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Original Article

Anesthetic efficacy of *Mentha piperita* and *Mentha spicata* essential oils on rainbow trout (*Oncorhynchus mykiss*)

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Abstract
In the present study, anesthetic effects of *Mentha piperita* and *Mentha spicata* essential oils were investigated on rainbow trout. For this purpose, five treatments of the essential oils (50, 100, 200, 300 and 500 mg L^{-1} concentrations) and one control (2-phenoxyethanol; 0.3 ml L^{-1} concentration) have been used for rainbow trout (*Oncorhynchus mykiss*; mean weight of 15 g). In addition, the mean lethal doses (10 minutes LC_{50} concentration) of the essential oils were also determined. *Mentha piperita* and *M. spicata* essential oils at 200, 300 and 500 mg L^{-1} doses showed better anaesthetic effect than control ($p < 0.05$). The ideal anesthetic dose of each essential oil was 200 mg L^{-1} ($p < 0.05$). At this concentration, deep anesthesia (Stage IV) induction time for *M. piperita* and *M. spicata* on rainbow trout were found as 169.66 s and 65.0 s, respectively. The recovery times at 200 mg L^{-1} concentration were 188.0 s for *M. piperita* and 65.33 s for *M. spicata*. The lethal dose of both essential oils in fish was 150 mg L^{-1}. This study suggests that the essential oils of *M. piperita* and *M. spicata* are both effective anesthetic agents for rainbow trout.

Keywords: essential oil; *Mentha piperita*; *Mentha spicata*; natural anesthesic; rainbow trout

1 | INTRODUCTION

Anesthetics are widely used to reduce stress, metabolic rate and oxygen demand, as well as to facilitate handling procedures such as biometric measurements and transportation in fish farming (Purbosari *et al.* 2019; Brandão *et al.* 2021). 2-phenoxyethanol, quinaldine, MS-222, benzocaine, etomidate and eugenol are the most commonly used anesthetics in aquaculture (Inoue *et al.* 2011; Priborsky and Velisek 2018). The use of synthetic anaesthetics is limited by side effects such as hyperactivity, tissue irritation, and increased mucus production in fish (Gilderhus and Marking 1987; Palić *et al.* 2006; Aydın and Barbas 2020). Therefore, there is a need to find reliable and effective natural anesthetic products for fish. In recent studies, more effective and safe herbal essential oils have been investigated as an alternative to synthetic agents (Inoue *et al.* 2003; Guénette *et al.* 2007; Metin *et al.* 2022; Yigit *et al.* 2022a 2022b). Essential oils such as clove (*Syzygium aromaticum*) and mint (*Mentha* spp.) have been recommended for fish anaesthesia due to their low costs, easy accessibility, efficacy and environmental safety (Pedrazzani and Neto 2016).

The genus *Mentha*, which includes many high value plants, belongs to the family Lamiaceae (Labiatae). The mentha essential oil is rich in menthol, carvone and menthone (Soković *et al.* 2009; Singh and Pandey 2018). These compounds have been reported to exhibit anesthetic, sedative, and muscle relaxant effects, making these plants promising candidates for use as natural anesthetic agents. The anesthetic properties of menthol have been suggested to be related to the activation of GABA-A receptors (Kasai *et al.* 2014). Similarly, carvone has been

reported to exert anesthetic effects on both the peripheral and central nervous systems, including central nervous system depression and sedative activity (Sousa *et al.* 2007).

Mentha piperita and *Mentha spicata* have economic importance due to their medicinal and aromatic values (Telci *et al.* 2011). *Mentha piperita*, named as "peppermint", is widely cultivated in temperate climates for essential oil production worldwide (Farnad *et al.* 2014). The main compounds of *M. piperita* essential oil are menthol, 1,8-cineole, menthofuran and menthone (Beigi *et al.* 2018). Menthol is responsible for the anesthetic effect in fish (de Pádua *et al.* 2018). The effects of *M. piperita* essential oil have been investigated in different fish species: tambaqui *Colossoma macropomum* (Facanha and Gomes 2005; Brandão *et al.* 2021), silver catfish *Rhamdia quelen* (Spanghero *et al.* 2019), blue dolphin cichlid *Cyrtocara moorii* (Can and Sümer 2019), European catfish *Silurus glanis* (Krasteva *et al.* 2021), and common carp (Rakhshani *et al.* 2018).

Mentha spicata, also known as spearmint, is grown commercially worldwide. Carvone is the major ingredient in the oil of the cultivated plant. However, wild populations of *M. spicata* contains menthone, piperitone oxide, piperitone, isomenthone, linalool, and 1,8-cineole (Telci *et al.* 2010; Şarer *et al.* 2011). *Mentha spicata* has a sedative effect (Mahendran *et al.* 2021). To the best knowledge of the authors, there is only one study on the use of *M. spicata* essential oil as an anesthetic in fish. The anesthetic effect of *M. spicata* has been investigated in common carp by Roohi and Imanpoor (2015). However, no studies are available on the anesthetic effect of *M. spicata* essential oil on rainbow trout.

The anesthetic effect of menthol, the active component of *M. piperita* essential oil, has also been studied in different fish species: *Salminus brasiliensis* (de Pádua *et al.* 2018), *Lophiosilurus alexandri* (Ananias *et al.* 2022), *Oligosarcus argenteus* (Uehara *et al.* 2019), *Astyanax altiparanae* (Pereira-da-Silva *et al.* 2016), angelfish (Romaneli *et al.* 2018), platy (Hoshiba *et al.* 2015), guppy (da Cunha *et al.* 2020), African cichlid (Ferreira *et al.* 2021), rainbow trout (Teta and Kaiser 2019), Nile tilapia (Simões and Gomes 2009), common carp (Hoshiba *et al.* 2015; Roohi and Imanpoor 2015; Mazandarani and Hoseini 2017) and tambaqui (Facanha and Gomes 2005). However, there is no study on the anesthetic efficacy of piperitone oxide, the major compound of wild-growing *M. spicata*, on fish. The aim of this study was to investigate the anaesthetic effects of *M. piperita* and *M. spicata* essential oils as new anaesthetic agents for rainbow trout (*Oncorhynchus mykiss*).

2 | METHODOLOGY

2.1 Essential oil isolation and analysis

In this study, *M. piperita* and *M. spicata* were taken in the experimental areas of Isparta University of Applied Sciences. The plants were dried in the shadow and the stem parts were separated after drying. The essential oils were isolated with the distillation process in the Industrial Crops laboratory in the Faculty of Agriculture, Isparta Applied Sciences University. 500 g plant samples in 1.5 L water were extracted by hydro-distillation for 3 hours using the Clevenger apparatus according to the standard procedure described in European Pharmacopoeia.

The compositions of essential oils were analyzed using gas chromatography-mass spectrometry (GC-MS) by modified method from Stein, 1990. GC-MS analysis of the essential oil samples was performed on Shimadzu (Japan) GC-2010 Plus equipped with Shimadzu GCMS-QP2010 SE detector. GC-MS analysis was performed under the following conditions: capillary column, Restek Rx-5Sil (30 m × 0.25 mm; film thickness = 0.25 µm); oven temperature program, 60°C raised to 250°C and then kept at 250°C for 15 min; total run time 60 min; injector temperature, 250°C; detector temperatures, 250°C; carrier gas and helium at flow rate of 20 ml min^{-1}. Identification of constituents was carried out with the help of retention times of standard substances by composition of mass spectra. Each component was identified by comparison from the Wiley, Nist, Tutor, FFNSC library of mass spectra.

2.2 Experimental design

This study was performed in the Egirdir Fisheries Faculty, Isparta Applied Sciences University in Turkey. Healthy rainbow trout (mean weight of 15 g) were obtained from a commercial trout production farm (Isparta, Turkey). In the experiment, a total of 150 fish were stocked into three tanks (400 L) in flowing water system and acclimated for 15 days. Fish 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. The water quality parameters were measured as dissolved oxygen 9 mg L^{-1} and temperature 10°C. After acclimation, the anesthetic efficacy of *M. piperita* and *M. spicata* essential oils was investigated. All fish were fasted for 24 h before the anesthesia experiment. The essential oils of *M. piperita* and *M. spicata* were dissolved in ethyl alcohol (95%) at 1 : 10 ratio. Fish were anesthetized with the essential oils at 50, 100, 200, 300, 500 mg L^{-1} concentrations and 2-phenoxyethanol (0.3 ml L^{-1}) as the control. Ten fish individuals were used to determine anesthesia induction and recovery times, and each fish was considered a replicate (n = 10 for each concentration). Induction and recovery stages during anesthesia experiment were evaluated following Keene *et al.* (1998) (Table 1). The times of each stage of anesthesia were determined by a stopwatch. For anesthesia induction, the fish (one at a time) were taken from adaptation tanks and transferred to the aquariums (10 L) containing the anesthetic solution with continuous aeration. The

time to reach deep anesthesia (Stage 4) in fish was recorded and then the fish were individually transferred to a clean-water aquarium (10 L) to determine the recovery time. After recovery, abnormal behavior (position in the water column, swimming, etc.) and mortalities were recorded during 30 min.

TABLE 1 Anesthesia stages in fish.

Stage	Description	Behavior exahibited
I	Light sedation	Equilibrium normal, slow swiming, decreased reactivity to externali stimuli, slight decrease in opercular rate
II	Deep sedation	Restlessness, equilibrium normal, voluntary swimming still possible; slight decrease in opercular rate no respond 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 of equilibrium and active swimming

2.3 Mean lethal concentrations (LC_{50})

Different concentrations of essential oils (50, 100, 200, 300 and 500 mg L^{-1}) were applied to fish for 10 minute (Velíšek *et al.* 2005). Ten fish (average weight of 15 g) were used for each concentration. Mortalities were recorded during the test period and when the fish were transferred to clean water. Then, LC_{50} values of essential oils were calculated from mortality rates.

2.4 Data analysis

The homogeneity of variances was assessed using Levene's test, and normality of the data was evaluated using the Shapiro-Wilk test. Differences between groups were analyzed using one-way ANOVA, followed by Duncan's post hoc test for multiple comparisons ($p < 0.05$) using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Regression equations were used to explain the relationship between induction and recovery times at different anesthetic concentrations.

3 | RESULTS

3.1 Chemical composition of essential oils

The components of *M. spicata* and *M. piperita* oils are given in Tables 2 and 3. The total components were determined as 55 in *M. spicata* and 44 in *M. piperita*. Piperitenone oxide (68.73%) in *M. spicata* and menthol (41.76%), isomenthone (24.37%) and menthyl acetate (10.46%) in *M. piperita* were found as major components.

TABLE 2 Components of *Mentha* spicata essential oil (%).

Component	Retention Time	Content (%)
Alpha.-Thujene	6.429	0.03
Alpha.-Pınene, (-)-	6.695	1.08
Hydroperoxide, 1-ethylbutyl	6.965	0.05
Linalyl acetate	7.287	0.14
Benzaldehyde (CAS) Phenylmethanal	7.635	0.07
Sabinene	8.108	0.45
2-.Beta.-Pınene	8.321	2.23
Methyl-5-hepten-2-one	8.519	0.10
beta.-Myrcene	8.753	0.56
n-Octan-3-ol	9.056	0.10
Butanoic acid, 2-methyl-, 2-methylpropyl ester	9.305	0.04
Pseudolimonene	9.395	0.03
Cymene <para->	10.334	0.11
Limonene	10.576	5.12
Eucalyptol (1,8-Cıneole)	10.715	0.28
Cis-Ocimene	10.890	0.32
Ocimene <(E)-, beta->	11.430	0.02
1-Methyl-4-isopropenylbenzene	13.686	0.13
Linalool	14.288	0.17
Butyrate < 2-methyl-, 3-methylbutyl->	14.481	0.07
1 Octen 3 Yl Acetate	14.772	5.06

TABLE 2 Continued.

Component	Retention Time	Content (%)
7-Methyl-4-octyl acetate	15.477	0.40
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	15.931	0.06
2-Isopropylidenecyclohexanone	16.194	0.13
1-Imıdazol-1-Yl-2,2-Dımethyl-Propan-1-One	16.808	0.11
Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis-	17.509	0.68
Isomenthone	18.073	0.06
L-(-)-Menthol	18.905	0.99
Trans-Sabinene hydrate	19.053	0.32
Dmbca	19.466	2.32
Beta. Fenchyl Alcohol	19.989	0.21
4,6-Dimethyltetrahydro-1,3-oxazine-2-thione	21.149	0.17
Phenol, 2-methyl-6-(2-propenyl)-	21.267	0.18
Butanoate <2-methyl-, 3(Z)-hexenyl-, cis- >	22.409	0.14
Cıs-3-Hexenyl Valerate	22.729	0.31
Benzaldehyde, 4-(1-methylethyl)-	22.920	0.06
2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (R)-	23.059	0.51
Benzene, 1-methoxy-4-(1-propenyl)-	25.853	0.52
Menthyl acetate	26.240	0.40
Piperitenone	29.144	0.21
Pıperıtenone Oxıde	30.977	68.73
Copaene <alpha->	31.716	0.06
Beta. Bourbonene	32.176	0.50
Beta. Elemene	32.636	0.12
Jasmone <(Z)>	32.775	1.49
Caryophyllene	34.400	0.60
Germacrene-D	35.084	0.08
2,6-Dimethyl-octa-2,6-dien-1-ol	35.611	0.22
Alpha.-Humulene	36.625	0.03
Farnesene <(E)-, beta->	36.831	1.36
Germacrene-D	38.272	0.28
Butyrate <2-methyl-, phenylethyl->	38.669	0.11
Delta.-Cadinene	40.701	0.05
(-)-Caryophyllene oxide	44.325	0.20
Ledene	45.098	2.24

TABLE 3 Components of *Mentha piperita* essential oil (%).

Component	Retention Time	Content (%)
Alpha.-Thujene	6.398	0.01
Alpha.-Pınene, (-)-	6.665	0.53
Hydroperoxide, 1-Ethylbutyl	6.931	0.04
L-Lınalool	7.260	0.05
Sabinene	8.073	0.27
Pinene <Beta->	8.284	0.70
Beta.-Myrcene	8.718	0.04
3-Octanol (CAS) N-Octan-3-Ol	9.025	0.08
Pseudolimonene	9.360	0.01
Cymene <Para->	10.298	0.30
Limonene	10.550	2.64
Eucalyptol (1,8-Cıneole)	10.686	4.94
Trans-Sabinene Hydrate	12.601	0.09
Alpha.-Pinene Oxide	14.140	0.03
Linalool	14.255	0.17

TABLE 3 Continued.

Component	Retention Time	Content (%)
Butyrate <2-Methylbutyl-, 2-Methyl->	14.450	0.05
Pentyl 3-Methylbutanoate	14.754	0.06
3-Acetoxytridecane	15.453	0.06
Trans-P-Mentha-1(7),8-Dien-2-Ol	16.117	0.09
Isomenthone	17.661	24.37
S) Isomenthone	18.084	2.97
(+)-Neomenthol	18.476	5.59
Isopulegone	18.724	0.28
Menthol	19.241	41.76
Beta. Fenchyl Alcohol	20.004	0.13
p-Allylanisole	20.200	0.02
3-(Cyclohex-3'-En-Yl) Propıonaldehyde	20.295	0.01
Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl-	21.225	0.03
Pulegone	22.685	1.27
Benzaldehyde, 4-(1-methylethyl)-	22.904	0.04
2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (R)-	23.037	0.06
Hexyl isovalerate	23.185	0.00
Piperitone	23.698	1.28
Neomenthol acetate	25.056	0.72
Anethole	25.839	0.11
Menthyl acetate	26.306	10.46
(+)-Menthylacetate	27.155	0.19
Isopulegol acetate	27.347	0.03
Dispiro[2.1.2.4]undecane, 8-methylene- (CAS)	28.099	0.01
Beta. Bourbonene	32.131	0.21
Farnesene <(E)-, beta->	36.810	0.04
Pentalene, Octahydro-1,4-Dıodo-	44.048	0.07
(-)-Caryophyllene oxide	44.315	0.10
Ledene	45.080	0.10

3.2 Anesthesia induction and recovery

In this study, anesthesia induction time reduced with rising of the concentration of *M. spicata* and *M. piperita* essential oils. However, recovery time rised with increasing of the concentration of these oils. Anesthesia induction and recovery times for *M. spicata* were found shorter than those for *M. piperita*. No abnormal behavior or mortality was observed after anesthesia for both *Mentha* species. The fish started feeding approximately two hours after anesthesia.

Mentha spicata oil at 100 – 500 mg L^{-1} displayed deep sedation at 29 – 11.66 s (Stage II) in rainbow trout. Anesthesia induction times at 100 mg L^{-1} concentration (29.0 s) for sedation were found to be shorter than 2-phenoxyethanol (49.0 s) ($p < 0.05$). *Mentha spicata* at concentrations of 200, 300 and 500 mg L^{-1} provided deep anesthesia (Stage IV) in fish. The lowest effective dose of *M. spicata* oil was determined as 200 mg L^{-1} (anesthesia induction: 65.0 s and recovery times: 65.33 s) (Table 4). *Mentha spicata* oil at 200 mg L^{-1} displayed better results than 2-phenoxyethanol in terms of anesthesia induction and recovery times ($p < 0.05$) (Table 4). There was a correlation between the anesthesia induction and recovery times (Stage IV) with *M. spicata* essential oil concentrations according to regression analysis (coefficient values: 0.94 for anesthesia induction time, 0.89 for recovery time) (Figures 1 and 2).

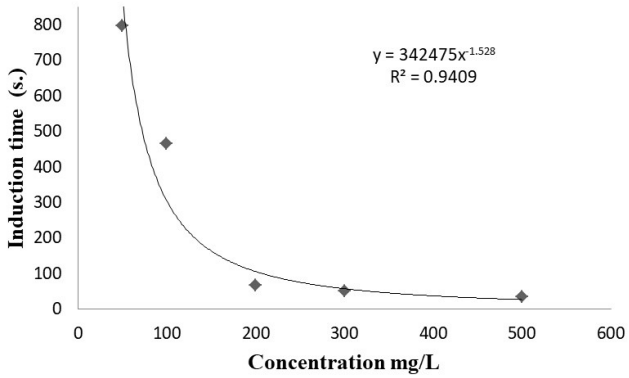

FIGURE 1 Relationship between *Mentha spicata* essential oil concentrations with anesthesia induction time of rainbow trout, *Oncorhynchus mykiss* (Stage IV).

Fish showed deep sedation with *M. piperita* essential oil at concentrations of 100 – 500 mg L^{-1}. Rainbow

trout reached to Stage II in 35.33 s at 500 mg L^{-1} concentration of *M. piperita*. Anesthesia induction times at 500 mg L^{-1} concentration for sedation were found to be shorter than 2-phenoxyethanol (50.0 s) ($p < 0.05$). Deep anesthesia (Stage IV) in fish was showed at 100 – 500 mg L^{-1} concentrations of *M. piperita*. At these concentrations, anesthesia induction times were significantly shorter than those of 2-phenoxyethanol (287 s) ($p < 0.05$). *Mentha piperita* essential oil at 200 mg L^{-1} concentration was found as the lowest effective dose (anesthesia induction: 169.66 s, recovery time: 188.0 s) (Table 5). There was a relationship between the anaesthesia induction and recovery times (Stage IV) with *M. piperita* oil concentrations according to regression analysis (coefficient values: 0.97 for anaesthesia induction time, 0.95 for recovery time) (Figure 3 and 4).

TABLE 4 Anesthetic effect of *Mentha spicata* essential oil (MSO) on rainbow trout.

Concentration (mg L^{-1})	Induction time (s) and anesthesia level				Recovery time (s)
	I	II	III	IV	
50 MSO	41.00±1.00^{a}	404.00±3.00^{a}	471.00±3.00^{a}	796.00±3.60^{a}	11.00±1.00^{f}
100 MSO	23.66±2.51^{b}	29.00±1.00^{c}	64.00±1.00^{c}	463.00±4.58^{b}	61.33±1.52^{e}
200 MSO	20.33±2.51^{b}	24.00±2.00^{d}	31.33±1.52^{d}	65.00±2.00^{d}	65.33±1.52^{d}
300 MSO	10.66±2.08^{cd}	18.66±2.08^{e}	24.00±1.00^{e}	51.00±1.00^{e}	80.00±2.00^{b}
500 MSO	8.66±1.52^{d}	11.66±1.52^{f}	21.66±3.05^{e}	32.66±1.52^{f}	95.00±1.00^{a}
Control (2-PE)	13.66±1.52^{c}	49.00±2.00^{b}	86.00±1.00^{b}	287.00±2.00^{c}	75.00±1.00^{c}
Equation*	$y = 574.23x^{-0,674}$	$y = 41027x^{-1,367}$	$y = 43858x^{-1,299}$	$y = 342475x^{-1,528}$	y = 33.148ln(x) - 108,07
R^2	0.94	0.79	0.85	0.94	0.89

Data are presented as mean ± SD. Values superscript with different letters at same column are significantly ($p < 0.05$) different. *: Relationships between concentration x anesthesia induction or recovery time in rainbow trout, exposed to the *Mentha spicata* essential oil.

TABLE 5 Anesthetic effect of *Mentha piperita* essential oil (MPO) on rainbow trout.

Concentration (mg L^{-1})	Induction time (s) and anesthesia level				Recovery time (s)
	I	II	III	IV	
50 MPO	49.33±2.51^{a}	109.00±2.64^{a}	191.66±4.72^{a}	441.66±6.11^{a}	92.00±2.00^{e}
100 MPO	47.00±2.64^{a}	87.00±2.00^{b}	165.00±2.00^{b}	225.33±2.08^{c}	107.66±1.52^{d}
200 MPO	34.00±3.00^{b}	68.00±3.00^{c}	129.33±3.05^{c}	169.66±2.51^{d}	188.00±1.00^{c}
300 MPO	30.00±3.00^{b}	57.66±2.51^{d}	72.50±2.50^{e}	103.33±1.52^{e}	263.33±0.57^{a}
500 MPO	23.00±2.64^{c}	35.33±1.52^{f}	41.00±3.00^{f}	64.00±2.64^{f}	231.33±1.52^{b}
Control (2-PE)	13.00±1.00^{d}	50.00±1.00^{e}	87.66±2.51^{d}	287.00±2.00^{b}	75.00±1.00^{f}
Equation*	$y = 0,0001x^{2} - 0,1266x + 56,363$	y = -30,86ln(x) + 230,21	$y = 0,0005x^{2} - 0,6321x + 224,03$	$y = 10250x^{-0,806}$	$y = -0,0016x^{2} + 1,2343x + 18,099$
R^2	0.98	0.99	0.98	0.97	0.95

Data are presented as mean ± SD. Values superscript with different letters at same column are significantly ($p < 0.05$) different. *: Relationships between concentration x anesthesia induction or recovery time in rainbow trout, exposed to the *Mentha piperita* essential oil.

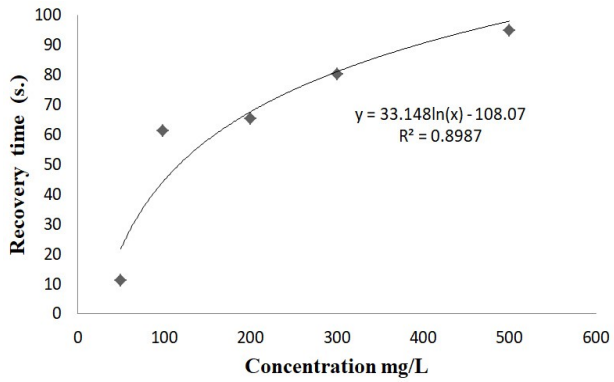

FIGURE 2 Relationship between *Mentha spicata* essential oil concentrations with recovery time of rainbow trout, *Oncorhynchus mykiss*.

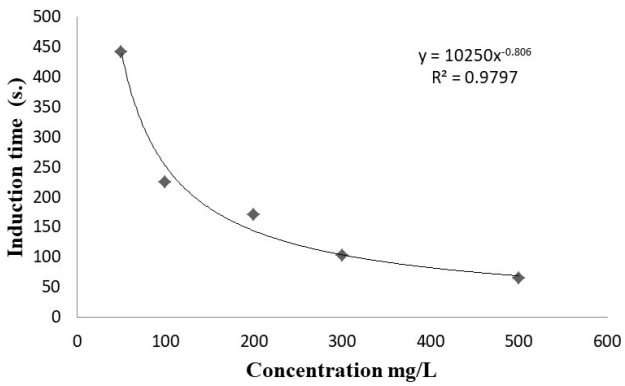

FIGURE 3 Relationship between *Mentha piperita* essential oil concentrations with anesthesia induction time of rainbow trout, *Oncorhynchus mykiss* (Stage IV).

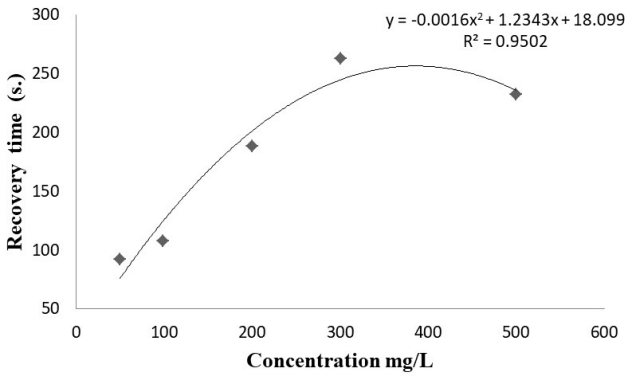

FIGURE 4 Relationship between *Mentha piperita* essential oil concentrations with recovery time of rainbow trout, *Oncorhynchus mykiss*.

3.3 Mean lethal concentrations (LC_{50})

The LC_{50} value of both essential oils was determined to be 150 mg L^{-1} in rainbow trout.

4 | DISCUSSION

Anesthetic effects of essential oils depend on their main component (da Cunha *et al.* 2010; Yigit *et al.* 2022a, 2022b). The major compounds of *M. piperita* essential oil are menthol, 1,8-cineole, menthofuran and menthone (Beigi *et al.* 2018). Similarly, in this study, menthol was determined to be the main component of *M. piperita*. The main component of cultivated *M. spicata* is carvone, whereas piperitone oxide has been reported as the main component in wild populations of *M. spicata* (Telci *et al.* 2010). Similarly, in the present study, piperitone oxide was identified as the main component of *M. spicata*.

The ideal anesthetic should provide rapid anesthesia induction (3 min) and recovery (5 min) in fish (Marking and Meyer 1985; Can *et al.* 2018). In this study, the lowest effective concentration of *M. piperita* essential oil required to induce deep anesthesia in rainbow trout (15 g) was determined to be 200 mg L^{-1} at 10°C water temperature. Brandão *et al.* (2021) observed an anesthetic effect at a concentration of 90 mg L^{-1} of *M. piperita* essential oil in *Colossoma macropomum* (130.55 g) at 28.7°C water temperature. Spanghero *et al.* (2019) reported that *M. piperita* essential oil was effective as an anesthetic at a concentration of 80 mg L^{-1} in silver catfish (3.08 g) at a water temperature of 26.2°C. Can and Sümer (2019) found that *M. piperita* essential oil at a concentration of 100 µL L^{-1} induced an anesthetic effect in juvenile blue dolphin cichlids (*Cyrtocara moorii*, 4.92 g) at a water temperature ranging from 27.2 – 28.39°C. Krasteva *et al.* (2021) noted that peppermint essential oil can be used to produce a sedative effect in silver catfish with a mean weight of 8.62 g, at the water temperature ranging from 26.2 – 27.5°C. The differences between these studies may be due to the differences in fish species, fish weight, water temperature and the chemical content of *M. piperita* essential oils.

There is one study on the anesthetic effect of *M. spicata* essential oil in fish. Roohi and Imanpoor (2015) reported that *M. spicata* oil, containing 28.4% carvone as the main component, showed an anaesthetic effect in common carp (16.59 g) at a concentration of 7 ml L^{-1} at 16°C water temperature. In the present study, *M. spicata* essential oil, containing piperitenone oxide (68.73%) as the main component, exhibited an anaesthetic effect at a concentration of 200 mg L^{-1} in rainbow trout (15 g) at 10°C water temperature. The difference between the two studies may be due to the differences in the main constituent of *M. spicata* essential oil, the fish species used, and water temperature.

In this study, menthol (41.76%) was found to be the primary component in *M. piperita* essential oil, and the anesthetic effect of *M. piperita* in rainbow trout can be attributed to menthol. Similarly, menthol has been recommended as an anesthetic at a concentration of 80 mg L^{-1} in rainbow trout (Teta and Kaiser 2019), 118 – 512 ppm in common carp (Mazandarani and Hoseini 2017), and 100 mg L^{-1} in *Oligosarcus argenteus* (Uehara *et al.* 2019). However, there are no studies available on the anesthetic efficacy of piperitenone oxide, one of the major components of *M. spicata*, in fish.

The LC_{50} values of both essential oils were observed to be 150 mg L^{-1} in rainbow trout for a 10 minute exposure. Spanghero *et al.* (2019) also determined 75.06 mg L^{-1} as LC_{50} value of *M. piperita* essential oil in silver cat fish juveniles for 4-hour exposure. Metin *et al.* (2022) noted that mean lethal concentration for 10 minutes was 500 mg L^{-1} for lavender and 450 mg L^{-1} for cumin in common carp. However, Yigit *et al.* (2022a) reported that *Ocimum basilicum* and *Eucalyptus globulus* essential oils did not cause mortality in rainbow trout in acute toxicity test (LC_{50} for 10 minutes). The differences may be due to differences in plant species, fish species, and the exposure duration to essential oils.

To the best of our knowledge, the anesthetic effect of *M. spicata* essential oil in rainbow trout was studied for the first time in this study. As a result, it was determined that the optimal concentration of *M. piperita* and *M. spicata* essential oils for deep anesthesia in trout was 200 mg L^{-1}. We conclude that *M. piperita* and *M. spicata* essential oils can be used as safe anesthetic agents in rainbow trout. In future studies, the effects of *M. piperita* and *M. spicata* essential oils on different fish species and developmental stages should be investigated along with their long-term physiological impacts and environmental effects.

ETHICAL STATEMENT

All the procedures used in this experiment were approved by the Isparta Applied Sciences University Animal Experi-

mentation Ethics Committee (approval number: HADYEK–02/2021).

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Secil Metin and Onur Ozdikyar designed and performed the experiment. Isa Telci provided *Mentha piperita* and *Mentha spicata*.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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