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Anesthetic and anthelminthic effects of clove basil (*Ocimum gratissimum*) essential oil for tambaqui (*Colossoma macropomum*)

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A B S T R A C T

Expensive imported drugs that are sometimes not available in the market are used to anesthetize and avoid handling stress in farmed tambaquis and to control and prevent parasites, especially monogeneans. Given that the use of these chemicals poses intoxication risks to fish, farm workers, consumers and the environment; the present study evaluated the anthelmintic and anesthetic properties of a natural product, clove basil (*Ocimium gratissimum*) essential oil (OgEO), in tambaqui farming. The first experiment assessed the anthelmintic effects of tambaqui exposure to OgEO at 0, 5, 10 and 15 mg L^{-1} in 15-min immersion baths; the second evaluated fish blood parameters after 15-min baths in 15 and 60 mg L^{-1} OgEO; and the final experiment assessed anesthesia induction time using OgEO at 20, 50, 100, 200 and 300 mg L^{-1}. OgEO showed anthelmintic and anesthetic effects at concentrations of at least 15 mg L^{-1}. Fish serum ammonia increased after the baths because of both handling and OgEO exposure, but after 24 h it returned to normal levels, which were exhibited by fish that were not subjected to stress conditions. The findings indicate that the essential oil of clove basil is a safe and efficient ingredient to be used in natural anthelmintic and anesthetic products for tropical fish farming.
Statement of relevance: Essential oils can anesthetize and treat ectoparasites of farmed fish at minimum stress level.

1. Introduction

Tambaqui is the main fish species farmed in the Amazon. Its meat is widely sold in large local markets, especially in Manaus. With easy fry production, good acceptance of artificial and complete feed, high growth rate and desirable feed conversion ratio, tambaquis are suitable for farming (Araújo-Lima and Gomes, 2005). Nevertheless, conditions such as high stocking density, excessive feeding, and stress management can compromise intensive tambaqui farming since they promote health problems and immunodeficiency, favoring infestation by parasites (Martins et al., 2001). In this scenario, therapeutic products need to be developed to treat fish and avoid economic losses.

Infestation by monogeneans is a serious health problem that has affected tambaqui farming (Varella et al., 2003). Monogeneans have an organ called haptor in the posterior part of their bodies, which consists of a set of hooks, bars and anchors that attach it to the host. The lesions caused by parasite attachment facilitate the penetration of secondary pathogens such as fungi and bacteria. As a response to eliminate the parasites, the host produces a large amount of mucus, which compromises gas exchange and causes fish death by asphyxia.

Chemical products such as parathion methyl, mebendazole, formaldehyde, potassium permanganate, sodium chloride, acetic acid and hydroperoxide, developed primarily for agricultural purposes, and can be used to treat monogenean-infested fish (Kabata, 1985; Pavanelli et al., 2002; Thatcher, 1991). However, when improperly used, these drugs can be toxic to fish and pose a risk to the health of workers and consumers and to the environment. In the search for safer products, investigations have focused on the development of natural products that can replace conventional drugs in aquaculture. Plant species with well-known medicinal activity can provide bioactive compounds to combat microorganism and gill parasites at low-cost without damaging the environment (Knaak and Fiúza, 2010). Essential oils from *Lippia sidoides* and *Mentha piperita* have effects against monogenean for tilapia (Hashimoto et al., 2016), and *Lippia alba* for tambaqui (Soares et al., 2016).

Several studies, however, showed that in anti-parasite treatment, the use of medicinal plants and their derivatives can be toxic to the host (Sutili et al., 2014; Hashimoto et al., 2016; Soares et al., 2016).

Abbreviations: OgEO, clove basil (*Ocimium gratissimum*) essential oil; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

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Therefore, the administration dose and toxic effects of these natural products must be evaluated to support their therapeutic use in fish farming. Essential oil of clove basil, *Ocimum gratissimum* (OgEO), is a promising product for aquaculture. OgEO was described as anesthetic for jundiás (*Rhamdia quelen*) and flounder (*Paralichthys orbignyanus*) (Silva et al., 2012, 2015; Benovit et al., 2012), and was also found to have antimicrobial properties (Matasyoh et al., 2007). The present study evaluated the use of OgEO baths to anesthetize and control monogenean infestation in tambaqui and assessed the effects of the treatments on fish blood parameters.

2. Material and methods

The study was approved by an Animal Experimentation Ethics Committee (CEUA/UFGD 003/2014).

2.1. Plant production, essential oil extraction and analysis

Clove basil production and collection were performed as routine procedures of the Medicinal Plants Division of Embrapa Western Amazon. The shoots of one year old plants at the reproductive stage were cut and dried in the shade until reaching constant weight. The leaves were separated in the laboratory and subjected to oil extraction by hydrodistillation in a Clevenger apparatus for 4 h. Oil composition was determined by gas chromatography at Embrapa Food Technology in Rio de Janeiro (Table 1).

2.2. Experiment I — anti-parasite baths

Juvenile tambaquis (16.3 ± 3.9 g mean weight) were obtained from the fish farming unit of the Balbina Hydropower Station in Presidente Figueiredo, Amazonas (AM) state, and transported to the facilities of the Embrapa Western Amazon, Manaus, AM. Fish were allowed to adapt to dugout pond conditions for 45 days before the beginning of the experiments. Fish were fed extruded commercial feed with 32% crude protein until apparent satiation. Feeding was suspended 24 h before the beginning of the experiment.

A few minutes before the experiment, 15 fish were collected from the pond and their gills were removed and fixed in 5% formaldehyde for further monogenean count under stereo microscope, and OgEO was diluted 1:20 with alcohol. The 15-min baths were performed in 150-L tanks supplied with water, aeration and aliquots of the OgEO alcohol solution at 0, 5, 10 and 15 mg L^{-1}. The treatments were carried out in triplicate, with 50 fish per tank. Next, fish were distributed in twelve 1000-L tanks supplied with constant aeration and total water exchange once a day. Seven and 14 days after the bath, 15 fish from each tank were sampled and their gills removed and fixed for further parasite inspection as described above. Based on the results, mean infestation rate (parasite count / number of fish) and parasite prevalence (number of infected hosts / number of fish × 100) were determined as recommended by Bush et al. (1997). Anti-parasite efficacy (in percentage) was determined by the difference between parasite count before and after the treatment divided by initial parasite count and multiplied by 100 (Martins et al., 2001). Data obtained as percentage values were arcsine transformed and subjected to ANOVA followed by the Dunnet test to compare the mean values between the treatments and the control group. The prevalence of parasites before and 7 and 14 days after the baths was compared using the t-test, and analyzed with Statistica 6.0 software.

Table 1
Predominant components of essential oil of clove basil from Western Amazon.

RI	Identification	Proportion (%)
938	alpha-pinene	1.0
975	sabinene	0.7
979	beta-pineno	2.8
991	myrcene	0.7
1032	1,8-cineole	28.2
1038	*cis*-ocimene	3.7
1049	*trans*-ocimene	0.0
1097	linalool	1.3
1166	delta-terpineol	0.4
1176	4-terpineol	0.4
1188	alpha-terpineol	1.1
1357	eugenol	43.3
1381	beta-bourbonene	0.9
1389	beta-elemene	0.8
1415	beta-caryophyllene	3.7
1450	alpha-humulene	0.6
1477	gamma-muurolene	0.9
1482	beta-selinene	5.5
1490	alpha-selinene	1.7
1513	7-*epi*-alpha-selinene	0.4

2.3. Experiment II — blood parameters

Juvenile tambaquis weighing 246 ± 58 g and 24.9 ± 1.9 cm long were held in twelve 310-L fiberglass tanks with a water recirculation system. Fish were kept at a density of 12 fish per tank, and water quality parameters were monitored daily early the in the morning (temperature 29.0 ± 1.2 °C and oxygen 6.6 ± 1.8 mg L^{-1} measured with YSI model 550 A; pH 6.9 ± 0.3 measured with YSI model 100; hardness 6.8 ± 1.4 mg L^{-1} and alkalinity 5.1 ± 1.4 mg L^{-1} analyzed by titration; ammonia 0.34 ± 0.2 mg L^{-1} analyzed by Nessler reagent; nitrite 0.09 ± 0.03 mg L^{-1} analyzed by colorimetric method). Fish were fed commercial feed (28% crude protein), which was provided near to satiation twice a day for 20 days. Feeding was suspended 24 h before the onset of the experiment. This experiment was done to evaluate the blood responses of the fish exposed to one concentration and time enough for anthelmintic effect (15 mg L^{-1} for 15 min) and other concentration for deep anesthesia (60 mg L^{-1} for 15 min). These fish blood responses were compared to fish from control and handling (only without essential oil) groups.

The 12 tanks were randomly allocated to 4 treatments (TC, T0, T15, T60), with 3 replications each. Fish from TC (control) were not subjected to handling nor immersion baths. Fish from T0, T15 and T60 were subject to handling and immersed in water containing OgEO at 0, 15 and 60 mg L^{-1}, respectively. To that end, fish from each tank from T0, T15 and T60 were respectively transferred to a bucket filled with 20 L of water and OgEO at the concentrations tested, and after 15 min fish were returned to the respective tank. Three fish from each tank were collected 0 and 24 h after the experimental procedures (TC, T0, T15, T60). Fish length and weight were measured, and blood harvested by caudal puncture with heparinized syringes. The sampled fish were discharged into other tanks and the remainder fish were counted 20 days after the baths to assess mortality.

Blood samples were analyzed to determine packed cell volume (PCV) (Goldenfarb et al., 1971), hemoglobin (Drabkin, 1948) and red blood cell count (RBC) (Lima et al., 1969). These parameters were used to calculate mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) (Lima et al., 1969). Blood plasma, separated by centrifugation of blood aliquots at 14,400*g* for 3 min, was used to determine glucose (Trinder, 1969), ammonia (Gentzkow and Masen, 1942), lactate (Harrower and Brown, 1972), chloride (APHA, 1980), Na^+ and K^+ (flame photometer).

2.4. Experiment III — anesthetic effects

Juvenile tambaquis (42.2 ± 6.6 g and 12.7 ± 1.4 cm) were stocked for 30 days in 3-m^3 tiled tanks with an open circuit water system and constant aeration. Fish were fed commercial pellets (containing 32% of crude protein), twice a day, near to satiation. Feeding was suspended 24 h before the beginning of the experiment. Anesthesia induction