Comparison of the anesthetic effect of lemongrass and clove oil on two-year old common carp (*Cyprinus carpio*, Linnaeus, 1758)

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Citation: Krasteva, V., Ivanova, A., & Yankova, M. (2022). Comparison of the anesthetic effect of lemongrass and clove oil on two-year old common carp (*Cyprinus carpio*, Linnaeus, 1758). *Zhivotnovadni Nauki*, *59*(4), 16-24 (Bg).

Abstract

The aim of the present study is to examine the efficacy of lemongrass oil (*Cymbopogon schoenanthus*) and clove oil (*Eugenia caryophyllata*) by establishing the time needed for induction and recovery from anesthesia of two-year old common carp (*Cyprinus carpio* L.). The fish treated with lemongrass oil have an average body weight (BW, g) of 614.20 ± 162.18 g and an average total length (TL, cm) of 36.10 ± 3.77 cm. The fish used in the experiment with clove oil have an average body weight of 572.32 ± 191.32 g and average total length of 36.19 ± 4.0 cm. For both experiments, five treatments are conducted with five experimental concentrations: 0.02 ml/l^{-1}, 0.04 ml/l^{-1}, 0.06 ml/l^{-1}, 0.08 ml/l^{-1} and 0.10 ml/l^{-1}. The method of individual treatment is applied. For each concentration, 10 fish are used to ensure statistical significance of the results. For both essential oils the fastest anesthetic effect is registered at the highest concentration of 0.10 ml/l^{-1} with a significant difference between the variants ($P \leq 0.05$). Based on the results, it can be concluded that the time required for anesthesia of two-year old common carp with lemongrass oil is significantly longer compared to the time required using clove oil. At the highest experimental concentration of lemongrass (0.10 ml/l^{-1}) anesthesia occurs after more than 10 min, which makes the use of this essential oil as an anesthetic ineffective in commercial or scientific activities.

During the experiment, it has been established that clove oil is significantly more effective as anesthetic agent for two-year old common carp compared to lemongrass oil.

Key words: anesthesia, lemongrass oil, clove oil, *Cyprinus carpio,* common carp

Сравнение на анестезиращия ефект на лимонена трева и карамфилово масло върху двугодишен обикновен шаран (*Cyprinus carpio* Linnaeus, 1758)

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Резюме

Целта на настоящото проучване е да се изследва анестезиращият ефект на масло от лимонена трева (*Cymbopogon citratus*) и масло от карамфил (*Eugenia caryophyllata*) чрез проследяване на времето необходимо за индукция и възстановяване от анестезия при двугодишен шаран (*Cyprinus carpio* L.). Рибите третирани с лимонена трева имат средно телесно тегло (BW, g) от 614,20 ± 162,18 g и средна обща дължина (TL, cm) от 36,10 ± 3,77 cm. При третирането с карамфиловото масло рибите са със средно телесно тегло от 572,32 ± 191,32 g и средна обща дължина от 36,19 ± 4,0 cm. При провеждането на експеримента са използвани пет концентрации лимонена трева и карамфилово масло: 0,02 ml/l^{-1}, 0,04 ml/l^{-1}, 0,06 ml/l^{-1}, 0,08 ml/l^{-1} и 0,10 ml/l^{-1}. Приложен е методът на индивидуално третиране. За всяка концентрация са използвани 10 риби, за да се осигури статистическа значимост на резултатите. При най-висока концентрация от 0,10 ml/l^{-1} е регистриран най-бърз анестезиращ ефект и при двете експериментални масла със значима разлика между вариантите ($P \leq 0{,}05$).

Въз основа на резултатите може да се заключи, че времето необходимо за анестезия на двугодишен шаран с масло от лимонена трева е значително по-дълго в сравнение с времето необходимо при използване на карамфилово масло. При най-високата експериментална концентрация на лимонената трева (0,10 ml/l^{-1}) анестезия настъпва за повече от 10 минути, което прави приложението на това етерично масло неефективно в производствени или научни дейности.

По време на експеримента е установено, че карамфиловото масло е по-ефективен анестетик за двугодишен шаран в сравнение с маслото от лимонена трева.

Ключови думи: анестезия, лимонена трева, карамфилово масло, *Cyprinus carpio,* обикновен шаран

Introduction

The aquaculture industry consists of stressful activities that can affect the well-being of the species, including handling, confinement, fertilization, transport, and different operations, from the hatchery to the final commercial stage (Ashley, 2007; Sampaio and Freire, 2016; Sneddon et al., 2016; Sánchez-Muros et al., 2017; Souza et al., 2019).

Essential oils have been used in aquaculture studies due to their diverse properties that can improve health, growth and welfare of animals, as well as to reduce stress processes (Azambuja et al., 2011; Zeppenfeld et al., 2014; Saccol et al., 2016, 2018; Souza et al., 2018; Souza et al., 2017; Souza et al., 2019). There are reviews of the effects of essential oils as sedatives, anesthetics, antioxidants, and antimicrobials (Cunha et al., 2018; Hoseini et al., 2018; Sutili et al., 2018; Souza et al., 2019).

Species belonging to genus *Cymbopogon* are perennial herbs of the Poaceae family, native to Asia and commonly found on the American continent (Oladeji et al., 2019). Essential oil from *C. citratus* is known for its immunomodulatory, anti-inflammatory, antiseptic, antimicrobial, and antifungal properties (Devi et al., 2011; Al-Sagheer et al., 2018; Souza et al., 2019).

The anesthetic effect of lemongrass in common carp (*Cyprinus carpio*) has not been studied so far. Research on the sedative and anesthetic effect of lemongrass has been done with species such as: freshwater angelfish, *Pterophyllum scalare* (Oliviera et al., in review), Nile tilapia juveniles, *Oreochromis niloticus* (Netto et al., 2017) and silver catfish, *Rhamdia quelen* (Santos et al., 2017). The efficacy of both clove oil and lemongrass oil as sedative in transportation has been tested in orange chromide, *Etroplus maculatus* (Dominic et al., 2016).

Many studies have been conducted to investigate the use of clove essential oil (*Eugenia aromatica* and *Eugenia caryophylata*) as a fish anesthetic and its effectiveness in different fish species (Javahery et al., 2012; Priborsky and Velisek, 2018; Aydın and Barbas, 2020). Besides its effectiveness, clove oil is widely used in aquaculture for being a lowcost, abundant, low toxic, and environmentally friendly product (Mitjana et al., 2014; Taheri Mirghaed et al., 2018; Aydın et al., 2019; Aydın and Barbas, 2020). However, some studies have reported negative effects, including decreased blood lymphocyte, neutrophilia, ventilatory failure, medullary collapse and partial fusion of lamellae after exposure to this oil (Sladky et al., 2001; Mazandarani et al., 2017; Kumari et al., 2018; Taheri Mirghaed et al., 2018, Aydın and Barbas, 2020).

The anesthetic effect of different essential oils have been tested on common carp, such as clove oil (Husen and Sharma, 2015); tea tree, *Melaleuca alternifolia* (Hajek, 2011); spearmint, *Mentha spicata* (Roohi and Imanpoor, 2015); basil, *Ocimum basilicum* (Khumpirapang et al., 2018, Krasteva et al., 2021 a); American basil, *O. canum* and holy basil, *O. sanctum* (Khumpirapang et al., 2018); lavender, *Lavandula angustifolia* and thyme, *Thymus vulgaris* (Krasteva et al., 2021 a) and rosemary, *Rosmarinus officinalis* (Krasteva et al., 2021 b).

The aim of the present research is to investigate the anesthetic effect of *Cymbopogon schoenanthus* and *Eugenia caryophyllata* in two-year old common carp in order to establish their effectiveness as sedative and anesthetic agents in aquaculture practice.

Material and methods

The study was conducted at the Institute of Fisheries and Aquaculture, Plovdiv in July 2021.

Subject of research

The subject of the experiment was two-year old *C. carpio*, in the size-weight group used for sport fishing, hatched by natural propagation in May 2019 and reared in the ponds of the experimental base of IFA, Plovdiv in polyculture with bighead carp, *Hypophthalmichthys nobilis* and grass carp, *Ctenopharyngodon idella*. For the purpose of the study, the fish were caught from the experimental ponds and transfered for storage in 3 m^3 tanks. The biometric characteristics of the experimental fish are presented in Table 1.

Table 1. Body weight (BW, g) and total length (TL, cm) of the fish in the experiment

Lemon grass		
Statistical value	BW (g)	TL (cm)
Mean ± SD	614.20 ± 162.18	36.10 ± 3.77
Min–max	330.0 ÷ 870.0	29.14 ÷ 41.50
CV, %	26.40	10.44
Clove oil		
Statistical value	BW (g)	TL (cm)
Mean ± SD	572.32 ± 191.32	36.19 ± 4.0
Min–max	190.0 ÷ 890.0	29.14 ± 41.64
CV, %	33.43	10.99

Essential oils

The lemongrass essential oil (*Cymbopogon schoenanthus*) and the clove oil (*Eugenia caryophyllata*) were purchased commercially with listed ingredients 100% pure lemongrass oil and 100% clove oil, produced in Plovdiv, Bulgaria by "Rivana" LTD. The experimental solutions were prepared by diluting the oil in ethyl alcohol (95%) in 1:9 ratio and were added to 10 l experimental tanks with vigorous stirring before treatment.

Experimental design

Due to the lack of published data, and in order to preserve the well-being of the treated fish and to prevent mortality, the lowest experimental concentration for both oils was 0.02 ml/l^{-1}. The applied concentrations were increased gradually in order to carefully observe the behavior and condition of the fish. The maximum applied concentration of clove oil was 0.10 ml/l^{-1}, as further increase in the concentration may lead to negative side effects and mortality.

The experimental concentrations of lemongrass oil were the same as clove oil, in order to compare the results.

The applied concentrations were five: 0.02 ml/l^{-1}, 0.04 ml/l^{-1}, 0.06 ml/l^{-1}, 0.08 ml/l^{-1} and 0.10 ml/l^{-1}. The method of individual treatment was applied. For each concentration, 10 fish were used to ensure statistical significance of the results. For each essential oil 100 fish were used, or a total of 200 fish for the experiment. In order to secure the welfare of the treated specimens, the biometric parameters, body weight (BW, g) and body length (TL, cm), were measured after treatment with the anesthetic solution.

When preparing the solutions for anesthesia and recovery, the temperature of the water was equalized to the temperature of the water in the storage tanks. Before adding the anesthetic solution, the temperature (T °C) and the level of dissolved oxygen (O_2, mg/l^{-1}) were measured.

To recover from anesthesia, the fish were transferred in tanks with the same volume of clean water (10 l) with placed microcompressors, where they were observed until complete recovery. The time required for the induction of anesthesia and subsequent recovery was measured with a stopwatch, taking into account the time of each phase. When processing the results, the data is converted into minutes according to the following formula: min = (min* 60 + sec)/60.

The behavior of the fish was described and analyzed according to the phases of anesthesia and recovery determined by Hamackova et al. (2006):

<u>Phases of anesthesia</u>

Phase 1. Accelleration of the opercular movements, increased respiratory activity.

Phase 2. Decreased respiratory activity accompanied by uncoordinated movements.

Phase 3. Loss of equilibrium, decreased opercular movements, the fish still react to strong external stimuli.

Phase 4. Complete immobilization, the fish lie on the bottom and do not react to handling.

<u>Phases of recovery</u>

Phase 1. Beginning of movements.

Phase 2. Weak, uncoordinated locomotor activity.

Phase 3. Normal position of the body. Normal locomotor activity is regained.

Statistical analysis

All data is presented as average values (mean ± SD). The results obtained for the induction of anesthesia and the period of recovery for each concentration and phase were analyzed at a confidence level of $P \leq 0.05$. Phase 4 at 0.04 ml/l^{-1} lemongrass oil was not included in the statistical analysis as anesthesia was not induced in all experimental individuals. The results were analyzed separately for each essential oil. For this purpose, a comparative Student T-test (Two sample for mean) was performed using Excel - Data Analysis.

Results and discussion

The main hydrochemical parameters dissolved oxygen (O2, mg/l^{-1}) and temperature (T °C), measured in the tanks for anesthesia and in the tanks for recovery are presented in Fig. 1 (A & B) and Fig. 2 (A & B). The measured oxy-

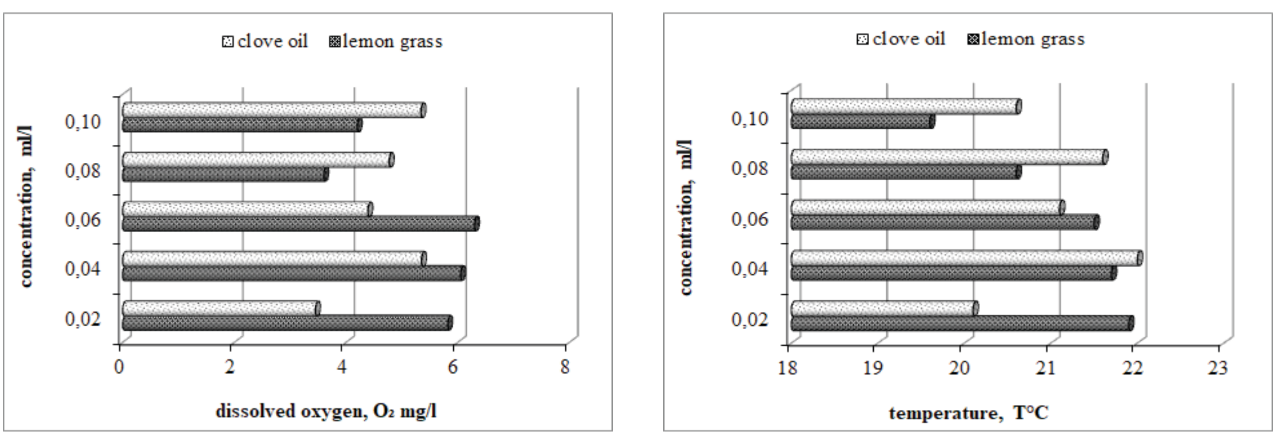
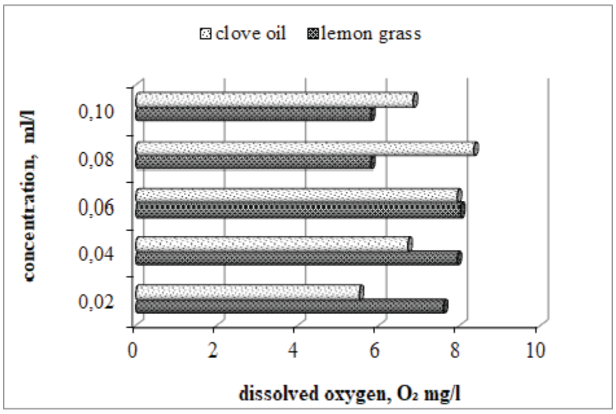


Fig. 1. Dissolved oxygen (O_2, mg/l^{-1}) (A) and water temperature (T °C) (B) in the tanks for anesthesia for lemongrass and clove oil

gen in the recovery tank, for both experimental oils, varies from 5.80 mg/l^{-1} to 8.53 mg/l^{-1}, and the measured temperature is in the range of 20.0 °C–22.3 °C. The oxygen measured in the anesthesia tanks, for both essential oils, is in the range from 3.60 mg/l^{-1} to 6.30 mg/l^{-1} and the measured is in the range from 19.6 °C to 21.9 °C.

During the whole duration of the experiment, the values of the dissolved oxygen and tempera-



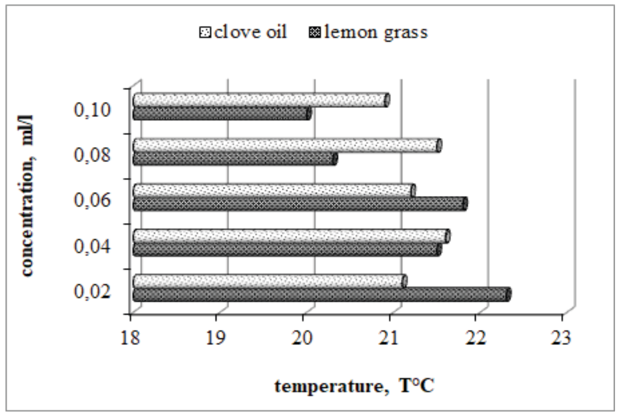


Fig. 2. Dissolved oxygen (O_2, mg/l^{-1}) (A) and water temperature (T °C) (B) in the tanks for recovery for lemongrass and clove oil

Table 2. Duration (min) of the phases of anesthesia and recovery of two-years common carp (K2), anaesthetized with two essential oils. Data are presented as average ± sd.

Phases	Concentration (ml/l^{-1})				
Lemon grass	0.02	0.04	0.06	0.08	0.10
A2	15.90 ± 0.97	9.68 ± 1.69^{bc}	5.76 ± 1.35^{cbde}	4.32 ± 0.65^{dce}	2.98 ± 0.44^{ecd}
A3	-	16.35 ± 0.92^{bc}	14.16 ± 1.14^{cb}	10.08 ± 0.77^{de}	8.58 ± 0.91^{ed}
A4	-	18.08 ± 1.30*	16.35 ± 1.28^{cd}	13.23 ± 2.11^{dce}	10.34 ± 0.65^{ed}
R1	-	2.78 ± 0.27^{a}	4.27 ± 0.64^{a}	4.58 ± 0.70^{a}	4.31 ± 0.60^{a}
R2	-	3.35 ± 0.71	5.77 ± 0.85^{ce}	6.83 ± 1.31	7.94 ± 1.71^{ec}
R3	-	4.40 ± 0.57^{bc}	6.45 ± 0.84^{cbd}	8.06 ± 1.30^{dce}	10.24 ± 1.32^{ed}
Phases	Concentration (ml/l^{-1})				
Clove oil	0.02	0.04	0.06	0.08	0.10
A2	2.97 ± 0.69^{ad}	2.03 ± 0.34^{bd}	1.26 ± 0.14	1.09 ± 0.35^{dabe}	0.51 ± 0.13^{ed}
A3	6.16 ± 1.25^{ab}	3.15 ± 0.66^{bad}	2.60 ± 0.75	2.00 ± 0.69^{db}	-
A4	7.63 ± 0.94	4.21 ± 0.65^{bc}	3.29 ± 0.60^{cbd}	2.42 ± 0.61^{dce}	1.45 ± 0.39^{ed}
R1	1.86 ± 0.59^{abc}	3.07 ± 0.82^{bade}	3.31 ± 0.27^{cae}	3.84 ± 0.82^{db}	4.35 ± 0.52^{ebc}
R2	2.49 ± 0.63^{abd}	5.00 ± 1.51^{bad}	4.57 ± 0.64^{ce}	6.02 ± 1.84^{dab}	6.39 ± 1.65^{ec}
R3	3.23 ± 0.75^{ab}	5.93 ± 1.84^{bade}	6.22 ± 0.75^{cd}	8.03 ± 2.04^{dbc}	9.15 ± 1.60^{ebc}

*70% of experimental individuals.

In all lines, values connected by different superscripts are significantly different from each other (P ≤ 0.05).

A2 – Phase 2 of anesthesia — *a – 0.02 ml/l^{-1}*
A3 – Phase 3 of anesthesia — *b – 0.04 ml/l^{-1}*
A4 – Phase 4 of anesthesia — *c – 0.06 ml/l^{-1}*
R1 – Phase 1 of recovery — *d – 0.08 ml/l^{-1}*
R2 – Phase 2 of recovery — *e – 0.10 ml/l^{-1}*
R3 – Phase 3 of recovery

ture varied within narrow limits and no critical values were registered.

The results of the experiment of the anesthetic effect of lemongrass and clove oil are presented in Table 2.

Lemon grass oil

At the lowest concentration of 0.02 ml/l^{-1}, for a period of 20 minutes, the fish reach only Phase 2 of anesthesia expressed in periods of static and active state of the individuals. No recovery period is observed due to the lack of anesthetic effect of the oil at this concentration.

At 0.04 ml/l^{-1}, Phase 4 of anesthesia occurs most slowly with an average induction time of 18.08 min in 70% of the fish, with the remaining 30% being in Phase 3 of loss of balance. The experimental individuals go through all phases of recovery for an average period of 4.40 min.

At 0.06 ml/l^{-1} anesthesia occurs with an average induction time of 16.35 min.

At the concentration of 0.08 ml/l^{-1} the period of anesthesia takes an average of 13.23 min.

At 0.10 ml/l^{-1}, Phase 4 of general anesthesia occurs after 10.34 min with a significant difference between the experimental variants ($P \leq 0.05$).

The recovery period for the higher concentrations (0.06 ml/l^{-1}, 0.08 ml/l^{-1} and 10 ml/l^{-1}) is respectively 6. 45 min, 8.06 min and 10.24 min.

Oliviera et al. (in review) applied 0.01 ml/l^{-1}, 0.025 ml/l^{-1}, 0.05 ml/l^{-1}, 0.075 ml/l^{-1}, 0.1 ml/l^{-1}, 0.15 ml/l^{-1}, 0.20 ml/l^{-1} and 0.25 ml/l^{-1} lemongrass to study the effects of anesthesia in freshwater angel fish (*Pterophyllum scalare*) of two different size groups – juveniles I (0.82 g) and II (2.40 g). The authors established that the best sedation and anesthesia times are obtained with 0.01 ml/l^{-1} and 0.025 ml/l^{-1} and 0.20 ml/l^{-1} and 0.25 ml/l^{-1} respectively.

A study by Netto et al. (2017) verified the sedative and anesthetic effect of *Cymbopogum flexuosus* in Nile tilapia juveniles at different concentrations of the essential oil: 0.01 ml/l^{-1}, 0.025 ml/l^{-1}, 0.05 ml/l^{-1}, 0.1 ml/l^{-1}, 0.2 ml/l^{-1}, 0.4 ml/l^{-1} and 0.6 ml/l^{-1}. The time of sedation ranged from 0.12 to 0.51 min and the recommended concentration was 0.01 ml/l^{-1} and 0.025 ml/l^{-1}. The best times for anesthesia and recovery were found for the concentrations of 0.6 ml/l^{-1} (5.45 and 6.25 min, respectively).

Santos et al. (2017) studied the time for anesthesia induction and recovery using different concentrations of *Cymbopogon flexuosus* in silver catfish (*Rhamdia quelen*). A total of 144 silver catfish, length 7.5 ± 1.1 cm, weighing 3.95 ± 0.85 g were used in the experiment. Essential oils were evaluated at concentrations of 0.025 ml/l^{-1}, 0.15 ml/l^{-1} and 0.3 ml/l^{-1}. The authors established that *C. flexuosus* induced effective sedation at 0.025 ml/l^{-1} and anesthesia at 0.15 ml/l^{-1} and 0.3 ml/l^{-1} without short-term mortality. As in the current experiment, time to anesthesia induction followed a negative concentration-dependent pattern, but the recovery time demonstrated a positive concentration-response relationship.

In the current research lemongrass oil induce anesthesia slower compared to previous studies of its anesthetic effect in other fish species. At the highest experimental concentration of 0.10 ml/l^{-1} anesthesia is induce after 10.34 min with long recovery period of 10.24 min which makes lemongrass essential oil not suitable for application in two-year old common carp.

Clove oil

Unlike lemongrass, the lowest concentration of clove oil (0.02 ml/l^{-1}) has anaesthetic effect on two-year old common carp with an average induction time of 7.63 minutes.

At 0.04 ml/l^{-1} anesthesia occurs after 4.21 minutes in 100% of the fish.

At the concentration of 0.06 ml/l^{-1} anesthesia is induced after an average of 3.29 minutes.

At 0.08 ml/l^{-1} the average time for induction of general anesthesia is 2.42 min.

The fastest anesthetic effect, with an average induction time of 1.45 min, is established at 0.10 ml/l^{-1} with significant difference between the experimental variants ($P \leq 0.05$). It has been observed that at the highest concentration of clove oil (0.10 ml/l^{-1}), the fish enter directly from Phase 2 to Phase 4 of general anesthesia, with Phase 3 of loss of balance being skipped.

The recovery period is the shortest (3.23 min) at 0.02 ml/l^{-1} and the longest at 0.10 ml/l^{-1} – 9.15 min ($P \leq 0.05$).

Husen and Sharma (2015) established that the best concentrations of clove oil in *Cyprinus carpio* are 0.005–0.0075 ml/l^{-1} for sedative effect, 0.025–0.075 for anesthetic effect and 0.05 ml/l^{-1} for best concentration response under 3 min. The authors used common carp fry with body weight of 0.53 ± 0.14 g. The results showed that the lowest effective doses that fulfil the most criteria of good anaesthetic characteristics is 0.05 ml/l^{-1} clove oil. The induction times were decreased significantly with increased concentrations.

Hajek et al. (2006) researched the lowest effective concentration of clove oil for the common carp, *Cyprinus carpio* L., and the safe working ranges for the anaesthetic. The fish were exposed to the concentrations of 0.01 ml/l^{-1}, 0.02 ml/l^{-1}, 0.03 ml/l^{-1}, 0.04 ml/l^{-1}, 0.05 ml/l^{-1}, 0.1 ml/l^{-1}, 0.15 ml/l^{-1} and 0.2 ml/l^{-1} of clove oil. Clove oil at the concentrations ranging from 0.03 ml/l^{-1} to 0.2 ml/l^{-1} induced general anaesthesia. The lowest concentration causing general anaesthesia with an average induction time below 3 min was 0.04 ml/l^{-1}. Hajek et al. (2016) established that recovery was concentration-independent and lasted for about 4 min. An increase in the concentration shortened the time of ventilation during anaesthesia and prolonged the recovery. The authors conclude that clove oil is a potent anaesthetic for carp, the safest and most effective at the concentrations of 0.03 ml/l^{-1} and 0.05 ml/l^{-1} and those solutions should be used, in the aquaculture practice, when the procedure requires more than 5 min of the exposure to the anaesthetic.

Based on different studies perfomed with cold and warm water fish species (Wagner et al., 2003; Velisek and Svobodova, 2004; Hajek et al., 2006; Gomulka et al., 2008) the optimal clove oil dosage to induce anaesthesia varies between 0.05 ml/l^{-1} and 0.1 ml/l^{-1} and is 0.01 ml/l^{-1} and 0.03 ml/l^{-1} for handling (Javahery et al., 2012).

The results in the current study correspond to previously reported data on the fast anesthetic effect of clove oil at low concentrations, both in common carp and other fish species, which makes it an affordable and effective sedative and anesthetic agent.

Conclusion

Based on the results, it can be concluded that the time required for the anesthesia of two-year old common carp with lemongrass oil is significantly longer compared to the time required using clove oil.

At the lowest experimental concentration of lemongrass (0.02 ml/l^{-1}) anesthesia is not observed for a period of 20 min, while in clove oil it occurs in 7.63 min. At 0.04 ml/l^{-1} of lemongrass only 70% of the fish reach Phase 4 of anesthesia for 18.08 min. At the same concentration of clove oil, anesthesia is induced after 4.41 min in all experimental individuals. At 0.06 ml/l^{-1} the difference in the induction time for lemongrass and clove oil is almost 4 times, respectively 16.36 min and 3.29 min. At 0.08 ml/l^{-1} clove oil the fish reach Phase 4 of anesthesia 10 min faster compared to lemongrass, respectively 2.42 min and 13.23 min. At the highest concentration of lemongrass (0.10 ml/l^{-1}), the average time required for general anesthesia is 10.34 min, while at the same concentration of clove oil anesthesia occurs in 1.45 minutes, which is 7 times faster.

The recovery period at the concentrations 0.06 ml/l^{-1}, 0.08 ml/l^{-1} and 0.10 ml/l^{-1} is faster when clove oils is applied as anesthetic.

The long induction time of anesthesia and slow recovery process make lemongrass essential oil not suitable for application as anesthetic agent in two-year old common carp. In the current study, it has been established that clove oil is significantly more effective as anesthetic agent for two-year old common carp than lemongrass oil.

References

Al-Sagheer, A. A., Mahmoud, H. K., Reda, F. M., Mahgoub, S. A., & Ayyat, M. S. (2018). Supplementation of diets for Oreochromis niloticus with essential oil extracts from lemongrass (Cymbopogon citratus) and geranium (Pelargonium graveolens) and effects on growth, intestinal microbiota, antioxidant and immune activities. *Aquaculture nutrition*, *24*(3), 1006-1014.

Ashley, P. J. (2007). Fish welfare: current issues in aquaculture. *Applied Animal Behaviour Science*, *104*(3-4), 199-235.

Aydın, B., & Barbas, L. A. L. (2020). Sedative and anesthetic properties of essential oils and their active compounds in fish: A review. *Aquaculture*, *520*, 734999.

Aydın, B., Akhan, S., Gümüş, E., & Özbaş, M. (2019). Anesthetic efficacy of clove oil and 2-phenoxyethanol on doctor fish, Garra rufa (Heckel, 1843). *Boletim do Instituto de Pesca*, *45*(4).

Azambuja, C. R., Mattiazzi, J., Riffel, A. P. K., Finamor, I. A., de Oliveira Garcia, L., Heldwein, C. G., ... & Llesuy, S. F. (2011). Effect of the essential oil of Lippia alba on oxidative stress parameters in silver catfish (Rhamdia quelen) subjected to transport. *Aquaculture*, *319*(1-2), 156-161.

Da Cunha, J. A., Heinzmann, B. M., & Baldisserotto, B. (2018). The effects of essential oils and their major compounds on fish bacterial pathogens–a review. *Journal of applied microbiology*, *125*(2), 328-344.

Devi, V., Ushanandhini, R., Kumar, K., Maheswari, M., Sivashanmugham, A., & Jagannath, P. (2011). Antioxidant activity of *Cassia auriculata* Linn flowers. *Pharmacology Online*, *2*, 490-498.

Dominic, A. D., Inasu, N. D., & Swapna, J. (2016). Efficiency of anaesthetics in combating stress in fish transportation of Etroplus maculatus (Bloch, 1975). *International Journal of Current Research*, *8*(7), 33850-33854.

Gomulka, P., Wlasow, T., Velíšek, J., Svobodová, Z., & Chmielinska, E. (2008). Effects of Eugenol and MS-222 Anaesthesia on Siberian Sturgeon Acipenser baerii Brandt. *Acta Veterinaria Brno*, *77*(3), 447-453.

Hajek, G. J. (2011). The anaesthetic-like effect of tea tree oil in common carp Cyprinus carpio L. *Aquaculture Research*, *42*(2), 296-300.

Hajek, G. J., Klyszejko, B., & Dziaman, R. (2006). The anaesthetic effects of clove oil on common carp, Cyprinus carpio L. *Acta ichthyologica et piscatoria*, *2*(36), 93-97.

Hamackova, J., Kouril, J., Kozak, P., & Stupka, Z. (2006). Clove oil as an anaesthetic for different freshwater fish species. *Bulgarian Journal of Agricultural Science*, *12*(2), 185-194.

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

Husen, M. A., & Sharma, S. (2015). Anaesthetics Efficacy of MS-222, Benzoak® vet, AQUI-S® and Clove oil on common carp (Cyprinus carpio) fry. *International Journal of Research in Fisheries and Aquaculture*, *5*, 104-114.

Javahery, S., Nekoubin, H., & Moradlu, A. H. (2012). Effect of anaesthesia with clove oil in fish. *Fish Physiology and Biochemistry*, *38*(6), 1545-1552.

Khumpirapang, N., Pikulkaew, S., Anuchapreeda, S., & Okonogi, S. (2018). Anesthetic activity of plant essential oils on Cyprinus carpio (koi carp). *Drug Discoveries & Therapeutics*, *12*(1), 21-30.

Krasteva, V., Yankova, M., & Ivanova, A. (2021). Comparison of the anesthetic effect of three essential oils on carp stocking material (Cyprinus carpio L. 1758). *Journal of Mountain Agriculture on the Balkans*, *24*(1), 42-63.

Krasteva, V., Yankova, M., & Ivanova, A. (2021). Anesthetic effect of rosemary essential oil (Rosmarinus officinalis L.) and its use for transport of Common carp (Cyprinus carpio L.) stocking material. *Zhivotnovadni Nauki*, *58*(6), 37-46.

Kumari, P., Abidi, S., & Parwez, I. (2018). Evaluation of anaesthetizing efficacy of clove oil in Channa punctatus. *International Journal of Fisheries and Aquatic Studies*, *6*(5), 262-266.

Mazandarani, M., Hoseini, S. M., & Dehghani Ghomshani, M. (2017). Effects of linalool on physiological responses of Cyprinus carpio (Linnaeus, 1758) and water physico□chemical parameters during transportation. *Aquaculture Research*, *48*(12), 5775-5781.

Mirghaed, A. T., Yasari, M., Mirzargar, S. S., & Hoseini, S. M. (2018). Rainbow trout (Oncorhynchus mykiss) anesthesia with myrcene: Efficacy and physiological responses in comparison with eugenol. *Fish Physiology and Biochemistry*, *44*(3), 919-926.

Mitjana, O., Bonastre, C., Insua, D., Falceto, M. V., Esteban, J., Josa, A., & Espinosa, E. (2014). The efficacy and effect of repeated exposure to 2-phenoxyethanol, clove oil and tricaine methanesulphonate as anesthetic agents on juvenile Angelfish (Pterophyllum scalare). *Aquaculture*, *433*, 491-495.

Netto, J. D. L., Oliveira, R. S., & Copatti, C. E. (2017). Efficiency of essential oils of Ocimum basilicum and Cymbopogum flexuosus in the sedation and anaesthesia of Nile tilapia juveniles. *Anais da Academia Brasileira de Ciências*, *89*, 2971-2974.

Oladeji, O. S., Adelowo, F. E., Ayodele, D. T., & Odelade, K. A. (2019). Phytochemistry and pharmacological activities of Cymbopogon citratus: A review. *Scientific African*, *6*, e00137.

de Oliveira, I. C., Oliveira, R. S. M., Lemos, C. H. D. P., de Oliveira, C. P. B., Felix e Silva, A., Lorenzo, V. P., ... & Copatti, C. E. (2022). Essential oils from Cymbopogon citratus and Lippia sidoides in the anesthetic induction and transport of ornamental fish Pterophyllum scalare. *Fish Physiology and Biochemistry*, 1-19.

Priborsky, J., & Velisek, J. (2018). A review of three commonly used fish anesthetics. *Reviews in fisheries science & aquaculture*, *26*(4), 417-442.

Roohi, Z., & Imanpoor, M. R. (2015). The efficacy of the oils of spearmint and methyl salicylate as new anesthetics and their effect on glucose levels in common carp (Cyprinus carpio L., 1758) juveniles. *Aquaculture*, *437*, 327-332.

Saccol, E. M., Jerez-Cepa, I., Ourique, G. M., Pês, T. S., Gressler, L. T., Mourão, R. H., ... & Martos-Sitcha, J. A. (2018). Myrcia sylvatica essential oil mitigates molecular, biochemical and physiological alterations in Rhamdia quelen under different stress events associated to transport. *Research in veterinary science*, *117*, 150-160.

Saccol, E. M., Toni, C., Pês, T. S., Ourique, G. M., Gressler, L. T., Silva, L. V., ... & Pavanato, M. A. (2017). Anaesthetic and antioxidant effects of Myrcia sylvatica (G. Mey.) DC. and Curcuma longa L. essential oils on tambaqui (Colossoma macropomum). *Aquaculture Research*, *48*(5), 2012-2031.

Sampaio, F. D., & Freire, C. A. (2016). An overview of stress physiology of fish transport: changes in water quality as a function of transport duration. *Fish and Fisheries*, *17*(4), 1055-1072.

Sánchez-Muros, M. J., Sánchez, B., Barroso, F. G., Toniolo, M., Trenzado, C. E., & Rus, A. S. (2017). Effects of rearing conditions on behavioural responses, social kinetics and physiological parameters in gilthead sea bream Sparus aurata. *Applied Animal Behaviour Science*, *197*, 120-128.

Dos Santos, A. C., Junior, G. B., Zago, D. C., Zeppenfeld, C. C., da Silva, D. T., Heinzmann, B. M., ... & da Cunha, M. A. (2017). Anesthesia and anesthetic action mechanism of essential oils of Aloysia triphylla and Cymbopogon flexuosus in silver catfish (Rhamdia quelen). *Veterinary anaesthesia and analgesia*, *44*(1), 106-113.

Sladky, K. K., Swanson, C. R., Stoskopf, M. K., Loomis, M. R., & Lewbart, G. A. (2001). Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (Piaractus brachypomus). *American journal of veterinary research*, *62*(3), 337-342.

Sneddon, L., Wolfenden, D., & Thomson, J. (2016). Stress management and welfare. In: *Fish Physiology: Biology of Stress in Fish*. Cambridge, MA: Academic Press, 463-539.

Souza, C., Baldissera, M., Salbego, J., Lopes, J., Vaucher, R., Mourão, R., Caron, B., Heinzmann, B., Silva, L., & Baldisserotto, B. (2017). Physiological responses of silver catfish to anesthesia withessential oils from two different chemotypes of *Lippia alba*. *Neotropical Ichthyology*, 15, e160083.

Souza, C. D. F., Baldissera, M. D., Baldisserotto, B., Heinzmann, B. M., Martos-Sitcha, J. A., & Mancera, J. M. (2019). Essential oils as stress-reducing agents for fish aquaculture: a review. *Frontiers in physiology*, *10*, 785.

de Freitas Souza, C., Baldissera, M. D., Bianchini, A. E., da Silva, E. G., Mourão, R. H. V., da Silva, L. V. F., ... & 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*(1), 21-34.

Sutili, F. J., Gatlin III, D. M., Heinzmann, B. M., & Baldisserotto, B. (2018). Plant essential oils as fish diet additives: benefits on fish health and stability in feed. *Reviews in Aquaculture*, *10*(3), 716-726.

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

Velíšek, J., & Svobodova, Z. (2004). Anaesthesia of common carp (Cyprinus carpio L.) with 2-phenoxyethanol: acute toxicity and effects on biochemical blood profile. *Acta Veterinaria Brno*, *73*(2), 247-252.

Wagner, G. N., Singer, T. D., & Scott McKinley, R. (2003). The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (Oncorhynchus mykiss Walbaum). *Aquaculture research*, *34*(13), 1139-1146.

Zeppenfeld, C. C., Toni, C., Becker, A. G., dos Santos Miron, D., Parodi, T. V., Heinzmann, B. M., ... & Baldisserotto, B. (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.