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Anesthetic and sedative efficacy of peppermint (*Mentha piperita*) and lavender (*Lavandula angustifolia*) essential oils in blue dolphin cichlid (*Cyrtocara moorii*)

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Abstract: The use of different essential oils (Eo_s), which are considered as a good alternative to synthetic materials, is increasing day by day in fish species. Nevertheless, studies on the anesthetic effect of peppermint Eo (*Mentha piperita*; MPEo) and lavender Eo (*Lavandula angustifolia*; LAEo) are scarce. In the present study, the effective concentrations of three anesthetic materials (MPEo, LAEo, and MS-222) were previously determined in the concentrations range of 100–400 ppm in *Cyrtocara moorii* juveniles (4.92 ± 1.03 g and 7.13 ± 1.22 cm) with the criteria of appropriate induction time (AI_t) and ready recovery time (RR_t) (Trial 1). Afterwards, four different groups were established at three different sedative concentrations (10, 20, and 30 ppm and a control group without anesthetic additive) for transporting (Trial 2). The study concluded that MPEo and LAEo can be used as anesthetic and sedative agents in tropical fish species safely. The use of 100 µL L^{-1} (183.5 s AI_t and 227.8 s RR_t) and 300 µL L^{-1} (109.2 s AI_t and 420.0 s RR_t) MPEo and LAEo, respectively, was sufficient for deep anesthesia, whereas concentrations in the range of 10 to 30 µL L^{-1} are recommended for fish transport.

Key words: Anesthesia, MS-222, lavender oil, mint oil, fish transport, *Cyrtocara moorii*

1. Introduction

The value of the ornamental fish trade has grown significantly over the past decades (1) and the annual worth of the world's widespread trade in tropical fish was reported at one billion USD in 2001 (2). The free-on-board export value was determined at 264,000,000 USD in 2005 and global exports of ornamental fish increased to 372 million USD in 2011. Most of the market supplies originate from Asia. Singapore as the major exporting country in the world exported about 56 million USD worth of ornamental fish to over 80 countries in 2013 and the trade in the world was growing at about an annual rate of 20% (3) to date with an impressive increase.

Cyrtocara moorii is a species of cichlid endemic to Lake Malawi in East Africa and it can grow to a total length of 20 cm. This species is popular among aquarists, where it is known as blue dolphin, Malawi dolphin, hump-head cichlid, or simply moorii (4). Anesthetics or sedatives have been used for many purposes, such as sedation and keeping fish in a stable form, and for comfortable handling, catching, transport, measurement, etc. (5–9). The transportation of fish is particularly common in the ornamental fish sector and involves the use of plastic or nylon bags. These systems have some limitations, such as a restricted oxygen supply and the build-up of ammonia and carbon dioxide levels (10,11). Aquaculture producers generally add pure oxygen to the nylon or plastic containers prior to fish transportation, which can cause variations in the dissolved oxygen levels. Use of sedatives reduces the metabolic activity and provokes blood flow rearrangement and efficient techniques of energy output (12).

The use of different essential oils (Eo_s), which are considered as a good alternative to synthetic anesthetics, is increasing day by day at present and studies on the identification of new herbal anesthetics are continuing at a rapid pace as a result of some regulations regarding animal welfare. Although anesthetic effects of Eo_s originating from plants, such as *Lippia alba* (13), *Ocimum gratissimum* (14), *Aloysia triphylla* (15), geranium (16), and camphor laurel (17), have been reported, excluding the commonly used herbal anesthetic clove Eo (18), the study of the anesthetic effects of peppermint Eo (*Mentha piperita*; MPEo) and lavender Eo (*Lavandula angustifolia*; LAEo) is scarce. Only one study was encountered about the anesthetic efficiency of mint Eo on aquarium fish species (19) until now; however, it was related to the anesthesia effect of *Mentha arvensis* on tropical fish. Only one study exists in aquaculture and it involves a cold-water fish species (rainbow trout, *Oncorhynchus mykiss*) (20). This is the first study in fish transportation with LAEo in aquaculture as

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well. In this study, MPEo and LAEo were assessed as new potential fish anesthetics for the ornamental fish sector.

2. Materials and methods

2.1. Fish and anesthetic agents

Blue dolphin (*Cyrtocara moorii*, 4.92 ± 1.03 g and 7.13 ± 1.22 cm) was obtained from aquarium fish producers and stocked in a 160-L glass aquarium with an inner filter. Fish were fed ad libitum twice daily with granular commercial feed containing 42.5% crude protein, 10.3% crude fat, 2.8% fiber, 5% moisture, and 4.9% crude ash (Sera Granured, Germany). pH, water temperature, and oxygen reduction potential were measured as 7.69–7.91, 27.20–28.39 °C, and 48.5–71.77 during holding, whereas oxygen concentration was between 6.23 and 6.47 mg L^{-1}. Total ammonia-N was measured every 3 days and never exceeded 0.2 mg L^{-1}. Total dissolved solids ranged between 250.1 and 270.3 mg L^{-1} while salinity was between 0.18% and 0.22%. Fish were fasted for 24 h prior to the trials (21).

MS-222 was used as a reference anesthetic agent in the concentration range of 100–400 mg L^{-1}. MS-222 (Sigma) and essential oils (Sigma, natural mint and lavender oil) were maintained according to CAS numbers. The concentrations were determined after a pretreatment trial. EO was diluted in 95% ethanol (1:10) to provide better dissolution in water during the process. To investigate the effect of ethanol exposure, ten fish were transferred to a tank including 10 ml L^{-1} ethanol and observed for 8 min, as adapted from Mylonas et al. (22). Eo_s are poorly soluble in water; therefore, predilution in ethanol was compulsory before using them for anesthetic baths. The solutions of Eo_s were prepared by diluting the oil in commercial alcohol (95%, Sigma) at a ratio of 1:10 at the required concentrations as in the methodology of Can et al. (16). Features of the anesthetic materials are available in the Table.

The Ethical Committee of Animal Experiments granted approval for the study (Decision No: 140, Protocol No: 2017-63).

2.2. Experimental design

2.2.1. Trial 1: Appropriate induction time and ready recovery time

In the present study, the effective concentrations of three anesthetic materials were previously determined in the range of 100–400 ppm (mg L^{-1} for MS-222 and µL L^{-1} for Eo_s) in *Cyrtocara moorii* juveniles with the criteria of appropriate induction time (AI_t) and ready recovery time (RR_t). In this trial, 10 fish for each concentration were separately anesthetized and recovered. The recovery tank was renewed with conditioned water in all experiments. After the recovery process, the fish were taken into the stock tank and kept under observation for 72 h. Each fish was evaluated as a repeat (10 replicates in total). A total of 40 fish were used in this trial. In order to register the cumulative time required to reach the different stages of induction and recovery from anesthesia, a digital chronometer was used.

Determination of anesthesia (A) and recovery (R) stages was modified from the previous literature (22,23) as follows: Induction A1 (partial loss of equilibrium), unresponsive to strong external stimulation, irregular swimming is seen, gill movements are accelerated; Induction A2 (deep anesthesia), opercular movements irregular and slow, no reflex; Recovery R1 (total recovery of equilibrium), starting of erratic swimming, total recovery of equilibrium; Recovery R2 (total behavioral recovery), starting of normal swimming behavior.

2.2.2. Trial 2: Fish transport

The anesthetic concentrations used in our study were about 10-fold lower than those causing deep anesthesia in *C. moorii* within AI_t and RR_t as identified in Trial 1 according to Becker et al. (11) and Cunha et al. (13). Thus, four different groups were established at three different sedative doses (10, 20, and 30 ppm and a control group without anesthetic additive) for transporting trials. The fish were put into nylon bags (each was filled with air at a 1:3 ratio of air to water and secured to be airtight) after adding the anesthetics and then they were transported for 6, 12, and 18 h while monitoring water quality criteria. The mortality and feeding behavior were then observed for 72 h after exposure to the anesthetics according to Carneiro et al. (10).

Nylon bags were placed in a 160-L tank prepared by adjusting it to 28 °C with a heater of 100 W in advance. In the tank, an air stone was used connected with air motors at different points of the tank in order to provide water movement. In this way it was ensured that the air in the nylon bags was mixed with the water so continuous movement was maintained, as well. In this trial, 15 fish (5 fish/bag and 3 replicates) were used for each concentration and a total of 160 fish were used.

Water parameters were measured before and after transportation. Dissolved oxygen (DO), pH, salinity, conductivity, total dissolved solids (TDS), oxygen reduction potential (ORP), and temperature were measured with a YSI Professional Plus oxygen meter (YSI Inc., USA). Unionized ammonia (NH_3) levels and nitrite (NO_2) and nitrate (NO_3) levels were measured by a Hanna photometer (DR900, Germany). Carbon dioxide (CO_2) was calculated by the method of Wurts and Durborow (24).

Mortality and feeding behaviors were then observed for 72 h after exposure to the anesthetics.

2.3. Statistical analysis

All data are presented as mean ± SE. To verify the normality and homogeneity of variances, data were submitted to the

Table. Features of anesthetic materials used in the study.

	MPEo *(Mentha piperita)*	LAEo *(Lavandula angustifolia)*	MS-222 (ethyl 3-aminobenzoate methanesulfonate)
CAS number	8006-90-4	8000-28-0	886-86-2
Density at 25 °C (g/mL)	0.898	0.879	-
Origin and purity level	Natural, Sigma-Aldrich	Natural, Sigma-Aldrich	Aldrich, 98%
Refractive index	*n*20/D 1.46	*n*20/D 1.46	-

Source: Sigma-Aldrich.

Shapiro–Wilk test. The Kruskal–Wallis test was used for data on anesthesia induction and recovery times. The results were analyzed separately for type and concentration of anesthetic agent. As a result, different anesthetic applications were compared in terms of water quality criteria. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., USA). The minimum significance level was set at $P < 0.05$.

3. Results

The induction times of all anesthetic agents used in the anesthesia tests decreased with increasing concentration in both A1 and A2 stages (Figure 1). Negative correlations were calculated for induction time and concentration ($R^2 = 0.904$, $R^2 = 0.8755$, and $R^2 = 0.7469$ for MPEo, LAEo, and MS-222, respectively), whereas a similar correlation was found in the recovery time of only Eo_s concentration ($R^2 = 0.8664$ and $R^2 = 0.6578$). No correlation was observed between recovery time and MS-222 concentration in the studied ranges ($R^2 = 0.2495$).

Induction occurred later in LAEo applications ($P < 0.05$). The final stage of induction was not achieved at 100 ppm in LAEo trials. No deaths were found in the fish during the treatments or the next 72 h after the tests.

Minimum effective concentrations were 200 ppm for MS-222, 100 ppm for mint, and 300 ppm for lavender. When the recovery times were examined, it was observed that the longest run was in the LAEo trial (Figure 1). At 300–400 ppm LAEo applications, there was no difference between concentrations, whereas differences were found in anesthetic materials. An important observation is that in the MS-222 experiment, the gill movements were very fast, but at the last stage they were irregular and fast (stopping after 3–4 quick strokes), almost as fast as the interesting strokes. This behavior was different from other anesthetics in full induction stage.

Relationships between time and MPEo, LAEo, and MS-222 concentrations in both full induction and total loss of reflex with box plots for *C. moorii* are presented in Figures 2–4.

When the water parameters were evaluated after 6 h of transfer, the increase in pH, CO_2, and NH_3 amounts was found to be significant in all groups according to baseline. However, there was no significant difference between the experimental and control groups in terms of concentration and anesthetic type. After 12 h of fish transport, the increase in pH, CO_2, and NH_3 amounts was found to be significant in all groups compared to initial values after 6 h. In addition, the pH and CO_2 values for the MS-222 at 20 and 30 ppm varied significantly from the control group and other groups and greatly reduced the deterioration in water parameters. At the end of the experiment, when NO_2 and NH_3 were examined, it was determined that anesthetic agents in all the experimental groups were effective in reducing water degradation compared to the control. The water parameters after 18 h of transfer differed in pH, CO_2, and NH_3 levels in all groups according to baseline. Interestingly, the amounts of NO_2 in lavender experiments were found to be quite high while the amounts of NH_3 decreased compared to the control (Figure 5). No mortality or adverse effects occurred and fish stayed calm during the trials and the next 72 h after the experiments. The effects of various anesthetic substances in different concentrations on water quality after fish transport are shown in Figure 5 (LAEo_10, 20, and 30 indicate 10, 20, and 30 ppm LAEo; MPEo_10, 20, and 30 indicate 10, 20, and 30 ppm MPEo; Ms-222_10, 20, and 30 indicate 10, 20, and 30 ppm Ms-222).

4. Discussion

Anesthetics are used with increasing frequency in aquaculture and environmentally friendly investigations for potential herbal anesthetics have garnered interest, as influenced by the 2010/63/EU Directive, where the significance of fish welfare is established in order to prevent animals from sensing distress and from experiencing lasting harm. The efficacy of LAEo and MPEo was assessed for blue dolphin *C. moorii* in this study. As a result, MPEo showed anesthetic effects on blue dolphin in the concentration range of 100–400 ppm. Mint is a sedative

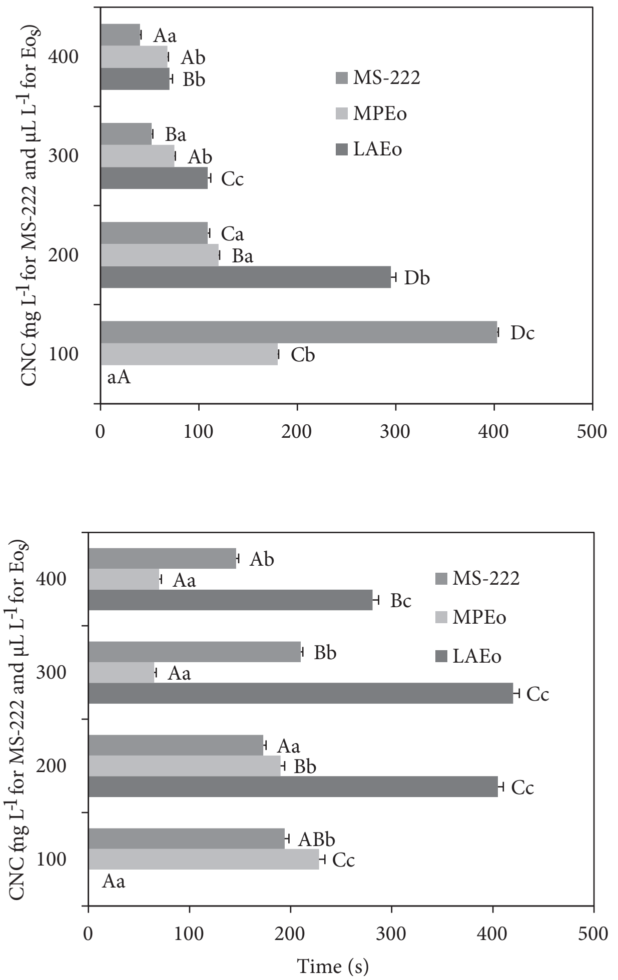

Figure 1. Comparisons of the induction time (full induction: A) and the recovery time (total behavioral recovery: B) at different concentrations of anesthetics. Uppercase letters indicate differences among the same concentration for different species; lowercase letters indicate differences among concentrations within the same species.

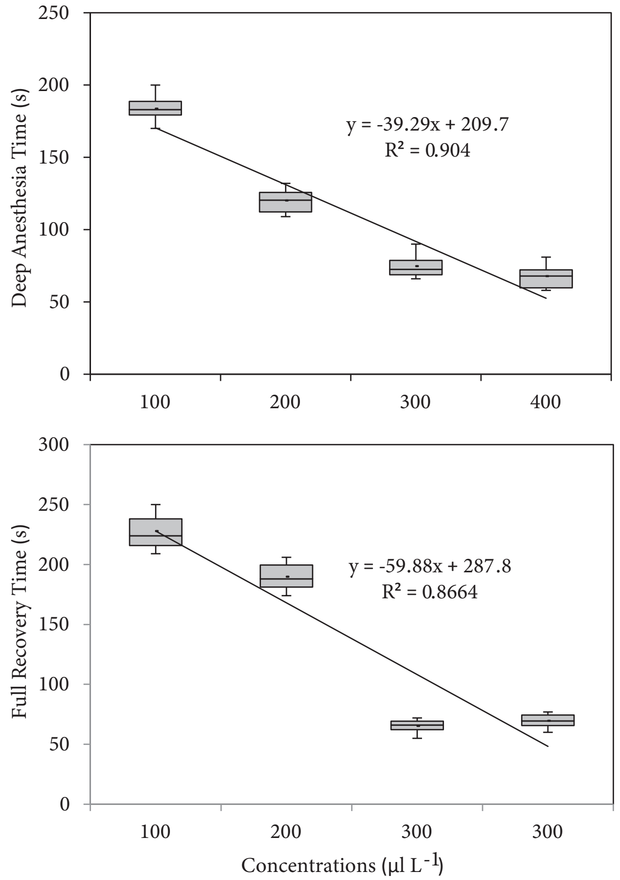

Figure 2. Relationships between time and MPEo concentrations in both full induction and total loss of reflex with box plots for *C. moorii*. Dots represent the median, the upper and lower lines of each plot indicate the max and min times observed, and inner box indicates the 25th and 75th percentiles.

(25) with the anesthetic effect of carvone as a component that adds flavor and odor to the plant. Carvone's anesthetic effects on the peripheral and central nervous systems include central nervous system depression, antinociceptive effects, sedation, and anticonvulsant-like activity (26). Authors recommended the use of mint oil (*Mentha arvensis*) at a concentration of 25 µL L^{-1} in transport (27) and at a concentration of 70 µL L^{-1} in deep anesthesia (19) for marine aquarium fish *A. ocellaris*. The AI_t is a bit different from our findings. AI_t was established at 15 min in their study, whereas it was about 3 min in the present work at the suggested concentration. The use of 100 µL L^{-1} (183.5 s AI_t and 227.8 s RR_t) MPEo is sufficient for deep anesthesia, whereas the concentration range of 10 to 30 µL L^{-1} is recommended for *C. moorii* transport. It is important to note that in the study with 15 min of induction time it could last with a lower concentration of the anesthetic than 3 min of induction.

Lavender oil previously showed sedative effects on rainbow trout *Oncorhynchus mykiss* above concentrations of 200 ppm (20). In this study, anesthetic effect was observed in the range of 200–400 ppm with rapid induction and ready recovery while showing a sedative effect at 100 ppm concentration in *C. moorii*. The difference may be attributed to the effect in different fish species, fish weight, water temperature, and salinity in addition to differences of EO_s. One of the major components of lavender, linalool, is a constituent of several Eo_s whose depressor activities on the central nervous system are well described in rodents (28), in addition to the anesthetic properties for aquatic animals (29).

Total ammonia is the main waste product of protein, and nonionizing ammonia is the most toxic form of metabolism in fish. Reduction in water pH in the first

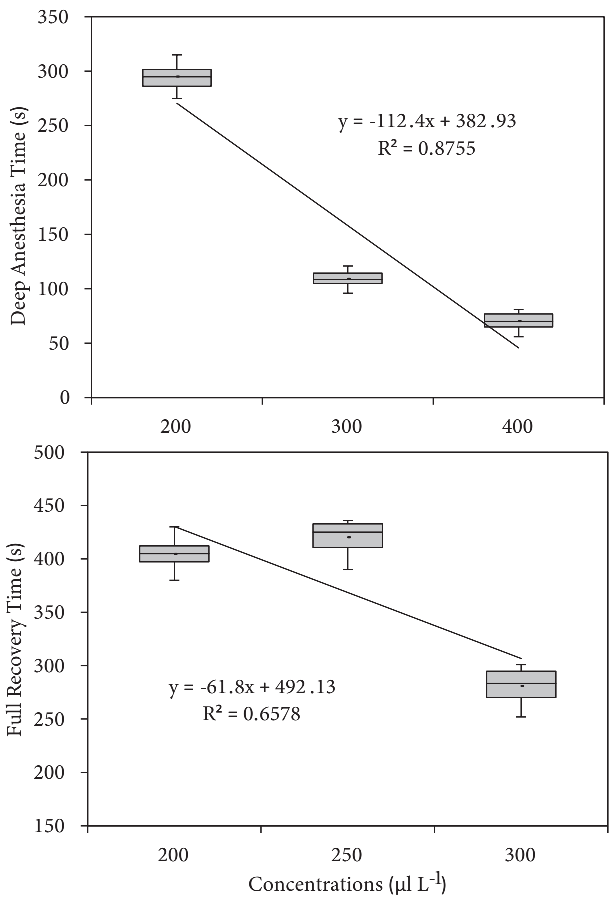

Figure 3. Relationships between time and LAEo concentrations in both full induction and total loss of reflex with box plots for *C. moorii*. Dots represent the median, the upper and lower lines of each plot indicate the max and min times observed, and inner box indicates the 25th and 75th percentiles.

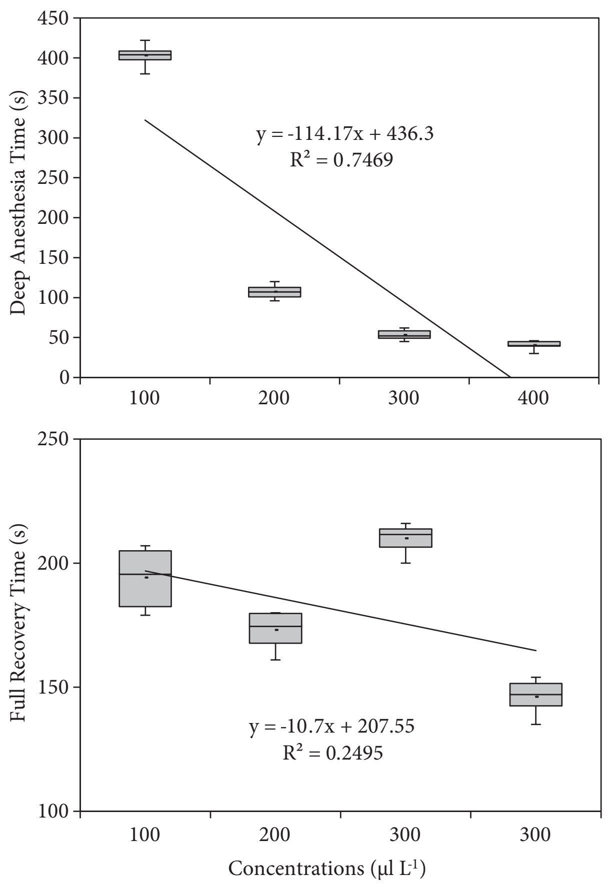

Figure 4. Relationships between time and MS-222 concentrations in both full induction and total loss of reflex with box plots in *C. moorii*. Dots represent the median, the upper and lower lines of each plot indicate the max and min times observed, and inner box indicates the 25th and 75th percentiles.

couple of hours of transport is due to carbonic acid formation resulting from carbon dioxide release during fish respiration (30,31). The pH and CO_2 values after the 6-h trial in our study were similar in all groups compared to baseline. Along with this, however, these changes are also possible with sedative agents (30,32). However, the pH decrease in the first hours may also be manipulated during the capture of the fish into the transfer bags, during capture from the aquarium, and during bagging. Every kind of manipulation in fish can cause immediate stress, and due to increased respiration, CO_2 is released and therefore pH can be increased. The present results show that the acidity in the transport medium can reduce the negative impacts of NH_3 considerably, thereby reducing the possibility of mass mortality during fish transport, as reported by Pramod et al. (33).

Increased concentrations led to shorter induction times in all anesthetic trials, compatible with previous studies (22,30), but there were also decreased recovery times, except in the MS-222 trials, in the present study. Weyl et al. (21) reported that the time taken for fish to recover from anesthesia was dependent on concentration, with those fish anesthetized at the highest concentration recovering later than those anesthetized at lower concentrations. Interestingly, recovery times were dose-independent in MS-222 trials (Figure 4) as reported by Küçük and Çoban (34) in goldfish. Differently, the recovery times shortened with increasing concentrations of Eo_s and this situation is explained as the fish exposed to the highest concentrations were not in contact with the Eo_s for long, which would allow for a faster recovery time. Similar results were also reported by Mylonas et al. (22) in their anesthesia study.

This study indicated that peppermint Eo (*Mentha piperita*) and lavender Eo (*Lavandula angustifolia*) can be safely used as anesthetic and sedative agents in tropical fish species as natural alternatives to synthetic anesthetics,

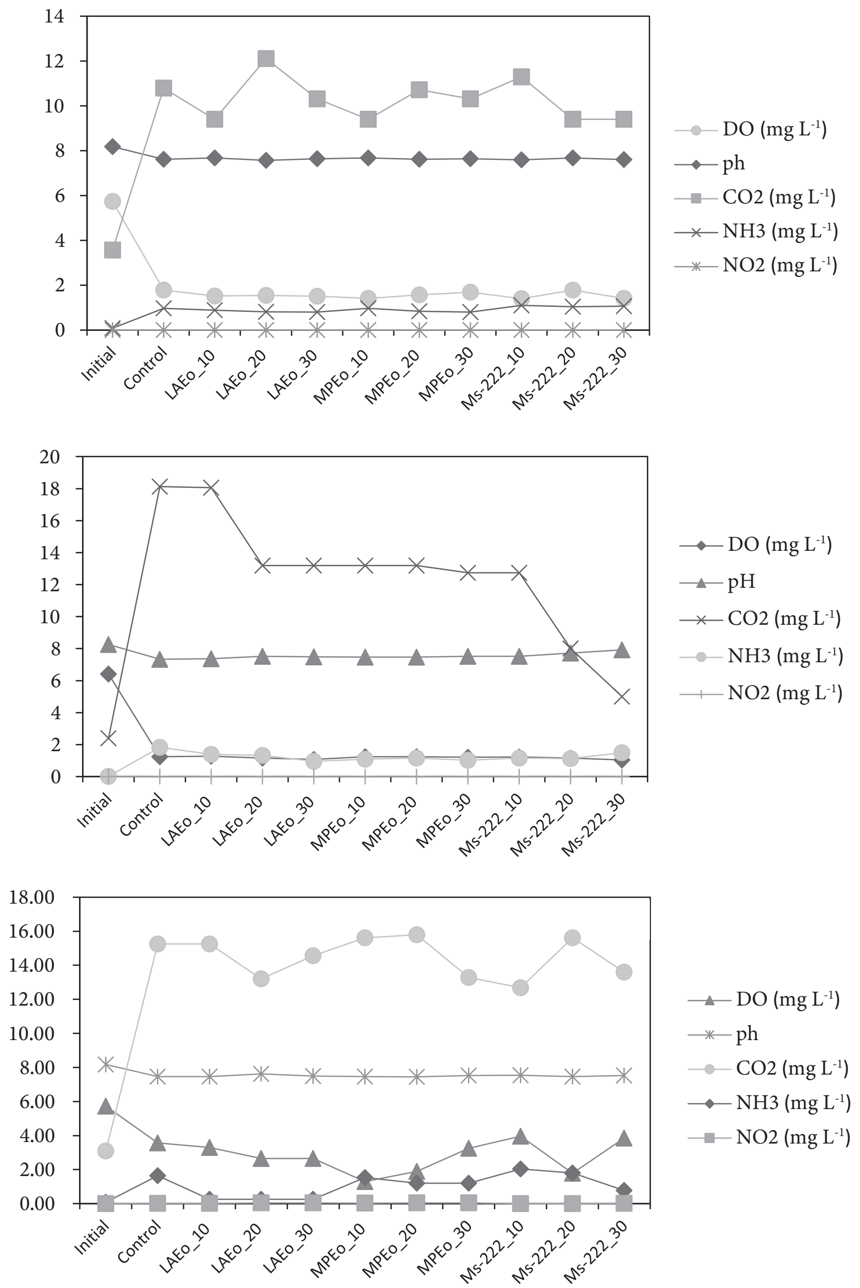

Figure 5. The effect of various anesthetic substances on water quality after 6 h (A), 12 h (B), and 18 h (C) of transfer at different concentrations.

with their pleasant smell. The use of 100 μL L^{-1} (183.5 s AI_t with 227.8 s RR_t) and 300 μL L^{-1} (109.2 s AI_t with 420.0 s RR_t) MPEo and LAEo, respectively, is sufficient for deep anesthesia, whereas the concentration range of 10–30 μL L^{-1} is suggested for fish transfer.

Results also showed that MPEo can be used with at least 3-fold lower concentrations than LAEo and 2-fold lower concentrations than MS-222 as a fish anesthetic. We conclude that both Eo_s are novel environmentally friendly herbal anesthetics suitable for use in aquaculture.

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