Arq. Bras. Med. Vet. Zootec., v.76, n.3, e13098, 2024

http://dx.doi.org/10.1590/1678-4162-13098

Communication - Veterinary Medicine

Communication

[Comunicação]

Essential oils from rosemary, lemongrass, and zinziba in the anesthetic induction of freshwater angelfish

[Óleos essenciais de alecrim, capim-limão e zinziba na indução anestésica de acará-bandeira]

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The freshwater angelfish (*Pterophyllum scalare*) is endemic to South America and widely distributed in the Amazon Basin. Due to its color pattern, easy reproduction in captivity, and high resistance, it is an ornamental fish in high demand in the aquarium market (Oliveira *et al.*, 2019, 2022). The capture and transport cause stress in ornamental fish. In this sense, anesthetics can improve the welfare of these fish during such activities (Limma-Netto *et al.*, 2017).

The capture and transport can cause stress in fish. Using essential oils (EO) has been recommended for fish sedation and anesthesia, improving their welfare during handling and transport activities and having low toxicity and biodegradability (Sena *et al.*, 2016; Limma-Netto *et al.*, 2017). EO of rosemary *Lippia sidoides* (EOLS) (synonymia for *Lippia origanoides*) has shown potential as an anesthetic in silver catfish (*Rhamdia quelen*) (Silva *et al.*, 2013) and freshwater angelfish (Oliveira *et al.*, 2022) and EO of lemongrass *Cymbopogon flexuosus* (EOCF) has demonstrated an anesthetic potential in silver catfish (Santos *et al.*, 2017) and Nile tilapia (*Oreochromis niloticus*) (Limma-Netto *et al.*, 2017). There is no study with the EO of zinziba *Lippia javanica* (EOLJ), but EO with *Lippia* species has been recommended for fish anesthesia (Sena *et al.*, 2016. Oliveira *et al.*, 2022).

This study aimed to verify the anesthetic potentials of EOLS, EOCF, and EOLJ in freshwater angelfish, as well as their effects on the survival of the fish and water quality after simulated transport in adverse conditions.

The EOs used in this study were purchased from Terra Flor Aromatherapy®. The primary chemical constituents of EOLS were carvacrol (26.55%), 1,8-cineole (22.90%), and para-cymene (9.91%), of EOCF were geranial (45.98%), neral (25.24%), and geranyl acetate (9.55%), and of EOLJ were limonene (37.50%), chrysanthenone (17.11%), and myrcenone (10.70%).

Freshwater angelfish juveniles (4.39±0.31 g) were transported to the Institute of Biology of the Federal University of Bahia (IBIO-UFBA) and placed in a 250 L tank with constant aeration for 15 days. Feeding was suspended 24 h before the beginning of the experiments. The ethics committee on the use of animals from IBIO-UFBA approved the project (02/2021 and 02/2022).

The following concentrations (n=6 fish per treatment) of EO (diluted 1:10 with ethanol) were evaluated: 0 (control group) 10, 25, 50, 75, 100, 200, 300, 400, and 500 μL L^{-1}. A second control group containing water with ethanol was also used (4,500 μL L^{-1}, which replicated the highest concentration of ethanol used for the EO dilution).

The animals (n=2 fish at a time) remained in the aquariums (2 L) until anesthesia or after 30 min had elapsed. The fish were considered sedated when they showed a partial loss of balance and

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Submitted: July 28, 2023. Accepted: December 11, 2023.

reduced response to stimuli, and they were anesthetized when presenting a total loss of balance and lack of response to stimuli (Sena *et al.*, 2016). Then, they were transferred to another aquarium (4 L) containing only water for anesthetic recovery assessment. The animals were considered to have partially recovered when they presented balance, similar to the control groups, and fully recovered when swimming, identical to the control groups. Additionally, survival for 72 h was evaluated.

The water quality parameters were measured using a commercial kit (Alfatecnoquímica, Florianópolis). They remained stable during the experiment for dissolved oxygen (8.50±0.20 mg O_2 L^{-1}), temperature (27.60±0.20°C), pH (6.25±0.25), total ammonia (0.25±0.01 mg L^{-1} N-NH_3), nitrite (0.00±0.01 mg L^{-1} N-NO_2), alkalinity (20.00±0.01 mg L^{-1} $CaCO_3$), and hardness (140.00±0.01 mg L^{-1} $CaCO_3$).

Levene's test verified the homoscedasticity of the variances. Anesthetic induction and recovery data were evaluated using power regression ($p<0.05$).

There was no mortality after the experiments. There was no sedation or anesthesia in the control groups. The angelfish exposed to 10 µL EOLS L^{-1}, 10-25 µL EOCF L^{-1}, and 10-100 µL EOLJ L^{-1} concentrations only reached the sedation stage. Concentrations above 25µL EOLS L^{-1}, 50 µL EOCF L^{-1}, and 200 µL EOLJ L^{-1} promoted anesthesia. There was a regression in the sedation and anesthesia for all tested EOs (Fig. 1). As the EO concentration increased, the sedation and anesthesia times were reduced ($p<0.05$). There was no regression for recovery from anesthesia (Table 1).

The anesthetic effect of an EO depends on its principal constituent or a synergistic effect between the different compounds present (Sena *et al.*, 2016). The major component of EOLS, EOCF, and EOLJ was carvacrol, citral (a mixture of geranial and neral isomers), and limonene, respectively. These compounds can act as positive allosteric modulators of GABA (gamma-aminobutyric acid) receptors, which are targets of anesthetic action, resulting in the inhibition of the central nervous system (Zhou *et al.*, 2009; Costa *et al.*, 2011; Nesterkina and Kravchenko, 2016), triggering the anesthetic responses verified in the present study.

EO concentrations promoting only a sedative effect should be used without affecting the fish balance. During our experiment, 10 µL L^{-1} of all EOs was safe for sedation and could be used in routine transport practices of these ornamental fish. In addition, an anesthetic must be able to provide rapid anesthesia and recovery (Santos *et al.*, 2017) to be suitable for use in fish. Therefore, in this study, a concentration of 100 µL L^{-1} of EOLS was the best, yielding induction (118.33 s) and anesthetic recovery (291.17 s) times within the range of the acceptable limits. For the EOCF and EOLJ, concentrations of 500 µL L^{-1} were most effective (induction and recovery times of 125.83 and 335.33 s and 864.50 and 310.00 s, respectively), where the times to achieve anesthesia in the fish were higher using EOLJ. In addition, due to the longer time to achieve anesthesia EOLJ is not a viable alternative as an anesthetic for fish management, including on the African continent, from which the plant is native and easier to cultivate (Maroyi, 2017).

Previous studies have found concentrations close to those found in our research. The use of 200-250 mg L^{-1} of EO from *Cymbopogon citratus* (73.56% α-citral) and EOLS (44.50% carvacrol) has been recommended for the anesthesia of freshwater angelfish (Oliveira *et al.*, 2022). In tambaqui, EO from *Lippia alba* with the chemotypes citral (55.28%) or linalool (59.66%) were effective in promoting anesthesia at concentrations of 100 and 200 µL L^{-1}, respectively (Silva *et al.*, 2019). In silver catfish, EO from *Aloysia triphylla* (50.19% citral and 11.90% limonene) and EOCF (86.37% citral) promoted sedation and anesthesia at concentrations of 25 and 300 µL L^{-1}, respectively (Santos *et al.*, 2017). For Nile tilapia, 25 and 600 µL L^{-1} of EOCF (90.45% citral) were indicated for sedation and anesthesia, respectively (Limma-Netto *et al.*, 2017). Limonene was the primary compound of EO from *Citrus x aurantium* (93.90%) and *Citrus x latifolia* (49.70%), which sedated silver catfish at concentrations between 100-300 µL L^{-1} and showed better anesthesia times in these fish with 800 and 500 µL L^{-1}, respectively (Lopes *et al.*, 2018).

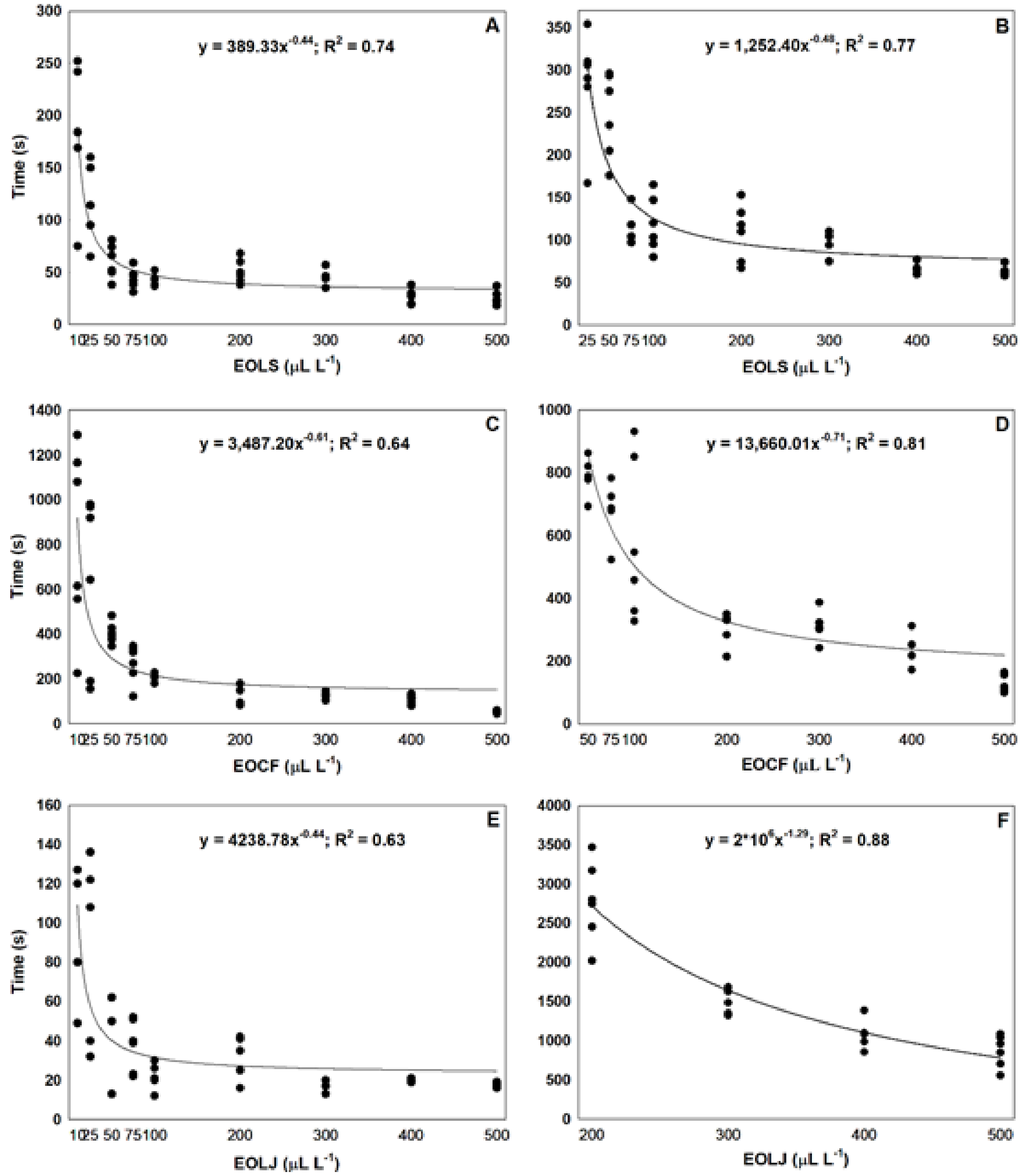

Figure 1. Sedation and anesthesia times (s) in freshwater angelfish (*Pterophyllum scalare*) (n=6 per treatment) with essential oils of *Lippia sidoides* (EOLS) (A and B), *Cymbopogon flexuosus* (EOCF) (C and D), and *Lippia javanica* (EOLS) (E and F). A, C, and E: sedation. B, D, and F: anesthesia.

Table 1. Anesthesia (Anes) and partial (Pt Rec) and total recovery (Tt Rec) times (s) (mean±SEM) in freshwater angelfish (*Pterophyllum scalare*) (n=6 per treatment) after anesthesia with essential oils of *Lippia sidoides* (EOLS), *Cymbopogon flexuosus* (EOCF), and *Lippia javanica* (EOLS)

Dose	EOLS			EOCF			EOLJ		
(µL L^{-1})	Anes	Pt Rec	Tt Rec	Anes	Pt Rec	Tt Rec	Anes	Pt Rec	Tt Rec
25	284.50	146.50	271.33	-	-	-	-	-	-
	±25.70	±14.15	±35.10						
50	246.67	164.33	346.17	788.50	199.67	295.00±	-	-	-
	±20.21	±48.27	±48.81	±22.93	±16.96	66.13			
75	118.67	246.33	387.33	679.17	184.17	301.67	-	-	-
	±9.79	±25.50	±25.15	±35.23	±36.88	±21.47			
100	118.33	210.83	291.17	579.00	136.50	290.50	-	-	-
	±13.23	±18.03	±30.81	±104.09	±52.15	±35.81			
200	109.00	264.67	373.50	308.17	237.50	288.17	2775.5	151.50	456.00
	±13.58	±46.09	±61.77	±20.96	±56.02	±32.65	±209.03	±18.11	±38.88
300	93.83	111.17	178.83	313.67	135.83	289.83	1548.5	288.00	375.00
	±6.32	±28.93	±30.40	±19.00	±27.50	±17.93	±68.33	±47.84	±44.13
400	66.01	242.00	319.08	237.33	135.00	263.67	998.33	245.83	369.66
	±2.60	±4.93	±6.14	±19.23	±20.98	±36.65	±139.92	±43.41	±51.18
500	63.33	232.22	329.22	125.83	228.50	335.33	864.50	214.17	310.00
	±2.29	±4.98	±5.06	±11.16	±8.29	±26.00	±83.59	±24.73	±27.72

The EOLS proved to be the most effective of the anesthetics tested in this study, as it achieved shorter anesthesia and anesthetic recovery times and used a fivefold lower concentration than the EOCF and EOLJ. For sedation, 10 µL L^{-1} of all the EOs of this study is recommended, as they promote sedation without causing anesthesia.

Keyword: Cymbopogon flexuosus, Lippia sidoides, Lippia javanica, Pterophyllum scalare

RESUMO

Este estudo avaliou o uso de óleos essenciais (OE) de alecrim (Lippia sidoides) (OELS), capim-limão (Cymbopogon flexuosus) *(OECF) e zinziba* (Lippia javanica) *(OELJ) para sedação e anestesia de acará-bandeira* (Pterophyllum scalare). *Uma concentração de 10 µL L^{-1} de todos os OE causou sedação. A concentração ideal para anestesia de OOLS foi de 100 µL L^{-1}, enquanto para OECF foi de 500 µL L^{-1}. OELJ não alcançou tempos ideais de anestesia mesmo com 500 µL L^{-1}. Todos os OE testados foram efetivos, sem causar mortalidade por até 72h após os experimentos. Em conclusão, os OE deste estudo podem ser usados para sedação dos peixes em uma concentração de 10 µL L^{-1}, e, para anestesia dos peixes, é recomendado OELS (100 µL L^{-1}), uma vez que ele foi mais efetivo que os demais OE.*

Palavras-chave: Cymbopogon flexuosus, Lippia sidoides, Lippia javanica, Pterophyllum scalare

ACKNOWLEDGMENTS

The authors are grateful to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil) for the research fellowships provided to Copatti, C.E. (PQ 304329/2021-5) and Silva. I.S., CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil) - Finance Code 001 - for a research grant provided for Lemos, C.H.P., and FAPESB (Fundação de Ampara à Pesquisa do Estado da Bahia) for a research grant provided for Castro-Neto, O.P.A.

REFERENCES

COSTA, C.A.; KOHN, D.O.; LIMA, V.M. *et al*. The GABAergetic system contributes to the anxiolytic-like effect of essential oil from *Cymbopogon citratus* (lemongrass). *J Ethnopharmacol.*, v.137, p.828-836, 2011.

LIMMA-NETTO, J.D.; OLIVEIRA, R.S.; COPATTI, C.E. Efficiency of essential oils of *Ocimum basilicum* and *Cymbopogum flexuosus* in the sedation and anaesthesia of Nile tilapia juveniles. *An. Acad. Bras. Ciênc.*, v.89, p.2971-2974, 2017.

LOPES, J.M.; SOUZA, C.F.; SCHINDLER, B. *et al*. Essential oils from *Citrus* x *aurantium* and *Citrus* x *latifolia* (Rutaceae) have anesthetic activity and are effective in reducing ion loss in silver catfish (*Rhamdia quelen*). *Neotr Ichthyol*, v.16, p.e170152, 2018.

MAROYI, A. *Lippia javanica* (Burm.f.) Spreng: traditional and commercial uses and phytochemical and pharmacological significance in the African and Indian subcontinent. *Evid. Based Compl. Altern. Med.*, v.2017, p.6746071, 2017.

NESTERKINA, M.; KRAVCHENKO, I. Synthesis and pharmacological properties of novel esters based on monocyclic terpenes and GABA. *Pharmaceuticals*, v.9, p.32, 2016.

OLIVEIRA, C.P.B.; LEMOS, C.H.P., FELIX E SILVA, A. *et al*. Use of eugenol for the anaesthesia and transportation of freshwater angelfish (*Pterophyllum scalare*). *Aquaculture*, v.513, p.734409, 2019.

OLIVEIRA, I.C.; OLIVEIRA, R.S.M.; LEMOS, C.H.P. *et al*. Essential oils from *Cymbopogon citratus* and *Lippia sidoides* in the anesthetic induction and transport of ornamental fish *Pterophyllum scalare*. *Fish. Physiol. Biochem.*, v.48, p.501-519, 2022.

SANTOS, A.C.; BANDEIRA JUNIOR, G.; ZAGO, D.C. *et al*. Anesthesia and anesthetic action mechanism of essential oils of *Aloysia triphylla* and *Cymbopogon flexuosus* in silver catfish (*Rhamdia quelen*). *Vet. Anaesth. Analg.*, v.44, p.106-113, 2017.

SENA, A.C.; TEIXEIRA, R.R.; FERREIRA, E.L. *et al*. Essential oil from *Lippia alba* has anaesthetic activity and is effective in reducing handling and transport stress in tambacu (*Piaractus mesopotamicus* x *Colossoma macropomum*). *Aquaculture*, v.465, p.374-379, 2016.

SILVA, H.N.; CARVALHO, B.C.; MAIA, J.L. *et al*. Anesthetic potential of the essential oils of *Lippia alba* and *Lippia origanoides* in tambaqui juveniles. *Ciênc. Rural*, v.49, p.e2018059, 2019.

SILVA, L.L.; SILVA, D.T.; GARLET, Q.I. *et al*. Anesthetic activity of Brazilian native plants in silver catfish (*Rhamdia quelen*). *Neotr. Ichthyol.*, v.11, p.443-451, 2013.

ZHOU, W.; YOSHIOKA, M.; YOKOGOSHI, H. Sub-chronic effects of s-limonene on brain neurotransmitter levels and behavior of rats. *J. Nutr. Sci. Vitaminol.*, v.55, p.367-373, 2009.