

# Essential oil of *Ocimum basilicum* and Eugenol as Sedatives for Nile Tilapia

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## Abstract

This study evaluated the modulation of the ventilatory frequency of Nile tilapia sedated with essential oil of *Ocimum basilicum* and eugenol. The fish were exposed to the following treatments: control (water only); ethanol 200  $\mu\text{l L}^{-1}$  (concentration used to dilute the anesthetic); eugenol 10  $\mu\text{l L}^{-1}$  and 20  $\mu\text{l L}^{-1}$ ; essential oil of *O. basilicum* at the concentration of 10  $\mu\text{l L}^{-1}$  and 20  $\mu\text{l L}^{-1}$ . After 90 minutes of exposure to the treatments, water quality, mortality and respiratory rate were determined. The concentration of 20  $\mu\text{l L}^{-1}$  of the essential oil of *O. basilicum* and eugenol showed a sedative effect and reduced the excretion of metabolic ammonia in Nile tilapia. There was no mortality in fish exposed to the treatments. The respiratory rate did not differ between the different treatments. It is concluded that the concentration of 20  $\mu\text{l L}^{-1}$  of the essential oil of *O. basilicum* and eugenol shows the best result in the inducing sedative effect for Nile tilapia, and that the essential oil of *O. basilicum* and eugenol in the concentrations of 10  $\mu\text{l L}^{-1}$  and 20  $\mu\text{l L}^{-1}$  in an exposure period up to 90 minutes do not alter the ventilatory frequency of Nile tilapia.

**Keywords:** opercular beat, eugenol, basil, essential oil

## 1. Introduction

Nile tilapia *Oreochromis niloticus* is one of the most important freshwater fish species for the global aquaculture (FAO, 2018). This fish species is characterized by being the most produced in Brazil, and in 2019 represented 57% (432,149 thousand tons) of all national production, which makes the country the fourth largest producer of tilapia in the world (Peixe BR, 2020).

Sedation is the first stage of anesthesia, in which sensory perception is reduced and there is no loss of equilibrium (Ross and Ross, 2008). The sedation stage is recommended for transportation, because it reduces the metabolism and, consequently, reduces excretion of metabolic ammonia, in addition to minimizing the physical damage and injuries caused by transport (Hohlenwerger et al., 2017). Essential oils have been showing to be effective for sedation in fish transportation, with advantages such as minimizing stress-inducing factors (Becker et al., 2012; Silva et al., 2013). Thus, the use of natural anesthetics can prevent losses, such as reduced growth, impaired immune system, in addition to transport mortality.

The assessment of ventilatory frequency is important to understand the physiological response of fish to anesthetics (Becker et al., 2012, 2018). Several studies have been carried

out to evaluate the sedative effect and the ventilatory frequency in different fish species exposed to essential oils, obtaining positive results for the modulation of the ventilatory frequency in Nile tilapia *O. niloticus* with essential oil of *L. alba* (Hohlenwerger et al., 2017) and *Aloysia triphylla* (Teixeira et al., 2018). However, the species and size of fish, as well as the dose, composition, and relative abundance of certain compounds of the essential oil are factors that influence the ventilatory response.

Eugenol (clove oil solution) is characterized for being an important anesthetic and sedative agent, widely used in aquaculture (Zahl et al., 2012). However, studies have revealed that the essential oil of *Ocimum basilicum* presents anesthetic and sedative effects in tambacu (hybrid of species *Piaractus mesopotamicus* x *Colossoma macropomum*) (Lima-Netto et al., 2016), Nile tilapia *O. niloticus* (Lima-Netto et al., 2017) and the clown fish *Amphiprion clarkii* (Correia et al., 2018). Despite this information, there are limit data showing the efficiency of the essential oil of *O. basilicum* and of eugenol for Nile tilapia to obtain sedation during handling procedures in aquaculture. According to Sena et al. (2016) the sedation concentration of an anesthetic agent should be kept to a minimum to avoid total loss of balance and inability to recover the vertical position, which characterize the stage 3 of anesthesia. Therefore, it is necessary to know the modulation of the ventilatory frequency to understand the effects of the essential oils on fish behavior and to establish concentrations capable of inducing sedation in different fish species. Thus, the objective of this study was to evaluate the modulation of the ventilatory frequency in Nile tilapia sedated with essential oil of *O. basilicum* and eugenol.

## 2. Material and Methods

### 2.1 Fish - Obtaining and acclimation

Fish were purchased from a commercial fish farming located in Dourados, Mato Grosso do Sul – Brazil (22° 9'57.98"S; 55°12'32.06"W) and were transported in 400 L capacity tanks (length 80cm x width 60cm x height 85cm Trevisan brand) to the experimental fish farming sector of the Federal University of Mato Grosso do Sul (UFMS) in the city of Campo Grande (20°29'59.04"S; 54°36'52.59"W) Mato Grosso do Sul-Brazil. The fish were acclimated for seven days in 5000 L tanks with constant oxygenation. They were fed until apparent satiation with a commercial diet (Do peixe Douramix®), with 7 to 9 mm pellets (28.0% crude protein, 5.0% ether extract, 3.5% crude fiber, 12.0% of moisture, 10.0% mineral matter, 3.0% calcium). Feeding was suspended 24 hours before the beginning of the experiment.

### 2.2 Preparation of the Anesthetic Solution

Essential oil of *Ocimum basilicum* (Phyoterápica®) and eugenol (Maquira®) used in the present study were purchased commercially. The anesthetic solutions were prepared at the time of the experiment with the dilution of 1:10 in ethanol, to guarantee the miscibility of the essential oil in water.

### 2.3 Ventilatory Frequency

To evaluate the respiratory frequency, Nile tilapia (n=48) with mean weight of  $344.58 \pm 28.49$  g and total length of  $23.84 \pm 1.39$  cm, were exposed to six different treatments (n=8): control (water), ethanol  $200 \mu\text{l L}^{-1}$  (concentration used to dilute the anesthetic), eugenol  $10 \mu\text{l L}^{-1}$  and  $20 \mu\text{l L}^{-1}$ ; essential oil of *O. basilicum* at a concentration of  $10 \mu\text{l L}^{-1}$  and  $20 \mu\text{l L}^{-1}$ . The experimental design was completely randomized with six treatments and four replicates, totaling 24 experimental units (aquariums), with two fish distributed individually in each aquarium, with eight fish per treatment. Fish were acclimated for 10 minutes in 8 L aquariums (width 40 cm x height 60 cm x length 65 cm) with constant oxygenation. The ventilatory

frequency was quantified in 0, 30, 60 and 90 minutes of exposure to the respective treatments, considering the time elapsed for the occurrence of 20 consecutive opercular beats and then transformed in frequency per minute (Alvarenga and Volpato, 1995). Each fish was used only once. After the experimental period the fish were allocated in net cages of 2.00 m x 2.00 m x 1.20 m (Max Telas®) and the mortality was assessed up to 48 hours. The experimental protocol was approved by the Animal Ethics and Welfare Committee (CEUA) of UFMS under the registration of 976/2018.

#### 2.4 Physical-Chemical Parameters of Water Quality

The physical-chemical parameters of water quality oxygen, temperature, pH and conductivity were measured in each aquarium after the end of the exposure period, with the aid of an YsiLife Sciences® multiparameter. The ammonia level was measured with a colorimetric kit (Alfakit®).

#### 2.4 Statistical Analysis

The results were evaluated using the Shapiro-Wilk test to assess normality and the Levene test to assess homoscedasticity. After observing that the data did not meet the assumptions of normality and homogeneity of variances, the Kruskal-Wallis non-parametric analysis was used, followed by the Dunn test. The data were analyzed using the software SAS® (Statistical Analysis System) at a significance level of  $p < 0.05$  (SAS, 2009).

### 3. Results and Discussion

The water quality parameters dissolved oxygen, temperature and conductivity did not differ between treatments. The water pH from the aquariums in which the fish were sedated with eugenol  $20 \mu\text{L L}^{-1}$  was lower ( $p < 0.05$ ) than the pH from the ones in which the fish were kept with water and ethanol only. According to Baldisserotto et al. (2008), the pH decrease occurs due to a reduction in the excretion of non-ionized ammonia, which can justify the results of the present work, considering that the total ammonia nitrogen concentration was lower in the treatment with eugenol  $20 \mu\text{L L}^{-1}$ . Similar results were obtained in the transport water of Nile tilapia with essential oil of *A. triphylla* at the concentrations of 20 or  $30 \mu\text{L L}^{-1}$  (Teixeira et al., 2018). It is important to highlight that the values of pH obtained in all treatments were within the range (6 to 9) considered adequate for tropical fish (Boyd, 1998).

The total ammonia nitrogen (TAN) content was lower ( $p < 0.05$ ) in the aquariums with essential oil of *O. basilicum* and eugenol  $20 \mu\text{L L}^{-1}$  in relation to those with ethanol and *O. basilicum*  $10 \mu\text{L L}^{-1}$  (Table 1). The reduction in the excretion of total ammonia nitrogen by fish can be possibly attributed to the induction of the sedation stage, which is characterized by the reduction of metabolism and decreased excretion of metabolic ammonia (Hohlenwerger et al., 2017). Similar results were obtained with eugenol and essential oil of *L. alba* in the water used to transport of *Rhamdia quelen* (Becker et al., 2012). It is interesting to notice that with  $10 \mu\text{L mL}^{-1}$  of eugenol a great reduction of total ammonia nitrogen was observed, while with *O. basilicum*, the double of the concentration was necessary for an accentuated decrease of total ammonia nitrogen to occur.

Table 1. Water quality parameters (mean  $\pm$  standard deviation) after 90 minutes of exposure of Nile tilapia to essential oil of *Ocimum basilicum* and eugenol added to the water

Parameters	Water	Ethanol 200 $\mu\text{l L}^{-1}$	<i>O. basilicum</i>		Eugenol	
			10 $\mu\text{l L}^{-1}$	20 $\mu\text{l L}^{-1}$	10 $\mu\text{l L}^{-1}$	20 $\mu\text{l L}^{-1}$
Oxygen $\text{mg L}^{-1}$	5.90 $\pm$ 0.55	6.01 $\pm$ 0.29	5.86 $\pm$ 0.29	5.65 $\pm$ 0.68	6.14 $\pm$ 0.98	6.08 $\pm$ 0.46
Temperature $^{\circ}\text{C}$	26.04 $\pm$ 0.60	26.08 $\pm$ 0.48	26.11 $\pm$ 0.47	26.18 $\pm$ 0.48	26.59 $\pm$ 0.04	26.65 $\pm$ 0.09
pH	8.47 $\pm$ 0.17 <sup>a</sup>	8.38 $\pm$ 0.13 <sup>a</sup>	8.29 $\pm$ 0.10 <sup>ab</sup>	8.25 $\pm$ 0.10 <sup>ab</sup>	8.23 $\pm$ 0.10 <sup>ab</sup>	8.17 $\pm$ 0.06 <sup>b</sup>
Conductivity $\mu\text{S cm}^{-1}$	48.70 $\pm$ 7.73	41.68 $\pm$ 14.59	52.39 $\pm$ 2.84	51.43 $\pm$ 4.54	50.99 $\pm$ 5.30	49.43 $\pm$ 2.85
Total Ammonia Nitrogen (TAN) $\text{mg L}^{-1}$	0.32 $\pm$ 0.07 <sup>ab</sup>	0.66 $\pm$ 0.05 <sup>b</sup>	0.44 $\pm$ 0.15 <sup>b</sup>	0.15 $\pm$ 0.07 <sup>a</sup>	0.19 $\pm$ 0.10 <sup>ab</sup>	0.16 $\pm$ 0.08 <sup>a</sup>

Different letters indicate significant difference between treatments Dunn test ( $p < 0.05$ ).

Fish recovered soon after removal from the aquarium with the anesthetic agent and there was no mortality during and after 48 hours of the experiment in all evaluated treatments. In the present study the use of ethanol at the concentration of 200  $\mu\text{l mL}^{-1}$  did not induce sedation, nor did it have an effect ( $p > 0.05$ ) on the ventilatory frequency of Nile tilapia when applied separately (Table 2). For *R. quelen*, ethanol increased the ventilatory frequency during the first four hours of exposure, when compared to the essential oil of *Citrus aurantium* and *Citrus latifolia* (Lopes et al., 2018). It was also observed for *R. quelen* that fish from the ethanol group showed lower ventilatory rate than the control group at 4 h of exposure (Cunha et al., 2017) and the fish exposed to the ethanol progressively reduced the ventilatory frequency up to 4 h (Becker et al., 2018). These changes did not occur in the present study with Nile tilapia, so we can infer that the ventilatory responses of fish to ethanol are quite variable.

A low ventilatory frequency can result in hypoxia, triggering the anaerobic metabolism in fish (Toni et al., 2014). Several fish anesthetics have an inhibitory effect on the respiratory system, which results in lower ventilatory frequency (Keene et al., 1998). However, this effect was not observed in the present work, as the ventilatory frequency did not differ between the different treatments up to 90 minutes of exposure (Table 2). A result similar to the present study was obtained by using increasing doses of the essential oil of *Lippia alba* (chemotype linalool) and *Lippia origanoides* (chemotype carvacrol) to induce sedation in tambaqui *Colossoma macropomum* (Silva et al., 2019). The non-occurrence of alteration in the ventilatory frequency of Nile tilapia exposed to eugenol and *O. basilicum* can be attributed to the doses used being low enough to have no depressant effect, capable of affecting the respiratory system.

Table 2. Ventilatory frequency of Nile tilapia *Oreochromis niloticus* (mean  $\pm$  standard deviation) maintained in different treatments and time

Time (min)	Water	Ethanol 200 $\mu\text{l L}^{-1}$	<i>Ocimum basilicum</i>		Eugenol	
			10 $\mu\text{l L}^{-1}$	20 $\mu\text{l L}^{-1}$	10 $\mu\text{l L}^{-1}$	20 $\mu\text{l L}^{-1}$
0	68.11 $\pm$ 10.54	68.10 $\pm$ 8.07	66.52 $\pm$ 7.77	63.35 $\pm$ 9.62	69.01 $\pm$ 10.09	75.26 $\pm$ 5.04
30	75.90 $\pm$ 7.98	80.44 $\pm$ 8.45	77.86 $\pm$ 16.26	74.36 $\pm$ 2.97	76.67 $\pm$ 5.33	73.51 $\pm$ 6.18
60	75.19 $\pm$ 8.07	79.30 $\pm$ 6.51	72.71 $\pm$ 8.50	72.77 $\pm$ 6.29	70.07 $\pm$ 2.64	72.38 $\pm$ 5.17
90	74.91 $\pm$ 11.85	71.43 $\pm$ 9.07	66.00 $\pm$ 0.95	66.23 $\pm$ 3.09	70.55 $\pm$ 2.54	70.78 $\pm$ 4.49

There was no significant difference between treatments Dunn test ( $p > 0.05$ ).

The ventilatory frequency in fish is influenced by species, fish size, tested anesthetic concentrations, time of exposure and the product used (Roohi and Imanpoor, 2015). In *R. quelen* exposed to eugenol and essential oil of *L. alba*, it was observed that after one hour of exposure there was no difference in the ventilatory frequency between treatments. However, after 2, 3 and 4 h, the ventilatory frequency was significantly lower in all treatments with anesthetics when compared to the control group (Becker et al., 2012). Nevertheless, the essential oil of *A. triphylla* reduced the ventilatory frequency of Nile tilapia after 30 minutes of exposure (Teixeira et al., 2018). Therefore, it is possible to infer that de modulation of the ventilatory frequency is easily changed, making it difficult to establish a pattern for a general response. In addition, the composition of the essential oil and the sedative potential of the plant are determining factors for the modulation of this response.

Although there was no effect of the anesthetic agents on the ventilatory frequency of Nile tilapia, a sedative effect of the essential oil of *O. basilicum* and eugenol was observed at the concentration of 20  $\mu\text{l L}^{-1}$ . The fish presented only mild sedation, maintained the ability to react to external stimuli, with reduced movements, but with normal balance, which is characterized as stage I as described by Small (2003). According to the same author, it is from stage II that there is a reduction of the opercular movement and of the reflexes to external stimuli, behavioral characteristics that were not observed for Nile tilapia. The variations in the sedative concentrations may be due to the composition and relative abundance of certain compounds of the essential oil (Bakkali et al., 2008), species and/or size of fish which influence the anesthetic response (Gomes et al., 2011; Zahl et al., 2012). However, the sedative efficacy of the essential oil of *O. basilicum* and eugenol was as expected (Becker et al., 2012; Lima-Netto et al., 2016, 2017).

In general, the results of modulation of the ventilatory frequency by natural anesthetics in fish are controversial. In tambaqui *C. macropomum* sedated with essential oils of *L. alba* (chemotype citral and linalool) and *L. origanoides* (chemotype carvacrol) it was not possible to establish a direct relationship between the increase in anesthetic concentration and reduction in ventilatory frequency (Silva et al., 2019). In *R. quelen* exposed to the essential oils of *L. alba* (chemotype citral) and *L. origanoides* no major differences in the ventilatory frequency were observed in relation to the control group (Becker et al., 2018). However, the essential oils of *Hesperozygis ringens* and *L. alba* (chemotype linalol) reduced the ventilatory frequency in *R. quelen* sedated at concentrations of 300 or 450  $\mu\text{l L}^{-1}$  (Toni et al., 2014). In Nile tilapia the essential oil of *L. alba* at the concentration of 20  $\mu\text{l L}^{-1}$  reduced the

ventilatory frequency over time of exposure, with bradypnea more effective between 4 and 5 h (Hohlenwerger et al., 2017). Therefore, the modulation of the ventilatory frequency in fish exposed to natural anesthetics is largely variable, being difficult to establish a general response, which suggests that this process can be better understood with a specific response.

#### 4. Conclusions

The 20  $\mu\text{l L}^{-1}$  concentration of the essential oil of *O. basilicum* and eugenol is capable of reducing the excretion of metabolic ammonia and inducing sedative effect in Nile tilapia. The essential oil of *O. basilicum* and eugenol at the concentrations of 10  $\mu\text{l L}^{-1}$  and 20  $\mu\text{l L}^{-1}$  during an exposure period of up to 90 minutes do not change the ventilatory frequency of Nile tilapia *O. niloticus*.

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