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Efficiency of *Ocimum basilicum* and *Eucalyptus globulus* essential oils on anesthesia and histopathology of rainbow trout, *Oncorhynchus mykiss*

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Abstract

In this study, anesthetic effects of *Ocimum basilicum* and *Eucalyptus globulus* essential oils were investigated on rainbow trout, *Oncorhynchus mykiss*. Fish (average 10 g) were exposed to 20, 50, 70, 100, 150, 200, 300, 400, 500, and 600 mg L^{-1} essential oils, anesthesia induction, and recovery times were recorded separately for each fish. Acute toxicities of essential oils (10 min LC_{50} concentration) on rainbow trout were determined at 70–400 mg L^{-1}. In addition, histopathological effects of the essential oils in fish tissues were determined after deep anesthesia. As a result of this study, *O. basilicum* at 300 mg L^{-1} dose and *E. globulus* essential oils at 400 mg L^{-1} dose showed ideal anesthetic effects on rainbow trout. Anesthesia induction (stage 4) and recovery times at this concentration for *E. globulus* were found as 186 and 117.5 s, respectively. Anesthesia induction (stage 4) and recovery times were 220.5 and 61 s for this concentration in *O. basilicum* essential oil, respectively. The toxic effects of essential oils for rainbow trout were not found. No pathological finding in gill, liver, and kidney was observed at the histopathological examination. Consequently, this study demonstrated that *O. basilicum* and *E. globulus* essential oils can be used as an effective and safe anesthetic in rainbow trout.

KEYWORDS
anesthesia, aquaculture, essential oil, histopathology, rainbow trout, sedation

1 | INTRODUCTION

Anesthetics are used in aquaculture to reduce stress and prevent physical injury during handling procedures and transport (Ananias et al., 2022; Hoseini et al., 2021; Yousefi et al., 2022). The most common anesthetics used in aquaculture are benzocaine, quinaldine, tricaine methanesulphonate (MS-222), and 2-phenoxyethanol (Ananias et al., 2022; Priborsky & Velisek, 2018). However, these anesthetics cause undesirable effects such as stressors, gill irritation, hyperactivity, excessive mucus secretion, and are also expensive (Aydın & Barbas, 2020; Gilderhus & Marking, 1987; Hoseini et al., 2021; Palić et al., 2006). Therefore, plant essential oils represent an excellent alternative to replace synthetic products. There is a growing interest in the use of herbal materials in fish anesthesia because of a wide range of beneficial health effects, including antioxidant, antimicrobial, stress-relieving, and immune-boosting effects (Hoseini, 2011; Hoseini et al., 2019; Salbego et al., 2015; Zahl et al., 2010; Zeppenfeld et al., 2014). Therefore, they have recently been widely tested as an anesthetic for fish (Hajek, 2011; Ribeiro et al., 2016; Silva et al., 2019).

Basil, *Ocimum basilicum*, is an important essential oil crop and belongs to the Lamiaceae family. It is an economically important plant distributed in several regions all over the world (Baritaux et al., 1992). The major components of *O. basilicum* essential oil were methyl chavicol and linalool (Ventura et al., 2020). *O. basilicum* essential oil also stands out for its anesthetic and sedative properties (Limma-Netto et al., 2016, 2017; Ventura et al., 2021). In fish, anesthetic and sedative effects of *O. basilicum* essential oil have been reported in the hybrid tambacu, *Piaractus mesopotamicus* × *Colossoma macropomum* (Limma-Netto et al., 2016), juvenile Nile tilapia, *Oreochromis niloticus* (Limma-Netto et al., 2017), and in the ornamental fish, *Amphiprion clarkii* (Correia et al., 2017). In addition, the active components of essential oils such as linaool in common carp, *Cyprinus carpio* (Taheri Mirghaed et al., 2016; Yousefi et al., 2018, 2019), in silver catfish (*Rhamdia quelen*) (Heldwein et al., 2014) were investigated.

Eucalyptus belongs to the Myrtaceae family and is cultivated in tropical regions (Santos et al., 1996). Essential oil of *Eucalyptus globulus* is rich in 1,8-cineole (Baptista-Silva et al., 2020). The anesthetic and sedative effects of *Eucalyptus* sp. have been monitored in catfish (Silva et al., 2021), sea bass, and meager (Bodur et al., 2018). In addition, the active components of essential oils such as 1,8-cineole in rainbow trout (Taheri Mirghaed et al., 2018), in Caspian trout, *Salmo caspius* (Taheri Mirghaed et al., 2022), in common carp (Hoseini et al., 2020; Mazandarani & Hoseini, 2017) were investigated.

Advantages and disadvantages of the different plant-based anesthetics should be clarified as it could impact fish health and welfare (Aydın & Barbas, 2020; Souza et al., 2018; Velisek et al., 2006). Histopathological effects of essential oils, especially the gills are one of the most affected organs because fish come into direct contact with essential oils (Bhuvaneswari et al., 2015; Brandão et al., 2021; Velisek et al., 2005).

No studies to date have investigated the anesthetic and histopathologic effect of basil and eucalyptus essential oil in rainbow trout. Therefore, the objective of the present study was to evaluate the anesthetic induction, recovery, and histopathology effect in rainbow trout exposed to different concentrations of the essential oil of *O. basilicum* and *E. globulus*.

2 | MATERIAL AND METHODS

2.1 | Chemical composition of essential oil

O. basilicum and *E. globulus* essential oils used in the study were obtained from a commercial company (Isparta, Turkey).

The compositions of essential oils were analyzed using gas chromatography–mass spectrometry (GC–MS). Each component was identified by comparison from the Wiley, Nist, Tutor, FFNSC library of Mass Spectra. The component amount was determined by proportioning the relative blocks of the peak areas to the total peak area.

2.2 | Experimental design

This study was conducted in the Egirdir Fisheries Faculty, Isparta Applied Sciences University in Turkey. Healthy rainbow trout (mean weight of 10 g) were obtained from a commercial farm. A total number of 400 rainbow trout were stocked in 2 tanks (450 L) with aeration. Fish were acclimated to experimental conditions for 15 days and were fed as ad libitum twice daily with commercial trout feed. The tanks' water was renewed daily by 50%. The tanks were cleaned by siphoning out the residual feed and feces. Water dissolved oxygen and temperature levels were measured using YSI 55A oxygen meter. Water temperature, dissolved oxygen, and pH were 8.2°C, 9.06 mg L^{-1}, and 7.9 during the experiment, respectively. After acclimation, the anesthetic efficacy effects of basil, *O. basilicum*, and eucalyptus, *E. globulus*, essential oils were investigated in the fish. All fish were starved for 24 h prior to the experiment. Each essential oil concentration was diluted with ethanol (95%) at 1:10 ratio (Limma-Netto et al., 2017). Different concentrations (20, 50, 70, 100, 150, 200, 300, 400, 500, and 600 mg L^{-1}) of the essential oils were used in the experiments. The control group contained only ethanol. Ten fish were used to determine anesthesia induction and recovery times. The fish were individually caught from the holding tanks and placed into the aquariums (10 L) with continuous aeration and this application was performed in two replicates. The determination of anesthesia stages were performed with modification from Keene et al. (1998) (Table 1). The duration of each stage was recorded with a stopwatch. The time to reach stage 4 anesthesia was recorded and then the fish was caught and placed into the recovery aquarium to record recovery time.

2.3 | Lethal concentration (LC_{50}) of essential oils

Acute toxicity of essential oils was determined according to Velisek et al. (2005). Fish were exposed to essential oils (70, 100, 150, 200, and 400 mg L^{-1}) for 10 min. Ten trout (average 10 g) were used for each concentration.

TABLE 1 Anesthesia stages in fish

Stage	Description	Behavior exhibited
I	Light sedation	Equilibrium normal, slow swimming, decreased reactivity to external stimuli, slight decrease in opercular rate
II	Deep sedation	Restlessness, equilibrium normal, voluntary swimming still possible; slight decrease in opercular rate no response to weak external stimulus
III	Light anesthesia	Partial loss of equilibrium; swimming erratic; increased opercular rate; reactive only to strong tactile and vibrational stimuli
IV	Deep anesthesia	Total loss equilibrium, lying on one side without movement, opercular movements slow and irregular; loss of all reflexes

Mortalities were recorded during the test period and when the fish were transferred to clean water. Mean lethal concentrations (LC_{50}) were calculated from mortality rates.

2.4 | Histopathological examination

Tissue samples of fish were taken from induction-recovery trial (stage 4) at 600 mg L^{-1} concentration for histopathological examinations. The fish (10 fish/each group) were euthanized by severing the spinal cords, and tissue samples (gill, liver and kidney) were obtained. Tissue samples were fixed in 10% neutral formalin and processed by automatic tissue processing equipment (Leica ASP300S; Leica Microsystem, Nussloch, Germany). The samples were embedded in paraffin, and 5 μm sections were taken by a Leica RM 2155 rotary microtome (Leica Microsystem, Nussloch, Germany). Then sections were stained with hematoxylin and eosin (HE). Microphotography was performed using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

2.5 | Statistical analysis

The data were analyzed using the SPSS software version 26.00. Homogeneity of the variances was confirmed by Levene's test. Normality of data was confirmed by Anderson–Darling test. Kruskal–Wallis test was used because of lack of preconditions. Nonparametric Bonferroni Dunn multiple range test was used to determine the significant variation ($p < 0.05$). Wilcoxon test was used to compare eucalyptus and basil separately for each stage and dose combination. Regression equations were used to explain the relationship between induction and recovery times of anesthetic concentrations.

3 | RESULTS

3.1 | Chemical composition

3.1.1 | GC/MS analysis

The components of *E. globulus* and *O. basilicum* essential oils by GC–MS are given in Tables 2 and 3. Components of *E. globulus* and O. *basilicum* essential oils were 16 and 23, respectively. 1,8-Cineole (85.44%) in *E. globulus* (Table 2) and methyl chavicol (estragole) (72.57%) and linalool (21.60%) in *O. basilicum* (Table 3) were determined as the main components.

3.2 | Anesthesia induction and recovery

Concentration increasing of the essential oils decreased anesthesia induction time in fish. However, recovery times increased by increasing the concentration of these oils. Neither mortality nor abnormal behavior in fish was recorded after anesthesia. Fish started to feed almost 2 h later anesthesia.

Eucalyptus essential oil had no anesthetic efficiency at 20–50 mg L^{-1} concentrations in trout. The essential oil at 70–150 mg L^{-1} concentrations showed only deep sedation (stage 2) on the fish. Exactly 200–600 mg L^{-1} concentrations of eucalyptus oil showed a deep anesthesia (stage 4) on fish (Table 4). The ideal induction time for deep anesthesia (stage 4) in fish was determined at 400 mg L^{-1} eucalyptus essential oil. Anesthesia induction and recovery times at this concentration were found as 186 and 117.5 s, respectively (Table 4). As a result of the Wilcoxon

TABLE 2 Essential oil components of *Eucalyptus globulus* (%)

Component	% Content
Alpha-pinene (−)-	4.32
Camphene	0.01
Sabinene	0.01
Beta-pinene	0.54
Beta-myrcene	0.65
l-Phellandrene	0.51
Alpha-terpinolene	0.13
Benzene, methyl(1-methylethyl)-(CAS) cymol	6.81
1,8-Cineole	85.44
1,3,6-Octatriene, 3,7-dimethyl-(e)-(CAS). Beta-Ocimene y	0.02
Gamma-terpinene	1.38
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	0.04
Benzene, 1-isopropenyl-?-methyl-	0.02
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	0.01
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-(CAS) 4-Terpineol	0.02
Beta Fenchyl alcohol	0.09
	100.00

test performed in terms of the times obtained in all dose and stages, the differences between basil and eucalyptus essential oils were not found to be statistically significant.

Basil essential oil had no anesthetic efficiency in trout at 20 mg L^{-1} concentration. The essential oil at 50–100 mg L^{-1} concentrations showed sedation (stage 2) on the fish. Basil essential oil at 200–600 mg L^{-1} concentrations showed anesthetic efficiency (stage 4) (Table 5). The lowest effective concentration for deep anesthesia (stage 4) in fish was 300 mg L^{-1} for basil essential oil. Anesthesia induction and recovery times were 220.5 s and 61 s at 300 mg L^{-1} concentration, respectively (Table 5).

3.3 | Acute toxicity of essential oils

Lethal concentrations (10 min LC_{50}) for both essential oils on fish were not determined at 70–400 mg L^{-1} concentration.

3.4 | Histopathological findings

At the histopathological examination, no pathological findings were observed in any fishes (Figures 1 and 2).

4 | DISCUSSION

In the current study, 1.8-cineole was found to be the main component in eucalyptus essential oil. Similarly, other studies have also reported that 1.8-cineole is the main component for eucalyptus oil (Bodur et al., 2018;

TABLE 3 Essential oil components of *Ocimum basilicum* (%)

Component	% Content
Alpha-pinene, (−)-	0.10
Beta-phellandrene	0.01
2-Beta-pinene	0.06
6-Methyl-5-hepten-2-one	0.13
Beta-myrcene	0.03
Benzene, methyl(1-methylethyl)-(CAS) cymol	0.03
L-Limonene	0.06
1,8-Cineole	0.16
1,3,6-Octatriene, 3,7-dimethyl-, (E)-(CAS). Beta Ocimene y	0.12
Linalool oxide cis	0.14
Linalool oxide trans	0.15
Linalool l	21.60
Methyl chavicol (estragole)	72.57
Acetic acid, octyl ester	0.02
Z-citral	0.36
E-citral	0.57
L-Menthyl acetate	0.01
Copaene <alpha->	0.03
Cıs 3 hexenyl lactate	0.08
Elemene <beta->	0.04
Benzene, 1,2-dimethoxy-4-(2-propenyl)-(CAS) Methyleugenol	0.02
Caryophyllene	0.34
Bergamotene <alpha-trans->	0.67
	100.00

Silva et al., 2021). Basil, *O. basilicum*, is rich in essential oils that mainly contain estragole and linalool (Sharopov et al., 2016). In the present study, it was determined that basil, *O. basilicum*, essential oil contain high levels of estragole (72.57%) and linalool (21.60%). Similarly, Ventura et al. (2020) noted that major constituents of the *O. basilicum* essential oil were methyl chavicol (estragole) (66.51%) and linalool (20.90%). Akgül (1989) reported that the main component of *O. basilicum* essential oil was linalool and other important components were estragole (2.70%), eugenol (13.4%), and methyl eugenol (9.57%). The differences in the chemical composition of basil essential oil can change depending on many factors such as the extraction method, the type of plant, or its geographical location.

Ideal deep anesthesia should occur in <3–5 min and recovery time should not be longer than 10 min (Ross & Ross, 2008; Tsantilas et al., 2006). According to these criteria, the optimal anesthetic concentration for eucalyptus essential oil was determined as 400 mg L^{-1} for rainbow trout in this study. Anesthesia induction time was 186 s (3.1 min) and the recovery time was 117.50 s (1.96 min) at a dose of 400 mg L^{-1}. Silva et al. (2021) noted that the lowest effective dose of *E. globulus* essential oil as anesthetic was 700 μL L−1 in catfish. Bodur et al. (2018) reported that *Eucalyptus* sp. essential oil at a concentration of 300 μL L^{-1} is suitable for anesthesia in sea bass, *Dicentrarchus labrax*, and meager, *Argyrosomus regius*. At this concentration, anesthesia induction times were 5 min for sea bass and 2 min 24 s for meager. Differences between these studies may be because of chemical variations in the plant and use of different fish species.

TABLE 4 Anesthetic effect of eucalyptus oil on trout

Dose (mg L^{-1})	Induction time (s) Anesthesia level I	II	III	IV	Recovery time (s)
20	217.50 ± 4.95[a*]	-	-	-	-
50	178.50 ± 9.19[ab]	-	-	-	-
70	110.50 ± 4.95[bc]	371.50 ± 4.95[a]	-	-	-
100	91.00 ± 8.49[c]	262.50 ± 6.36[b]	-	-	-
150	56.00 ± 5.66[d]	166.00 ± 5.66[c]	-	-	-
200	55.50 ± 6.36[d]	146.50 ± 6.36[d]	438.50 ± 4.95	585.50 ± 6.36	51.00 ± 5.66
300	51.50 ± 4.95[d]	128.50 ± 4.95[e]	185.50 ± 6.36	260.00 ± 8.49	61.50 ± 10.61
400	40.00 ± 7.07[e]	105.50 ± 6.36[f]	128.50 ± 6.36	186.00 ± 5.66	117.50 ± 3.54
500	28.50 ± 2.12[ef]	66.00 ± 5.66[g]	116.50 ± 4.95d	153.50 ± 7.78	131.50 ± 4.95
600	23.00 ± 5.66[f]	40.50 ± 4.95[h]	107.00 ± 4.24	131.50 ± 6.36	175.00 ± 7.07
p-value	0.030	0.038	0.068	0.068	0.078
R^2	0.959	0.930	0.914	0.960	0.947
Equation	$y = 1851.6x^{-0.661}$	$y = 15{,}888x^{-0.883}$	$y = 318{,}497x^{-1.276}$	$y = 625{,}611x^{-1.34}$	$y = 26.625e^{0.0032x}$

Note: Data are presented as mean ± SD. Values superscript with different letters in the same column are significantly ($p < 0.05$) different. -, no anesthetic effect.

TABLE 5 Anesthetic effect of basil oil on trout

Dose (mg L^{-1})	Induction time (s) Anesthesia level I	II	III	IV	Recovery time (s)
20	60.00 ± 11.31[a]	-	-	-	-
50	52.00 ± 5.66[a]	279.50 ± 7.78[a]	-	-	-
70	44.50 ± 4.95[ab]	182.00 ± 4.24[ab]	-	-	-
100	38.00 ± 5.67[bc]	166.50 ± 6.36[bc]	-	-	-
150	34.00 ± 5.66[bcd]	71.00 ± 9.90[c]	221.50 ± 4.95	-	-
200	25.00 ± 2.83[cde]	67.50 ± 6.36[c]	180.00 ± 8.49	411.50 ± 4.95	52.00 ± 4.24
300	24.00 ± 4.24[de]	56.00 ± 5.66d[d]	113.50 ± 6.36	220.50 ± 3.54	61.00 ± 5.66
400	21.50 ± 4.95[ef]	55.50 ± 6.36[d]	91.50 ± 9.19	176.00 ± 5.66	68.50 ± 9.19
500	13.50 ± 3.54[f]	39.50 ± 2.12[e]	87.50 ± 7.78	161.00 ± 9.90	76.00 ± 5.50
600	12.50 ± 3.54[f]	50.00 ± 5.66[de]	61.50 ± 6.36	75.50 ± 6.36	86.00 ± 5.66
p-value	0.037	0.038	0.061	0.068	0.093
R^2	$R^2 = 0.946$	$R^2 = 0.903$	$R^2 = 0.944$	$R^2 = 0.920$	$R^2 = 0.994$
Equation	$y = 53.284e^{-0.003x}$	$y = 4119.5x^{-0.736}$	$y = 297.63e^{-0.003x}$	$y = 796.86e^{-0.004x}$	$y = 41.446e^{0.0012x}$

Note: Data are presented as mean ± SD. Values superscript with different letters in the same column are significantly ($p < 0.05$) different. -, no anesthetic effect.

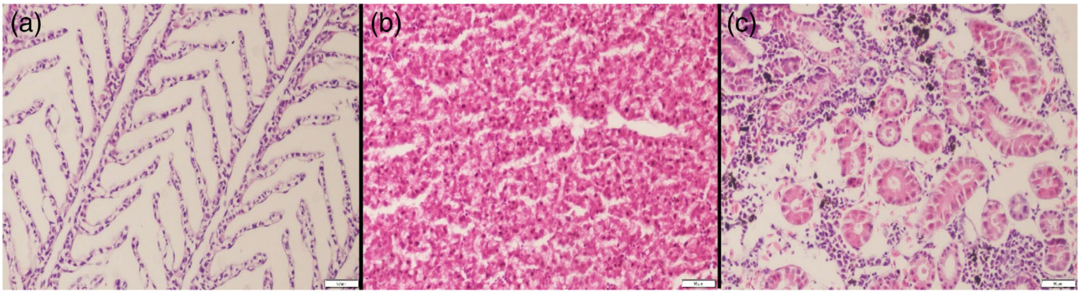

FIGURE 1 Representative microphotographs of the eucalyptus-treated fish organs (a) gill, (b) liver, and (c) kidney, hematoxylin and eosin, scale bars = 50 μm

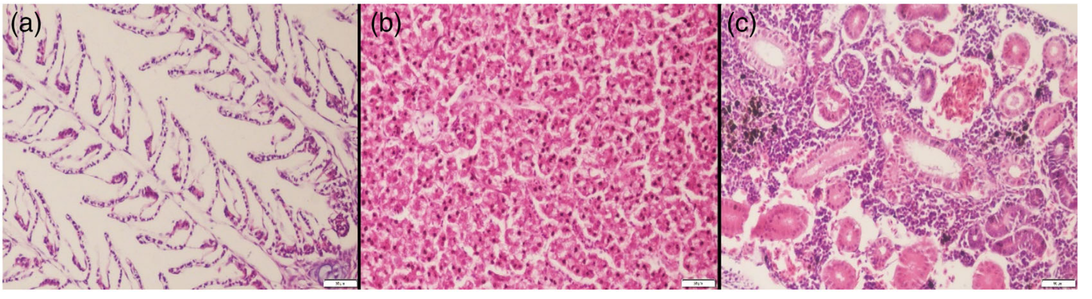

FIGURE 2 Microscopical appearance of the basil-treated fish organs (a) gill, (b) liver, and (c) kidney, hematoxylin and eosin, scale bars = 50 μm

In the present study, cineole (85.44%) was found to be the main component in eucalyptus essential oil. Taheri Mirghaed et al. (2018) reported that cineole was efficient in anesthetizing rainbow trout at concentrations of 200–800 μL L^{-1}. Hoseini et al. (2020) noted that 1000 μL L^{-1} cineole proved suitable for rapid sampling in common carp. Mazandarani and Hoseini (2017) informed that 300 ppm cineole was capable of inducing all anesthesia stages in common carp. Our findings were relatively similar to these studies.

In the current study, optimal anesthetic concentration for basil essential oil was observed as 300 mg L^{-1}. Anesthesia induction time was 220.50 s (3.67 min) and recovery time was 61 s (1.01 min) at a dose of 300 mg L^{-1}. Ventura et al. (2021) reported that the essential oil of *O. basilicum* in Tambaqui, *C. macropomum*, provides rapid entry into anesthesia (222 s) at a concentration of 1000 μL L^{-1}. Limma-Netto et al. (2016) informed that the best times for anesthesia and recovery were found for the concentrations of 300 μL L^{-1} for *O. basilicum* (113.90 and 152.12 s, respectively) in tambacu, *P. mesopotamicus* × *C. macropomum*. De Lima Silva et al. (2012) reported that 150 and 300 mg L^{-1} *Ocimum gratissim*um essential oil effectively performed rapid anesthesia (<4 min) in juvenile silver catfish. At 150 and 300 mg L^{-1} concentrations, anesthesia induction times were 153 s and 65.5 s and the recovery times were 1031 and 1181 s, respectively. Ferreira et al. (2021) reported that concentrations between 90 and 150 mg L^{-1} essential oil of *O. gratissimum* were efficient for anesthesia and recovery in tilapia, *O. niloticus*. Differences between these studies may be because of chemical variations in the plant and use of different fish species.

In our study, linalool (21.60%) was found to be one of the main active ingredients in basil laurel essential oil. In previous studies, the anesthetic efficacy of linalool has been demonstrated in different fish species. Yousefi et al. (2018) informed that linalool at concentrations of 400–2000 mg L^{-1} induced deep anesthesia within 467–118 s with recovery time of 174–215 s in common carp. Taheri Mirghaed et al. (2016) reported that the required linalool concentration to induce stage 4 anesthesia within 180 s was calculated to be 753 ppm. Heldwein et al. (2014) noted that use of linalool as a sedative and anesthetic for silver catfish was effective at 30 and 180 μL L^{-1}, respectively.

In this study, both plant essential oils did not cause pathological effects on the gills, liver, and kidneys of the trout. Similarly, the use of clove essential oil as an anesthetic in common carp (*C. carpio*) did not show histopathological changes in liver and kidneys (Velisek et al., 2005). However, the use of essential oils of *Aloysia triphylla*, *Lippia sidoides*, and *Mentha piperita* as anesthetics in juvenile tambaqui resulted in hypertrophy and hyperplasia of the gill lamellar epithelium (Brandão et al., 2021).

In this study, it was determined that the ideal anesthetic dose for rainbow trout was 400 and 300 mg L^{-1} for eucalyptus and basil essential oils, respectively. No toxic effect had been observed with the use of these essential oils in fish. In addition, no histopathological findings were found in the gill, liver, and kidney tissues. The results of this study showed that basil and eucalyptus essential oils are suitable for use as anesthetics in rainbow trout. In future studies, the anesthetic effects of these oils in different fish species and their effects on health parameters can be studied.

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CONFLICT OF INTEREST

There is no conflict of interest in this study.

DATA AVAILABILITY STATEMENT

This study is available from the corresponding author on request.

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REFERENCES

Akgül, A. (1989). Volatile oil composition of sweet basil (*O. basilicum* L.) cultivating in Turkey. *Food/Nahrung*, *33*(1), 87–88.

Ananias, I. D. M. C., de Melo, C. L., Costa, D. C., Ferreira, A. L., Martins, E. D. F. F., Takata, R., & Luz, R. K. (2022). Menthol as anesthetic for juvenile *Lophiosilurus alexandri*: Induction and recovery time, ventilatory frequency, hematology and blood biochemistry. *Aquaculture*, *546*, 737373.

Aydın, B., & Barbas, L. A. L. (2020). Sedative and anesthetic properties of essential oils and their active compounds in fish: A review. *Aquaculture*, *520*, 1–9.

Baptista-Silva, S., Borges, S., Ramos, O. L., Pintado, M., & Sarmento, B. (2020). The progress of essential oils as potential therapeutic agents: A review. *Journal of Essential Oil Research*, *32*(4), 279–295.

Baritaux, O., Richard, H., Touche, J., & Derbesy, M. (1992). Effects of drying and storage of herbs and spices on the essential oil. Part I. Basil, *Ocimum basilicum* L. *Flavour and Fragrance Journal*, *7*(5), 267–271.

Bhuvaneswari, R., Manickam, N., & Bhavan, P. S. (2015). Calamus oil as an anesthetic for *Cyprinus carpio* (Ornamental Koi). *International Journal of Pure and Applied Bioscience*, *3*(1), 18–26.

Bodur, T., Afonso, J. M., Montero, D., & Navarro, A. (2018). Assessment of effective dose of new herbal anesthetics in two marine aquaculture species: *Dicentrarchus labrax* and *Argyrosomus regius*. *Aquaculture*, *482*, 78–82.

Brandão, F. R., Farias, C. F. S., Souza, D. C. M., Oliveira, M. I. B., Matos, L. V., Majolo, C., Oliveira, M. R., Chaves, F. C. M., O'Sullivan, F. L. A., & Chaga, E. C. (2021). Anesthetic potential of the essential oils of *Aloysia triphylla*, *Lippia sidoides* and *Mentha piperita* for *Colossoma macropomum*. *Aquaculture*, *534*, 736275. https://doi.org/10.1016/j.aquaculture.2020.736275

Correia, A. M., Pedrazzani, A. S., Mendonça, R. C., Massucatto, A., Ozório, R. A., & Tsuzuki, M. Y. (2017). Basil, tea tree and clove essential oils as analgesics and anesthetics in *Amphiprion clarkii* (Bennett, 1830). *Brazilian Journal of Biology*, *78*, 436–442.

De Lima Silva, L., Parodi, T. V., Reckziegel, P., de Oliveira Garcia, V., Bürger, M. E., Baldisserotto, B., Malmann, C. A., Pereira, A. M. S., & Heinzmann, B. M. (2012). Essential oil of *Ocimum gratissimum* L.: Anesthetic effects, mechanism of actio and tolerance in silver catfish, *Rhamdia quelen*. *Aquaculture*, *350*, 91–97. https://doi.org/10.1016/j.aquaculture.2012.04.012

Ferreira, A. L., Favero, G. C., Boaventura, T. P., de Freitas Souza, C., Ferreira, N. S., Descovi, S. N., Baldisserotto, B., Heinzmann, B. M., & Luz, R. K. (2021). Essential oil of *Ocimum gratissimum* (Linnaeus, 1753): Efficacy for anesthesia and transport of *Oreochromis niloticus*. *Fish Physiology and Biochemistry*, *47*(1), 135–152.

Gilderhus, P. A., & Marking, L. L. (1987). Comparative efficacy of 16 anesthetic chemicals on rainbow trout. *North American Journal of Fisheries Management*, *7*, 288–292.

Hajek, G. J. (2011). The anesthetic-like effect of tea tree oil in common carp *Cyprinus carpio* L. *Aquaculture Research*, *2011*(42), 296–300.

Heldwein, C. G., de L Silva, L., Gai, E. Z., Roman, C., Parodi, T. V., Bürger, M. E., Baldisserotto, B., de M Flores, É. M., & Heinzmann, B. M. (2014). S-(+)-linalool from *Lippia alba*: Sedative and anesthetic for silver catfish (*Rhamdia quelen*). *Veterinary Anesthesia and Analgesia*, *41*(6), 621–629.

Hoseini, S. M. (2011). Efficacy of clove powder solution on stress mitigation in juvenile common carps, *Cyprinus carpio* (Linnaeus). *Comparative Clinical Pathology*, *2*, 359–362.

Hoseini, S. M., Rajabiesterabadi, H., Khalili, M., Yousefi, M., Hoseinifar, S. H., & Van Doan, H. (2020). Antioxidant and immune responses of common carp (*Cyprinus carpio*) anesthetized by cineole: Effects of anesthetic concentration. *Aquaculture*, *520*, 734680.

Hoseini, S. M., Taheri Mirghaed, A., Pagheh, E., Hoseinifar, S. H., & Van Doan, H. (2021). Anesthesia of rainbow trout with citronellal: Efficacy and biochemical effects. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *337*, 227–237.

Hoseini, S. M., Taheri Mirghaed, A., & Yousefi, M. (2019). Application of herbal anesthetics in aquaculture. *Reviews in Aquaculture*, *11*(3), 550–564.

Keene, J. L., Noakes, D. L. G., Moccia, R. D., & Soto, C. G. (1998). The efficacy of clove oil as an anesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research*, *29*(2), 89–101.

Limma-Netto, J. D., Oliveira, R. S. M., & Copatti, C. E. (2017). Efficiency of essential oils of *Ocimum basilicum* and *Cymbopogum flexuosus* in the sedation and anesthesia of Nile tilapia juveniles. *Anais da Academia Brasileira de Ciências*, *89*, 2971–2974. https://doi.org/10.1590/0001-3765201720170001

Limma-Netto, J. D., Sena, A. C., & Copattı, C. E. (2016). Essential oils of *Ocimum basilicum* and *Cymbopogon flexuosus* in the sedation, anesthesia and recovery of tambacu (Iaractus mesopotamicus male x *Colossoma macropomum* female). *Boletim do Instituto de Pesca*, *42*(3), 727–733. https://doi.org/10.20950/1678-2305.2016v42n3p727

Mazandarani, M., & Hoseini, S. M. (2017). Menthol and 1, 8-cineole as new anesthetics in common carp, *Cyprinus carpio* (Linnaeus, 1758). *Aquaculture Research*, *48*(6), 3041–3051.

Palić, D., Herolt, D. M., Andreasen, C. B., Menzel, B. W., & Roth, J. A. (2006). Anesthetic efficacy of tricaine methanesulfonate, metomidate and eugenol: Effects on plasma cortisol concentration and neutrophil function in fathead minnows (*Pimephales promelas* Rafinesque, 1820). *Aquaculture*, *254*(1–4), 675–685.

Priborsky, J., & Velisek, J. (2018). A review of three commonly used fish anesthetics. *Reviews in Fisheries Science & Aquaculture*, *26*(4), 417–442.

Ribeiro, A. S., Batista, E. D. S., Dairiki, J. K., Chaves, F. C. M., & Inoue, L. A. K. A. (2016). Anesthetic properties of *Ocimum gratissimum* essential oil for juvenile matrinxã. *Acta Scientiarum. Animal Sciences*, *38*(1), 1–7. https://doi.org/10.4025/actascianimsci.v38i1.28787

Ross, L. G., & Ross, B. (2008). *Anesthetic and sedative techniques for aquatic animals* (3rd ed., pp. 41–88). Blackwell Publishing.

Salbego, J., Becker, A. G., Parodi, T. V., Zeppenfeld, C. C., Gonçalves, J. F., Loro, V. L., Morsch, V. M. M., Schetinger, M. R. C., Maldaner, G., Morel, A. F., & Baldisserotto, B. (2015). Methanolic extract of *Condalia buxifolia* added to transport water alters biochemical parameters of the silver catfish *Rhamdia quelen*. *Aquaculture*, *437*, 46–50.

Santos, L. M. A., Craveiro, S. C., & Calado, A. J. (1996). Silica-scaled chrysophytes from threeα mesosaprobic water bodies of Central Portugal. *Nova Hedwigia Beih.*, *114*, 171–191.

Sharopov, F. S., Satyal, P., Ali, N. A. A., Pokharel, S., Zhang, H., Wink, M., Kukaniev, W. N., & Setzer, W. N. (2016). The essential oil compositions of *O. basilicum* from three different regions: Nepal, Tajikistan, and Yemen. *Chemistry & Biodiversity*, *13*(2), 241–248.

Silva, E., Deschamps, G. T., Matter, F. D. L., Bertoldi, F. C., Aldegunde, M., da Silva, D. F., Lopes, C., Jatobá, A., & Weber, R. A. (2021). The anesthetic efficacy of *Eucalyptus globulus* essential oil on silver catfish (*Rhamdia quelen*). *Aquaculture Research*, *52*(11), 5190–5197.

Silva, H. N. P. D., Carvalho, B. C. F., Maia, J. L. S., Becker, A. G., Baldisserotto, B., Heinzmann, B. M., Mourão, R. H. V., & da Silva, L. V. F. (2019). Anesthetic potential of the essential oils of *Lippia alba* and *Lippia origanoides* in Tambaqui juveniles. *Ciência Rural*, *49*(6), 1–6. https://doi.org/10.1590/0103-8478cr20181059

Souza, C. F., Baldissera, M. D., Bianchini, A. E., da Silva, E. G., Mourão, R. H. V., da Silva, L. V. F., Schmidt, D., Heinzmann, B. M., & Baldisserotto, B. (2018). Citral and linalool chemotypes of *Lippia alba* essential oil as anesthetics for fish: A detailed physiological analysis of side effects during anesthetic recovery in silver catfish (*Rhamdia quelen*). *Fish Physiology and Biochemistry*, *44*, 21–34.

Taheri Mirghaed, A., Ghelichpour, M., & Hoseini, S. M. (2016). Myrcene and linalool as new anesthetic and sedative agents in common carp, *Cyprinus carpio*-comparison with eugenol. *Aquaculture*, *464*, 165–170.

Taheri Mirghaed, A., Ghelichpour, M., Zargari, A., & Yousefi, M. (2018). Anesthetic efficacy and biochemical effects of 1,8-cineole in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792). *Aquaculture Research*, *49*(6), 2156–2165.

Taheri Mirghaed, A., Hoseini, S. M., Aydın, B., Paolucci, M., Hoseinifar, S. H., & Van Doan, H. (2022). Effects of anesthesia with 1,8-cineole on haematological and plasma stress responses in Caspian trout, *Salmo caspius*, subadults. *Aquaculture Research*, *53*(3), 893–900.

Tsantilas, H., Galatos, A. D., Athanassopoulou, F., Prassinos, N. N., & Kousoulaki, K. (2006). Efficacy of 2-phenoxyethanol as an anesthetic for two size classes of white sea bream, *Diplodus sargus* L., and sharp snout sea bream, *Diplodus puntazzo* C. *Aquaculture*, *253*, 64–70. https://doi.org/10.1016/j.aquaculture.2005.07.034

Velisek, J., Svobodova, Z., Piackova, V., Groch, L., & Nepejchalova, L. (2005). Effects of clove oil anesthesia on common carp (*Cyprinus carpio* L.). *Veterinary Medicine*, *50*(6), 269–275.

Velisek, J., Wlasow, T., Gomulka, P., Svobodová, Z., Novotný, L., & Ziomek, E. (2006). Effects of clove oil anesthesia on European catfish (*Silurus glanis L.*). *Acta Veterinaria*, *75*, 99–106.

Ventura, A. S., Corrêa Filho, R. A. C., Teodoro, G. C., Laice, L. M., Barbosa, P. T. L., Stringhetta, G. R., & Povh, J. A. (2020). Essential oil of *Ocimum basilicum* and eugenol as sedatives for Nile tilapia. *Journal of Agricultural Studies*, *8*(2), 657–665.

Ventura, A. S., Jerônimo, G. T., Corrêa Filho, R. A. C., de Souza, A. I., Stringhetta, G. R., da Cruz, M. G., dos Santos Torres, G., Gonçalves, L. U., & Povh, J. A. (2021). *Ocimum basilicum* essential oil as an anesthetic for tambaqui *Colossoma macropomum*: Hematological, biochemical, non-specific immune parameters and energy metabolism. *Aquaculture*, *533*, 736124.

Yousefi, M., Hoseini, S. M., Aydın, B., Mirghaed, A. T., Kulikov, E. V., Drukovsky, S. G., Seleznev, S. B., Rudenko, P. A., Hoseinifar, S. H., & Van Doan, H. (2022). Anesthetic efficacy and hemato-biochemical effects of thymol on juvenile Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, *547*, 737540.

Yousefi, M., Hoseinifar, S. H., Ghelichpour, M., & Hoseini, S. M. (2018). Anesthetic efficacy and biochemical effects of citronellal and linalool in common carp (*Cyprinus carpio* Linnaeus, 1758) juveniles. *Aquaculture*, *493*, 107–112.

Yousefi, M., Vatnikov, Y. A., Kulikov, E. V., & Ghelichpour, M. (2019). Change in blood stress and antioxidant markers and hydromineral balance of common carp (*Cyprinus carpio*) anesthetized with citronellal and linalool: Comparison with eugenol. *Aquaculture Research*, *50*(4), 1313–1320.

Zahl, I. H., Kiessling, A., Samuelsen, O. B., & Olsen, R. E. (2010). Anesthesia induces stress in Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*). *Fish Physiology and Biochemistry*, *36*, 719–730.

Zeppenfeld, C. C., Toni, C., Becker, A. G., dos Santos Miron, D., Parodi, T. V., Heinzmann, B. M., Barcellos, L. J., Koakoski, G., da Rosa, J. G., Loro, V. L., & da Cunha, M. A. (2014). Physiological and biochemical responses of silver catfish, *Rhamdia quelen*, after transport in water with essential oil of *Aloysia triphylla* (L'Herit) Britton. *Aquaculture*, *418*, 101–107.

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