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Comparing the anesthetic effect of clove and peppermint oil in juvenile European catfish, *Silurus glanis* (Linnaeus, 1758)

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Abstract

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In this research, the effect of two anaesthetic agents (clove oil and peppermint oil) is studied in juvenile European catfish (*Silurus glanis*) with an average body weight of 8.62 ± 1.61 g. Three concentrations of clove oil are tested: 0.02 $ml.l^{-1}$, 0.04 $ml.l^{-1}$ and 0.06 $ml.l^{-1}$, while the concentrations of peppermint oil are six: 0.02 $ml.l^{-1}$, 0.04 $ml.l^{-1}$, 0.06 $ml.l^{-1}$, 0.08 $ml.l^{-1}$, 0.1 $ml.l^{-1}$ and 0.12 $ml.l^{-1}$. The induction and recovery phases of anesthesia are measured for every fish and are described according to Hamackova et al. (2006). The induction of anesthesia and the recovery time from it, depend significantly on the concentration of the anaesthetic used ($P<0.05$). A linear exponential correlation is observed between concentration and recovery time for clove oil ($r=0.93$) and mint oil ($r=0.72$). Higher concentration values correspond to a shorter induction time for both anesthetics ($r=0.97$; $r=0.85$). Based on the results from the study, it can be concluded that clove oil is more suitable anesthetic agent, compared to mint oil, for juvenile European catfish.

Keywords: clove oil; peppermint oil; anesthesia; *Silurus glanis*; European catfish

Introduction

The use of anesthesia in the commercial fish sector and in the scientific studies can prevent injuries and reduce stress in fish. Currently, an increasing number of anaesthetic agents are being applied in the fish aquaculture (Hajek et al., 2006; Hamachkova et al., 2006; Velisek et al., 2006; Zaikov et al., 2008c; Brown, 2011; Mikodina et al., 2011). Some of them have a synthetic origin, such as MS-222, benzocaine and 2-phenoxyethanol (Marking & Mayer, 1985; Gilderhus & Marking, 1987; Coyle et al., 2004; Hamachkova et al., 2004; Githukia et al., 2016; Priborsky & Velisek, 2018). Others are natural products, like essential oils with plant origin (Hoseini et al., 2018). They are considered as an alternative to synthetic preparations (Readman et al., 2013), mainly due to lower prices, easy access, efficiency and safety of the environment.

The most commonly used anesthetic of natural origin in aquaculture is the clove oil, which Anderson et al. (1997) determines as an alternative to MS-222. It covers completely the qualities of a good anesthetic described by Coyle et al. (2004) and Brown (2011). Some of them include: the application of low concentrations, quick induction and recovery of anesthesia, soluble in water and alcohol, etc.

The clove oil is a natural product, which is distilled from the buds, leaves and stems of the clove tree *Eugenia caryophyllata*. It has application in medicine, cosmetics and food industry (Bullerman et al. 1977; Taylor & Roberts, 1999; Mikodina et al., 2011). Clove oil is the most common anesthetic in aquaculture with increasing interest. Consequently, there are many studies on its effects with different species of fish: rainbow trout (*Oncorhynchus mykiss*) (Anderson et al., 1997; Hamackova et al., 2006; Yildiz et al., 2013), carp (*Cyprinus carpio)* (Velisek et al., 2005a; Hajek et al., 2006; Kamble et al., 2014), pike (*Esox lucius*) (Hamackova et al., 2006; Zaikov et al., 2008c;), channel catfish (*Ictalurus* *punctatus*) (Small, 2003; Zaikov et al., 2009), african catfish (*Clarias gariepinus)* (Ogretmen & Gokçek, 2013; Diyaware et al., 2017) etc.

The research on the anesthetic effect of the clove oil on European catfish are relatively few (Velisek et al., 2006; Zaikov&Iliev, 2007; Gokcek et al., 2016; Krasteva&Zaikov, 2019) with no comparative analysis with other essential oil.

Another essential oil that has anesthetic effect on fish is peppermint oil, a natural product with some antiseptic, analgesic and bactericidal action (Tsuchiya, 2017). It is produced in Bulgaria and it is obtained from different types of mint (*Mentha* spp.), most often from *Mentha arvensis*. This species is native to Europe, Asia and North America (Hoseini et al., 2018). Other mint species are *Mentha spicata*, native to Europe and Asia, but common in other parts of the world and *Mentha piperita*, native to Europe and the Middle East, cultivated in other countries and regions (Hoseini et al., 2018).

The efficacy of peppermint oil and its main component (menthol) as an anesthetic has been tested in a variety of predominantly non-European freshwater and marine fish, like clownfish *Amphiprion ocellaris* (Pedrazzani & Neto, 2014), *Piaractus mesopotamicus* (Goncalves et al., 2008), *Colossoma macropomum* (Facanha & Gomes, 2005), *Centropomus parallelus* (Souza et al., 2012), Nile tilapia *Oreochromis niloticus* (Teixeira et al., 2011; Mello et al., 2012; Rezende et al., 2017). American trout *Oncorhynchus mykiss* (Metin et al., 2015), Persian sturgeon *Acipenser persicus* (Mazandarani & Hoseini, 2017), Silver catfish *Rhamdia quelen* (Gressler et al., 2014; Santos et al., 2017), African catfish *Clarias gariepinus* (Popoola et al., 2015.There is no information, in the scientific literature, for the anesthetic effect of peppermint oil in European catfish, regardless of the stage of its individual development, which determined its use in the present study.The aim of this study is to establish the efficacy of two anesthetics: clove oil and peppermint oil, at different concentrations and the time needed for induction and recovery from anesthesia of juvenile European catfish .

Material and Methods

For the aim of the study, 90 juvenile European catfish, reared in flow-through system at the Institute of Fisheries and Aquaculture-Plovdiv, with an average body weight of 8.62 ± 1.61 g, are anesthetized with clove and peppermint oil. During the experiment, the water temperature is 26.2-27.5ºC and the dissolved oxygen level is 6.2 – 6.5 mg.l^{-1}.

All phases of anesthesia and recovery are described following Hamackova et al. (2006) with a change, listed in Table 1.

Three concentrations of clove oil are tested: 0.02 ml.l^{-1}, 0.04 ml.l^{-1} and 0.06 ml.l^{-1}, while the concentrations of peppermint oil are six: 0.02 ml.l^{-1}, 0.04 ml.l^{-1}, 0.06 ml.l^{-1}, 0.08 ml.l^{-1}, 0.1 ml.l^{-1} and 0.12 ml.l^{-1}. of the experimental minimum and maximum concentrations of clove oil are based on previously published information for European catfish (Zaikov & Iliev, 2007; Krasteva & Zaikov, 2019). Due to the lack of data for European catfish, peppermint concentrations are determined according to these of clove oil. Peppermint oil has been tested at three higher concentrations (0.08 ml.l^{-1}, 0.1 ml.l^{-1}, 0.12 ml.l^{-1}), because its anesthetic effect on the fish is significantly lower compared to the clove oil. Prior to the preparation of the working solution, the clove and peppermint oil are diluted in ethyl alcohol (95%), in a 1: 9 ratio, and then added to 10 l experimental baths without water aeration. All experimental variants are replicated twice. Five fish are put in each tub with the corresponding concentration of clove and peppermint. The time to achieve anesthesia and subsequent recovery of each specimen is measured with stopwatch. In order to recover from the anesthesia, the fish are transferred in tubs with clean and aerated water, where they are observed for 24 hours.

Student`s t-test (Two sample for means under the null hypothesis) is used to assess the differences in induction and recovery time of the different concentrations of each anesthetic agent. The significance difference is established and presented at level of $P < 0.05$. Correlation analyses (Pearson coefficient) and non-linear regression analyses are used to

Table 1. Phases of induction and recovery from anesthesia

Phase	Anaesthetizing	Phase	Recovery
	Behavior of the fish		Behavior of the fish
1	Acceleration of the opercula movements increased respiratory activity.	1	Weak, uncoordinated locomotion.
2	Decreased respiratory activity accompanied by uncoordinated locomotion.	2	Decreased locomotor activity.
3	Loss of equilibrium, decreased opercula movements, the fish still react to strong external stimuli.	3	Normal position of the body. Normal locomotor activity is regained.
4	Complete immobilization, the fish lie on the bottom and do not react to handling.		
5	Complete cessation of opercula movements, the fish die if they remain in the solution.		

establish the correlation between concentration and induction time, as well as the concentration and recovery time. The results are presented as mean ± S.E.M. and are analyzed via Data Analysis (Excel 2010).

Results and Discussion

The results of the experiment with clove and peppermint oil are presented in Table 2 as an average values (± sd).

Clove oil

The time for induction of anesthesia is the longest for the fish placed at the lowest concentration, respectively the shortest period of induction is observed at the highest concentration. The transition from Phase 2 of reduced respiratory activity and uncoordinated movements to Phase 3 of equilibrium loss is relatively fast, taking an average of 27 s at a concentration of 0.02 ml.l^{-1} and 2 sec at 0.04 ml.l^{-1} ($P \leq 0.01$). The fish exposed to the highest concentration of 0.06 ml.l^{-1}, directly entered Phase 4 from Phase 2, skipping Phase 3 of sedation. In Phase 4, the fish lost their balance and lay still on the bottom, but responded to an external stimulus (Figure 1).

The recovery period was the shortest – 1.18 min at the lowest concentration – 0.02 ml.l^{-1} and the longest at the highest concentration, respectively 7.9 min – 0.06 ml.l^{-1} ($P \leq 0.05$). This tendency in catfish is confirmed by other authors (Zaikov & Iliev, 2007; Zaikov et al., 2009), as well as for other species (Hoskonen & Pirhonen, 2004; Keene et al., 1998; Zaikov et al., 2008).

The transition from Phase 1 to Phase 2 of recovery is relatively short process, taking an average of 4 s at concentrations of 0.02 and 0. 04 ml.l^{-1} and 14 s at a concentration of 0.06 ml.l^{-1}. During the recovery from anesthesia, a directly proportional correlation between the time and the concentration parameters is observed, the lower the concentration, the shorter the time required for recovery and vice versa (Figure 1).

Peppermint oil

The anesthetic time for peppermint oil is the longest at the lowest concentration and shortest at the highest concentration with significant differences between the parameters ($P \leq 0.05$). With the increasing concentration of peppermint oil, the time required for anesthesia to occur decreases. The transition period from Phase 2 of reduced respiratory activity to Phase 3 of equilibrium loss is the shortest at all six experimental concentrations. The period between Phase 3 of sedation and Phase 4 of complete immobilization is the longest at all experimental concentrations of peppermint oil. The fish at the bottom of the tub were still but responsive to an external stimulus for 25.4 min – 0.02 ml.l^{-1}, 22.4 min – 0.04 ml.l^{-1}, 19.25 min – 0.06 ml.l^{-1}, 14.2 min – 0.08 ml.l^{-1}, 7.9 min – 0.10 ml.l^{-1}, 3.8 min – 0.12 ml.l^{-1}. In Phase 3, the same tendency

Table 2. Induction and recovery times (min) for European catfish, anaesthetized with two anaesthetic agents. Data are presented as average ± sd

Clove oil	Concentration (mg.l^{-1})					
Stages	0.02	0.04	0.06			
I_2	5.15±1.03^{a}	2.26±0.12^{a}	1.27±0.03^{a}			
I_3	5.42±0.82^{b}	2.28±0.11^{b}				
I_4	15.76±3.59^{a}	2.31±0.12^{b}	1.38±0.31^{ab}			
R_1	1.10±0.30^{a}	3.51±1.80^{b}	6.91±2.42^{ca}			
R_2	1.15±0.25^{bc}	3.55±1.80^{b}	7.05±2.50^{c}			
R_3	1.18±0.25^{a}	4.04±1.89^{b}	7.9±2.04^{ca}			
Peppermint oil	Concentration (mg.l^{-1})					
Stages	0.02	0.04	0.06	0.08	0.10	0.12
I_2	4.86±2.75^{a}	4.71±3.73^{a}	4.37±1.32^{c}	3.51±1.20^{de}	2.96±0.52^{e}	2.23±0.68^{f}
I_3	7.27±2.97^{a}	5.47±3.14^{a}	5.08±1.48^{cd}	3.97±0.59^{d}	3.02±0.52^{ea}	2.45±0.69^{fc}
I_4	32.68±2.38^{a}	27.89±1.32^{b}	24.33±1.37^{ca}	18.13±3.03^{d}	10.86±1.15^{e}	6.23±1.11^{e}
R_1	1.44±0.83^{b}	2.06±0.70^{b}	2.41±0.61^{cd}	3.07±2.65^{db}	3.41±0.63^{ef}	5.87±0.88^{f}
R_2		2.08±0.80^{b}	2.43±0.64^{cd}	3.10±2.64^{db}	3.47±0.59^{ef}	5.89±0.89^{f}
R_3	1.45±0.83^{a}	2.08±0.70^{b}	2.43±0.61^{cd}	3.27±2.66^{db}	3.50±0.59^{ea}	5.95±0.88^{e}

In all lines, values connected by different superscripts are significantly different from each other ($P \leq 0.05$)

I_2 – induction to anesthesia, stage 2, I_3 – induction to anesthesia, stage 3, I_4 – induction to anesthesia, stage 4, R_1 – recovery from anesthesia, stage 1, R_2 – recovery from anesthesia, stage 2, R_3 – recovery from anesthesia, stage 3 is observed as in Phase 2, the anesthetic effect increases with the increasing concentration. During the recovery phase, the same tendency is observed as in clove oil, it is the shortest at the lowest concentration (1.45 min – 0.02 ml.l^{-1}) and the longest at the highest concentration ($P \leq 0.05$). At a concentration of 0.02 ml.l^{-1}, the fish regain their normal position immediately after starting the move, thus they did not enter Phase 2 of impaired movement activity. The same effect is observed at

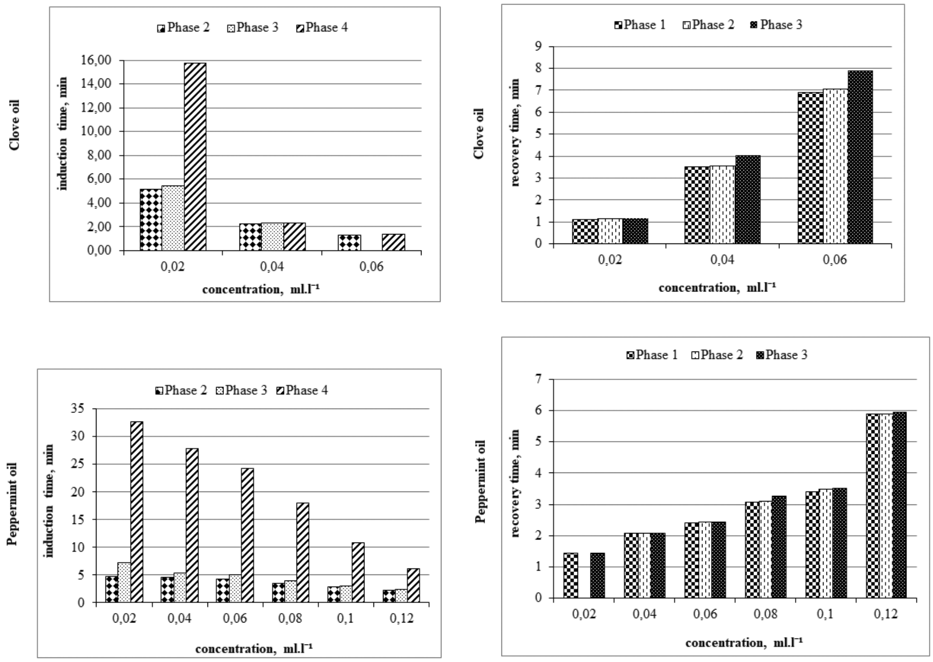

Fig. 1. Induction and recovery time (min) of anesthesia for clove and peppermint oil

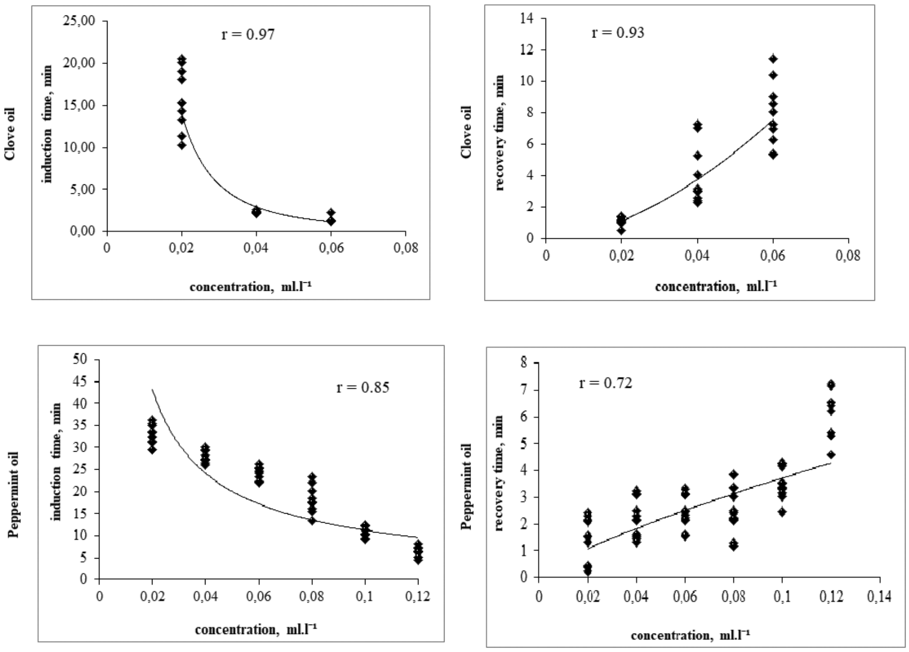

Fig. 2. Correlation of induction and recovery time and concentration of clove and peppermint oil (n = 10)

the concentrations of 0.04 ml.l^{-1} and 0.06 ml.l^{-1}, where Phase 2 and Phase 3 of recovery occured at the same time, without being clearly distinguishable. The tendency between the time required for anesthesia and the concentration of peppermint oil is directly proportional, as with clove oil (Figure 2).

A linear exponential correlation is observed between concentration and recovery time of clove oil (r = 0.93) and peppermint oil (r = 0.72). Higher values of concentration correspond to longer recovery time for both tested oils.

Inverse exponential correlation is observed between the concentration of the anesthetics and the induction time. Higher concentration values correspond to shorter induction time for both oils, which is confirmed by the Pearson coefficient (r = 0.97; r = 0.85).

Conclusions

Based on the results, it can be concluded that the time required for the anesthesia of juvenile European catfish with peppermint oil is significantly longer compared to the time required using clove oil. Anesthesia at the lowest concentration of 0.02 ml.l^{-1} occurred in 15.8 min with clove oil and in 32.7 min with peppermint. At concentration of 0.06 ml.l^{-1} clove oil, anesthesia occured in 1.4 min, and at concentration of peppermint oil of 0.12 ml.l^{-1} – for 6.2 min. Peppermint oil can be used to produce a sedative effect, while high doses and long exposure are required for anesthesia, making it difficult to use in commercial or scientific activities.

During the experiment, it has been established that the clove oils is significantly more effective as anesthetic agent for juvenile European catfish than peppermint oil.

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