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Short communication

Lippia alba essential oil as anesthetic for tambaqui

Erix dos Santos Batista[a], Franmir Rodrigues Brandão[b], Cláudia Majolo[c], Luís Antonio Kioshi Aoki Inoue[d], Patricia Oliveira Maciel[e], Marcelo Róseo de Oliveira[c], Francisco Célio Maia Chaves[c], Edsandra Campos Chagas[b,c,*]

[a] Uninorte Laureate International Universities, Av. Leonardo Malcher, 715 – Centro, 69020-010 Manaus, AM, Brazil
[b] Programa de Pós-graduação em Ciências Pesqueiras nos Trópicos, Universidade Federal do Amazonas, Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, 69067-005, Manaus, AM, Brazil
[c] Embrapa Amazônia Ocidental, Rodovia AM 010-km 29, Caixa postal 319, 69010-970, Manaus, AM, Brazil
[d] Embrapa Agropecuária Oeste, Rodovia BR 163, Km 253,6 - Cx. Postal 449, Zona Rural, 79804-970, Dourados, MS, Brazil
[e] Embrapa Pesca e Aquicultura, Caixa postal 90, 77008-900, Palmas, TO, Brazil

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ABSTRACT

The study evaluated the anesthetic activity of *Lippia alba* essential oil (EO) in tambaqui (*Colossoma macropomum*). For this, two assays were realized. The first experiment determined the time to induce tambaqui anesthesia using *L. alba* EO at 20, 50, 100, 200 and 300 mg L^{-1}. In the second were evaluated the biochemical response of fish to the stress caused by handling and anesthesia with 0, 50 and 100 mg L^{-1} of *L. alba* EO. In tambaquis the anesthetic action of *L. alba* EO was obtained using doses of 50 to 300 mg L^{-1}. Doses from 200 to 300 mg L^{-1} caused the faster anesthesia (< 4 min), whereas 100 mg L^{-1} induced anesthesia in < 10 min. Glucose, lactate and ammonia levels increased immediately after 10 min of fish anesthesia with 50 and 100 mg L^{-1} of *L. alba* EO and handling stress, but they recovery from the procedures after 24 h. The results indicated that *L. alba* EO can be used to induced faster anesthesia in tambaquis (200–300 mg L^{-1}), but the tambaqui exposure to doses of 50 and 100 mg L^{-1} of *L. alba* EO for 10 min did not prevent the effects of handling stress.

1. Introduction

Tambaqui (*Colossoma macropomum*) is the main native fish species produced in Brazil, yielding 136,990 tons in 2016 (IBGE, 2016). Its body weight reaches 2.62 kg, and under intensive and aerated farming, generates 18,530 kg ha^{-1} in a 10-month rearing period (Izel et al., 2013). Different procedures are used for growth and health monitoring during tambaqui farming. However, intense and long-term protocols eventually cause stress, which can compromise the immune system, promote pathogen dissemination and ultimately cause partial or even total loss of production (Valladão et al., 2016; Tavares-Dias and Martins, 2017).

A number of techniques are applied to attenuate stress during routine fish farming procedures, including the use of anesthetics (Sink and Neal, 2009). Anesthetics in particular have been widely used in fish management to prevent body injuries and facilitate handling (Velisek and Svobodova, 2004). In addition, low anesthetic doses are applied to reduce negative stress and mortality during juvenile fish transportation (Gressler et al., 2017). The most commonly used anesthetics in fish farming are tricaine methanesulfonate (MS 222), benzocaine, quinaldine, 2-phenoxyethanol and eugenol (Velisek and Svobodova, 2004; Inoue et al., 2011; Stringhetta et al., 2017).

In recent years, essential oils (EO) extracted from medicinal plants have been tested as potential anesthetics for fish. Some of the essential oils studied were obtained from *Lippia alba* (da Cunha et al., 2010; Toni et al., 2014; Souza et al., 2018), *Ocimum gratissimum* (Boijink et al., 2016; Ribeiro et al., 2016), *Hesperozygis ringens* (Toni et al., 2014, 2015a), *Nectandra grandiflora* (Barbas et al., 2017a), *Cymbopogon nardus* (Barbas et al., 2017b), among others. The major components of EO extracted from *L. alba*, commonly known as bushy matgrass (or “erva cidreira” in Brazil), are citral, carvone and linalool (Tavares et al., 2005). This plant has been frequently used as analgesic, sedative and anxiolytic medication, as well as mucolytic and antimicrobial agents (Lorenzi and Matos, 2008).

Considering the abovementioned facts and the lack of information on the use of EO in Amazonian fish, the present study aimed at assessing the anesthetic activity of *Lippia alba* in tambaqui (*Colossoma macropomum*) by determining the anesthetic induction time and

* Corresponding author at: Embrapa Amazônia Ocidental, Manaus, AM, Brazil.
E-mail address: edsandra.chagas@embrapa.br (E.C. Chagas).

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Table 1
Predominant components of *Lippia alba* essential oil.

Components	*L. alba* (%)	RI[a]
Myrcene	2.0	992
Linalool	1.5	1102
Terpinen-4-ol	1.2	1178
Neral	16.6	1254
Geranial	25.4	1286
β-Elemenene	2.0	1394
β-Caryophyllene	6.6	1417
β-Selinene	1.7	1490
α-Selinene	1.3	1496
Caryophyllene oxide	16.0	1579

[a] Retention Index in HP-5.

physiological stress response to EO exposure and fish handling during biometric procedures.

2. Material and methods

2.1. Animals

The juvenile tambaqui (n = 255; 70.4 ± 1.9 g and 15.6 ± 0.4 cm) were obtained from the Santo Antônio Farm (Rio Preto da Eva, AM) and transported to the experimental field at Embrapa Amazônia Ocidental (Manaus, AM), where they were held in 1000-L fiberglass tanks supplied with water recirculation and constant aeration for 30 days. The water parameters were monitored and the mean values were: temperature 28.7 ± 0.5 °C, dissolved oxygen 6.3 ± 0.3 mg L^{-1}, pH 7.4 ± 0.5, alkalinity 17.2 ± 3.3 mg L^{-1} and total ammonia 0.76 ± 0.1 mg L^{-1}. In this period, fish were fed commercial food for omnivorous fish containing 32% crude protein (CP).

2.2. Plant species, extraction and chemical composition of the essential oil

The plants of *L. alba* were cropped in the Medical Plants and Vegetables Division of Embrapa Amazônia Ocidental, Manaus, Amazonas state (AM). The branches were cut and the leaves separated and dried in the shadow until reaching constant weight. In the Laboratory of Medical Plants and Phytochemistry, essential oil from the dried leaves was extracted by hydro distillation for 2 h in a Clevenger-type apparatus. The chemical composition of *L. alba* EO was determined by gas chromatography and mass spectrometry at Embrapa Agroindústria de Alimentos (Table 1).

2.3. Experiment 1. Anesthetic induction and recovery

Five concentrations of *L. alba* EO (20, 50, 100, 200 e 300 mg L^{-1}) were established to evaluated the anesthetic induction and recovery of fish. Fifteen fish were used for each concentration of EO evaluated and the fish was used only once. The anesthetic induction was conducted in aquaria of 4 L and the recovery in tanks of 310 L. The EO was diluted in ethanol (1:10). The time of anesthesia induction (in seconds) was recorded according to anesthetic stages established by Woody et al. (2002). After anesthesia induction, fish were transferred to anesthetic-free tanks to evaluate the time of fish recovery (in seconds) that was characterized by return to normal swimming and reaction to external stimuli. Fish were observed for 10 days, counted and the experiment finished.

2.4. Experiment 2. Evaluation of stress response during handling

Tambaquis were transferred to 20 L boxes and exposed to anesthetic concentrations (50 and 100 mg L^{-1}) and the control treatment for 10 min and then simulated the procedure for handling biometrics. These concentrations led to sedation and anesthesia and were chosen based on the previous experiment, since in those concentrations there were no mortalities, even after exposures longer than 10 min. Fish from the basal group were not handled or exposed to *L. alba* EO. After this, blood samples were collected from nine fish per treatment in two times: 1) immediately after anesthesia and handling, 2) 24 h after the procedures, for evaluation of recovery. The fish sampled left the experiment and the remaining fish remained in the same initial conditions for observation for 10 days, when mortality data were recorded.

Blood collection was performed by caudal vein puncture with heparinized syringes (5.000 UI) and the plasma aliquots were separated for determinations of glucose and lactate using commercial kits (Labtest, MG, Brazil) and ammonia as described by Gentzkow and Mansen (1942).

2.5. Statistical analysis

The results obtained are expressed as mean ± standard error. Anesthetic induction and recovery were evaluated by analysis of variance (one-way ANOVA) followed by Tukey's test ($P < 0.05$). Blood parameters of fish subjected to handling stress were evaluated by analysis of variance (two-way ANOVA) followed by Tukey's test ($P < 0.05$).

3. Results

3.1. Anesthetic induction and recovery

In all concentrations of *L. alba* EO evaluated, changes in fish behavior were observed. After 6 h of exposure at the 20 mg L^{-1} *L. alba* EO the fish presented only moderate loss of balance with some difficulty in maintaining the normal swimming position, remaining in stage 2 of anesthesia (Table 2). The fish reached stage 3 of anesthesia, characterized by total loss of equilibrium and inability to regain the upright position in concentration of 50 mg L^{-1} or higher. In addition, concentrations of 200 and 300 mg L^{-1} induced faster anesthesia (< 4 min), while the concentration of 100 mg L^{-1} promotes tambaquis anesthesia in < 10 min (Table 2).

The time required to equilibrium reestablishment of fish after anesthesia induction with *L. alba* EO was similar for all treatments (Table 2). No fish mortality was observed even 10 days after the tests.

Table 2
Time required for tambaqui (*Colossoma macropomum*) anesthesia induction and recovery using different concentrations of *Lippia alba* essential oil.

Lippia alba (mg L^{-1})	Time (seconds)				
	Stage 1	Stage 2	Stage 3	Stage 4	Recovery
20	285,9 ± 18,7^{a}	631,3 ± 32,4^{a}	–	–	–
50	159,9 ± 8,6^{ab}	352,2 ± 20,9^{ab}	2505,3 ± 87,9^{a}	3120,9 ± 94,5^{a}	173,7 ± 20,1^{a}
100	75,7 ± 4,2^{bc}	215,8 ± 15,3^{bc}	502,3 ± 40,4^{a}	743,5 ± 38,2^{a}	134,0 ± 13,7^{a}
200	63,8 ± 0,5^{cd}	95,9 ± 6,3^{cd}	207,4 ± 17,0^{ab}	265,8 ± 13,7^{ab}	127,9 ± 9,8^{a}
300	40,6 ± 2,9^{d}	56,9 ± 2,6^{d}	100,4 ± 12,5^{b}	153,6 ± 17,3^{b}	115,4 ± 10,9^{a}

Different letters indicate significant differences between the treatments as determined by one-way ANOVA and Tukey's test ($P < 0.05$).