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Short communication

Is monoterpene terpinen-4-ol the compound responsible for the anesthetic and antioxidant activity of *Melaleuca alternifolia* essential oil (tea tree oil) in silver catfish?

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A R T I C L E I N F O

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A B S T R A C T

The aim of this study was to evaluate the anesthetic efficacy of tea tree oil (TTO; *Melaleuca alternifolia*) and its three main compounds: terpinen-4-ol, γ-terpinene, and α-terpinene, in silver catfish (*Rhamdia quelen*). We also aimed to investigate which of these compounds may be responsible for the anesthetic effect, as well as the effect of prolonged exposure on oxidative parameters. Fish were exposed to 100, 300, 500, 800, and 1000 μL L^{-1} of TTO, and the corresponding concentrations of terpinen-4-ol, γ-terpinene, and α-terpinene in TTO; anesthesia induction time was recorded based on fish behavior. Anesthesia induction time with 300–1000 μL L^{-1} TTO was 185–1.512 s, and with terpinen-4-ol was 103–630 s; for both, the recovery time was 134–673 s. The monoterpenes α-terpinene and γ-terpinene were incapable of causing sedation and anesthesia in *R. quelen*, but presented with an antagonistic effect when used in association with terpinen-4-ol. Based on this evidence, it can be concluded that terpinen-4-ol itself caused an anesthetic effect, and therefore, can be considered the compound responsible for the anesthetic effect of TTO. Long-term (6 h) exposure to TTO (25 μL L^{-1}) and terpinen-4-ol (42 μL L^{-1}) reduced thiobarbituric acid-reactive substances levels in the liver, and terpinen-4-ol (42 and 20 μL L^{-1}) reduced lipid hydroperoxide in the liver and kidney. Glutathione-S-transferase activity demonstrated an increase in the liver of silver catfish exposed to terpinen-4-ol (20 and 42 μL L^{-1}), and increased in the kidney of silver catfish exposed to TTO at concentration of 25 μL L^{-1} compared to the control group. In summary, TTO and terpinen-4-ol may be recommended to induce faster anesthesia, and may be suitable as sedatives for transport, because they improved the antioxidant status of silver catfish.

1. Introduction

Farmed fish are frequently exposed to a variety of stressors that can affect their health and survival during aquaculture practices. These stressors usually cause biochemical and physiological disorders, as well as behavioral changes as a result of coping mechanisms (Barton et al., 2000; Davis, 2006). In order to minimize these stress-induced changes, anesthetics have been widely used during handling procedures and as sedatives added to water for transport (Azambuja et al., 2011).

Numerous factors are considered when choosing an ideal anesthetic agent, such as efficacy, availability, cost, ease of use, and safety for the user and the environment. In addition, an ideal anesthetic should produce anesthesia within 180 s, allow recovery within 300 s, and cause no toxicity to fish at treatment levels (Marking and Meyer, 1985). In recent decades, several products have been recommended as fish anesthetics; the most frequently recommended products include tricaine methane–sulfonate (MS –222), quinaldine, benzocaine, and 2–phenoxyethanol. However, these synthetic anesthetics are expensive and can, paradoxically, have side effects that decrease fish welfare (Zahl et al., 2010) through elevated cortisol levels, acidosis, and ionoregulatory stress (Palić et al., 2006). Based on this evidence, it is necessary to develop new alternative products that do not present such side effects. For this purpose, numerous researchers have tested the potential anesthetic effects of natural compounds in fish, such as the essential oils (EOs) of *Lippia alba* and *Spilanthes acmella* (Silva et al., 2013; Saccol et al., 2016; Barbas et al., 2017), and the successful fish anesthetization

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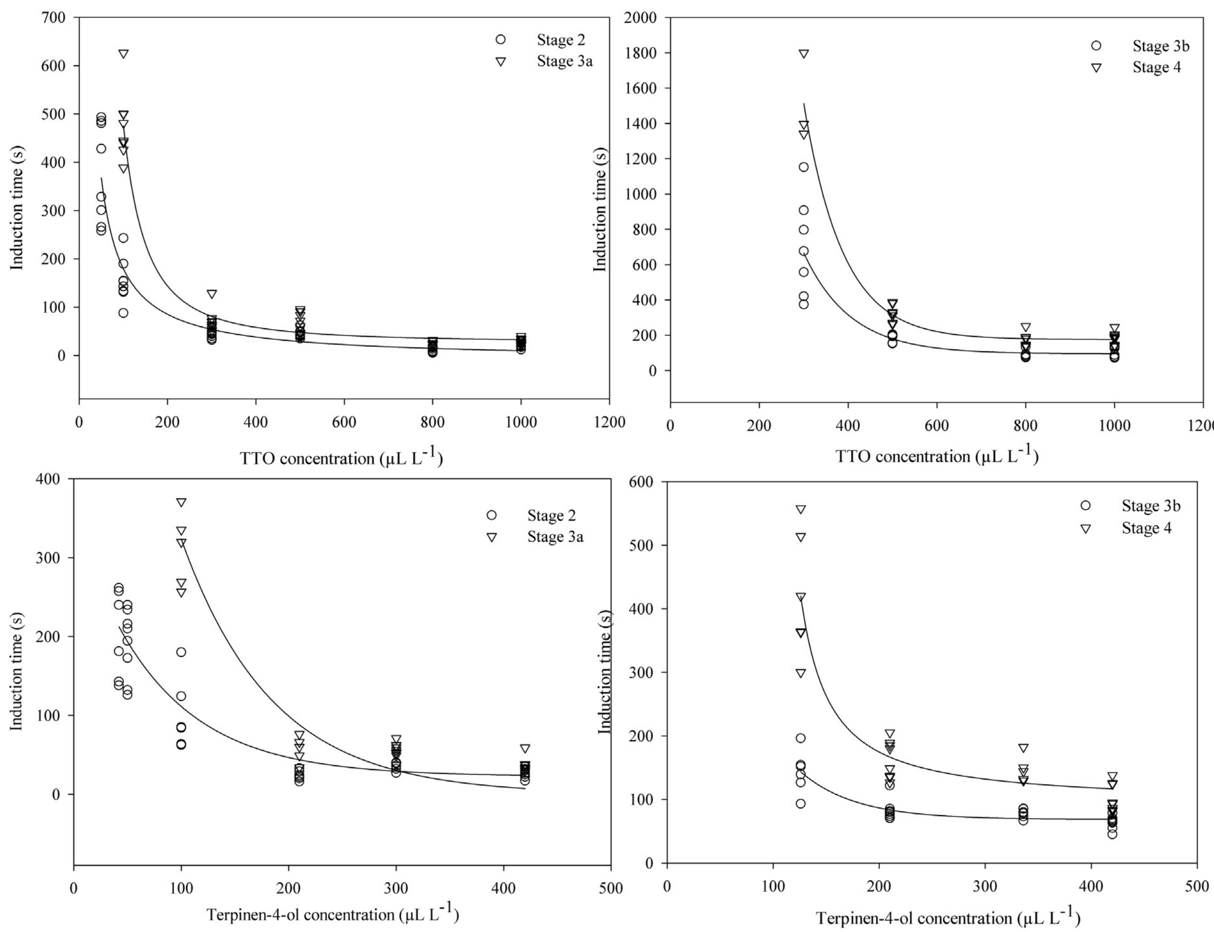

Fig. 1. Time required for the induction of sedation and anesthesia in silver catfish (*Rhamdia quelen*) with *Melaleuca alternifolia* essential oil (TTO) and terpinen4-ol. [A] stage 2: $y = 26.46 + (1923.75/x) + (4{,}299{,}242.17/x^2)$, $r^2 = 0.979$; and stage 3a: $y = -8.91 + (18{,}818.58/x)$, $r^2 = 0.9431$. [B] stage 3b: $y = 94.76 + 9926.45e^{-0.009x}$, $r^2 = 0.8908$; and stage 4: $y = 175.82 + 39{,}644.07e^{-0.011x}$, $r^2 = 0.9825$. [C] stage 2: $y = 22.83 + 329.45e^{0.0131x}$, $r^2 = 0.913$; and stage 3a: $y = 1057.56e^{-0.01x}$, $r^2 = 0.9623$. [D] stage 3b: $y = 92.42e^{81.18/(x - 72.35)}$, $r^2 = 0.9257$; and stage 4: $y = 1119.88 + 5037.94e^{-0.022x}$, $r^2 = 0.9237$. The equations fitted above represent a relationship between the time of anesthesia and concentration of TTO, where y = time to reach the stage and x = concentration of TTO (in $\mu L\ L^{-1}$).

is obtained from appropriate anesthetic concentration and exposure time (Hoseini and Nodeh, 2013). Therefore, anesthetic efficacy of the potential agents must be investigated in different fish species, to measure the optimum concentrations for each.

Melaleuca alternifolia is a native plant of Australia, and its essential oil (tea tree oil; TTO) is widely used for medicinal purposes due to its excellent antiseptic activity; it is a potent agent against a wide range of microorganisms (fungi, viruses, bacteria, and parasites) and also presents with antioxidant properties (Baldissera et al., 2014; Baldissera et al., 2017). In aquaculture, TTO can be used successfully as an antiparasitic and antibacterial agent (Valladão et al., 2016; Souza et al., 2016). In addition, studies have demonstrated that TTO can also be used as an anesthetic for common carp (*Cyprinus carpio*) (Hajek, 2011) and gilthead seabream (*Sparus aurata*) (Golomazou et al., 2016). TTO has a complex chemical composition, containing > 100 components; terpinen-4-ol, α-terpinene, and γ-terpinene are the major constituents (Baldissera et al., 2014). Terpinen-4-ol is the main active constituent of TTO, and study has demonstrated that terpinen-4-ol, when used alone, produces similar or more effective results to pure TTO in relation to antimicrobial activity (Mondello et al., 2006).

The composition of EOs can vary according to chemotype, plant age, extraction method, and harvest time (Souza et al., 2017a), affecting their anesthetic efficacy. Thus, it is of interest to determine the anesthetic efficacy of compounds present in these EOs. In this sense, it is worth noting that there are many plant-derived terpenoids in EOs that possess analgesic and anesthetic effects, e.g., myrcene, linalool, menthol, limonene, and 1,8-cineole (Taheri Mirghaed et al., 2016; Mazandarani et al., 2017). For example, terpinen-4-ol was tested as a sedative for silver catfish (*Rhamdia quelen*), but only up to the concentration of 10 $mg\ L^{-1}$ (Silva et al., 2013).

Thus, the aim of this study is to evaluate the efficacy of TTO and its main isolated compounds (terpinen-4-ol, γ-terpinene, and α-terpinene) in order to define the best compound and its ideal concentrations for anesthesia and sedation of *R. quelen*. In addition, plasma biochemical indices and oxidative stress biomarkers in the liver and kidney of fish were investigated after long-term exposure (6 h).

2. Material and methods

2.1. Preparation of anesthetics

The TTO was obtained from Delaware (Brazil) and its composition was analyzed by gas chromatography-flame ionization detection using an Agilent Technologies 6890 N system, equipped with a DB-5 capillary column connected to a flame ionization detector, as recently described in detail by Baldissera et al. (2017). Fifteen compounds were identified in TTO, representing 95.86% of the total composition; terpinen-4-ol (41.98%) was the most abundant compound, followed of γ-terpinene (20.15%), and α-terpinene (9.85%) (Baldissera et al., 2017). Terpinen-4-ol, γ-terpinene, and α-terpinene were purchased from Sigma Aldrich Corporation (St. Louis, United States), and their purity was 97%, 95%, and 85%, respectively. All agents were dissolved in ethanol before tests.