Original article

Anesthesia of tambaqui *Colossoma macropomum* (Characiformes: Serrasalmidae) with the essential oils of *Aniba rosaeodora* and *Aniba parviflora* and their major compound, linalool

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The aim of this study was to determine the anesthetic efficacy of the essential oils (EOs) of *Aniba rosaeodora* (EOAR) and *Aniba parviflora* (EOAP) and one of their main compounds, linalool, in two forms: synthetic and extracted from EOAR (linalool-AR) in tambaqui (*Colossoma macropomum*). In the first experiment, the anesthetic induction and recovery of juveniles exposed to 25- 200 µL L^{-1} of EOAR or 50- 300 µL L^{-1} of EOAP or synthetic linalool or linalool-AR was evaluated. The second experiment observed the behavioral effects of long-term exposure (12h) of these EOs and linalools (5 and 10 µL L^{-1}). Fish exposed to 50- 200 µL L^{-1} of EOAR and 100-300 µL L^{-1} of EOAP and both linalools reached deep anesthesia between 1-10 min. Induction time for all anesthesia stages decreased with the increasing concentration of the anesthetics. Linalool-AR showed lengthier time for anesthesia induction in some stages and for recovery at 100 and 200 µL L^{-1} in comparison to synthetic linalool. Normal equilibrium and swimming behavior was observed in fish exposed to the EOs and linalools throughout the 12 h of exposure. In conclusion, both EOs and linalools can be used as anesthetics and sedatives in tambaqui.

Keywords: Anesthetics, Linalool isomers, Rosewood oil, Sedation, Swimming behavior.

O objetivo deste trabalho foi determinar a eficácia anestésica dos óleos essenciais (OEs) de *Aniba rosaeodora* (OEAR) *Aniba parviflora* (OEAP) e um de seus compostos majoritários, linalol, em duas formas: sintética e extraída a partir de OEAR (linalol-AR) em tambaqui (*Colossoma macropomum*). No primeiro experimento avaliou-se a indução anestésica e a recuperação de juvenis expostos a 25- 200 µL L^{-1} de EOAR ou 50- 300 µL L^{-1} de EOAP ou linalol sintético ou linalol-AR. No segundo experimento observaram-se os efeitos comportamentais de uma longa exposição (12h) a estes OEs e linalois (5 e 10 µL L^{-1}). Os peixes expostos a 50-200 µL L^{-1} de EOAR e 100-300 µL L^{-1} de OEAP e ambos os linalois alcançaram anestesia profunda entre 1-10 min. Tempo de indução a todos os estágios de anestesia diminuiu com o aumento na concentração dos anestésicos. Linalol-AR levou um maior tempo para induzir anestesia e para recuperação com 100 e 200 µL L^{-1} em comparação ao composto sintético. Peixes expostos aos OES e linalois por 12 h apresentaram equilíbrio e comportamento natatório normais. Em conclusão, tanto os OEs como o linalol sintético ou natural de AR podem ser usados como anestésicos e sedativos em tambaqui.

Palavras-chave: Anestésicos, Isômeros de linalool, Natação, Óleo de palisandro, Sedação.

Introduction

The anesthetic efficacy of several vegetable extractives and essential oils (EO) have been demonstrated in fish (Keene *et al.*, 1988; Cunha *et al.*, 2010; Pádua *et al.*, 2013; Silva *et al.*, 2013a, b; Parodi *et al.*, 2014; Benovit *et al.*, 2015; Barbas *et al.*, 2016, 2017; Pedrazzani, Neto, 2016; Cunha *et al.*, 2017; Saccol *et al.*, 2017; Souza *et al.*, 2017; Bodur *et al.*, 2018), as well as some of their main compounds, as menthol (Façanha, Gomes, 2005; Mazandarani, Hoseini, 2017), globulol (Silva *et al.*, 2013a), (+)-spathulenol (Benovit *et al.*, 2015), myrcene (Mirghaed *et al.*, 2016), (+)-dehydrofukinone (Garlet *et al.*, 2016), thymol, carvacrol (Bianchini *et al.*, 2017), and 1,8-cineole (Mazandarani, Hoseini, 2017).

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Synthetic linalool (a mix of S-(+) and R-(-) isomers) presented anesthetic efficacy in common carp (*Cyprinus carpio*) (Mirghaed *et al.*, 2016) and S-(+)-linalool isolated from the EO of *Lippia alba* had a similar sedation profile to the EO of this plant at a proportional linalool concentration in silver catfish (*Rhamdia quelen*) (Heldwein *et al.*, 2014). The sedation was induced in silver catfish without differences between the compounds; however, R-(-)-linalool promoted faster anesthesia (Silva *et al.*, 2017).

Experiments with rodents demonstrated the sedative effect of the EO of *Aniba rosaeodora* Ducke (EOAR, rosewood oil), a large tree native to the Amazon region (Almeida *et al.* 2009). The major component of EOAR is linalool (90.0%, with 80% being S-(+) and 20% being R-(-) isomers) (Lupe *et al.*, 2008; Fidelis *et al.*, 2013). The EO of *Aniba parviflora* (Meisn.) Mez. (EOAP), another tree morphologically similar to *A. rosaeodora* (Galaverna *et al.*, 2015), also contains linalool (29.6%) as main compound, as well as E-caryophyllene (10.9%), α-phellandrene (10.5%), β-selinene (8.0%), jinkoh eremol (7.2%) and *p*-cymene (6.3%) (Souza, 2010).

Tambaqui *Colossoma macropomum* (Cuvier, 1816) is the most raised native species in Brazil, mainly in the Amazon region (Valladão *et al.*, 2016), where both *Aniba* trees can be found. Tambaqui has been used as a model of Amazonian fish in studies of anesthesia with vegetable extractives and isolated compounds (Façanha, Gomes, 2005; Roubach *et al.*, 2005; Inoue *et al.*, 2011; Pádua *et al.*, 2013; Barbas *et al.*, 2016, 2017; Saccol *et al.*, 2017). Consequently, the aim of the present study is to verify the anesthetic efficacy of EOAR, EOAP and one of their main compounds, linalool, in two forms: synthetic and extracted from EOAR (linalool-AR).

Material and Methods

Essential oils and linalool. The EOAR and EOAP extraction from the leaves and their analysis was performed as described by Fidelis *et al.* (2013) and Souza (2010), respectively. The synthetic linalool [(±)-3,7-Dimethyl-1,6-octadien-3-ol, (±)-3,7-Dimethyl-3-hydroxy-1,6-octadiene] was purchased from Sigma-Aldrich. Linalool-AR was extracted from EOAR as described by Zellner *et al.* (2006).

Fish and culture conditions. The experiments were conducted in a fish farm (Santarém, Pará, Brazil) under authorization of Secretaria Estadual de Desenvolvimento Agropecuário e de Pesca (SEDAP, Pará, Brazil) and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio)/ Sistema de Autorização e Informação em Biodiversidade (SISBIO, nº 35427-1). Capture and maintenance of juveniles of tambaqui (voucher number UFOPA-I 000143) was performed as described by Saccol *et al.* (2017). The experimental protocol was approved by the Committee on Ethics in Research of Universidade do Estado do Pará (Campus XII, Santarém) under registration nº 42/2012. All animal manipulations were carried out in compliance with the guidelines of ethics committee mentioned above.

Anesthesia induction and recovery. Tambaqui juveniles (2.0 ± 0.2 g, 5.0 ± 0.2 cm) were individually transferred to continuously aerated aquaria containing 500 mL of water and 25, 50, 100 or 200 µL L^{-1} of EOAR or 50, 100, 200 or 300 µL L^{-1} of EOAP or synthetic linalool or linalool-AR, first diluted in ethanol (1:10) to enable better dilution in water. Previous tests were made with 200 µL L^{-1} of both EOs and compounds, based on studies of Cunha *et al.* (2010) with the EO of *Lippia alba* chemotype linalool (around 47% linalool) and Heldwein *et al.* (2014) and Silva *et al.* (2017) with linalool in silver catfish. Based on the time to reach deep anesthesia using this concentration, the concentration range to be tested with each EO or compound was chosen. The maximum evaluation time was 30 min, and 10 juveniles were used for each concentration tested; each juvenile was only used once. Ethanol alone did not produce any anesthetic effect in tambaqui (Saccol *et al.*, 2017). The evaluation of each anesthesia stage was an adaptation of Small (2003): stage I - light sedation, with decreased reactivity to external stimuli; stage II - deep sedation, with partial loss of equilibrium and erratic swimming; and stage III - deep anesthesia, with a total loss of equilibrium, cessation of locomotion and no response to tactile stimuli (pressure of a glass rod on the caudal peduncle).

Long-term exposure. The long-term exposure was performed to analyze the use of the EOs and linalool for fish transport. Juveniles were maintained individually for 12 h (n=10 each concentration tested) in continuously aerated 1 L aquaria containing 5 or 10 µL L^{-1} of each tested EO or linalool. Tambaquis exposed to 25 µL L^{-1} EOAR reached deep sedation within 30 min (see results) and consequently the concentrations chosen for log-term exposure were lower than this concentration to avoid fish reaching anesthesia as time went by, which is not recommended for transportation (Becker *et al.*, 2012). Swimming behavior was observed every hour up to the end of the experiment, to check if fish showed agitation (sudden swimming bursts) or any loss of equilibrium. The temperature was 25.6-26.4ºC and dissolved oxygen levels (YSI multiparameter- Professional plus-) were above 6.0 mg L^{-1} throughout the experiment.

Statistical analyses. The data are expressed as mean ± SEM. The evaluation of anesthetic activity was performed by regression analysis (concentration x time of anesthesia induction; concentration x time of recovery from anesthesia) using Sigma Plot 11.0 software. When no relationship was found, homogeneity of variances between concentrations was tested with the Levene test. Data were log transformed to obtain homoscedasticity and then were analyzed using one-way ANOVA and Tukey's test. Comparisons between synthetic linalool and linalool-AR were tested by two-way ANOVA (linalool type x concentration). All statistical analyses were performed using the software Statistica 7.0. Minimum significance level was $P<0.05$.

Results

Anesthesia induction and recovery. No fish died during the course of the anesthesia experiment. Induction time for all anesthesia stages decreased as the concentrations of EOAR, EOAP, synthetic and linalool-AR were raised, but a significant relationship for concentration x anesthesia induction time was not observed in the stage of light sedation for EOAR and linalool-AR (Figs. 1-2). Fish exposed to 25 µL L^{-1} EOAR and 50 µL L^{-1} of EOAP and both linalools did not reach deep anesthesia (Tabs 1-2). Linalool-AR showed a longer time for anesthesia induction in some stages and for recovery at 100 and 200 µL L^{-1} (Tab. 2) in comparison to the synthetic mixture. Recovery time was lengthened as the concentration of EOAR and synthetic linalool increased and from 50 to 100 µL L^{-1} linalool-AR. The recovery time was not affected significantly by EOAP concentration (Tabs. 1-2). A significant relationship for concentration x recovery time was observed only in fish anesthetized with synthetic linalool (Fig. 2a).
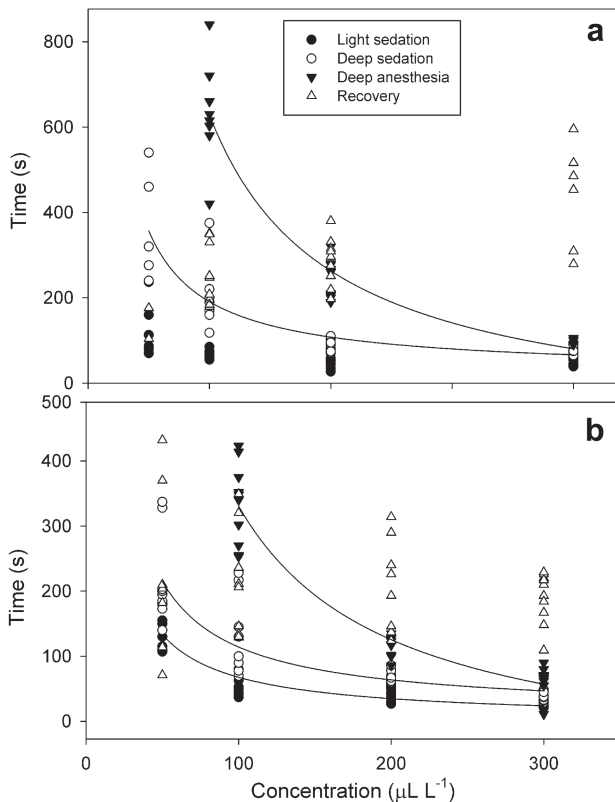

Fig. 1. Relationships of concentration x anesthesia induction or recovery time in tambaqui, *Colossoma macropomum*, exposed to the essential oils. **a.** *Aniba rosaeodora* (EOAR); deep sedation: y=24.8+(8300/x), r^2=0.705, deep anesthesia: y=-102.3+(36527/x), r^2=0.913. Light sedation and recovery: no significant relationship. **b.** *Aniba parviflora* (EOAP); light sedation: y=2.08+(6563/x), r^2=0.832, deep sedation: y=13.2+(10098/x), r^2=0.703, deep anesthesia: y=-79.8+(41067/x), r^2=0.906. Recovery: no significant relationship. y = time to reach stage or recovery (s) and x = concentration (µL L^{-1}).
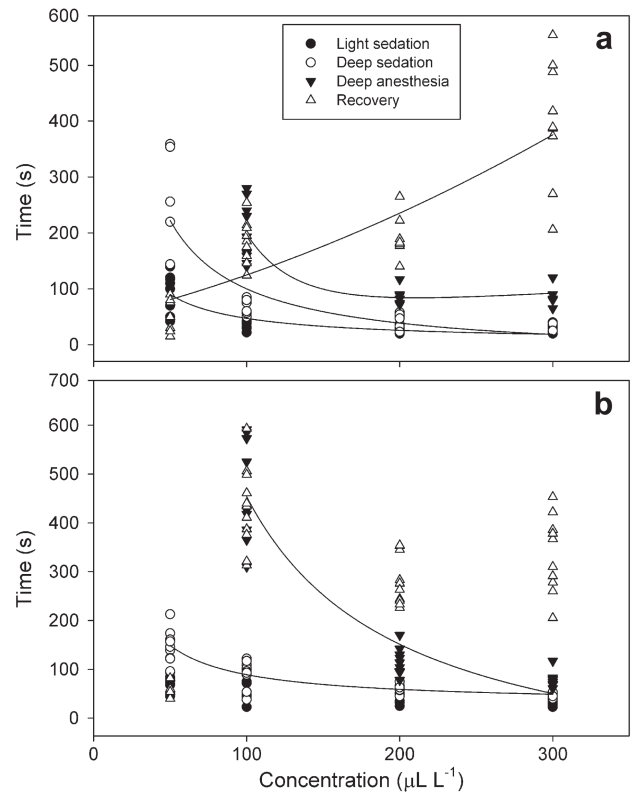

Fig. 2. Relationships concentration x anesthesia induction or recovery time in tambaqui, *Colossoma macropomum*, exposed to the linalools. **a.** synthetic linalool; light sedation: y=4.4+(4281/x), r^2=0.716, deep sedation: y=-22.5+(12252/x), r^2=0.773, deep anesthesia: y=15.3+(17878/x), r^2=0.669, recovery: y=43.9+0.66x+0.0015x^2, r^2=0.712. **b.** linalool extracted from *Aniba rosaeodora*; deep sedation: y=29.0+(6010/x), r^2=0.784, deep anesthesia: y=-151.3+(60541/x), r^2=0.873. Light sedation and recovery: no significant relationship. y = time to reach stage or recovery (s) and x = concentration (µL L^{-1}).

Tab. 1. Time required for induction and recovery from anesthesia using the essential oils of *Aniba rosaeodora* (EOAR) and *Aniba parviflora* (EOAP). Different letters in the column indicate significant difference between concentrations. One-way ANOVA and Tukey's test (P<0.05). Stages in which there was a significant relationship concentration x time of anesthesia induction, no statistical comparison between concentrations was made.

Concentration (µL L^{-1})	Induction (s)			
	Light sedation	Deep sedation	Deep anesthesia	Recovery (s)
	EOAR			
25	123.0±11.8[a]	355.1±41.1	-	144.0±12.6[a]
50	68.3±3.4[b]	204.1±26.8	636.4±37.5	264.6±23.2[b]
100	50.5±5.3[c]	87.8±4.6	240.9±14.3	285.9±19.0[b]
200	48.6±2.4[c]	78.6±3.1	98.7±2.1	419.4±36.5[c]
	EOAP			
50	140.9±10.5	212.2±20.8	-	226.1±49.4[a]
100	46.4±3.0	120.8±18.7	333.2±19.6	199.5±25.7[a]
200	42.9±2.3	67.6±2.7	116.4±4.9	212.2±22.5[a]
300	27.0±1.7	39.5±2.2	64.0±6.6	190.3±12.3[a]

Tab. 2. Time required for induction and recovery from anesthesia using synthetic linalool and linalool extracted from the essential oil of *Aniba rosaeodora* (linalool-AR). *significantly different from synthetic linalool. Different letters in the column indicate significant difference between concentrations. One-way ANOVA and Tukey's test (P<0.05). Stages in which there was a significant relationship concentration x time of anesthesia induction, no statistical comparison between concentrations was made.

Concentration (µL L^{-1})	Induction (s)			
	Light sedation	Deep sedation	Deep anesthesia	Recovery (s)
Synthetic linalool				
50	95.6±9.5	234.7±25.2	-	48.8±9.2
100	32.7±2.4	68.9±4.0	198.7±16.5	184.6±3.1
200	27.7±2.2	41.5±4.3	84.3±4.8	195.6±11.9
300	25.6±1.6	34.5±1.7	92.1±6.7	386.4±35.8
Linalool-AR				
50	69.7±5.1[a]	147.7±10.1*	-	60.8±5.1[a]
100	61.8±7.1[a]*	92.1±8.4	464.3±33.4*	431.0±25.0[b]*
200	40.7±3.4[b]	61.8±2.4*	114.6±8.6	281.8±16.1[c]*
300	30.7±2.5[b]	44.7±1.6	78.1±4.8	335.0±24.8[bc]

Long-term exposure. Fish exposed to both concentrations of EOs and linalools did not show any loss of equilibrium or sudden swimming bursts throughout the 12 h of exposure.

Discussion

Time for tambaqui to reach anesthesia reduced as the concentration of EOAR, EOAP and both linalools increased. The same pattern was observed for juveniles of this species with the same size of the present study when exposed to the essential oils from the leaves of *Myrcia sylvatica*, *Curcuma longa* (Saccol *et al.*, 2017) and *Nectandra grandiflora* (Barbas *et al.*, 2017), as well as 56-88 g specimens with the compounds eugenol (Roubach *et al.*, 2005) and menthol (Façanha, Gomes, 2005). The increased concentration of synthetic linalool and the S-(+) and R-(-) isomers also decreased anesthesia induction time in common carp (4-5 g) (Mirghaed *et al.*, 2016) and silver catfish (4-9 g) (Heldwein *et al.*, 2014; Silva *et al.*, 2017), respectively. The increase in time for anesthesia recovery with the increase in EOAR and synthetic linalool concentrations in tambaqui was also observed in this species when anesthetized with the essential oil of *N. grandiflora* (Barbas *et al.*, 2017), eugenol (Roubach *et al.*, 2005) and menthol (Façanha, Gomes, 2005) and in silver catfish anesthetized with both linalool isomers (Silva *et al.*, 2017). However, recovery time showed no relationship with synthetic linalool concentration in common carp (Mirghaed *et al.*, 2016) with either concentration of the essential oils of *M. sylvatica* and *C. longa* in tambaqui (Saccol *et al.*, 2017). Recovery time from anesthesia varies with the anesthetic and the species analyzed; the present study demonstrated that the EOAP concentration did not change recovery time in tambaqui.

Exposure to 25 and 50 µL L^{-1} EOAR was sufficient to induce sedation and deep anesthesia in tambaqui; therefore, this essential oil was more efficient than EOAP, synthetic linalool and linalool-AR because they need two-fold concentrations to provoke the same effects. Apparently, some minor compounds of EOAR (all less than 10%) contribute to improve the anesthetic efficacy of linalool. EOAP anesthetic induction time is within the synthetic linalool and linalool-AR. As EOAP contains only 29.6% linalool, the other compounds are probably involved in its anesthetic effect. E-caryophyllene is a major compound of the essential oil of *L. alba* linalool chemotype, which anesthetizes silver catfish (Souza *et al.*, 2017), and *p*-cymene is an important compound of the essential oil of *Cinnamomum camphora*, which has anesthetic activity in clown anemonefish (*Amphiprion ocellaris*) (Pedrazzani, Neto, 2016). α-phellandrene and *p*-cymene are major compounds of the essential oil of *Foeniculum vulgare*, which reduces anxiety in mice (Mesfin *et al.*, 2014), β-selinene is a major compound of the essential oil of *M. sylvatica* with anesthetic properties (Saccol *et al.*, 2017). Peritoneal and intracerebroventricular injection of jinkoh eremol decreased both methamphetamine- and apomorphine-induced spontaneous motility in mice (Okugawa *et al.*, 1996).

The essential oil of *M. sylvatica* needed the same concentrations as EOAP to induce sedation and deep anesthesia in tambaqui (Saccol *et al.*, 2017), but the essential oils of *C. longa* and *N. grandiflora* were less efficient in specimens of the same size (Saccol *et al.*, 2017; Barbas *et al.*, 2017). The waxy extract of the flowers of *Spilanthes acmella* presented a better anesthetic efficiency in 46 g tambaqui because 5 mg L^{-1} was sufficient to induce sedation and deep anesthesia (Barbas *et al.*, 2016, 2017).

Exposure to 50 µL L^{-1} synthetic linalool also induced light sedation in common carp, but only fish exposed to 200 µL L^{-1} reached deep anesthesia (Mirghaed *et al.*, 2016), and in both situations induction time was longer than for tambaqui. Time to induce light sedation with 60 µL L^{-1} S-(+) and *R*-(-)-linalool in silver catfish (Silva *et al.*, 2017) is similar to that for synthetic and linalool-AR in tambaqui, but silver catfish took longer to reach deep anesthesia with both linalool isomers. However, in this context, it is noteworthy that tambaqui experiments were carried out at higher water temperatures than those used in silver catfish, which can explain the differences observed in the recovery time. The fish size and water parameters from these studies were also different, and as anesthesia induction time may be affected by these parameters (Gomes *et al.*, 2011), they may also explain these differences.

The *R*-(-)-linalool promoted faster anesthesia in silver catfish within the 180-500 µL L^{-1} range compared to the S-(+) isomer (Silva *et al.*, 2017). The same authors also demonstrated that synthetic linalool contains almost equal concentrations of both isomers. Thus, the higher percentage of the *R*-(-)-linalool isomer in the synthetic sample explains why linalool-AR (20% of the *R*-(-)-isomer) took slightly longer to induce some stages of anesthesia in tambaqui.

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The transport of common carp for 3 h with 50-200 µL L^{-1} synthetic linalool was not recommended because it decreased dissolved oxygen levels, has no benefits in preventing ion loss and increased stress compared to the control fish (Mazandarani *et al.*, 2017). However, exposure of tambaqui for 12 h to 5 or 10 µL L^{-1} of EOAR, EOAP, synthetic linalool and linalool-AR did not change equilibrium and swimming behavior. Therefore, these concentrations may be tested in the transport of this species evaluating ionoregulatory parameters and stress response.

In conclusion, considering anesthesia induction and recovery time, EOAR, EOAP, synthetic linalool and linalool-AR can be used as anesthetics and probably as sedatives for the transport in tambaqui. Additional studies must investigate whether these anesthetics can avoid or reduce stress provoked by handling and/or transportation.

Acknowledgments

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