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Efficacy of lemongrass essential oil as an anesthetic for common carp (*Cyprinus carpio,* Linnaeus 1758) stocking material

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Abstract

Krasteva, V., Yankova, M. & Ivanova, A. (2023). Efficacy of lemongrass essential oil as an anesthetic for common carp (Cyprinus carpio, Linnaeus 1758) stocking material. *Bulgarian Journal of Animal Husbandry, 60*(5), 43-50.

The purpose of the present study is to examine the efficacy of lemongrass oil (*Cymbopogon schoenanthus*) by establishing the time needed for induction and recovery from anesthesia of common carp (*Cyprinus carpio*, Linnaeus 1758) stocking material. The fish have an average body weight (BW, g) of 14.15±4.68 g and an average total length (TL, cm) of 10.92±4.17 cm.

Ten treatments are conducted with ten experimental concentrations: 0.02 ml/l^{-1}, 0.04 ml/l^{-1}, 0.06 ml/l^{-1}, 0.08 ml/l^{-1}, 0.10 ml/l^{-1}, 0.12 ml/l^{-1}, 0.14 ml/l^{-1}, 0.16 ml/l^{-1}. 0.18 ml/l^{-1} and 0.20 ml/l^{-1}. For each concentration 10 fish (five treatments with two fish) are used or a total of 100 fish for the whole experiment.

At concentration of 0.20 ml.l^{-1} the induction of anesthesia is the fastest (2.14±0.20 min), thus the recovery time at this concentration is the longest (7.06±0.59 min) ($P \leq 0.05$). From all tested concentrations the recovery time is the shortest at concentration 0.04 ml.l^{-1} – 2.61±0.43 min ($P \leq 0.001$). The lowest concentration (0.02 ml.l^{-1}) has no anesthetic effect on carp stocking material.

Based on the results obtained from the current experiment it can be concluded that lemongrass essential oil is effective as anesthetic agent for common carp stocking material, which makes it applicable in the fish farming practice.

Keywords: anesthesia; lemongrass oil; *Cyprinus carpio*; common carp stocking material

Introduction

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., 2017, 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).

Because of the high variability observed among fish species (size, natural habitat, behavior and physiology), the choice of type, dose of and the length of exposure to anesthetic or sedative drugs should be considered with utmost care. Thus, further research is needed to increase the knowledge about the effects of anesthetics on fish used in research, to develop appropriate

anesthetic protocols and specific procedures for each species (Martins et al., 2018).

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).

There are few studies on the anesthetic effect of lemongrass in common carp (*Cyprinus carpio*) so far. Krasteva et al. (2022) compare the anesthetic effect of lemongrass and clove oil on two-year old common carp (*Cyprinus carpio*, Linnaeus, 1758). Research on the sedative and anesthetic effect of lemongrass has been done with species such as: freshwater angelfish, *Pterophyllum scalare* (Oliviera et al., 2022), 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).

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., 2021a); American basil, *O. canum* and holy basil, *O. sanctum* (Khumpirapang et al., 2018); lavender, *Lavandula angustifolia* and thyme, *Thymus vulgaris* (Krasteva et al., 2021a) and rosemary, *Rosmarinus officinalis* (Krasteva et al., 2021b).

The aim of the present study is to determine the anesthetic effect of lemongrass essential oil in common carp stocking material, which will contribute to the improving of the data on the effect of anesthetics in *C. carpio*.

Material and Methods

The study is conducted at the Institute of Fisheries and Aquaculture, Plovdiv in May 2022.

Study object

The object of the experiment was *C. carpio* stocking material hatched by natural propagation in May 2021 and afterwards grown in the ponds in 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 transferred for storage in 3 m^3 tanks. The biometric characteristics of the experimental fish are presented in Table 1.

Essential oil

The lemongrass essential oil (*Cymbopogon schoenanthus*) was commercially purchased with listed ingredients 100% pure lemongrass 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 setup

Due to the lack of published data, and in order to preserve the well-being of the treated fish, the lowest experimental concentration was 0.02 ml/l^{-1}. The applied concentrations were increased gradually in order to carefully observe the behavior and the condition of the fish.

The applied concentrations were ten: 0.02 ml/l^{-1}, 0.04 ml/l^{-1}, 0.06 ml/l^{-1}, 0.08 ml/l^{-1}, 0.10 ml/l^{-1}, 0.12 ml/l^{-1}, 0.14 ml/l^{-1}, 0.16 ml/l^{-1}, 0.18 ml/l^{-1} and 0.20 ml/l^{-1}. For each concentration, 10 fish (five treatments with two fish) are used or a total of 100 fish for the whole experiment. In order to ensure the welfare of the treated specimens, the biometric parameters, body weight (BW, g) and

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

Statistical value	BW (g)	TL (cm)
mean±SD	14.15±4.68	10.92±4.17
min-max	8.12-22.83	4.70-31.12
CV, %	33.07	38.17

body length (TL, cm) were measured after exposure to 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 micro compressors, 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):

Phases of anesthesia

Phase 1. Acceleration 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.

Phases of recovery

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, are analyzed at a confidence level of $P \leq 0.05$. For this purpose a comparative Student T-test (paired two sample for means) was performed using Excel Data analysis.

At 0.02 ml/l^{-1}of lemongrass oil no induction of anesthesia or recovery process was observed and no data was statistically processed.

Results and Discussion

The main hydrochemical parameters, dissolved oxygen (O_2, mg/l^{-1}) and temperature (T°C), measured in the tanks for anesthesia and in the tanks for recovery are presented in Figure 1 and Figure 2.

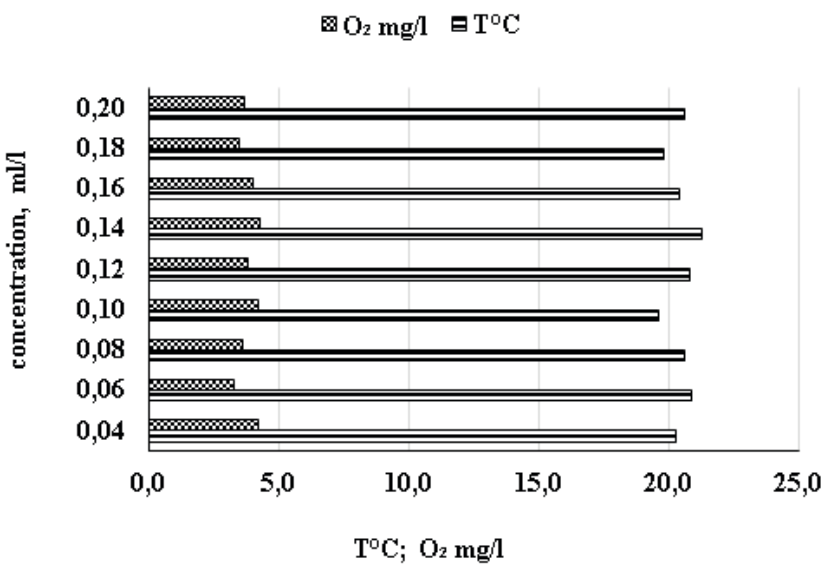

Figure 1. Dissolved oxygen (O2, mg/l-1) and water temperature (T°C) in the tanks for anesthesia

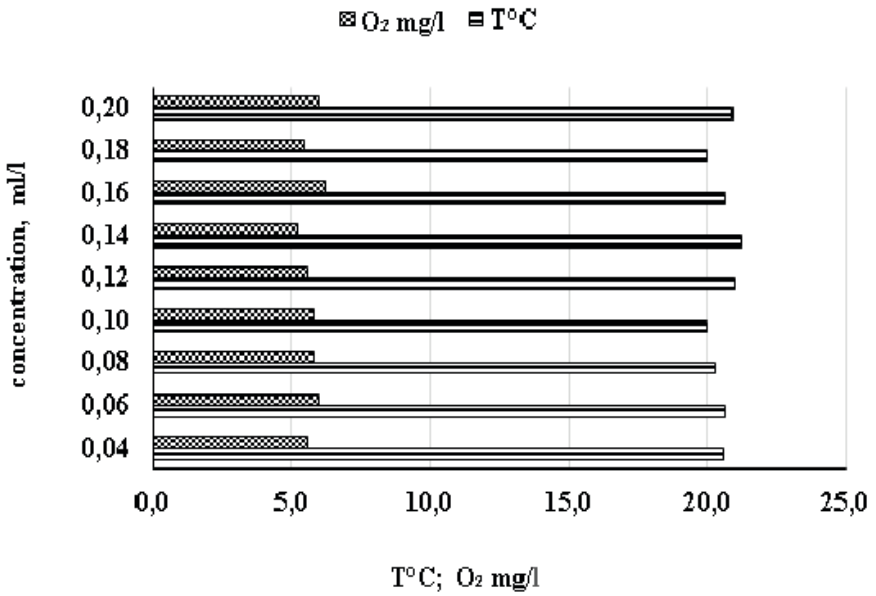

Figure 2. Dissolved oxygen (O2, mg/l-1) and water temperature (T°C) in the tanks for recovery

The measured oxygen in the tank for anesthesia with lemongrass oil varied from 3.3 mg/l^{-1} to 4.4 mg/l^{-1} with mean value of 3.9 mg/l^{-1}. Dissolved oxygen in the recovery tank varied from 5.2 to 6.2 mg/l^{-1} with mean value 5.7 mg/l^{-1}. The mean values of dissolved oxygen are higher in the recovery tank rather than in the anesthesia tank, due to the installed microcompressors for support of the recovery process.

The measured temperature in the anesthetic tank varied from 19.6°C to 21.3°C with mean value of 20.4°C. In the recovery tanks the temperature is in the range of 20.0°C-21.2°C with average value 20.6°C. The difference in the mean values in both experimental sets being only 0.2°C.

During the experiment, the values of the dissolved oxygen and temperature varied within narrow limits and no critical values were registered.

Lemongrass essential oil

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

Duration (min) of the phases of anesthesia and recovery of common carp stocking material (K1), anaesthetized with lemongrass essential oil.

The lowest concentration of 0.02 ml/l^{-1}, for a period of 20 min, has no anesthetic effect on *C. carpio* stocking material. The fish reach only Phase 2 of anesthesia expressed in slow, uncoordinated movements. This behavior is registered until the end of the exposure. A period of recovery is not observed as no fish have been anesthetized.

In contrast to the previous concentration, at 0.04 ml/l^{-1} an anesthetic effect is recorded in all individuals for 14.85±1.97 min. The period of recovery is the shortest at 0.04 ml/l^{-1} compared to all other concentrations ($P \leq 0.05$).

At the concentration of 0.06 ml/l^{-1} anesthesia is reached for an average period of 10.73±2.06 min, which is 4.12 min faster compared to 0.04 ml/l^{-1} ($P \leq 0.05$). The recovery period at 0.06 ml/l^{-1} is 1.20 minutes longer compared to 0.04 ml/l^{-1} ($P \leq 0.05$).

At 0.08 ml/l^{-1}, Phase 4 anesthesia occurs for an average period of 7.83±1.20 min, as the fish go

Table 2. Duration (min) of the phases of anesthesia and recovery of common carp stocking material, anaesthetized with lemongrass essential oil. Data is presented as mean±SD

Phases	Concentration (ml/l^{-1})				
Lemon grass	0.02	0.04	0.06	0.08	0.10
A2	15.63±1.16	7.55±0.95^{ab}	4.97±1.35^{ba}	2.94±0.68^{ceg}	2.11±0.34^{dgi}
A3	-	14.49±2.02^{ab}	10.45±2.07^{ba}	7.50±1.21^{ce}	5.96±0.95a
A4	-	14.85±1.97^{ab}	10.73±2.06^{ba}	7.83±1.20	6.15±0.90^{de}
R1	-	2.08±0.44^{abc}	3.29±0.72^{baf}	3.52±1.05^{ca}	3.77±0.69a
R2	-	2.22±0.42^{abc}	3.40±0.74^{baf}	4.02±1.04^{ca}	3.92±0.70a
R3	-	2.61±0.43^{abcd}	3.80±0.76^{baef}	4.29±1.19^{cae}	4.42±1.06^{dai}
Phases	Concentration (ml/l^{-1})				
Lemon grass	0.12	0.14	0.16	0.18	0.20
A2	2.03±0.29^{ec}	1.86±0.20^{a}	1.81±0.23^{gcd}	1.78±0.19a	1.56±0.34^{id}
A3	5.40±0.62^{ec}	4.25±0.30^{a}	3.06±0.84^{gih}	2.37±0.47^{hgi}	2.00±0.20^{igh}
A4	5.52±0.74^{ed}	4.39±0.31^{a}	3.23±0.78^{gi}	3.00±0.64^{hi}	2.14±0.20^{igh}
R1	3.80±0.94a	5.46±2.16^{fb}	6.17±0.99^{a}	6.43±0.73^{a}	6.35±0.68^{a}
R2	4.00±0.97a	5.60±2.16^{fb}	6.28±0.99^{a}	6.53±0.73^{a}	6.45±0.65^{a}
R3	4.85±1.06^{ebc}	5.96±2.20^{fb}	6.73±1.00^{a}	6.92±0.84^{a}	7.06±0.59^{id}

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

A2 – Phase 2 of anesthesia
A3 – Phase 3 of anesthesia
A4 – Phase 4 of anesthesia
R1 – Phase 1 of recovery
R2 – Phase 2 of recovery
R3 – Phase 3 of recovery

a – 0.04 ml/l^{-1}
b – 0.06 ml/l^{-1}
c – 0.08 ml/l^{-1}
d – 0.10 ml/l^{-1}
e – 0.12 ml/l^{-1}
f – 0.14 ml/l^{-1}
g – 0.16 ml/l^{-1}
h – 0.18 ml/l^{-1}
i – 0.20 ml/l^{-1}

through all the phases of anesthesia. No significant difference in the fish behavior, compared to the previous concentrations, was recorded.

At 0.10 ml/l^{-1}, anesthesia occurs in 6.15±0.90 min, which is 0.63 min slower compared to 0.12 ml/l^{-1} (P≤0.05). The recovery period at 0.10 ml/l^{-1} is longer than at the lower concentrations (P≤0.05).

At 0.12 ml/l^{-1} Phase 4 of anesthesia is reached after 5.52±0.74 min. Recovery period at 0.12 ml/l^{-1} is 0.43 min slower compared to 0.10 ml/l^{-1} (P≤0.05).

At concentration of 0.14 ml/l^{-1}, anesthesia occurs in 4.39±0.31 min. During the experiment the fish reacted with accelerated movements when placed in the anesthetic solution, after which the individuals go through all phases of the anesthesia. The recovery takes an average of 5.96±2.20 min, with Phase 1 occuring after 5 min.

At 0.16 ml/l^{-1}, anesthesia occurs in an average of 3.23±0.78 min, with no negative reaction towards the anesthetic solution being recorded. The recovery process takes 6.73±1.00 min, which is the slowest period compared to all previous concentrations.

At concentration of 0.18 ml/l^{-1} anesthesia occurs 0.86 min slower (3.00±0.64 min induction time) compared to 0.20 ml/l^{-1} – 2.14±0.20 min (P≤0.05).

Lemongrass essential oil had the fastest anesthetic effect at the highest concentration (0.20

ml/l^{-1}) compared to the concentrations 0.16 ml/l^{-1} and 0.18 ml/l^{-1} (P≤0.05).

As in previously conducted experiments, in the current study the time for induction of anesthesia followed a negative concentration-dependent pattern, but the recovery time demonstrated a positive concentration-response relationship. Accordingly, at the highest concentration of 0.20 ml/l^{-1} *C. carpio* stocking material had the longest recovery period of 7.06 min, with significant difference being established when compared to 0.10 ml/l^{-1} (P≤0.05).

When it comes to anesthesia in fish it is difficult to compare the result from different experiments due to the high variability observed among fish species (size, natural habitat, behavior and physiology), the choice of type, dose of and the length of exposure to anesthetic or sedative drugs should be considered with utmost care. Thus, further research is needed to increase the knowledge about the effects of anesthetics on fish used in research, to develop appropriate anesthetic protocols and specific procedures for each species (Martins et al., 2018).

Krasteva et al. (2021a) studied the anesthetic effect of three essential oils: lavender (*Lavandula anqustifolia*), thyme (*Thymus vulgaris*) and basil (*Ocimum basilicum*) on carp stocking material. The authors applied 6 concentrations (0.02 ml/l^{-1}, 0.04 ml/l^{-1}, 0.06 ml/l^{-1}, 0.08 ml/l^{-1}, 0.10 ml/l^{-1} and 0.12 ml/l^{-1}) for each essential oil. at concentration of 0.12 ml/l^{-1} and an exposure of 02.22 min, all individuals enter Phase 4 of anesthesia, with recovery time being the longest of the three oils (14.40 min) The anesthetic effect of basil oil (0.12 ml/l^{-1}) is observed at an exposure of 04.12min with recovery time of 10.32 min. Lavender oil (0.12 ml/l^{-1}) had the slowest anesthetic effect – 07.28 min, but the shortest recovery period – 06.30 min. When comparing the results from the current experiment with those established by Krasteva et al. (2021a) it can be concluded that lemongrass essential oil is more effective anesthetic for *C. carpio* stocking material compared to lavender, thyme and basil essential oils.

Krasteva et al. (2021b) examined the efficacy of rosemary oil as an anesthetic for common carp stocking material with six experimental concentrations: 0.20 ml/l^{-1}, 0.30 ml/l^{-1}, 0.40 ml/l^{-1}, 0.50 ml/l^{-1}, 0.60 ml/l^{-1} and 0.70 ml/l^{-1}. The authors state that at concentration of 0.70 ml/l^{-1} the induction of anesthesia is the fastest (4.24 min), thus the recovery time at this concentration is the longest (4.86 min). From all tested concentrations the recovery time is the shortest at the lowest concentration (0.20 ml/l^{-1}) – 0.95 min (P ≤ 0.05). The results from the current experiment show that lemongrass oil is again more effective anesthetic agent for *C. carpio* stocking material compared to rosemary essential oil.

It is interesting to be noted that when anesthetizing two-year old common carp with lemongrass essential oil, Krasteva et al. (2022) found that the time required for anesthesia 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 lemongrass essential oil as an anesthetic ineffective in commercial or scientific activities with grown *C. carpio* specimens, but the results from the current experiment established that it is good anesthetic for *C. carpio* stocking material.

Conclusions

At concentration of 0.20 ml.l^{-1} the induction of anesthesia is the fastest (2.14±0.20 min), thus the recovery time at this concentration is the longest (7.06±0.59 min) (P≤0.05).

From all tested concentrations, the recovery time is the shortest at concentration 0.04 ml.l^{-1} – 2.61±0.43 min (P≤0.001). The lowest concentration (0.02 ml.l^{-1}) has no anesthetic effect on carp stocking material.

Based on the results obtained from the current experiment, it can be concluded that lemongrass essential oil is effective anesthetic agent for *C. carpio* stocking material and it can be applied in different fish farming activities.

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