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Original Article

Essential oil of *Lippia origanoides* as an anesthetic for *Piaractus mesopotamicus*: implications for induction and recovery times, ventilatory frequency and blood responses after biometric management

Óleo essencial de *Lippia origanoides* como anestésico para *Piaractus mesopotamicus*: implicações nos tempos de indução e recuperação, frequência ventilatória e respostas sanguíneas após manejo biométrico

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Abstract

This study evaluated the use of the essential oil of *Lippia origanoides* (EOLO) as an anesthetic for juvenile pacu, *Piaractus mesopotamicus*. Two experiments were performed. In Experiment I, anesthetic induction and recovery times and ventilatory frequency (VF) were determined for fish (n= 48; 29.94 ± 6.69 g) exposed to different EOLO concentrations [0 (control - 4000 µL alcohol L^{-1}), 25, 50, 100, 200 and 400 µL L^{-1}]. From the responses obtained in Experiment I, the EOLO concentrations of Experiment II (n= 36; 29.25 ± 5.90 g) were determined, which evaluated the effects of 0 (control - 2000 µL alcohol L^{-1}), 50 (outside recommended induction and recovery times) and 200 µL L^{-1} (within recommended induction and recovery times) by analyzing immunohematological and biochemical parameters at different collection times (1 h and 24 h post-induction). Fish survival was 100% in both experiments. The EOLO concentration of 25 µL L^{-1} was not able to induce deep anesthesia in the animals, while the concentration of 200 µL L^{-1} showed induction and recovery times within the range indicated for fish and was efficient at reducing VF during induction. No differences were observed in immunohematological and biochemical parameters among concentrations, but differences were observed in comparisons between collection times. Nonetheless, blood variables returned to levels considered normal for the species in approximately 24 hours. Therefore, an EOLO concentration of 200 µL L^{-1} can be considered safe for use prior to biometric management of *P. mesopotamicus*.

Keywords: phytotherapeutic anesthetic, well-being, carvacrol, hematology, pacu.

Resumo

Este estudo avaliou o uso do óleo essencial de *Lippia origanoides* (EOLO) como anestésico para juvenis de pacu, *Piaractus mesopotamicus*. Foram realizados dois experimentos. No Experimento I foram determinados os tempos de indução e recuperação anestésica e a frequência ventilatória (FV) para peixes (n= 48; 29,94 ± 6,69 g) expostos a diferentes concentrações de EOLO [0 (controle - 4000 µL álcool L^{-1}), 25, 50, 100, 200 e 400 µL L^{-1}]. A partir dos resultados obtidos no Experimento I foram determinadas as concentrações de EOLO do Experimento II (n= 36; 29,25 ± 5,90 g), na qual avaliou os efeitos de 0 (controle - 2.000 µL álcool L^{-1}), 50 (fora da indução recomendada e tempos de recuperação) e 200 µL L^{-1} (dentro dos tempos de indução e recuperação recomendados), sob os parâmetros imunohematológicos e bioquímicos em diferentes tempos de coleta (1 hora e 24 horas pós-indução). A sobrevivência dos peixes foi de 100% em ambos os experimentos. A concentração de EOLO de 25 µL L^{-1} não foi capaz de induzir anestesia profunda nos animais, enquanto a concentração de 200 µL L^{-1} apresentou tempos de indução e recuperação dentro da faixa indicada para peixes e foi eficiente na redução da FV durante a indução. Não foram observadas diferenças nos parâmetros imunohematológicos e bioquímicos entre as concentrações, mas foram observadas diferenças nas comparações entre os tempos de coleta. Porém, a maioria das variáveis sanguíneas

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retornaram a níveis considerados normais para a espécie 24 horas após a anestesia. Portanto, uma concentração EOLO de 200 µL L^{-1} pode ser considerada segura para uso antes do manejo biométrico de *P. mesopotamicus*.

Palavras-chave: anestésico fitoterápico, bem-estar, carvacrol, hematologia, pacu.

1. Introduction

Pacu, *Piaractus mesopotamicus*, is a native species of the Paraguay, Paraná and Uruguay river basins (Valladão et al., 2018). It has aquacultural importance, as it presents rapid growth performance, rusticity to different types of management, easy adaptation to artificial feeding (feed) and good acceptance by the consumer market (Urbinati and Gonçalves, 2005).

With the increase in fish production, aquaculture systems have become intensified, especially through the use of greater stocking densities. This densification, combined with inappropriately used management practices (handling, reproduction, biometrics and transport, among others), can lead to physiological fluctuations that can compromise the health and survival of fish (Costa et al., 2019; Pellegrin et al., 2023; Luz and Favero, 2024). Thus, the adoption of improvements in management practices, combined with the concept of stress mitigation measures within production systems, has become essential for successful aquaculture (Boyd et al., 2008; Ferreira and Barcellos, 2008). In this context, the use of anesthesia has become an extremely important practice to favor the homeostasis and well-being of these animals during and after manipulation actions (Ross and Ross, 2008; Teixeira et al., 2017; Hoseini et al., 2019; Ferreira et al., 2021a, b; Moreira et al., 2024).

Synthetic or natural substances can be used for anesthesia (Purbosari et al., 2019; Luz and Favero, 2024). The ideal concentration of a given anesthetic is associated with the species and size of the fish (Ross and Ross, 2008; Readman et al., 2017; Ferreira et al., 2020, 2021a; Silva et al., 2023). Commercially available synthetic anesthetics for fish include benzocaine, tricaine methanesulfonate (MS-222), quinaldine, metomidate, etomidate, barbiturates, and propofol (Neiffer and Stamper, 2009; Bolasina et al., 2017; Readman et al., 2017; Souza et al., 2019a; Uehara et al., 2019). However, undesirable side effects with the use of synthetic anesthetics have already been observed in fish, such as depression of cardiovascular and respiratory function, elevation of lactate levels, increased levels of catecholamines, and inhibition of cortisol synthesis (Carter et al., 2011; Zahl et al., 2012).

Many aquaculture researchers have investigated the anesthetic efficacy of products extracted from aromatic plants, such as essential oils (Souza et al., 2019b; Aydin and Barbas, 2020). Anesthetic efficiency is related to rapid induction of anesthesia (< 180 s) and rapid recovery (< 300 s) (Keene et al., 1998; Ross and Ross, 2008). Furthermore, a good anesthetic is one that is practical to use, cheap, soluble in water, and does not leave residues on employees, fish or in the environment (Park et al., 2018; Bolasina et al., 2017; Purbosari et al., 2019). Many aquaculture researchers have investigated these characteristics in products extracted from aromatic plants, such as essential oils (Souza et al., 2019b; Aydin and Barbas, 2020).

In this sense, essential oils from plant species of the family Verbenaceae have been investigated for their biological activity, such as *Lippia origanoides*, an aromatic plant found throughout South America (Stashenko et al., 2010). The essential oil of *L. origanoides* (EOLO) has three chemotypes, characterized according to their main components: α- and β-phellandrenes, p-cymene, and limonene distinguish chemotype A; while carvacrol and thymol are the major components of chemotypes B and C, respectively (Stashenko et al., 2010). The use of EOLO in aquaculture has demonstrated antimicrobial (Oliveira et al., 2024), antioxidant (Santos-Filho et al., 2023) and anesthetic (Silva et al., 2019) effects.

Thus, the objective of the present study was to evaluate the anesthetic potential of the essential oil of *L. origanoides* (EOLO) for juvenile *P. mesopotamicus* as determined by induction and recovery times, ventilatory frequency and blood responses, after biometric management.

2. Material and Methods

2.1. Ethical approval

The experiment was conducted at the Centro de Excelência em Ciência Animal do Cerrado e Pantanal (CECA), of the Universidade Estadual de Mato Grosso do Sul (UEMS), in the municipality of Aquidauana, Mato Grosso do Sul, Brazil. All procedures were approved by the Comitê de Ética no Uso de Animais (CEUA) of UEMS, under registration number 008/2023.

2.2. EOLO extraction and composition, and preparation of stock solutions

The cultivation of *L. origanoides* and the extraction of its essential oil were carried out in the Plantas Medicinais e Hortaliças sector of Embrapa Amazônica Ocidental, Manuas, Amazônia, Brazil. The essential oil was obtained from fresh leaves of *L. origanoides* using a Clevenger-type device (2 h), as also used by Soares et al. (2017). Following the same authors, the chemical composition of EOLO was determined by a gas chromatograph coupled to a mass spectrometer. The three main compounds found in EOLO were carvacrol (49.7%), p-cymene (13.3%) and thymol (9.9%), with smaller amounts of other components (Figure 1).

Prior to the experiments, all concentrations of EOLO were diluted in ethyl alcohol 1:10 (V:V), to assure complete dissolution in water. A volume of ethyl alcohol equivalent to the highest concentration of EOLO, in each experiment, was used for the control group (0 µL L^{-1}), as also adopted by Moreira et al. (2024).

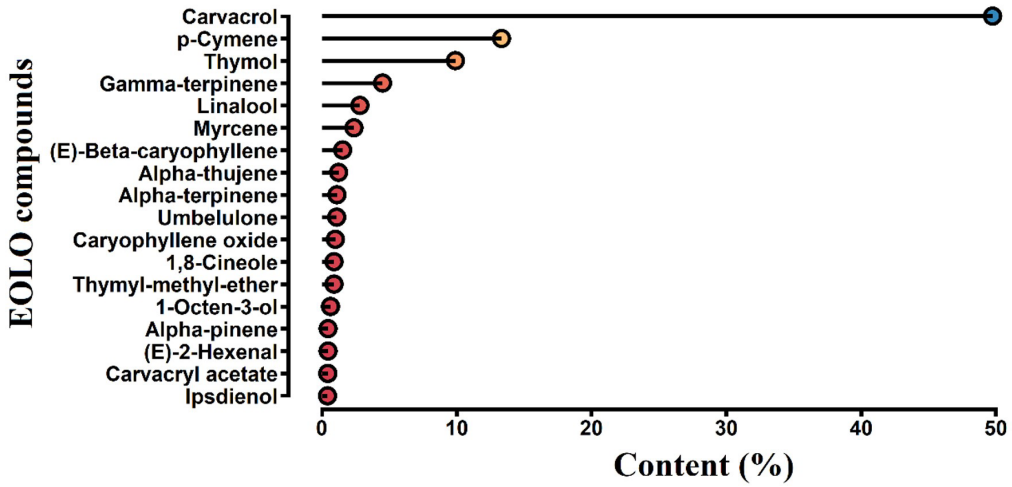

Figure 1. Chemical composition of the essential oil of *Lippia origanoides* (EOLO) determined through gas chromatography-mass spectrometry (GC-MS).

2.3. Animals and acclimatization

The fish were purchased from the Piscicultura sector of UEMS. The animals were distributed in 12 polyethylene tanks (400 liters of useful volume) in a system of continuous water circulation and constant aeration, where they were acclimatized for two weeks. The fish were fed twice a day (08:00 and 16:00) until apparent satiety, with a commercial diet (Supra, ACQUAline) of 2.5 mm diameter and 42% crude protein. All animals were fasted for 24 hours prior to the experiments to empty the gastrointestinal tract. Water quality parameters were measured daily, approximately 30 minutes before the provisioning of feed. The water temperature remained at 23.85 ± 1.78 °C, pH at 6.87 ± 0.57, dissolved oxygen at 5.90 ± 0.57 mg L^{-1} (measured with a Hanna HI98194 multiparameter probe), and total ammonia at 0.02 ± 0.0003 mg L^{-1} (determined using a Alfakit Labcon Test colorimetric kit).

2.4. Experiment 1 – Anesthetic effect of EOLO on juvenile Piaractus mesopotamicus

Forty-eight juvenile *P. mesopotamicus* (29.94 ± 6.69 g; and 11.52 ± 0.91 cm) were distributed in a completely randomized design among six different concentrations of EOLO, namely: 0 (Control – 4000 µL L^{-1} of ethyl alcohol), 25, 50, 100, 200 and 400 µL L^{-1}, according to Silva et al. (2019). Each concentration received eight fish (n = 8), and each animal was used only once and considered a replicate (Figure 2).

Anesthetic effect was evaluated by exposing the fish to pre-established EOLO concentrations in 1-L beakers (water from the cultivation system itself, with supplementary aeration). The time for anesthesia induction was timed with a digital stopwatch (Vollo VI510), from the fish's first contact with the anesthetic solution until it reached the stage of deep anesthesia, characterized by loss of balance and absence of swimming, implying slow and irregular opercular movements and absence of reflexes (Small, 2003; Ross and Ross, 2008; Silva et al., 2023). The ventilation frequency (VF) of the animal was monitored from the beginning of anesthetic induction by counting the number of opercular movements per minute until the deep anesthesia stage (Alvarenga and Volpato, 1995; Ferreira et al., 2021a, b). After deep anesthesia, biometrics (measurement of weight and length) of the animals were performed using a semi-analytical scale (Shimadzu – BL320H) and a ruler. This procedure lasted approximately 45 s, after which the animals were transferred to 1-L beakers (with clean water, without anesthetic) for recovery time measurement and VF determination. Fish were considered recovered when they reached the full recovery stage, which was basically characterized by balance, normal swimming, and responsiveness to visual stimuli (Small, 2003; Ross and Ross, 2008; Silva et al., 2023).

At the end of the experiment, fish from each EOLO concentration were relocated to their original experimental units to observe their return to feeding and survival for seven days.

2.5. Experiment 2 – Blood responses after biometric management of juvenile Piaractus mespotamicus anesthetized with EOLO

Based on the results obtained in Experiment 1, Experiment 2 evaluated three concentrations of EOLO, namely: 0 (Control – only ethyl alcohol), 50 and 200 µL L^{-1} of EOLO. Keene et al. (1998) and Ross and Ross (2008) emphasize that the ideal concentration of a given anesthetic is related to rapid anesthetic induction (up to 180 s) and recovery (up to 300 s). Based on this assumption, the concentrations used in this experiment were chosen because they include one that is outside the time range considered ideal for fish anesthesia (50 µL L^{-1}), one that is within the time range considered ideal for fish anesthesia (200 µL L^{-1}), and a control group (2000 µL L^{-1} of alcohol).

Experiment 2 used 36 juvenile *P. mesopotamicus* (n = 12 fish for each concentration) with an average weight of 29.25 ± 5.90 g and total length of 11.30 ± 0.93 cm, and the same procedures adopted in Experiment 1. However, in

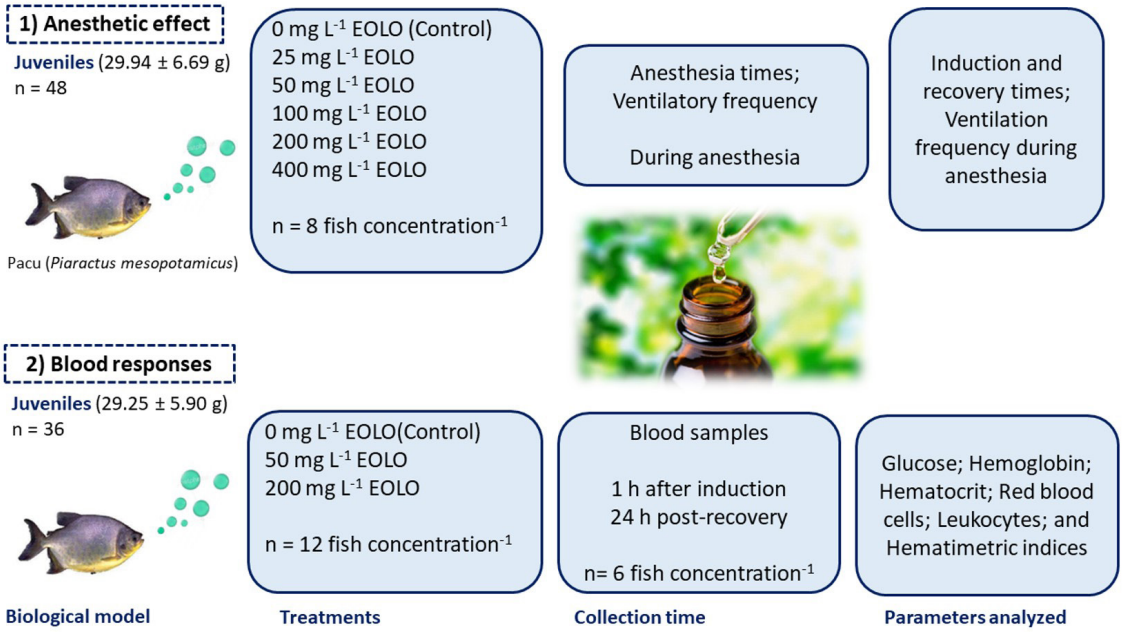

Figure 2. Schematic diagram of experimental design (Experiment 1 and 2).

Experiment 2, blood samples were collected from six fish of each concentration at 1 h after anesthesia and 24 h after recovery. The animals were distributed in a completely randomized design in a factorial arrangement (3×2) (Figure 2). Blood extraction (300 µL L^{-1}) was performed by caudal puncture (3-mL syringes previously bathed in 3% EDTA) close to the lateral line of fish that were carefully captured and contained in damp cloths.

2.6. Blood analyses

Aliquots of blood were used to quantify blood glucose (mg dL^{-1}) using a digital glucometer (G-tech, Lite); determine hematocrit percentage (Ht) using the microhematocrit method of Goldenfarb et al. (1971); and determine hemoglobin (Hb) using the cyanmethemoglobin method proposed by Collier (1944). The number of red blood cells (RBCs) was counted in a Neubauer chamber under an optical microscope (Axio Scope.A1 Zeiss®; 40 x) using a 1:200 dilution in formaldehyde-citrate solution. The following hematimetric indices were then calculated: mean corpuscular volume (MCV) = Ht x (10/RBC's), mean corpuscular hemoglobin concentration (MCHC) = Hb x (100/Ht) and mean corpuscular hemoglobin (HCM) = 10 x (Hb/RBC's).

For the total leukocyte count, blood smears were prepared in duplicate from each animal, air-dried and stained panchromically with May Grünwald-Giemsa-Wright as described by Tavares-Dias and Moraes (2006).

2.7. Statistical analyses

All data were subjected to the Shapiro-Wilk normality test and Levene's test of homoscedasticity of variances. Data on time for anesthesia induction and for anesthesia recovery were submitted to ANOVA followed by regression analyses ($P < 0.05$). Data for VF during induction and recovery did not meet the assumptions of ANOVA, so they were subjected to the non-parametric Kruskal-Wallis test ($P < 0.05$). Blood variables were submitted to two-way ANOVA followed by Tukey's post-test ($P < 0.05$). Data analyses were performed using Infostat software.

3. Results

No mortality was observed during, nor for seven days after, the experiment and all fish returned to feeding within 48 h after the test.

3.1. Experiment I

Exposure to 25 µL L^{-1} EOLO did not induce deep anesthesia in the animals. For the other concentrations evaluated, both induction time and recovery time showed a quadratic effect ($P < 0.05$), with minimum points at 333.87 µL L^{-1} (73.71 s) (Figure 3a), and 93.02 µL L^{-1} (253 s) (Figure 3b), respectively.

The highest VF observed during anesthesia induction was for fish exposed to 400 µL L^{-1} EOLO, in relation to those subjected to the other evaluated concentrations ($P < 0.05$). The lowest VF observed during recovery was for fish exposed to 400 µL L^{-1} EOLO, compared to fish subjected to the other evaluated concentrations (Table 1).

3.2. Experiment 2

There was no interaction between EOLO concentration and blood collection time for all the evaluated variables ($P > 0.05$) (Table 2). Nevertheless, the highest values for RBC

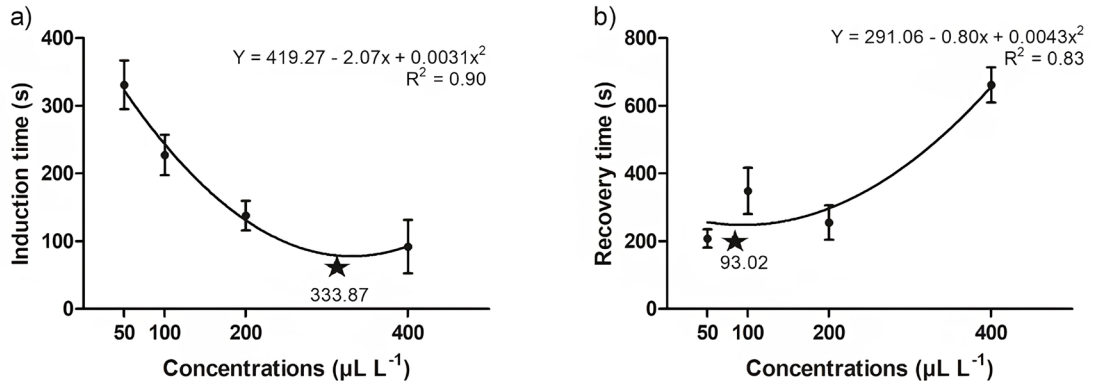

Figure 3. Time required for anesthetic induction (a) and recovery (b) of juvenile *Piaractus mesopotamicus* exposed to different concentrations of the essential oil of *Lippia origanoides* (EOLO). Data were subjected to regression analyses ($p < 0.05$). Stars represent values estimated by the derived equation.

Table 1. Values (mean ± standard deviation) for ventilation frequency (opercular beats per minute) during anesthesia induction and recovery of juvenile *Piaractus mesopotamicus* submitted to different concentrations of the essential oil of *Lippia origanoides* (EOLO).

Concentrations EOLO (µL L^{-1})	Induction ventilation frequency (beats min^{-1})	Recovery ventilation frequency (beats min^{-1})
50	65.55 ± 4.62 b	64.01 ± 2.92 a
100	65.00 ± 2.00 b	62.94 ± 1.70 a
200	64.46 ± 2.23 b	62.92 ± 1.67 a
400	84.08 ± 14.61 a	60.64 ± 0.78 b

Means in the same column followed by different letters differ significantly by the Kruskal-Wallis test ($p < 0.05$).

Table 2. Values (mean ± standard deviation) of biochemistry and hematological variables at two blood collection times for juvenile *Piaractus mesopotamicus* exposed to different concentrations of the essential oil of *Lippia origanoides* (EOLO).

		Glucose (mg dL^{-1})	Hemoglobin (g dL^{-1})	Hematocrit (%)	Red blood cells (x10^6 mL^{-1})	Leukocytes (x10^3 mL^{-1})
Concentrations (µL L^{-1})	0 (Control)	83.80 ± 24.61	12.01 ± 4.99	24.00 ± 5.60	2.04 ± 0.09	28.04 ± 5.61
	50	92.83 ± 33.16	11.61 ± 3.19	26.00 ± 3.35	2.02 ± 0.21	23.36 ± 7.09
	200	86.09 ± 31.55	11.14 ± 4.94	25.91 ± 6.77	1.98 ± 0.18	28.57 ± 7.77
Collection time (h)	1	104.25 ± 30.06 a	14.48 ± 3.20 a	27.00 ± 6.04	2.08 ± 0.15 a	31.03 ± 5.24 a
	24	72.41 ± 19.50 b	8.79 ± 3.36 b	23.82 ± 4.60	1.94 ± 0.15 b	21.79 ± 5.08 b
Overall average		88.33 ± 24.78	11.64 ± 3.28	25.41 ± 5.32	2.01 ± 0.15	26.41 ± 5.16
p-value Concentrations (A)		0.7691	0.8411	0.6165	0.6962	0.3542
p-value Collection time (B)		0.0020	0.0001	0.1119	0.0218	0.0003
p-value Interaction (A × B)		0.8728	0.6622	0.6616	0.0659	0.3651
CV (%)		30.23	29.87	21.78	7.23	19.22

Means in the same column followed by different letters differ significantly by Tukey's test ($p < 0.05$); Control group: 2000 µL alcohol L^{-1}; CV = Coefficient of variation.

were observed at 1 h after anesthesia, being reduced at 24 h after ($P < 0.05$). Glucose, hemoglobin and leukocyte values did not differ significantly among EOLO concentrations ($P > 0.05$). However, the highest values for these variables were observed at 1 h after anesthesia, being reduced at 24 h after ($P < 0.05$).

There was no interaction between EOLO concentration and blood collection time for the studied hematimetric indices ($P > 0.05$) (Table 3). CHCM values did not differ significantly between EOLO concentrations ($P > 0.05$); however, the highest values were observed 1 h after anesthesia, being reduced at 24 h after ($P < 0.05$).

Table 3. Values (mean ± standard deviation) of hematimetric indices at two blood collection times for juvenile *Piaractus mesopotamicus* exposed to different concentrations of the essential oil of *Lippia origanoides* (EOLO).

		MCV (fL)	MCHC (g dL^{-1})	MCH (*pg*)
Concentrations (µL L^{-1})	0 (Control)	124.14 ± 18.16	45.47 ± 15.27	59.01 ± 25.40
	50	133.95 ± 16.32	44.08 ± 14.42	60.22 ± 16.01
	200	115.80 ± 29.80	43.62 ± 20.42	54.05 ± 20.69
Collection time (h)	1	129.23 ± 26.80	54.98 ± 11.65 a	69.26 ± 14.85
	24	118.92 ± 16.54	34.41 ± 14.37 b	43.39 ± 17.27
Overall average		124.08 ± 21.67	44.69 ± 13.01	56.32 ± 16.06
p-value Concentrations (A)		0.1869	0.8781	0.2665
p-value Collection time (B)		0.1290	0.0005	0.0002
p-value Interaction (A × B)		0.9589	0.1333	0.3795
CV (%)		18.06	29.01	27.20

Means in the same column followed by different letters differ significantly by Tukey's test ($p < 0.05$); Control group: 2000 µL alcohol L^{-1}; Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), and Mean corpuscular hemoglobin (MCH); CV = Coefficient of variation.

4. Discussion

The main compound of *Lippia origanoides* essential oil (EOLO) identified in the current study was carvacrol (49.7%), characterizing the carvacrol chemotype, as described by Stashenko et al. (2010). The anesthetic effect of EOLO in fish was expected, as Becker et al. (2018) reported that EOLO, containing 47.2% carvacrol, effectively induced anesthesia in silver catfish (*Rhamdia quelen*). However, other studies indicate that the carvacrol chemotype of *L. origanoides* essential oil may cause adverse effects in fish. For example, Aydin and Orhan (2021) observed an increase in swimming speed and mobility in *R. quelen* anesthetized with carvacrol, while Bianchini et al. (2017) reported strong muscle contractions and even mortality. Given these findings, it is crucial to exercise caution when extrapolating the ideal EOLO (carvacrol) concentrations for different fish species, considering potential variations in physiological responses.

According to Keene et al. (1998) and Ross and Ross (2008), the ideal concentration of a given anesthetic for fish should induce anesthesia within 180 s and promote recovery within 300 s. In the present study, the anesthetic action of EOLO was obtained using concentrations of at least 50 µL L^{-1}. Furthermore, the anesthetic effect occurred in 212 s with the EOLO concentration of 100 µL L^{-1}, and rapid anesthesia was achieved in 145 and 92 s using 200 and 400 µL L^{-1}, respectively. Likewise, Silva et al. (2019), working with the essential oil of *L. origanoides*, obtained anesthesia of *C. macropomum* using 200 µL L^{-1}, with a quick induction time (120 s), but with long recovery times. Becker et al. (2018) reported that concentrations between 50 and 200 µL L^{-1} EOLO cause anesthesia in *R. quelen* and can be used for rapid anesthesia (< 180 s), but they also reported a long time for full recovery. In the current study, only the concentration of 200 µL L^{-1} EOLO promoted recovery of *P. mesopotamicus* within the time limit recommended by Ross and Ross (2008).

Although EOLO requires dilution in ethyl alcohol, this solvent, when at low concentrations, does not have an inductive effect and does not cause mortality in fish (Becker et al., 2018; Silva et al., 2019), as observed for the control animals of the present study. Furthermore, the different EOLO concentrations studied did not cause mortality during, nor seven days after, the experiment, and all animals returned to feeding. Similarly, Ferreira et al. (2021a) reported that, when anesthetizing two size classes of juvenile pirapitinga (*Piaractus brachypomus*) with different concentrations of menthol and eugenol, they observed 100% survival and all animals resumed feeding after the end of the experiments. These results suggest that the studied individuals of *P. mesopotamicus* subjected to different concentrations of EOLO recovered well from the adopted management (anesthesia and biometry).

Ventilatory frequency (VF) is a non-invasive assessment tool that can be useful to understand the physiology of fish in response to anesthetics (Alvarenga and Volpato, 1995; Ferreira et al., 2021a, b; Silva et al., 2023). In the present study, the concentration of 200 µL L^{-1} EOLO, which corresponds to the recommended concentration for this species, caused a reduction in the VF of juvenile *P. mesopotamicus* during induction. In this way, it was possible to immobilize the fish in a safe and calm manner, both for the animal and the handler, during biometrics. Hypoventilation and, consequently, decreased oxygen consumption in fish can occur due to anesthesia (Houston et al., 1971), as observed in the present study.

In general, fish in stressful situations release catecholamines and corticosteroids that activate the processes of glycogenolysis and gluconeogenesis, in order to mobilize energy reserves. The result is an increase in glucose in the bloodstream so that the animal body can escape or adjust to the new physiological conditions imposed by the environment (Barton and Iwama, 1991; Pankhurst, 2011; Jerez-Cepa and Ruiz-Jarabo, 2021). Hyperglycemia was observed in fish 1 h after anesthesia and biometry. This finding can be understood as a result of the triggering of

glycogenolysis and an increase in blood glucose levels during fish handling, as also observed by Iwama et al. (2004). On the other hand, the fasting period to which the animals were subjected may have reduced glycogen stores and caused an increase in glucose levels, as reported by Ribeiro et al. (2013). Similarly, when anesthetizing juvenile pirapitinga (*P. brachypomus*) with different concentrations of menthol, Ferreira et al. (2021a) observed an increase in plasma glucose values 1 h after anesthesia.

In the present study, the increase in hemoglobin levels observed 1 h after anesthesia indicates greater oxygen transport capacity by erythrocytes. Different studies have demonstrated that these changes occur in response to the increased physiological demand imposed by a stressful situation (Ventura et al., 2020, 2021). Therefore, these observed hematological changes indicate that EOLO was not effective in mitigating the physiological effects of induced stress caused by the adopted management (anesthesia and biometrics), requiring adaptive response mechanisms from the animals after manipulation. However, hemoglobin levels returned to homeostasis after 24 h of recovery.

Elevations in leukocyte values can occur in stressful situations for most fish species, being considered an attempt to recover homeostasis (Ranzani-Paiva et al., 2013). This response was observed in the fish of the present study 1 h after anesthesia and biometric handling. On the other hand, when evaluating the effect of therapeutic baths with the essential oil of *Alpinia zerumbet* (300 mg L^{-1}) for juvenile *C. macropomum*, Luz et al. (2021) found a decrease in the number of leukocytes and reported that this was due to the anesthetic effect of this oil. It is important to emphasize that oscillations in the hematological responses of different fish species may be related to exogenous and endogenous factors (Ahmed et al., 2020), as well as stress level and exposure time.

5. Conclusions

A concentration of 200 µL L^{-1} EOLO can be recommended for deep anesthesia of juvenile *P. mesopotamicus*, as it causes anesthesia within the induction and recovery time ranges considered ideal for fish. Furthermore, this concentration was able to reduce the VF of fish during anesthesia induction. However, EOLO was not able to mitigate the induced stress responses caused by biometric management, but most blood variables returned to normal values within 24 h.

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