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Original Research Article

Tea Tree (*Melaleuca alternifolia*) and Geranium (*Pelargonium graveolens*) Essential Oils as new Anesthetics in Rainbow Trout (*Oncorhynchus mykiss*)

Key words

Oncorhynchus mykiss;
anesthesia;
histopathology;
tea tree;
geranium

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Abstract: In the present study was investigated anesthetic and histopathological effects of tea tree (*Melaleuca alternifolia*) and geranium (*Pelargonium graveolens*) essential oils in rainbow trout. These essential oils were applied at 20, 50, 70, 100, 150, 200, 300, 400, 500 and 600 mg L-1 concentrations to determine the anesthetic effect. As a result of this study, major components were found as beta-citronellol (27.52%) for geranium oil and 4-terpineol (40.77%) for tea tree oil. The lowest effective doses of geranium and tea tree oils were 400 mg L^{-1} and 300 mg L^{-1} for deep anesthesia of fish, respectively. At these doses, anesthesia induction and recovery times were 129.00 and 247.00 s for geranium essential oil, 118.67 and 65s for tea tree essential oil, respectively. No histopathological findings in liver, kidney and gill tissues were observed in anesthetized fish for each essential oil. In conclusion, geranium and tea tree oils can use as safe and effective anesthetics for rainbow trout.

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Introduction

Anesthetics are used for preventing physical injury and stress during aquaculture procedures (1, 2). Drugs, such as quinaldine, etomidate, phenoxyethanol and MS-222 have been often used for anesthesia in aquaculture (3). These agents cause side effects such as excessive mucus and secretion gill irritation (4). Therefore, studies on natural anesthetics in fish have increased recently.

Tea tree (*Melaleuca alternifolia*) is a native plant of Australia. Tea tree is mainly composed of terpinen-4-ol, g-terpinene, α-terpinenene and 1.8-cineole (5,6). The anesthetic activity of *M. alternifolia* essential oil has been investigated in different fish species: common carp (*Cyprinus carpio*) (6), silver catfish (*Rhamdia quelen*) (7), *Astyanax bimaculatus* (8) and gilthead seabream (*Sparus aurata*) (9) In addition, tea tree essential oil was recommended as anesthetic agent for transport in tambaqui (*Colossoma macropomum*) (10) and nil tilapia (*Oreochromis niloticus*) (11).

Geranium (*Pelargonium graveolens*) exists naturally in South Africa and is generally cultivated in many countries of the world (12,13). The major components of geranium are citronellol, geranoil and citronellal. These components have sedative and antidepressant effects (14,15,16). There is a limited number of studies about the anesthetic efficiency of geranium essential oil in fish. Can et al. (17) showed that geranium oil was an effective anesthetic in *Labidochromis caeruleus* and *Sciaenochromis fryeri*.

In this study, use of geranium (*P. graveolens*) and tea tree (*M. alternifolia*) essential oils in rainbow trout as anesthetic agents and their histopathologic effects were investigated for the first time.

Table 1: Anesthesia stages in fish

Stage	Description	Behaviour 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
	Recovery	Regaining equilibrium and active swimming

Materials and methods

Eessential oil

Tea tree (*Melaleuca alternifolia*) and geranium (*Pelargonium graveolens*) essential oils were purchased from Botalife company (Isparta, Turkey). Analysis of essential oils for determination of their components was performed by GC-MS (Gas chromatography–mass spectrometry).

Experimental design

Fish (10 g) were provided from a rainbow trout farm (Isparta, Turkey). 200 trout were placed in two tanks (450 L) in flowing water system. Fish were fed ad libitum with commertial feed for 15-day adaptation period. Water temperature, pH and dissolved oxygen were measured as 8.2°C, 7.9 and 9.06 mg/L, respectively. Essential oils of tea tree (*M. alternifolia*) and geranium (*P. graveolens*) were dissolved in ethyl alcohol (95%) at 1:10 ratio. After adaptation, anesthetic effects of these essential oils at 20, 50, 70, 100, 150, 200, 300, 400, 500 and 600 mg L^{-1} concentrations were determined in fish. Induction and recovery stages were evaluated according to Keene et al. (18) (Table 1). Each fish was considered replicate (n=10 for each concentration). For anesthesia induction, each fish (one at a time) were caught with hand net from the adaptation tanks and taken in to the aquariums (10 L). After anasthesia, fish were placed to the aquarium (10 L) containing clean water for recovery times. Each stage was recorded with a stopwatch. After recovery, abnormal behavior (swimming, position in the water column, etc.) and mortalities were observed. This study was taken permission by Isparta Applied Sciences University, Animal Experimentation Ethics Committee (No: 77211729-804.01/694).

Histopathological examination

Five fish anesthezed with both essantial oils at 600 mg L^{-1} concentration were euthanized by severing the spinal cords and sampled from tissues (gill, kidney, liver). Tissue samples were fixed in 10% neutral formalin and processed. The samples were embedded in paraffin, and 5µm sections were taken by a Leica RM 2155 rotary microtome (Leica Microsystem). Then, sections were stained with hematoxylin and eosin (HE) examined under a light microscope and microphotographed.

Statistical analysis

The homogeneity of variances by Levene's test and normality of data by Shaphiro-Wilk test were controlled. Then, data were analyzed by one-way ANOVA and significant variations were tested with the Duncan test ($p < 0.05$). Regression equations were used to determination of a relationship between anesthetic concentrations and induction/ recovery times.

Results

Essential oil compounds

The compounds of tea tree (*Melaleuca alternifolia*) and geranium (*Pelargonium graveolens*) essential oils were given in Table 2, 3. Beta.-citronellol (27.52%), citronellyl formate (13.92) in geranium and 4-terpineol (40.77%), gama terpinene (20.18 %) and alpha. terpinene (10.96 %) in tea tree were found as major compounds.

Anesthesia induction and recovery times

Anesthesia induction time decreased with rising of the concentration of tea tree (Melaleuca alternifolia) and geranium (Pelargonium graveolens) essential oils. Recovery time rised with increasing of dose of these oils. These essential oils did not cause any abnormal behavior or mortalities in rainbow trout.

The lowest effective concentration for deep anesthesia in fish for tea tree essential oil was found as 300 mg L-1 (Table 4). Anesthesia induction and recovery times at this concentration were 118.67 and 65 s respectively . There is a correlation between the anesthesia induction and recovery times with tea tree essential oil concentrations (R2: 0.86 for

Table 2: Components of tea tree essential oil (%)

Name	Retention time	Area%
Furan. Tetrahydro-2.5-dimethyl-	2.509	0.02
Alpha.-pinene. (-)-	6.684	4.30
Camphene	7.249	0.05
Beta.-phellandrene	8.086	0.23
2-.beta.-pinene	8.293	0.30
3-menthene	8.567	0.05
L-Phellandrene	9.479	0.11
Alpha. Terpinene	10.011	10.96
Benzene. Methyl(1-methylethyl)- (CAS) Cymol	10.341	4.79
L-Limonene	10.566	2.86
1.8-cineole	10.716	1.96
Beta. Ocimene y	11.403	0.51
Gama terpinene	12.101	20.18
3.8-p-Menthadiene	12.667	0.41
3-Methyl-4-cyclohexene-1.2-dicarboxylic anhydride	12.765	0.19
Alpha.-terpinolene	13.457	2.63
Alpha.-fenchone	13.600	0.24
Dimethylstyrene <alpha-para->	13.690	0.07
Cyclopentanol. 1.2-dimethyl-3-(1-methylethenyl)-. [1R-(1.alpha..2.beta..3.beta.)]	16.959	
4-Terpineol (terpinen-4-ol)	19.377	40.77
3.6-Dimethyl-2.3.3a.4.5.7a-hexahydrobenzofuran	19.545	0.01
Alpha. Terpıneol	20.142	6.33
Terpineol <gamma->	20.403	0.30
7-Oxabicyclo[4.1.0]heptane. 1-methyl-4-(2-methyloxiranyl)-	23.759	0.16
Gurjunene <alpha->	33.601	0.41
Alpha.-guaiene	38.669	0.59
Viridiflorene	38.891	1.03
Alpha.-selinene	39.139	0.16
Alloaromadendrene	44.591	0.19
		100.00

Table 3: Compounds of geranium (Pelargonium graveolens) essential oil (%)

Name	Retention time	Area%
Alpha-pinene. (-)-	6.663	1.10
Beta-myrcene	8.720	0.25
L-Phellandrene	9.457	0.11
Benzene Methyl(1-methylethyl)- (CAS) Cymol	10.296	0.25
Limonene	10.529	0.36
3.6-dimethyl-3.5-cycloheptadienone	10.610	0.07
1.3.6-Octatriene. 3.7-dimethyl-. (E)- (CAS) .BETA. OCIMENE Y	11.377	0.13
Linalool oxide cıs	12.666	0.24
Linalool l	14.319	7.33
Rose oxide A	14.849	1.77
Rose oxide A	15.789	0.70
6-Octenal. 3.7-dimethyl-. (R)-	17.337	0.29
P-Menthan-3-one. (1R.4R)-(+)-	18.086	10.70
Beta. Fenchyl alcohol	20.013	0.38
Beta.-citronellol	22.451	27.52
Z-citral	22.880	2.29
2.6-Octadien-1-ol. 3.7-dimethyl-. (Z)-	23.942	4.07
E-citral	24.810	0.64
6-Octen-1-ol. 3.7-dimethyl-. Formate (CAS) Citronellyl formate	25.251	13.92
Geranyl bromide	26.830	6.49
Alpha.-cubebene	29.801	0.27
Citronellyl acetate	30.182	0.37
Copaene <alpha->	31.585	0.98
Beta. Bourbonene	32.064	2.80
Caryophyllene	34.310	2.36
Beta.-cubebene	34.996	0.19
Aristolen	35.786	0.30
Citronellyl propionate	35.973	0.19
Gurjunene <alpha->	36.214	0.77
Alpha.-humulene	36.565	0.32
Cadina-1(6).4-diene <10betah->	37.695	0.20
Germacrene-d	38.202	2.35
Bicyclogermacrene	39.124	0.51
Cadinene <delta->	40.628	0.90
Citronellyl butyrate	41.282	0.47
Isospathulenol	42.168	0.26
Geranyl butyrate	43.134	1.04
2-Butenoic acid. 2-methyl-. 2-phenylethyl ester. (E)-	44.560	1.01
Eudesmol <epi-gamma->	46.728	5.87
Citronellyl tiglate <(E)->	49.331	0.22
		100.00

Table 4: Anesthesia induction and recovery times for tea tree essential oil

Concentration (mg L^{1})	Induction Time (s) AnaeAnesthesiael				Recovery Time (s)
	I	II	III	IV	
20	58.33±7.51^{a}	-	-	-	
50	52.67±5.51^{a}	236.00±4.58^{a}	-	-	
70	50.00±6.24^{a}	120.33±7.51^{b}	-	-	
100	35.67±4.04^{b}	87.33±5.51^{c}	-	-	
150	34.67±5.51^{bc}	85.33±4.51^{c}	129.33±6.66^{a}	-	
200	28.00±2.65^{abc}	55.00±7.21^{d}	116.67±4.04^{b}	300.67±7.37^{a}	66.00±4.58^{a}
300	26.33±4.04^{cd}	51.00±6.24^{d}	103.00±7.21^{c}	118.67±6.03^{b}	65.00±8.19^{b}
400	25.67±4.51^{d}	49.00±8.19^{d}	75.33±4.51^{d}	117.67±3.21^{bc}	149.00±7.00^{c}
500	22.00±3.00^{d}	48.00±4.58^{de}	67.67±4.04^{d}	107.33±6.51^{c}	237.67±3.21^{d}
600	20.67±2.52^{d}	38.00±3.00^{e}	46.67±5.51^{e}	64.67±5.51^{d}	330.33±7.09^{d}
R^2	0.95	0.89	0.92	0.87	0.99
Equation	$y=175.18x^{-0.33}$	$y=1826.2x^{-0.614}$	$y=0.0005x^{2}-0.5158x+198.05$	$y=148218x^{-1.198}$	$y=0.0014x^{2}-0.3971x+81.4$

Table 5: Anesthesia induction and recovery times for geranium essential oil

Concentration (mg L^{1})	Induction Time (s) AAnesthesialevel				Recovery Time (s)
	I	II	III	IV	
20	67.33±5.69^{a}	-	-	-	
50	63.00±6.08^{a}	145.00±8.89^{a}	272.33±10.97^{a}	-	
70	61.00±9.54^{a}	147.00±4.36^{a}	261.67±5.69^{a}	-	
100	60.00±3.00^{ab}	144.67±7.51^{a}	259.67±8.50^{a}	572.67±12.01^{a}	94.67±9.07^{d}
150	59.00±7.21^{ab}	94.67±10.21^{b}	220.00±9.17^{b}	534.67±7.51^{b}	122.0±12.12^{c}
200	48.00±6.00^{bc}	65.00±8.89^{c}	133.67±10.07^{c}	225.00±11.14^{c}	222.67±9.29^{b}
300	47.67±8.62^{bc}	64.67±13.50^{c}	105.33±6.66^{d}	227.67±9.29^{c}	245.33±11.02^{a}
400	38.33±10.02^{d}	58.00±8.19^{c}	94.33±11.72^{de}	129.00±9.54^{d}	247.00±9.17^{a}
500	21.00±6.00^{e}	57.33±9.29^{c}	84.67±7.09^{e}	115.33±11.93^{de}	246.33±17.56^{a}
600	19.33±3.79^{e}	34.67±7.51^{d}	56.00±7.55^{f}	100.33±12.01^{f}	264.33±10.21^{a}
R^2	0.97	0.90	0.95	0.93	0.89
Equation	$y=-2E-05x^{2}-0.0701x+67.362$	$y=1463.2x^{-0.551}$	$y=0.0009x^{2}-0.9692x+326.75$	$y=74097x^{-1.04}$	$y=-0.0014x^{2}+1.2283x-7.8595$

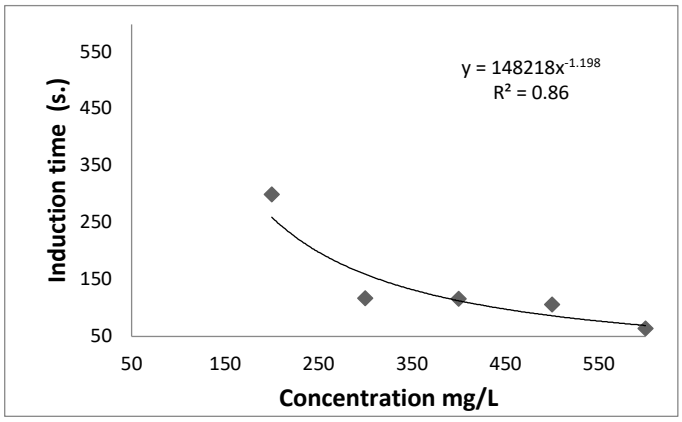

Figure 1: Relationship between tea tree oil concentration with anesthesia induction time of rainbow trout (stage 4).

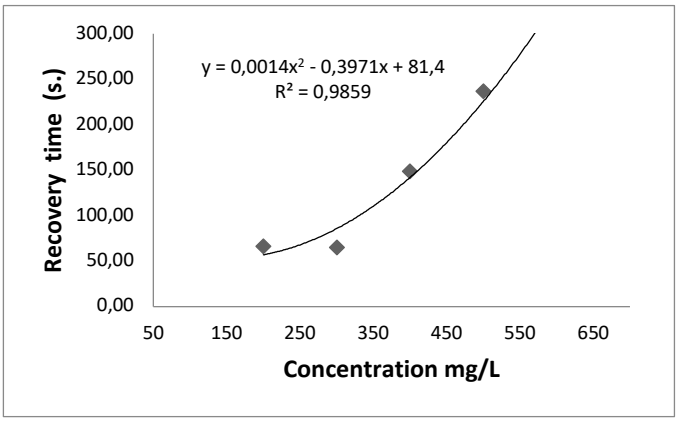

Figure 2: Relationship between tea tree oil concentration with recovery time of rainbow trout

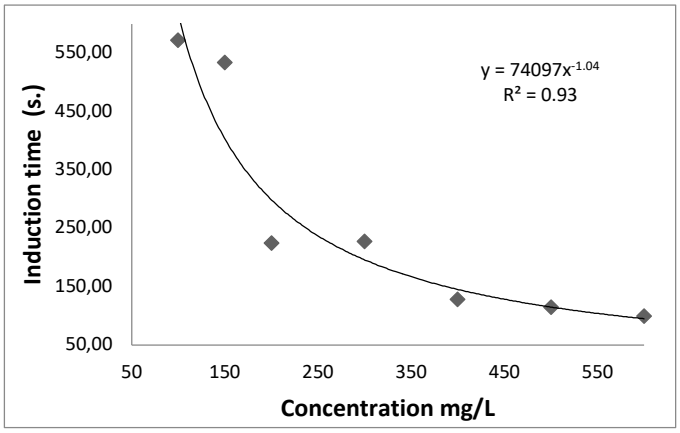

Figure 3: Relationship between geranium oil concentration with anesthesia induction time of rainbow trout (stage 4).

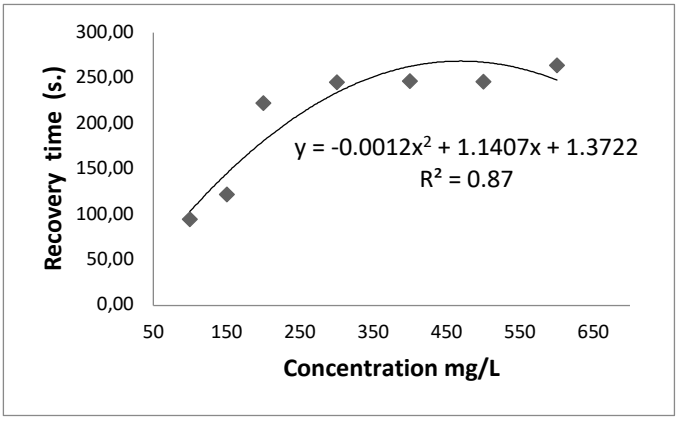

Figure 4: Relationship between geranium oil concentration with recovery time of rainbow trout

anesthesia induction time; 0.99 for recovery time) (Figures 1 and 2).

The lowest effective dose of geranium essential oil in trout was found as 400 mg L -1 for deep anesthesia (anesthesia induction: 129s s and recovery times: 247s) (Table 5). There is a correlation between the anesthesia induction and recovery times with geranium essential oil concentrations (R2: 0.93 for anesthesia induction time; 0.87 for recovery time) (Figures 3 and 4).

Histopathological results

No histopathological findings were detected in gill, liver and kidney of fish anesthetized with each of the essential oils (Figure 5 and 6).

Discussion

Beta citronellol (27.52%), citronellyl formate (13.92%) for geranium (*Pelargonium graveolens*) essential oil and 4-terpineol (40.77%), gama terpinene (20.18 %) and alpha terpinene (10.96 %) for tea tree were found as the primary components in this study. Simalarly, the major components of essential oils have been reported as citronellol (33.49%), geraniol (*synonym* citronellyl formate) (15.08%) for geranium (*Pelargonium graveolens*) (17) and terpinen-4-ol for tea tree *Melaleuca alternifolia*. (7).

Ideal deep anesthesia should occur in approximately 5 minutes and recovery time should not be longer than 10 minutes (19). According to these criteria, the lowest effective dose for deep anesthesia of geranium essential oil in rainbow trout was found as 400 L^{-1} (induction: 129s and recover time: 247s) in the present study. There was one study on the anesthetic effect of geranium essential oil in fish. Can et al. (17) determined that optimal concentration of geranium oil for deep anesthesia in *Sciaenochromis fryeri* and *Labidochromis caeruleus* was 75 µl L^{-1}. Differences can be due to different fish species.

Tea tree (*Melaleuca alternifolia*) essential oil at 300 mg L^{-1} (induction time: 118,67 s and recovery time: 65s) was ideal concentration for deep anesthesia in this study. Simalarly, da Silva et al. (8) noted that *M. alternifolia* at 300

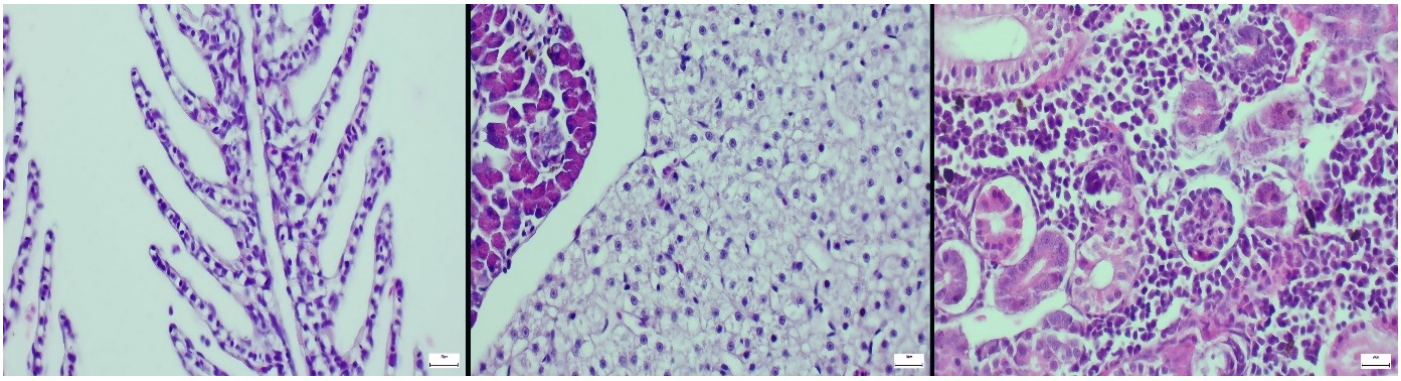

Figure 5: Gill, liver and kidney histology of fish exposed to 600 mg L tea tree essantial oil HE, scale bars =50µm, x20

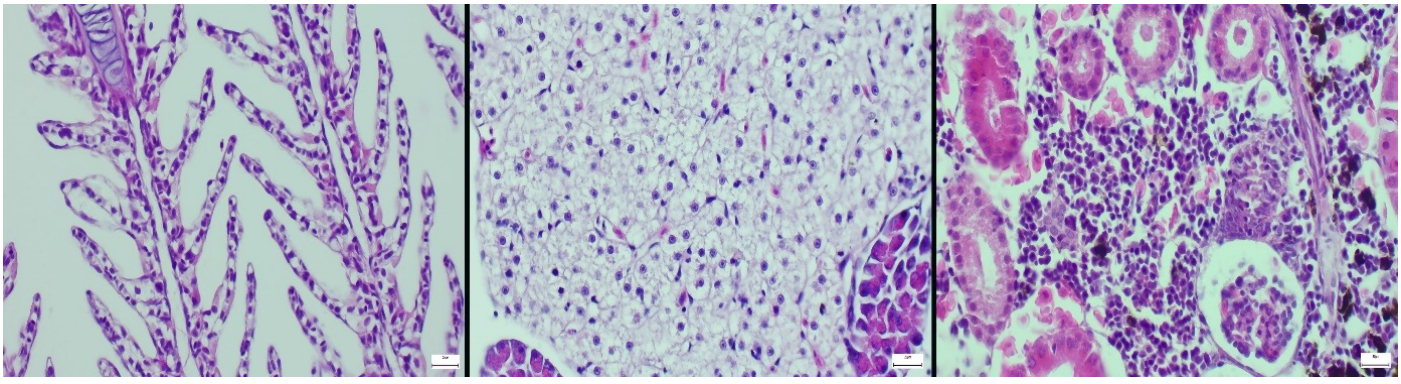

Figure 6: Gill, liver and kidney histology of fish exposed to 600 mg L geranium essantial oil. HE, scale bars =50µm, x20

µl L^{-1} concentration has effective anesthetic properties in Astyanax bimaculatus. Golomazou et al. (9) also observed anesthetic effect at 200 µl L^{-1} concentration (induction time: 691.25 s and recovery time: 302.00) of *M. alternifolia* oil in gilthead seabream (*Sparus aurata*). Hajek (6) informed that *M. alternifolia* at 0.2 to 0.6 ml L^{-1} concentrations provided sedation and immobilization in common carp. Souza et al. (7) showed that tea tree (*Melaleuca alternifolia*) essential oil provided deep anesthesia at concentrations ranging from 500 (316 s) to 1000 µL L^{-1} (185 s) in silver catfish.

In this study, no pathological finding in gill, liver and kidney was observed in fish anesthetized with *Melaleuca alternifolia* and *Pelargonium graveolens* essential oil. Similarly, Santos et al. (10) reported that harmful morphological alterations to the gill epithelium did not observed in tambaqui anesthtezed with tea tree oil during transport. No histopathological effects also reported in liver, kidney and gill of rainbow trout anesthetized with basil (*Ocimum basilicum*), eucalyptus (*Eucalyptus globulus*), coriander (*Coriandrum sativum*), lavender (*Lavandula angustifolia*) and laurel (*Laurus nobilis*) essential oils at 600 mg L^{-1} concentration (20,21,22). Metin et al. (23) reported no lesion in gill, skin, hepatopancreas in common carp anesthetized with lavender (*L. angustifolia*) and cumin (*Cuminum cyminum*) essential oils at 400 and 500 mg L^{-1} concentrations. However, unlike these studies, Yigit and Kocaayan (22) observed marked hyperemia, edema, inflammatory cell infiltrations and desquamation in gills of rainbow trout anesthezied with thyme (*Origanum onites*) essential oil at 600 mg L^{-1} concentration. Brandão et al. (24) also noted that hypertrophy and hyperplasia in gills were observed with use as anesthetics of *Mentha piperita*, *Lippia sidoides* and *Aloysia triphylla* essential oils in tambaqui. Differences among these studies may be due to differences in essential oils and main components in essential oils.

In the present study, the lowest effective concentrations for deep anesthesia in rainbow trout were 400 mg L^{-1} for geranium essential oil and 300 mg L^{-1} for tea tree essential oil. Additionally, these essential oils do not cause tissue damage in fish. Concequently, geranium and tea tree essential oils are suitable using as safely and effectively herbal anesthetics for rainbow trout.

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Authorship Contributions. Hakan DIDINEN designed and performed the experiment.

Conflict of Interest. No conflict of interest was declared by the authors.

Data Availability Statement. Data are available on request due to privacy or other restrictions.

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Eterična olja čajevca (*Melaleuca alternifolia*) in pelargonije (*Pelargonium graveolens*) kot nova anestetika pri šarenki (*Oncorhynchus mykiss*)

H. Didinen

Izvleček: Študija je preučevala anestetične in histopatološke učinke eteričnih olj čajevca (*Melaleuca alternifolia*) in pelargonije (*Pelargonium graveolens*) na šarenko. Ti eterični olji sta bili uporabljeni v koncentracijah 20, 50, 70, 100, 150, 200, 300, 400, 500 in 600 mg L^{-1} za določitev anestetičnega učinka. Na podlagi te študije so bile ugotovljene naslednje glavne sestavine: beta-citronelol (27,52 %) za olje geranije in 4-terpineol (40,77 %) za olje čajevca. Najnižji učinkoviti odmerki olja geranije in čajevca so bili 400 mg L^{-1} oziroma 300 mg L^{-1} za globoko anestezijo rib. Pri teh odmerkih sta bila čas indukcije anestezije in čas prebujanja iz anestezije 129,00 in 247,00 s za eterično olje geranije ter 118,67 in 65 s za eterično olje čajevca. Pri anesteziranih ribah pri nobenem eteričnem olju niso opazili histopatoloških sprememb v tkivih jeter, ledvic in škrg. Iz tega sledi, da se olji geranije in čajevca lahko uporabljata kot varna in učinkovita anestetika za šarenko.

Ključne besede: *Oncorhynchus mykiss*; anestezija; histopatologija; čajevec; geranija