Anesthetic effect of rosemary essential oil (*Rosmarinus officinalis* L.) and its use for transport of common carp (*Cyprinus carpio* L.) stocking material

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Abstract

The aim of the present study is to examine the efficacy of rosemary oil as an anesthetic for common carp (*Cyprinus carpio* L.) and as an additional agent for transport of carp stocking material. The fish used in the research have an average body weight (BW, g) of 12.72 ± 5.87 and an average total length (TL, cm) of 10.19 ± 1.43. For the experiment of the anesthetic effect of *R. officinalis*, six treatments are conducted with six experimental concentrations: 0.20 ml/l, 0.30 ml/l, 0.40 ml/l, 0.50 ml/l, 0.60 ml/l and 0.70 ml/l. For each concentration 10 individuals are used or a total of 60 fish. For the transport experiment, 3 concentrations are used: 0.02 ml/l, 0.04 ml/l and 0.06 ml/l. The applied stocking density is 8 fish/l. A total of 80 fish are used for each concentration or a total of 240 fish.

Based on the results it can be concluded that at concentration of 0.70 ml/l 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) – 0.95 min ($P \leq 0.05$).

The behavior of the fish and the phases in which they enter (Phase 0 and Phase 2) change every hour in all experimental variants of the transport experiment. All three experimental concentrations (0.02 ml/l, 0.04 ml/l and 0.06 ml/l) of rosemary oil are applicable for the transport of carp stocking material from 1 to 4 hours.

Key words: *Cyprinus carpio,* common carp, *Rosmarinus officinalis*, rosemary oil, transport, anesthesia

Анестезиращ ефект на етерично масло от розмарин (*Rosmarinus officinalis* L.) и приложението му при транспорт на зарибителен материал от обикновен шаран (*Cyprinus carpio* L.)

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Резюме

Целта на настоящото проучване е да се изследва ефикасността на маслото от розмарин като анестетик при обикновен шаран (*Cyprinus carpio* L.) и като добавъчно вещество при транспортиране на зарибителен материал от шаран. Рибите, използвани в изследването, имат средно телесно тегло (BW, g) 12,72 ± 5,87 и средна голяма дължина (TL, cm) 10,19 ± 1,43. При експеримента, изследващ анестетичния ефект на *R. Officinalis,* са проведени шест третирания с шест експериментални концентрации: 0,20 ml/l, 0,30 ml/l, 0,40 ml/l, 0,50 ml/l, 0,60 ml/l и 0,70 ml/l. За всяка концентрация се използват 10 индивида или общо 60 риби. При експеримента за транспорт са приложени 3 концентрации: 0,02 ml/l, 0,04 ml/l и 0,06 ml/l с гъстота на посадката 8 риби/л. За всяка концентрация са използвани общо 80 риби или общо 240 риби.

На база на получените резултатите може да се заключи, че при концентрацията от 0,70 ml/l настъпването на анестезия е най-бързо (4,24 min), като съответно времето за възстановяване при тази концентрация е най-дълго (4,86 min). От всички тествани концентрации времето за възстановяване е най-кратко при най-ниската концентрация (0,20 ml/l) – 0,95 минути ($P \leq 0,001$).

Поведението на рибите и фазите, в които встъпват (Фаза 0 и Фаза 2) се променят всеки час и в трите експериментални варианти при експеримента за транспорт. На базата на получените резултати от експеримента може да се твърди, че и трите концентрации (0,02 ml/l, 0,04 ml/l и 0,06 ml/l) на маслото от розмарин са напълно приложими за транспорт на зарибител шаран от 1 до 4 часа.

Ключови думи: *Cyprinus carpio,* обикновен шаран, *Rosmarinus officinalis*, масло от розмарин, транспорт, анестезия

Introduction

The use of fish anesthesia provides fish welfare, prevents physical injuries of the individuals and facilitates fishfarming and research activities (Zahl et al., 2012; Benovit et al., 2015; Aydın and Barbas, 2020). Natural anesthetics are more environmental friendly, cost-effective and safer than synthetic drugs for the management of aquatic organisms (Aydın and Barbas, 2020). A good anesthetic should induce rapid anesthesia and result in quick recovery, and minimize the effects of stress on fish. Moreover, it should be widely available, cost-effective, and have low or no toxicity (Aydın and Barbas, 2020).

Anesthetics should not build up in fish tissues and organs and pose problems for human or animal consumption, thus the excretion of the anesthetics from the fish body should be fast (Mylonas et al., 2005; Javahery et al., 2012; Azad et al., 2014; Roohi and Imanpoor, 2015; Aydın and Barbas, 2020).

Clove oil is the most commonly used plant-based anesthetic in aquaculture and several other studies have been conducted on the use of essential oils of basil, thyme, mint, rosemary, lavender, citronella, verbena and camphor for different fish species. In recent years, active compounds of different essential oils, such as eugenol, menthol, myrcene, 1.8-cineole, linalool, limonene, citronellal, thymol, carvacrol, spathulenol, α- and β-pinene, 4-allylphenyl acetate and globulol, have also been investigated for sedation and anesthesia purposes in fish (Aydın and Barbas, 2020).

Common rosemary (*Rosmarinus officinalis* L.) is a perennial herbaceous plant with evergreen coniferous leaves, belonging to family *Lamiaceae*. The plant is native to the Mediterranean region, but it is currently found in all parts of the world (European Medicines Agency, 2010; Gonzales-Minero, 2020). The main active ingredients of rosemary essential oil are alpha-pinene and eucalyptol (1.8-cineole) (Bauer et al., 1997).

Up to date the anesthetic and sedative effect of rosemary essential oil has not been well studied. Only one experiment has been conducted with rosemary essential oil for anesthesia of *C. carpio* (mean weight = 652 g) with concentrations from 0.25 to 1 ml/l which resulted in effective sedation (< 3 min) and recovery (< 10 min) (Ghazilou and Chenary, 2011).

Other essential oils that have been studied for anesthesia of carp include clove oil, *Syzygium aromaticum*, with most effective concentration of 0.05 ml/l (Husen and Sharma, 2015); tea tree oil, *Malaleuca alternifolia*, with effective concentration of 0.4–0.6 ml/l (Hajek, 2011); spearmint, *Mentha spicata* – 5 ml/l (Roohi and Imanpoor, 2015); basil, *Ocinum basilicum* – 0.3 ml/l; American basil, *O. canum* – 0.2 ml/l and tulsi, *O. sanctum* – 0.1 ml/l (Khumpirapang et al., 2018).

Very few experiments have been conducted regarding the application of essential oils or their active substances for transport of live fish, with no studies for rosemary essential oil. Transported fish can die from shock caused by stress during transport (Bulgarian Food Safety Agency, 2011). In this regard, the addition of an anesthetic with a certain concentration in the transport tank could relieve stress and prevent mortality. According to Mirghaed et al. (2016) results, 0.05 ml/l myrcene and 0.05 and 0.1 ml/l linalool are suitable to keep *C. carpio* at stage 2 of anesthesia, i.e, at a sedation state, for at least 2 h during transportation. On the other hand, Mazandarani et al. (2017) reported that because of increased stress, linalool (0.05–0.2 ml/l) is not suitable for *C. carpio* transportation in plastic bags.

The aim of the present study is to investigate the anesthetic effect of rosemary essential oil on stocking material of common carp and to establish its effective use in fish farming practice and research activities. The application of rosemary essential oils as sedative agent for transport of live fish has also been investigated in the current research.

Material and methods

The study is conducted at the Institute of Fisheries and Aquaculture, Plovdiv in April 2021.

Subject of research

The subject of the experiment is *C. carpio* stocking material hatched by natural propagation in May 2020 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 are 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

Statistical value	BW (g)	TL (cm)
Mean ± SD	12.72 ± 5.87	10.19 ± 1.43
Min–max	5.72–29.73	7.84–13.54
CV, %	46.15	14.08

Essential oil

The *R. officinalis* essential oil is purchased commercially with listed ingredients 100% pure rosemary oil, produced in Plovdiv, Bulgaria by "Rivana" LTD. The experimental solutions are prepared by diluting the oil in ethyl alcohol (95%) in 1:9 ratio and added to 10 l experimental tanks with vigorous stirring before treatment.

Preliminary test

Due to the lack of sufficient data, a preliminary test is performed to study different concentrations of rosemary oil. Treatment of 5 fish, with double repetition, is used since the size of the fish allows observations of more than one specimen.

In order to preserve the well-being of the treated fish and to prevent mortality, the lowest experimental concentration is 0.02 ml/l. The preliminary test is performed with 7 experimental concentrations: 0.02 ml/l, 0.04 ml/l, 0.06 ml/l, 0.08 ml/l, 0.10 ml/l, 0.12 ml/l, and 0.14 ml/l, with an exposure of 20 min so that the effect of the oil can be monitored for a longer period of time.

Experiment of the anesthetic effect of R. officinalis

Based on the preliminary test, 6 experimental concentrations are used in the study of the anesthetic effect of rosemary oil: 0.20 ml/l, 0.30 ml/l, 0.40 ml/l, 0.50 ml/l, 0.60 ml/l and 0.70 ml/l. For each concentration, 10 fish (2 fish with five repetitions) are used or a total of 60 fish for the experiment. In order to ensure the welfare of the treated specimens, the biometric parameters, body weight (BW, g) and body length (TL, cm), are measured after treatment with the anesthetic solution (Table 1).

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

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

Phases of anesthesia

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.

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.

Transport experiment

The experiment for transport is carried out in laboratory conditions according to the ”Instruction for the application of the requirements for the transport of live fish” (BFSA, 2011). Prior to the beginning of the experiment, the fish are not fed for 24 hours and an assessment of their health is made. The experiment is conducted with three variants of concentrations of rosemary oil, in 10 l tanks, based on the preliminary test: Variant 1 – 0.02 ml/l, Variant 2 – 0.04 ml/l and Variant 3 – 0.06 ml/l. The applied density is 8 fish/l calculated relative to the average weight of the fish and a standard of 100 kg/m^3 density without being further increased (Table 2). For each variant 80 fish are used or a total of 240 fish.

Microcompressors are placed in the water 1 hour before the introduction of the fish in the experimental variants. Hydrochemical measurements (T °C and O_2 mg/l) are performed every hour during the experiment.

The behavior of the fish in the three variants is observed every hour according to the table of Hamackova et al. (2006). The fish in each phase are counted and their percentage is calculated according to their total number for each variant. The following formula is used: Phase, % = (num-

Table 2. Requirements for density and duration of transport of live fish

Fish species	Density	Duration of transport, hours
Herbivorous fish		
Stocking material	70 – 130 kg/m^3 depending on the weight	from 3 to 6
The amount of fish with an average body weight below 100 g can be increased by 60–80%		

Source: Instruction for application of the requirements for transport of live fish (BFSA, 2011)

ber of fish in the phase *100) / total number of fish.

At the end of the experiment, the water from the tanks is drained slowly so that the fish do not stay dry. Immediately after that, clean water of the same volume is added. After 3 to 5 minutes the fish are released into their natural environment.

Statistical analysis

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) is performed using Excel - Data analysis.

Results and discussion

Preliminary test

At all studied concentrations in the preliminary test (0.02 ml/l, 0.04 ml/l, 0.06 ml/l, 0.08 ml/l, 0.10 ml/l, 0.12 ml/l, and 0.14 ml/l) the fish reach only Phase 2 of the anesthesia (Fig. 1).

In Phase 2 the fish remain static, do not move or perform very slow, uncoordinated movements. The fish enter Phase 2 in 5.95 min at the highest concentration of 0.14 ml/l and in 15.85 min at 0.02 ml/l.

A recovery process is not observed at any of the experimental concentrations in the preliminary test as the fish did not reach phase of anesthesia.

Experiment of the anesthetic effect of *R. officinalis*

The results of the anesthesia with the experimental concentrations (0.20 ml/l, 0.30 ml/l, 0.40 ml/l, 0.50 ml/l, 0.60 ml/l and 0.70 ml/l) are presented in Table 3. Phase 4 of anesthesia, at concentrations of 0.20 ml/l and 0.30 ml/l, is not included in the statistical analysis, because in the first case the fish do not enter anesthesia phase, and in the second – 80% of the fish reach Phase 4. Each phase of the corresponding concentration is compared with the same phase of all other concentrations in a horizontal order, with a significant difference $P \leq 0.05$ between the mean values, indicated with different superscripts.

At 0.20 ml/l, after 18.95 min the fish reach only Phase 3 of anesthesia with short recovery process being observed (0.95 min). At concentration of 0.30 ml/l, the fish react to the solution with rapid movements, which gradually subside. 80% of the treated fish reach Phase 4 of anesthe-

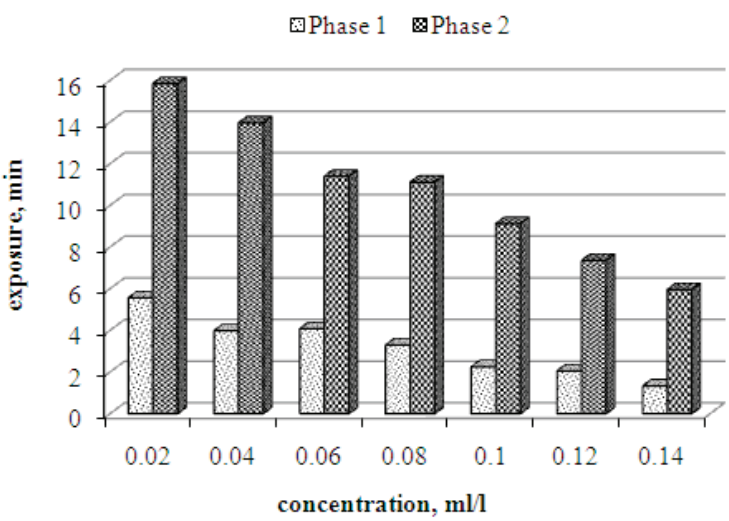

Fig. 1. Time to reach Phase 1 and Phase 2 at different experimental concentrations from the preliminary test

Table 3. Duration (min) of the phases of anesthesia and recovery of common carp stocking material (K1)

Phases	Concentration (ml/l)					
	0.20	0.30	0.40	0.50	0.60	0.70
A2	4.89 ± 0.36	4.90 ± 0.53	3.44 ± 0.35	2.54 ± 0.32^{def}	2.24 ± 0.27^{ed}	1.98 ± 0.33^{fd}
A3	18.95 ± 0.63	10.99 ± 2.63^{bcd}	13.69 ± 2.07^{cb}	5.80 ± 0.54^{dbe}	5.34 ± 0.53^{ed}	3.59 ± 0.63
A4	-	16.71 ± 2.47	13.98 ± 1.98	6.13 ± 0.50^{de}	5.53 ± 0.55^{ed}	4.24 ± 0.59
R1	0.53 ± 0.13	1.69 ± 0.17^{bcde}	2.13 ± 0.30^{cbf}	2.25 ± 0.82^{db}	2.08 ± 0.52^{ebf}	2.57 ± 0.29^{fce}
R2	0.61 ± 0.13	2.50 ± 0.58^{bf}	2.26 ± 0.28^{cf}	2.45 ± 0.83	2.22 ± 0.52^{ef}	2.80 ± 0.35^{fbce}
R3	0.95 ± 0.14^{ab}	2.69 ± 0.51^{bade}	2.70 ± 0.30^{cdef}	3.54 ± 0.57^{dbcef}	4.16 ± 1.13^{ebcdf}	4.86 ± 1.17^{fcde}

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 – 0.20 ml/l
b – 0.30 ml/l
c – 0.40 ml/l
d – 0.50 ml/l
e – 0.60 ml/l
f – 0.70 ml/l

sia and 20% remain in Phase 3. The experimental individuals are sedated after 16.71 min and recover from anesthesia for 2.69 min. At 0.40 ml/l the induction of anesthesia takes 13.98 min with a recovery period of 2.70 min. At the concentrations of 0.50 ml/l and 0.60 ml/l, the fish enter Phase 4 of anesthesia for 6.13 min and 5.53 min ($P \leq 0.05$), with a recovery period of 3.54 min and 4.16 min, respectively ($P \leq 0.05$). At the highest concentration of 0.70 ml/l the induction of anesthesia required 4.24 min and the fish are fully recovered after 4.86 min with significant difference being established when compared with the recovery period of the concentrations 0.60 ml/l ($P \leq 0.05$), 0.50 ml/l ($P \leq 0.05$) and 0.40 ml/l ($P \leq 0.05$).

Ghazilou and Chenary (2011) investigated the anesthetic effect of rosemary essential oil in *C. carpio*, with an average weight of 625 g. The authors established that at concentrations from 0.25 to 1.00 ml/l, Phase 4 of complete anesthesia is achieved within 3 min, with an increase in concentration resulted in a significant shortening of anesthesia induction time.

In the present study, Phase 4 of anesthesia is achieved in 4.24 min at the highest concentration of 0.70 ml/l, while in the study of Ghazilou and Chenary (2011) the induction of anesthesia takes 28 sec at 1 ml/l. At 0.50 ml/l, the authors achieved anesthesia in 40 sec, while in the current study, at the same concentration of rosemary oil, anesthesia occurred after 6.13 min. The reason for the different effect of rosemary oil may be due to range of factors, such as different stage of individual development, different methods of preparation of the anesthetic solution, etc.

Husen and Sharma (2015) evaluated the anaesthetic efficacy of MS-222, Benzoak vet, AQUI-S and clove oil on common carp fry with mean body weight of 0.53 ± 0.14 g and mean length of 3.49 ± 0.32 cm. The authors stated that all four anaesthetics could be used in aquaculture purposes for handling and transportation of common carp fry. The lowest effective doses were 0.15 ml/l of MS-222, 0.05ml/l of AQUI-S, 0.075 ml/l of benzoak vet and 0.05 ml/l of clove oil.

Hajek (2011) investigated the efficacy of tea tree oil as an anaesthetic for common carp. All applied concentrations (from 0.2 to 0.6 ml/l) resulted in sedation and immobilization. The lowest effective concentration (induction time ≤ 3 min, recovery time ≤ 10 min after 15 min of exposure) was 0.5 ml/l. Exposure in excess of 30 min at a concentration of 0.5 ml/l caused mortality.

Roohi and Imanpoor (2015) evaluated the effects of two anesthetics of spearmint oil and methyl salicylate oil on *Cyprinus carpio* (16.59 ± 0.43 g). Fish were exposed to different concentrations of the spearmint oil (3, 5 and 7 ml/l)

and the methyl salicylate oil (1, 2 and 3 ml/l) for induction of anesthesia. Results showed that induction time decreased significantly with increasing of the concentration of the experimental oils. However, recovery time increased significantly with increasing of the concentration of anesthetics. Opercular rate first increased and then slowly decreased with increasing the concentration of anesthetics. These findings suggested that spearmint oil and methyl salicylate oil are useful anesthetics for common carp juveniles.

Khumpirapang et al. (2018) investigate the anesthetic and cytotoxic effects of essential oils of *Ocimum basilicum*, *O. canum*, and *O. sanctum* on koi carp. *O. sanctum* at concentrations of 0.1, 0.2, and 0.3 ml/l had induction time of 169.5 ± 10.2, 62.8 ± 2.3, 45.3 ± 2.2 sec, respectively, significantly shorter than *O. canum*, and *O. basilicum*. *O. canum* showed the longest recovery time of 313.0 ± 8.1, 420.7 ± 12.6, 616.6 ± 12.1 sec for concentrations of 0.1, 0.2, and 0.3 ml/l, respectively, followed by *O. sanctum* and *O. basilicum*.

The main hydrochemical parameters are presented in Fig. 2.

The temperature in the tank for anesthesia is higher than the temperature in the tank for recovery and the minimum and maximum values vary within narrow limits. In contrast to temperature, the oxygen values are lower in the tank for anesthesia than in the tank for recovery. The reason for this are the installed microcompressors which support the recovery process.

Transport experiment

During the transport experiment, the behavior of the fish varied between normal motor activity and Phase 2 of the anesthesia, expressed in decreased locomotor activity and slow uncoordinated movements. Upon external stimuli the fish immediately regain their normal body position (Table 4).

The results show that as the transport time increases, the number of fish in a state of normal motor activity decreases in relation to the number of fish that have entered Phase 2 of uncoordinated movements which increases. This trend is observed in all experimental variants.

Based on the conducted experiment, it can be recommended when transporting stocking material of common carp for 1 hour to apply concentration of 0.06 ml/l, with highest percentage of fish with decreased locomotor activity. The concentrations of 0.04 ml/l and 0.06 ml/l can be used for transport of bighead carp from 1 to 2 hours, with concentration of 0.04 ml/l being applied for transport from 2 to 3 hours. The highest percentage of fish is in Phase 2, without any fish entering Phase 3 of loss of equilibrium. At concentration of 0.02 ml/l, the percentage of fish in a state of normal motor activity remains the highest in all three variants.

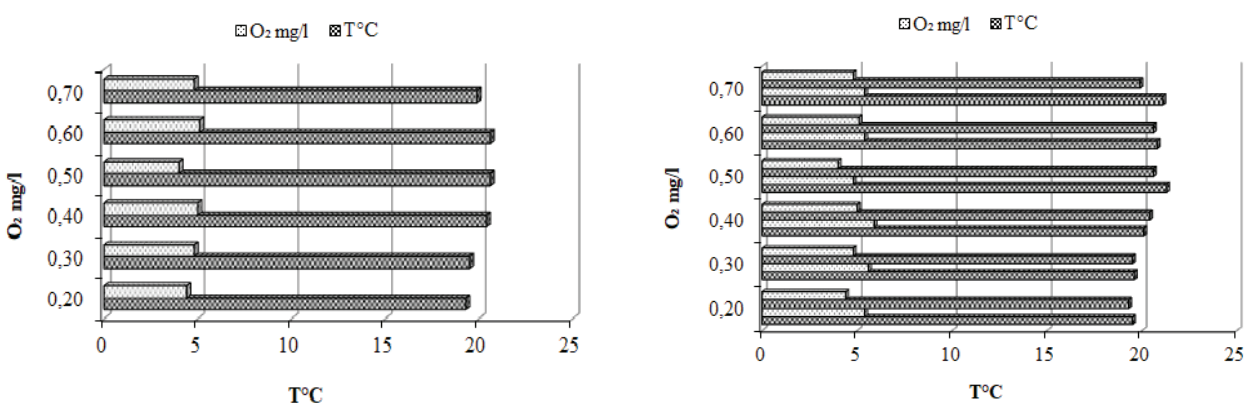

Fig. 2. Water temperature (T °C) (A) and dissolved oxygen (O_2, mg/l) (B) in the tanks for anesthesia and recovery

Table 4. Results of the experiment for transport of bighead carp stocking material with rosemary oil in three experimental variants

hour	Variant 1 (0.02 ml/l)		Variant 2 (0.04 ml/l)		Variant 3 (0.06 ml/l)	
	Phase 0	Phase 2	Phase 0	Phase 2	Phase 0	Phase 2
1	40%	60%	35%	65%	25%	75%
2	35%	65%	25%	75%	15%	85%
3	20%	80%	15%	85%	10%	90%
4	25%	75%	30%	70%	13%	87%

**The number of fish in the different conditions and phases of anesthesia is represented by a percentage*
Phase 0 – normal locomotor activity
Phase 2 – uncoordinated movements (static position)

Mirghaed et al. (2016) established that myrcene at 0.05 ml/l and linalool at 0.05 ml/l and 0.1 ml/l were able to keep the fish at stage 2 anesthesia for 2 h and suggested that these substances can be used in transport of *C. carpio*, although not as efficacious as eugenol.

Mazandarani et al. (2017) investigated the potential of linalool as an anaesthetic during transportation of common carp. The fish were transported at a loading density of 103 g/l for 3 h in 12 plastic bags (3 L water and 6 L pure oxygen) divided into four triplicated treatments: control (without linalool), L 50 (0.05 ml/l), L 100 (0.1 ml/l) and L 200 (0.2 ml/l). After 3 h transportation, serum physiological responses and water physico-chemical parameters were compared among the treatments. The results showed that linalool is not beneficial for carp transportation in plastic bags, because it reacts with water oxygen, increases stress in fish, interferes with ammonia excretion and has no benefits in preventing ion loss.

Similar experiment is performed by Zhao et al. (2017) with grass carp and the synthetic substance eugenol at concentrations of 0.005 ml/l, 0.01 ml/l, 0.015 ml/l, 0.02 ml/l, 0.03 ml/l and 0.04 ml/l and exposure for 8 hours, with the aim being to determine the minimumeugenol concentration that keeps the fish in deep sedation without causing mortality and stress. The results obtained show that the optimal concentration is 0.010 ml/l, which induced rapid anesthesia with minimum stress within 10 min and maintained deep sedation for 8 hours. The fish then return to normal activity in a few minutes with zero mortality. At concentration of 0.040 ml/l eugenol the authors registered 100% mortality.

In the present study, the higher experimental concentrations of rosemary oil (0.04 ml/l and 0.06 ml/l) are the most effective for transport of common carp up to 3 hours, while the established effective concentration of other agents for the same fish species are 0.05 ml/l myrcene, 0.05 ml/l and 0.1 ml/l linalool (Mirghaed et al., 2016), and 0.015 ml/l and 0.020 ml/l MS-222, 0.0025 ml/l and 0.005 ml/l AQUI-S, 0.015 ml/l Benzoak vet, and 0.005 ml/l and 0.0075 ml/l of clove oil (Husen and Sharma, 2015).

The hydrochemical measurements are presented in Fig.3 and Fig.4.

The measured temperature varies from 12.3 °C to 12.8 °C. According to the official instructions of Bulgarian Food Safety Agency (BFSA), temperature should not exceed 21 °C. The amount of dissolved oxygen is in the range of 6.9–7.4 mg/l which is within the permissible values according to BFSA.

Conclusion

Based on the results it can be concluded that concentrations from 0.20 ml/l of rosemary essential oil, within an exposure of 20 min, have no anesthetic effect on common carp stocking material. At 0.30 ml/l, 80% of the fish reach

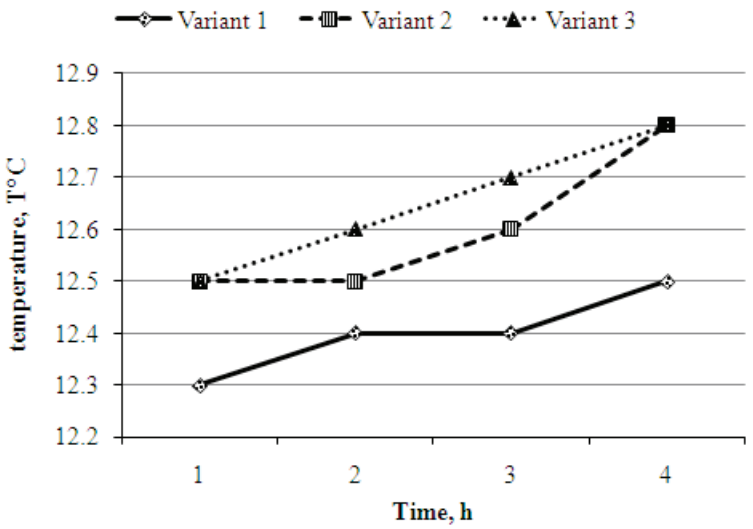

Fig. 3. Measured temperature per hour in the three experimental variants

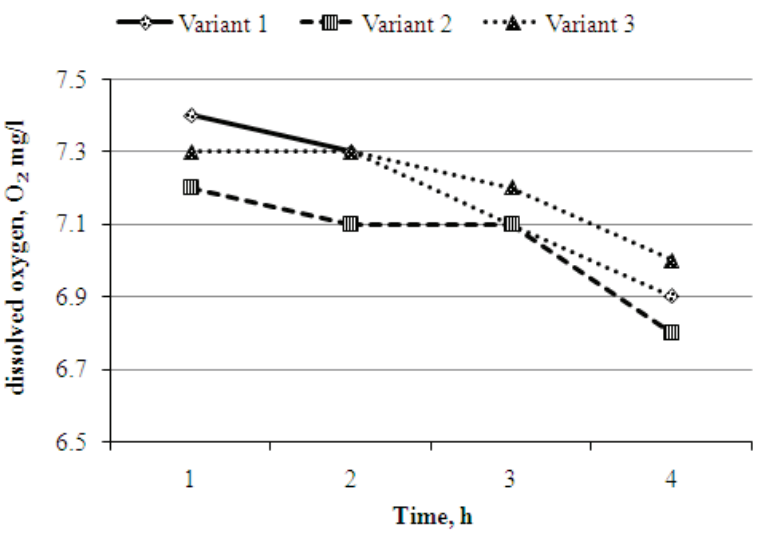

Fig. 4. Amount of dissolved oxygen per hour in the three experimental variants

Phase 4 of anesthesia and 20% remain in Phase 3. As the concentration increases, the time required for anesthesia decreases, with the fastest induction time of anesthesia and slowest period of recovery being observed at the highest concentrations of 0.50 ml/l, 0.60 ml/l and 0.70 ml/l. It can be concluded that rosemary essential oil has weak anesthetic effect in *C. carpio* stocking material.

For transport of common carp stocking material for 1 hour, the optimal concentration of rosemary oil is 0.06 ml/l, at which the highest percentage of fish is in a state of decreased locomotor activity. For 1 to 2 hour transport, the con-

centrations 0.04 ml/l and 0.06 ml/l can be used. For transport from 2 to 3 hours, the most optimal concentration is 0.06 ml/l, at which the highest percentage of fish remain in Phase 2. During the transport experiment no fish enter Phase 3 of loss of equilibrium.

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