 2020).

The anthelmintic efficacy of EOOG in tambaqui was first evaluated in the control of *N. buttnerae* infections. A decrease in the mean intensity of infection was observed, with approximately 60% efficacy after 30 days of feeding with this EO. In tambaqui, anthelmintic efficacy against *N. buttnerae* using 0.86 and 1.72 g EOLG kg^{-1} (62.1% and 50.7%, respectively) and with 0.76 and 1.52 g EOLO kg^{-1} (61.8% and 43.8%, respectively) was similar. These efficacies were also similar to those described by Costa et al. (2020) for tambaqui fed with feed

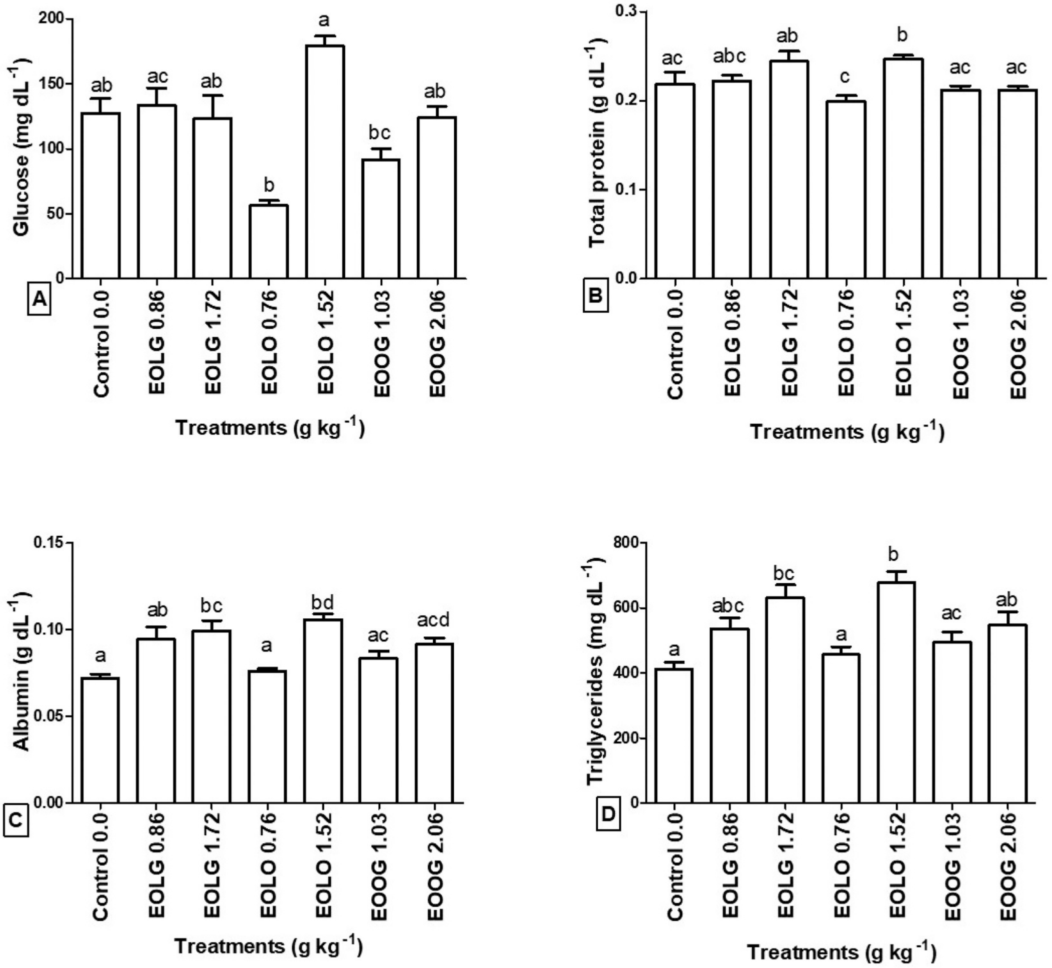

Fig. 1. Plasma glucose (A), total protein (B), albumin (C) and triglycerides (D) concentration in *Colossoma macropomum* infected with *Neoechinorhynchus buttnerae*, after 30 days of feeding with diets containing the essential oils from *Lippia grata* (EOLG), *Lippia origanoides* (EOLO) and *Ocimum gratissimum* (EOOG). Different letters indicate differences according to the Dunn test ($p > 0.05$).

supplemented with 1.44 and 2.88 g kg^{-1} of *Lippia alba* EO. Studies with EOOG have shown also significant anthelmintic activity against monogeneans in fish (Bandeira-Júnior et al., 2017; Boijink et al., 2016). Therefore, EOOG, EOLG and EOLO added to the diets of tambaqui can be an alternative for the control of acanthocephaliasis caused by *N. buttnerae*. These medicinal plant-based products are sustainable and effective alternatives when compared to conventional chemical drugs used for the control of diseases caused by parasitic helminths in aquaculture.

The maintenance of the growth performance of tambaqui after 30 days of feeding with EOOG, EOLG and EOLO was observed. In addition, the growth performance of tambaqui was not affected by the abundance of *N. buttnerae*. In tambaqui fed a diet containing *Zingiber officinale* EO, the growth performance was also unaffected (Chung et al., 2021). However, Gayatri and Rajani (2014) reported improvements in the growth performance of *Clarias batrachus* fed for 30 days with diets supplemented with the extract from the leaves of *O. gratissimum*. Improvements in growth performance or immune parameters in tambaqui supplemented with the EO of *Mentha piperita* and *Z. officinale* (Costa et al., 2020), in *Pseudoplatystoma reticulatum* supplemented with the EO of *Z. officinale* (Almeida et al., 2021), in *Arapaima gigas* and tambaqui supplemented with the EO of *Piper aduncum* (Corral et al., 2018; Queiroz et al., 2022) and in *Oncorhynchus mykiss* supplemented with EO of *Myrtus communis* and *Satureja khuzistanica* (Saei et al., 2016) have already been reported. Therefore, the use of these different EOs in dietary supplementation of farmed fish is recommended; however, these EOs at the concentrations evaluated in this study should not exceed the indicated treatment period for control the infection of *N. buttnerae*.

Regarding hematological parameters, no significant change in the levels of Hct, Hb, RBC, MCV and MCHC were observed with the use of EOOG, EOLG and EOLO in the diet of tambaqui. The negative correlation between the mean value of RBC and the mean values of the constant MCV and MCH, observed in fish fed with 1.03 g EOOG kg^{-1} indicates the occurrence of morphological adjustments, in which a reduction in the number of RBCs is compensated by an increase in the volume of these cells and, consequently, there is more space for hemoglobin. Therefore, this does not impair the exchange of respiratory gases, which is the primary function of the RBCs in the circulating blood (Tavares-Dias and Moraes, 2010; Ranzani-Paiva et al., 2013) and in the maintenance of homeostasis. However, in tambaqui fed with diets containing *Z. officinale* EO, the hemoglobin levels increased (Chung et al., 2021). These compensatory mechanisms have also been reported in fish fed with the addition of EOs, for example in tambaqui fed with *L. alba* EO (Costa et al., 2020) and in *C. batrachus* fed with diets supplemented with the extract from the leaves of *O. gratissimum* (Gayatri and Rajani, 2014).

In tambaqui, an increase in plasma total protein and alkaline phosphatase levels with higher concentrations of L. *origanoides* EO was observed. Increase in plasma protein concentrations in tambaqui has been also reported by Soares et al. (2016, 2017) after therapeutic baths with L. *origanoides* EO and L. *alba* EO. Souza et al. (2022) evaluated the immune responses associated with parasite-host relationship, and also reported an increase in serum alkaline phosphatase, total protein levels

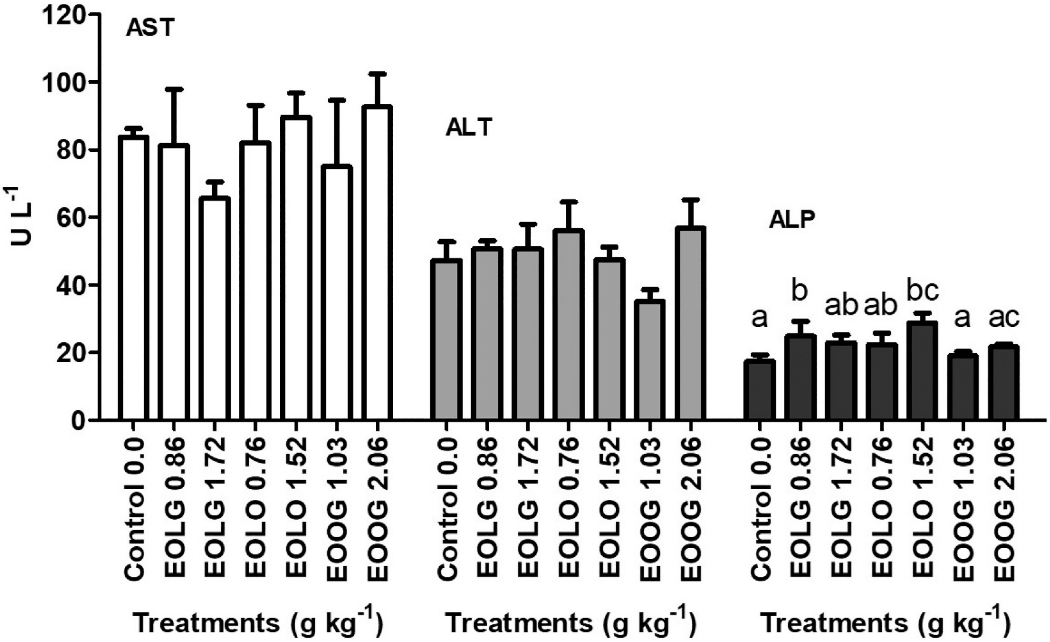

Fig. 2. Activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) concentrations in *Colossoma macropomum* infected with *Neoechinorhynchus buttnerae*, after 30 days of feeding with diets containing the essential oils from *Lippia grata* (EOLG), *Lippia origanoides* (EOLO) and *Ocimum gratissimum* (EOOG). Different letters indicate differences according to the Dunn test ($p > 0.05$).

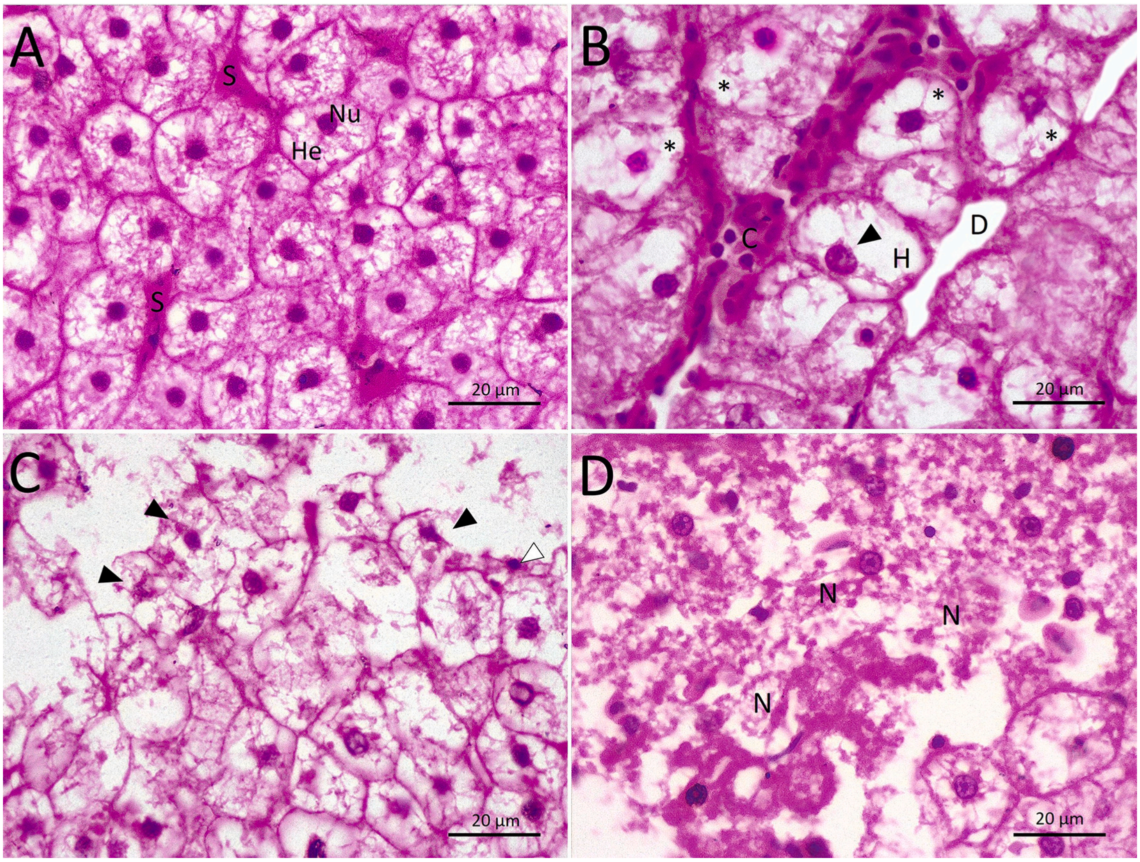

Fig. 3. Histopathological alterations in the liver of *Colossoma macropomum* after 30 days of feeding with diets containing different essential oils (*Lippia grata, Lippia origanoides* and *Ocimum gratissimum*). A. Histological structure of the liver parenchyma: polyhedral hepatocytes (He) organized in cords with a central nucleus (Nu) around the sinusoid capillaries (S). B. Most common level 1 damage: hypertrophy (H), vacuolization of hepatocytes (*), dilatation (D) and congestion (C) of sinusoid capillaries. C. Most frequent level 2 damage: cell disruption (black arrow) of hypertrophied hepatocytes with pyknotic nuclei (white arrow). D. Level 3 damage: dissolution of small groups of hepatocytes and their nuclei and loss of the normal architecture of the liver parenchyma, corresponding to focal necrosis (N).

Table 4
Frequency of occurrence, by degree of damage, of histopathological alterations in the liver of *Colossoma macropomum* infected with *Neoechinorhynchus buttnerae*, after 30 days of feeding with diets containing essential oils from *Lippia grata, Lippia origanoides* and *Ocimum gratissimum*.

Type of damage	Target constituent of liver tissue	Level of damage	Relative frequency of occurrence (%)*
Cell/nuclear hypertrophy of the hepatocyte; cytoplasmic vacuolization; deformation in the cell contour; dilatation of sinusoid vessels	hepatocyte; capillary bed in the hepatic stroma	I	57.12
Pyknosis/nuclear vacuolization; vascular congestion; cell disruption	hepatocyte; capillary bed in the hepatic stroma	II	40.62
Focal necrosis	liver parenchyma	III	2.26
Total			100

[*] the frequency of occurrence value is the same for all EOs.

and intestinal mucus of tambaqui infected by *N. buttnerae*, thus reflecting an influence of this infection on the innate immune response of this host. In addition, plasma albumin levels also increased in tambaqui fed with 1.62 g EOLO kg^{-1} and 1.52 g EOLG kg^{-1}. Plasma proteins and albumin are produced by the hepatocyte cords that make up the hepatic parenchyma (Ross and Pawlina, 2016), and variations in the levels of these proteins may reflect the functional status of this important organ. In these treatments with EOLO and EOLG, a greater occurrence of necrosis was observed in liver tissue of tambaqui, which was indicated by the histopathological alteration index, and cell destruction of hepatocytes, and which lead to decoupling of protein synthesis machinery and the consequent reduction in the levels of these proteins (Bera et al., 2020). However, the mean alteration value (MAV) of tambaqui fed with a diet containing EOOG, EOLG and EOLO indicated the specific and non-extensive location of the foci of necrosis, which probably does not compromise liver function, and which is compatible with the healthy macroscopic aspect of the liver.

It has been reported that the decrease in plasma levels of aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) enzymes is due to the effect of liver activity in response to dietary supplementation with different herbal products in different fish species (Akrami et al., 2015; Karataş et al., 2020; Yousefi et al., 2020). In tambaqui fed with diets containing *Z. officinale* EO, the plasma levels of AST and ALT levels increased (Chung et al., 2021). However, in tambaqui fed with feed containing EOOG, a decrease in plasma levels of AST and ALT was observed, thus indicating a possible toxicity of this EO. Notwithstanding, in tambaqui, feeding with diets based on EOLO and EOLG had a hepatoprotective effect, since the plasma levels of AST and ALT were not altered, which indicates the non-impairment of normal liver function; the serum activity of these aminotransferases is increased when injuries occur in liver tissue (Contreras-Zentella and Hernández-Muñoz, 2016).

Due to the current demand for alternative sources for treatment of the acanthocephaliasis in tambaqui, the main compounds of these EOs should be also tested in an isolated manner and in combinations in order to understand possible synergistic effects and improve their therapeutic efficacy against *N. buttnerae*. Furthermore, the use of 60 days of supplementation may be tested for improving the efficacy of these EOs in the control of *N. buttnerae* infections.

5. Conclusions

EOLG (0.86 g kg^{-1}), EOLO (0.76 g kg^{-1}) and EOOG (1.03 and 2.06 g kg^{-1}) may be used in supplementation of diets for tambaqui for the control and treatment of *N. buttnerae* infections, since these EOs besides be themselves to be efficient, did not caused significant physiological alterations.

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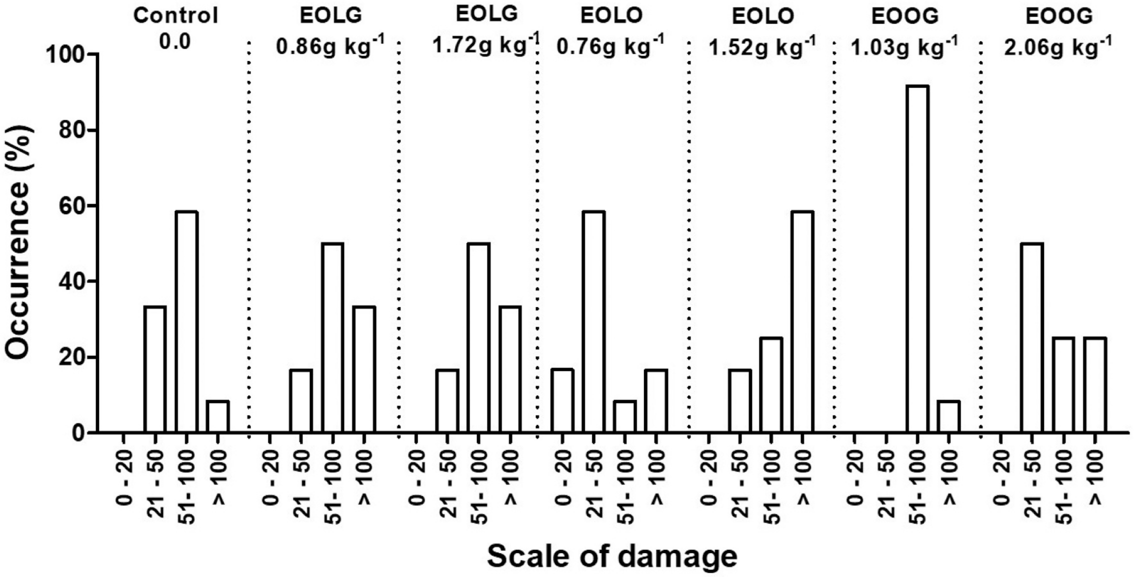

Fig. 4. Histopathological alteration index (HAI) according to scale of damage (0–20 mild damage; 21–50 mild to moderate damage; 51–100 moderate to severe damage; >100 severe and irreparable damage) in the liver tissue of *Colossoma macropomum* infected with *Neoechinorhynchus buttnerae*, after 30 days of feeding with diets containing the essential oils of *Lippia grata* (EOLG), *Lippia origanoides* (EOLO) and *Ocimum gratissimum* (EOOG).

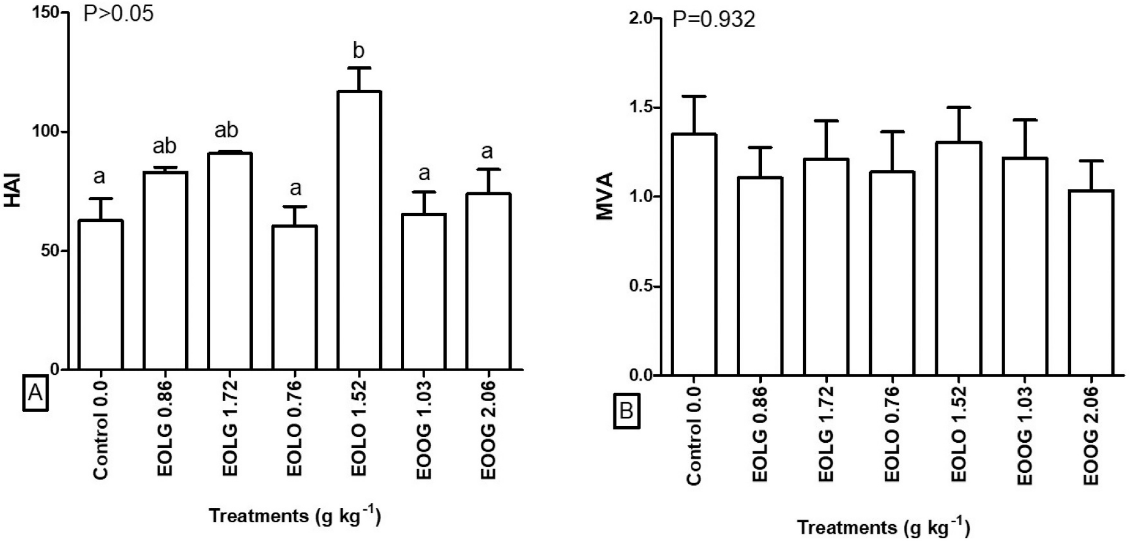

Fig. 5. Mean histopathological alteration index (HAI) (A) and mean alteration value (MAV) (B) in the liver tissue of *Colossoma macropomum* infected with *Neoechinorhynchus buttnerae*, after 30 days of feeding with diets containing the essential oils of *Lippia grata* (EOLG), *Lippia origanoides* (EOLO) and *Ocimum gratissimum* (EOOG). Different letters indicate differences according to the Tukey test ($p > 0.05$).

Francisco Célio Maia Chaves - Methodology - Essential oil production and chemical characterization.

Edsandra Campos Chagas - Conceptualization, methodology, writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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