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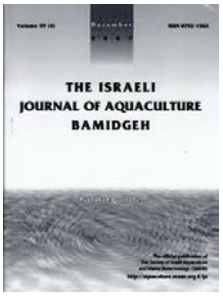

The Efficacy of Chamomile (*Matricaria chamomilla*) Oil as a Promising Anaesthetic Agent for Two Freshwater Aquarium Fish Species

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Abstract

The efficacy of anesthetic chamomile oil (from *Matricaria chamomilla L.*) was evaluated in two freshwater aquarium fish species, Electric Blue Hap (*Sciaenochromis fryeri*) and Yellow Princess (*Labidochromis caeruleus*). Fish were exposed to ten concentrations of anesthetic (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml/L). The optimal concentrations identified as 0.6 ml/L for deep anesthesia (A5) for both two species. The minimal sedative concentration at the stage of loss of equilibrium (A3) was found to be 0.3 ml/L. The induction time generally decreased significantly with increasing concentrations of chamomile oil for all treatment groups. Recovery time tended to increase with increased chamomile oil at concentrations lower than 0.7 ml/L, but after this concentration it decreased. Chamomile oil proved to be effective as an anesthetic for both ornamental fish species. These findings suggest that chamomile oil is a promising anesthetic agent for aquaculture. However, further studies should be focused on species based investigations on the effect of temperature, transfer of fish, and their effect on antioxidant and oxidant status, in order to gather further information.

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Introduction

To facilitate the handling of fish without causing injury or stress requires some form of sedation or anesthesia (Summerfelt and Smith, 1990; Ross and Ross, 1999). When choosing an anesthetic, it is important to consider aspects such as efficacy, cost, availability, and ease of use, as well as toxicity to fish, humans, and their impact on the environment (Soto and Burhanuddin, 1995). Up to now, various anesthetics have been used for aquaculture (Gilderhus and Marking, 1987; Summerfelt and Smith, 1990; Stoskopf, 1993; Ross and Ross, 1999; Altun and Danabaş, 2006). The major ones are tricaine methanesulphonate (MS-222), 2-phenoxyethanol, quinaldine, and benzocaine. Studies have been conducted and have demonstrated success in employing essential oils with anesthetic properties in aquaculture; these include clove oil from *Syzygium aromaticum* Linn (Soto and Burhanuddin 1995; Cooke et al. 2004; Bressler and Ron, 2004; Gullian and Villanueva 2009; Pedrazzani and Neto 2016), bushy lippia oil from *Lippia alba* (Cunha et al. 2010), rosemary *Rosmarinus officinalis* oil (Ghazilou and Chenary 2011), tree basil *Ocimum gratissimum* oil (Silva et al. 2012), spearmint *Mentha spicata* oil (Roohi and Imanpoor 2015), as well as camphor *Cinnamomum camphora* oil, and mint *Mentha arvensis* oil (Pedrazzani and Neto 2016) (Table 1).

Table 1. Effective concentrations reported for some essential oils in aquaculture

Species for anesthetic agent	*Species*	*Weight (g)*	*Concentration (µl/L)*	*Reference*
Clove oil *(Eugenia caryophylatta)*	Rainbow trout (*Oncorhynchus mykiss*)	20± 1,25	40-50	Metin et al., (2015)
Clove oil (*Syzygium aromaticum*)	Clown Anemonefish (*Amphiprion ocellaris)*	0.48 ±0.21	27	Pedrazzani and Neto., (2016)
Mint oil *(Menta piperita)*	Rainbow trout (*Oncorhynchus mykiss*)	20± 1,25	200	Metin et al., (2015)
Mint oil (*Mentha arvensis)*	Clown Anemonefish (*Amphiprion ocellaris*)	0.48±0.21	70	Pedrazzani and Neto., (2016)
Lavender oil *(Lavandula angustifolia)*	Rainbow trout (*Oncorhynchus mykiss*)	20± 1,25	>200	Metin et al., (2015)
Rosemarine oil *(Rosmarinus officinalis)*	Common carp, (*Cyprinus carpio)*	652 g ± 3.1	250-1000	Ghazilou and Chenary (2011)
Camphor oil (*Cinnamomum camphora*)	Clown Anemonefish (*Amphiprion ocellaris)*	0.48 ± 0.21	500	Pedrazzani and Neto., (2016)
Lemon verbena *(Aloysia triphylla)*	Silver catfish (*Rhamdia quelen)*	2.6-3.0	200	Parodi et al., (2014)
Basil oil *(Ocimum americaum*)	Silver catfish (*Rhamdia quelen)*	8.09 ± 0.22	200-500	Silva et al., (2015)

The ideal anesthetic should permit a reasonable duration of exposure, produce anesthesia within 3 min or less, allow recovery within 5 min or less, cause no toxicity to fish at treatment levels, present no safety problems, leave low tissue residue after a withdrawal time of 1 h or less, and be reasonable in cost (Marking and Meyer, 1985). Additionally, correct concentration of anesthetic must ensure the survival of treated fish when exposed to anesthetic for 30 min (Chambel et al., 2015).

Chamomile is a well-known medicinal plant species from the Asteraceae family, often referred to as the “star among medicinal species.” It is a highly favored and much used medicinal plant in folk and traditional medicine (Singh et al., 2011). International demand for chamomile oil has grown steadily in different areas such as food, agriculture, medicine etc. As a result, the plant is widely cultivated in Europe and

has been introduced in some Asian countries for production of its essential oil. *M. chamomilla* L., *Anthemis nobilis* L. *Ormenis multicaulis* which belongs to the family Asteraceae is a natural major source of “blue oil” and flavonoids. The oil is used as a sedative and for digestion (Gould et al., 1973; Sharma et al. 1983) and is also antibacterial and fungicidal (Gould et al., 1973). Other properties include sedative, anti inflammatory, antiseptic, healing, carminative, and spasmolytic activity (Salamon, 1992). Chamomile is widely regarded as a mild tranquillizer and sleep-inducer. Sedative effects may be due to a flavonoid that binds to benzodiazepine receptors in the brain (Avallone et al., 1996). Compounds present in extracts of chamomile can also bind BDZ and GABA receptors in the brain and may be responsible for some sedative effect (Park et al. 1999). However, many of these compounds are as yet unidentified (Srivastata et al., 2010).

The Electric Blue Hap, *Sciaenochromis fryeri,* has long been a favorite among African cichlid keepers because of its intense electric blue coloring. It comes from Lake Malawi, Africa, and is found in many color morphs throughout the lake (Konings, 1993). The Yellow Princess, *Labidochromis caeruleus*, is a freshwater perciform fish, a cichlid, also called the electric yellow, lemon yellow lab, the blue streak hap and the electric yellow. The Yellow Princess is a bright yellow freshwater cichlid and one of the most commercially valuable aquarium fish species (Maréchal, 1991; Ergün et al., 2010).

Essential oils obtained from plants such as clove, mint, rosemary, lemon verbena, lavender have been studied, however oils from chamomile have not. Thus, the aim of present study was to investigate efficacy and determine the optimum concentration of the chamomile oil as a new anesthetic agent for two ornamental fish species *Sciaenochromis fryeri,* and *Labidochromis caeruleus*.

Material and Methods

Experimental layout. Experiments were conducted on *S. fryeri* (mean length 4.29 ± 0.14 cm, mean body weight 1.13 ± 0.12 g) and on *L. caeruleus* (mean length 3.9 ± 0.13 cm, mean body weight 1.02 ± 0.12 g). Fish were stocked in separate 160 L glass aquaria, water temperature 25°C, and pH 7.84, with an inner filter, and fed ad libitum once daily with granulate commercial feed containing 42.5% crude protein, 10.3 % crude fat, 2.8% fibre, 5% moisture and 4.9% crude ash (Sera Granured, Germany). All the fish were starved for 24 h prior to the experiment (Weyl et al., 1996) which was conducted at the Laboratory of Bioengineering Department, Munzur University Engineering Faculty in Turkey.

Anesthethic agent and GC Analysis. Chamomile oil *(Matricaria chamomilla L.)* was obtained from a commercial company (Defne Doga, Turkey). The EO was obtained from fresh leaves using the hydrodistillation process with a Clevenger type apparatus (European Pharmacopoeia, 2007). The essential oil component was analyzed at the Ege University Drug Development and Pharmacokinetic Research Center (ARGEFAR).

GC–MS TIC analysis was performed using an Agilent-6890 gas chromatograph coupled with an Agilent 5973 mass selective detector under the following conditions: HP innovax column (1:100 hegzane, 60 m×0.32 mm×0.25 µm); split inlet 1:100; Databank NIST 2002 and ARGEFAR. The constituents of the chamomile oil were identified by comparing their mass spectra with a mass spectral library (NIST, 2002). Results are shown in Table 2.

Table 2. Compounds of chamomile essential oil used in the study

Essential Oil Analysis of Chamomile	
Compounds	*Result (% Area)*
Linalool	8.40
Alpha terpinenyl acetate	1.65
Benzyl acetate	4.34
Geranyl acetate	4.05
Beta citronellol	3.70
1.1 oxbis 2-propanol	7.35
Geraniol	10.04
Alpha ionone	5.65
3.3 oxybis 2-buthanol	3.62
Benzenethanol	6.40
Hidroxycitronellal	4.47
Anisaldehyde	1.47
Alpha bisabolol oxide	28.53
Anisaldehyde propylene glycol acetal	6.05
Unidentified	4.28

Experimental design. Ten different concentrations of the chamomile oil were 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml/L (N = 6 for each group) after a pre-treatment trial. All treatments were conducted in triplicate. The determination of induction (A) and recovery (R) stages after anesthesia were modified from Summerfelt and Smith (1990)**;** Keene et al. (1998). See Table 3.

Table 3. Anesthesia stages in the present study (modified from Summerfelt and Smith 1990; Keene *et al., 1998;* Mylonas et al., 2005)

Phase	*Code*	*Fish Behavior Characteristics*
Induction		
Total loss of equilibrium	A3	Total loss of equilibrium (first occurrence), regular opercular movement, pectoral fins moving
Deep anesthesia	A5	No reflex, opercular movements irregular and slow, no response to strong external stimulus, pectoral fin movements depend on the opercular movement or no movement
Recovery		
Total recovery of equilibrium	R1	Total recovery of equilibrium, swimming erratic at onset
Total behavioral recovery	R3	Normal Swimming began, responsiveness to visual stimuli

The anesthetic effects were recorded by seconds. Different concentrations of chamomile oil previously dissolved in ethanol 95% ethanol (at a rate of 1:10). Once stage A5 was reached, the fish were removed, weighed, and placed individually in a recovery aquarium within <1 min. When reaching stage A5 exceeded 5 min the observations were discontinued. Induction and recovery times were recorded to the nearest second using an electronic stop-watch (Mylonas et al., 2005). Once recovered, fish were transferred into stock aquariums, and were monitored for survival and abnormal behavior for the following 24 hours. The control groups contained only ethanol at a concentration that was equivalent to the highest concentration used in the experimental conditions (10 ml/L) and were observed for 5 min. (Adapted from Mylonas et al., 2005). The chosen concentrations are based on preliminary tests.

Reliability test for optimal anesthetic concentration. The reliability test is important because the duration of exposure should always be kept within an adequate margin of safety since practical situations often involve anesthesia of a large number of fish in one tank. To identify optimal concentrations of anesthetic, fish were exposed for 30 min at a concentration of 0.6 ml/L (adapted from Chambel et al., 2015).

Statistical analysis. Prior to testing, normality and homogeneity of data were checked to comply with the assumptions of ANOVA. Significant differences between concentrations of chamomile oil were determined by one-way ANOVA followed by the Duncan Multiple Range test. Statistically significant differences are expressed as $p<0.05$. Analysis of data was carried out using SPSS 15.0. The results are presented as means ± standard error (standard error of the mean, SEM).

Results

Induction and recovery. To determine the efficacy of each concentration of chamomile oil, induction times (as A3 and A5) and recovery times (as R3 and R5) were observed. Induction times of anesthesia varied with anesthetic concentrations, decreasing with the increase of chamomile oil concentrations. Recovery time tended to increase with the increase of the chamomile oil concentration at lower concentrations than 0.7 ml/L, but at upper concentrations of 0.7 ml/L it decreased. The concentration of 0.6 ml/L was found to be the correct concentration for both cichlid species. At the concentration of 0.1-0.2 ml/L, fish in all the groups maintained their equilibrium while fish exposed to 0.3 and 0.4 ml/L of chamomile oil reached the A3 stage. Deep anesthesia (A5) was induced at a more rapid rate at higher concentrations of anesthetic than 0.4 ml/L for all fish ($p<0.05$) (Tables 4 & 5).

Table 4. The timing of anesthesia phases at different concentrations of chamomile oil for the Electric Blue Hap (*Sciaenochromis fryeri*)

Concentration (ml/L)	Induction Time (sec)		Recovery Time (sec)	
	A3	A5	R1	R3
0.1	n.o.	n.o.	n.t.	n.t.
0.2	n.o.	n.o.	n.t.	n.t.
0.3	174±13.52 ^{a}	n.o.	n.t.	n.t.
0.4	163±10.33 ^{a}	n.o.	n.t.	n.t.
0.5	155±8.61 ^{a}	210±5.41 ^{a}	155±7.65 ^{ab}	190±10.01 ^{a}
0.6	114±6.11 ^{b}	168±9.12 ^{b}	140±7.22 ^{a}	173±9.06 ^{a}
0.7	105±8.61^{bc}	140±5.91 ^{c}	170±8.13 ^{b}	283±10.23 ^{b}
0.8	99±4.14 ^{c}	112±8.61 ^{d}	160±8.61 ^{ab}	230±8.61 ^{c}
0.9	80±3.88 ^{d}	125±7.25 ^{d}	140±8.44 ^{a}	175±8.31 ^{a}
1.0	50±7.98 ^{e}	60±6.45 ^{e}	95±9.11 ^{c}	150±5.03 ^{d}

*Values (mean± SE) with different superscripts within the same column are significantly different ($p<0.05$).
$^{n.o.}$ indicates the phase was not observed
$^{n.t.}$ indicates fish were not treated

The shortest time for both induction (A5) and recovery (R3) occurred at the same concentration (1.0 ml/L) at 60±6.45 sec and 90±5.46 sec, respectively. However, the highest induction time for A5 stage was observed at the concentration of 0.6 ml/L (240±9.56 sec) while the highest recovery time for R3 stage was seen at the concentration of 0.7 ml/L (283±10.23 sec).

No mortality occurred in all treated groups during the anesthetic exposure and during observation of 24 hours after exposure. Tables 4 & 5 present the results of induction and recovery times of *Sciaenochromis fryeri* and *Labidochromis caeruleus*, exposed to different concentrations of chamomile oil.

Table 5. The timing of anesthesia stage at different concentrations of chamomile oil for Yellow Princess (*Labidochromis caeruleus*)

Concentration	*Induction Time (sec)*		*Recovery Time (sec)*	
(ml/L).	*A3*	*A5*	*R1*	*R3*
0.1	n.o.	n.o.	n.t.	n.t.
0.2	n.o.	n.o.	n.t.	n.t.
0.3	183±12.08 [a]	n.o.	n.t.	n.t.
0.4	175±9.11 [a]	n.o.	n.t.	n.t.
0.5	135±8.21b [b]	240±9.56 [a]	135±7.01 [a]	178±8.86 [a]
0.6	118±6.44 [bc]	172±9.12 [b]	100±7.32 [b]	150±10.23 [b]
0.7	115±6.66 [c]	160±8.11 [b]	150±5.69 [c]	202±12.03 [c]
0.8	110±9.21 [c]	123±8.99 [c]	200±11.12 [d]	245±14.33 [d]
0.9	100±6.51 [c]	120±9.39 [c]	125±8.64 [a]	160±9.79[a]
1.0	48±7.65 [d]	61±8.11 [d]	65±3.92 [e]	90±5.46[e]

*Values (mean± SE) with different superscripts within the same column are significantly different ($p<0.05$).

[n.o.] indicates the phase was not observed

[n.t.] indicates fish were not treated

The reliability test. Additionally, fish were exposed to anesthetic for 30 min at a concentration of 0.6 ml/L. No death was observed either during the treatment, or after observation of 24 hours.

In the control, the exposure of the two ornamental fish species to ethanol (i.e., the anesthetic solvent) did not induce anesthesia, or any apparent modifications in the behavior of the fish. The concentration of ethanol used in the study had no effect on the fish at maximum exposure time of this study (10 ml for 5 min).

Discussion

An ideal anesthetic should produce anesthesia rapidly (e.g., less than 3 min), allow for speedy recovery, should not be toxic to fish and users, leave low tissue residues, and be inexpensive (Marking and Meyer, 1985). According to these criteria, the optimal identified concentration was 0.6 ml/L for both cichlid species. In addition, we observed that recovery times are related to the anesthetic concentration. The induction time generally decreased significantly with increasing concentrations of chamomile oil for all species studied (Tables 4 & 5). Recovery time was positively correlated with concentration of anesthetic (Weyl et al. 1996; Mylonas et al., 2005). Interestingly, when concentration was greater than 0.7 ml/L, recovery time decreased in both fish species studied. Recovery time increased with increasing concentrations of MS-222 for three ornamental fish species, *D. rerio*, *P. reticulata,* and *S. discus* while recovery time decreased with increasing concentrations of the same anesthetic in another species, *X. helleri* (Chambel, et al., 2015). A possible explanation for these results is probably related to the fact that increasing concentrations are also associated with a shorter time of contact between fish and the anesthetic and a lower uptake of the anesthetic agent (Chambel, et al., 2015). Thus, during recovery, the anesthetic is cleared more rapidly from the bloodstream (Weber et al. 2009; Chambel, et al., 2015). However, the molecular characteristics of the anesthetics, as well as the physiological and metabolic characteristics probably affect recovery time as well (Chambel, et al., 2015). Therefore, chamomile oil proved to be effective as anesthesia, and safe for both ornamental fish species.

A correct concentration of anesthetic for fish should allow fish to survive for 30 min after being applied (Chambel et al., 2015). In our study the fish did not die when treated with an optimal concentration of anesthetic, and reacted favorably after 30 min induction. The reliability test is particularly important and relevant because in practice, anesthesia involves a large number of fish in one tank and their exposure beyond these limits can result in major mortalities (Chambel et al., 2015).

“The ideal anesthetic should permit a reasonable duration of exposure, produce anesthesia within 3 min or less, allow recovery within 5 min or less, cause no toxicity to fish at treatment levels, present no mammalian safety problems, leave low tissue residues after a withdrawal time of 1 h or less, and be reasonable in cost” (Marking and Meyer 1985). We also claim that “the anesthetic is conceived acceptable if it has a good smell and is also environmental friendly for aquaculture” as reported in anesthetic experiments for two other herbal essential oil, *Eucalyptus* sp. and *Origanium*

sp. (Bodur et al., 2018).

Our findings suggest that chamomile oil (from *M. chamomilla* L.) is a promising anesthetic agent for aquaculture. However, further studies are needed to establish its effect on fish of different sizes, held in different temperatures.

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