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Peppermint Oil Administration Can Stabilizes Blood Glucose and Improves Survival of Bonylip Barb in Closed Transportation System

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Abstract

Transportation of Bonylip barb fish is important for supports the production of cultivation and economic markets distribution. The high mortality of Bonylip barb seeds is an problem for Bonylip barb fish farmers. An addition of peppermint oil (*Mentha piperita* L.) attempts to suppress respiratory response and minimize mortality. The purposes the research was to test the effect of peppermint oil on Bonylip barb seeds and find out the best dosage of peppermint oil on Bonylip barb seeds. The research design is Completely Randomized Design consisting of 4 treatments and 3 replications, i.e. A (0 mL/L); B (0.1 mL/L); C (0.2 mL/L); and D (0.3 mL/L). This study used Bonylip barb seeds (3.33 ± 0.58 g) with density of 20 fish/L. Research procedures included seed preparation and acclimatization, fasting, adding peppermint oil, packaging, fish transportation for 8 hours, and rearing for 7 days. The results showed that peppermint oil had a significant effect on induction time, recovery time, blood glucose after transportation and maintenance, and survival life of Bonylip barb seeds. The best dose of peppermint oil was shown by 0.1 mL/L, which produced an induction time of 8.14 ± 5.07 minutes and a sedative time of 11.54 ± 2.60 minutes; fish blood glucose after transportation and maintenance was 68.33 ± 3.06 mg/dL and 64.67 ± 0.58 mg/dL, and the survival rate of 88.33 ± 5.77%. Result of this research is expected to provide essential guidance for fish farmers during the transportation of Bonylip barb, helping to enhance the safety and well-being of the fish throughout the shipping process.
Keywords: *anesthesia, behavior, endemic, induction, sedative*

INTRODUCTION

Bonylip barb fish (*Osteochilus vittatus*) is an endemic variety native to Indonesia. The benefits of cultivating Bonylip barb fish are to meet the needs of community consumption and as a mixture of ornamental fish feed. The protein content of Bonylip barb fish is 38.83% (Utami *et al*., 2019). Bonylip barb fish cultivation has the advantages of a fast growth cycle, easy adaptation to various environmental conditions, and high survival. Based on statistical data from the Ministry of Maritime Affairs and Fisheries (2022), Bonylip barb cultivation national production was 32,854 tons in 2021.

Seed transportation plays an important role in expanding businesses and meeting national consumption. The problems of Bonylip barb seed transportation is increased mortality due to competition for space and oxygen, decreased water quality, and shipping time. Factors that affect the transportation of Bonylip barb seeds include size, time, density, and fish transportation techniques. One of the methods to suppress seed mortality is carried out by reducing fish activity through anesthesia (Park *et al*., 2018).

Peppermint Oil Anesthesia (*Mentha piperita* L.) contains the active ingredient menthol as a sedative, strong analgesic, and pain reliever (Tafrihi *et al*., 2021). The efficacy of peppermint oil anesthesia has been tested on goldfish with a dose of 0.2 mL/L resulting in a induction time of 10 seconds and a consciousness time of 5 minutes (Rakhshani *et al*., 2018). Administration of a dose of 0.12 mL/L of peppermint oil to catfish according to research by Krasteva *et al*. (2021) produced a sedative effect after 6.2 minutes. Peppermint oil dose of 7.4 mL/L oil allows a sedative effect after 8 minutes in tilapia (Rezende *et al*., 2017). Based on those, it is known that different doses of peppermint oil anesthesia produce different results. The study of adding peppermint oil anesthesia to Bonylip barb seeds aims (1) to test the effect of peppermint oil anesthesia on Bonylip barb fish seeds, and (2) to determine the best dose of peppermint oil anesthesia on Bonylip barb seeds (*Osteochius vittatus*).

MATERIALS AND METHODS

Research Design

The research design used Completely Randomized Design. The addition of peppermint oil anesthesia to Bonylip barb seeds used four test dose treatments, i.e. 0 mL/L (A); 0.1 mL/L (B); 0.2 mL/L (C); and 0.3 mL/L (D). Repetition was carried out three times for each treatment. Preparation of the research container was carried out using 12 aquariums measuring 80x40x40 cm^3. The container was cleaned of dirt and filled with water with a height of 25 cm. The fish for the research were Bonylip barb seeds measuring 3.33±0.58 g as many as 240 fish. The seeds were acclimatized for 24 hours before transportation. The plastic packing was filled with 1 L of water, then peppermint oil was added according to the treatment dose and stirred evenly slowly. Bonylip barb seeds were inserted with a density of 20 fish/L. The oxygenated Bonylip barb seeds were then placed in a polystyrene box. Transportation was carried out for 8 hours using a pick-up truck. Resuscitation was carried out by placing the fish in a new container filled with water, assisted by an aerator to speed up the resuscitation time.

Bonylip barb seeds were maintained for 7 days after transportation. Commercial feed with 30% protein was given with a frequency of 2 times a day in the morning at 08.00 and in the afternoon at 16.00 WIB. Measurement of water quality, temperature, pH, and DO (dissolved oxygen) twice a day which was observed in the morning and evening.

Observation Parameter

The induction time of the Bonylip barb seeds is calculated using a stopwatch starting from the addition of peppermint oil anesthesia evenly into the water in the plastic packing, then the Bonylip barb seeds were put into the bag and the response of the fish's induction behavior was observed (Firdaus *et al*., 2022). The sedative time was calculated from when the Bonylip barb seeds were placed in the maintenance container with the help of aeration until the fish are conscious and swim actively on the surface of the water (Bolasina *et al*., 2017). Observations of the behavior of the Bonylip barb seeds were carried out 2 times, namely during the induction time and the time of consciousness.

Blood glucose calculations in Bonylip barb seeds were carried out 3 times before transportation, after transportation, and maintenance. Blood glucose measurements used test strips with a minimum sample of 4 µL. Test strips compared to the blood glucose range of 30-600 mg/dL (Nugraha *et al*., 2022). The water quality parameters observed include temperature, pH, and DO. Observations were carried out twice a day in the morning and evening. Measurement of the survival rate of Bonylip barb seeds was obtained through the percentage of the final number of seeds compared to the number of seeds at the beginning (Dewi *et al*., 2023). Measurement of the survival rate of Bonylip barb seeds was carried out 3 times: after transportation, after maintenance, and the total survival rate as a whole. Peppermint oil testing was carried out using the GC-MS (Gas Chromatography-Mass Spectrometry) method.

Data analysis

Data testing used statistical analysis for blood glucose levels, induction time, sedative time, and survival rate (SR). The ANOVA test was carried out with a 95% confidence interval, followed by the Duncan test using SPSS 16. Water quality data were described using descriptive methods.

RESULTS AND DISCUSSION

Induction Time

The results of the fastest induction time measurements of Bonylip barb seeds was in treatment D (2.79 ± 1.43 minutes), while the longest was in treatment B (8.14 ± 5.07 minutes) (Table 1). An induction time of less than 3 minutes indicates that the anesthetic works very well (Gajutos and Aurelia, 2023). Rakhshani *et al*., (2018) stated that the best induction time for goldfish at a dose of 200 ppm (0.2 mL/L) resulted in a induction time of 10 seconds. The same anesthetic material produces different induction time effects on different species.

The longer the total time of fainting, the better the effect of anesthesia. Menthol content accelerates the fainting time of the seeds. The fainting condition is caused by an uncontrolled central nervous system, so that the sensitivity of the fish to external stimuli decreases. The induction process is attempted so that the fish become faint faster, so that the stress time of the fish is reduced (Daud *et al*., 2017).

Table 1. Induction Time of Bonylip barb seeds with different dosage. Number with different superscript letter in the same column indicate significantly different (P<0.05).

Treatment	Induction time (minutes)	Sedative time (minutes)
A (0 mL/L)	-	-
B (0.1 mL/L)	8.14±5.07[a]	11.54±2.60[a]
C (0.2 mL/L)	5.00±3.24[ab]	13.98±3.41[ab]
D (0.3 mL/L)	2.79±1.43[b]	18.60±5.36[b]

Sedative Time

The best results of the measurement of the consciousness time of the Bonylip barb seeds observed in treatment B (11.54±2.60 minutes), while the longest sedative time was in treatment D (18.60±5.36 minutes) (Table 1). The anesthesia sedation time is classified as good if the fish can be regained consciousness in less than 10 minutes. (Chao *et al*., 2018). Rakhshani *et al*. (2018) mentioned that peppermint oil in goldfish with a dose of 200 ppm (0.2 mL/L) produced a consciousness time of 5 minutes. The sedative time of Bonylip barb fish was much slower than that of goldfish when using peppermint oil anesthesia.

Sedative time is influenced by body length and anesthetic dose. Small fish recover faster than large fish when using the same anesthetic dose. The concentration of peppermint anesthetic causes the fish to faint faster and the recovery time to be longer. Low concentrations of anesthetics have a slow working power in inhibiting the body's ability to bind oxygen (Puspito *et al*., 2023).

Fish Behavior

The results of behavioral observations on Bonylip barb seeds are shown at the time of fainting and consciousness of the fish (Table 2). Behavior during induction time and sedative time showed the same characteristics in treatments B, C, and D. The reaction time span to peppermint oil anesthesia depends on the dose. The speed of the reaction of fainting and consciousness of fish is directly proportional to the amount of peppermint oil dose given to Bonylip barb fish seeds.

The administration of peppermint oil anesthesia affects the induction time of Bonylip barb seeds marked by a decrease in the respiratory response. The aromatic content of fish anesthesia contains the active ingredient menthol in anesthesia to accelerate the immotile condition which is marked by being unreactive to the response, following the flow, slowing operculum movements, and the body being in an unconscious state (Arlanda *et al*., 2018). Fainting in fish is marked by panic as the beginning of the response phase of changes in induction behavior. Panic is characterized by fish flailing, moving erratically and very fast mouth and operculum movements, slowing down, until entering an unconscious state (Suwetja *et al*., 2016).

Table 2. Behavior during induction time of Bonylip barb seeds after giving peppermint anesthesia with different dosage

Treatment	Induction Time (min)	Behaviors
A (0 mL/L)	-	-
B (0.1 mL/L)	2.42	decreased reflexes, slow movements
	4.17	operculum slows down, stimulation decreases
	8.13	faint
C (0.2 mL/L)	1.57	operculum movement slows down
	3.55	tilted movement, unresponsive
	5.00	faint
D (0.3 mL/L)	0.77	starting to get restless, tilted/unfocused movements
	1.53	operculum slows down, body begins to turn upside down
	2.78	faint

Administration of peppermint oil affects the time of consciousness of Bonylip barb seeds during 8-hour transportation. The behavior of fish consciousness is influenced by the dose of anesthesia. Bonylip barb seeds recovery occurs most quickly when given a dose of 0.1 mL/L of peppermint oil (Table 3). The behavior of Bonylip barb seeds during consciousness includes fish movement against the current, reflection to shocks, active gill movement, and active body movement. The mechanism of fish recovery occurs through oxygen entering the gills, then flowing in the bloodstream. This functions to clean the remaining anesthetic in the fish's body (Wijaya *et al*., 2023). Factors that affect how quickly fish faint are the dose of anesthetic and skin thickness. The difference in fish fainting time is influenced by the adaptive nature of the fluctuating transportation environment. Catecholamine hormones, such as epinephrine, affect fish recovery, increasing fish blood glucose back to normal (Mahasri *et al*., 2022).'

Table 3. Behavior during sedative time of Bonylip barb seeds after giving peppermint anesthesia with different dosage

Treatment	Induction Time	Behaviors
A (0 mL/L)	-	-
B (0.1 mL/L)	6.00	movement follows the current, operculum calm
	7.85	recovery appears, movement follows the current
	11.52	conscious, against the current
C (0.2 mL/L)	8.22	movement follows the current, operculum calm
	12.28	recovery appears, movement follows the current
	13.98	conscious, against the current
D (0.3 mL/L)	9.35	Still, has no reflect
	15.15	passive operculum movement, movement follows the current
	18.60	conscious, against the current

Blood Glucose Levels

The results of blood glucose in Bonylip barb fish (*Osteochilus vittatus*) seeds before transportation showed no significant differences between treatments. The results of blood glucose of Bonylip barb seeds after transportation with the best value in treatment B were 68.33±3.06 mg/dL after transportation and 64.67±0.58 mg/dL after maintenance. While the highest blood glucose value was in treatment D of 92.33±4.04 mg/dL after transportation and 85.67±1.53 mg/dL after maintenance. The standard blood glucose of fish in normal conditions is 40-90 mg/dL (Widiastuti *et al*., 2022). Fish need enough energy to maintain homeostasis (Yudhistira *et al*., 2020). The full results are presented in Figure 1.

The results of further tests of fish blood glucose after transportation showed that there was a significant effect in giving peppermint oil on the blood glucose levels of Bonylip barb seeds after transportation. The highest blood glucose value in treatment D was 92.33±4.04 mg/dL after transportation indicating a stress response. The stress response in fish indicates decreased blood sugar, disrupting the energy supply to the brain (Wiyoto *et al*., 2022). Factors that affect fish stress include: sudden temperature changes, hormones, density, and shocks during the journey (Yustiati *et al*., 2017).

Water Quality

The results of the transportation temperature measurement were in the range of 27.4±0.23 and 28.6±0.85ºC and the maintenance temperature after transportation had a temperature range of 27.4±0.35 and 28.4±0.78ºC. The temperature parameter value meets the optimum temperature value of 28 ºC (NSA 8296.1, 2016). The temperature in the fish bladder affects the homeostatic condition of the fish's body. The adjustment of tilapia fish to adapt to physical shocks is carried out by adjusting the appropriate density, sufficient O2, and short travel time during wet transportation (Nani *et al*., 2015). The temperature in the transportation media is not constant and shows dynamic changes. An increase in temperature has an impact on the rate of metabolism due to enzymatic activity (Maruli *et al*., 2023).

The results of measuring the pH during transportation were 7.0 ± 0.05 - 7.2 ± 0.00 and the maintenance pH after transportation were 7.2 ± 0.05 - 7.3 ± 0.05. Those values are in the neutral pH range and are good for maintaining Bonylip barb seeds. The optimum pH standard value is 6.5 - 8 (Dinata *et al*., 2021). The increase in pH that will occur is balanced by the level of dissolved CO_2 in the water. The effect of pH can affect fish behavior (Mila and Rahayu, 2023). Unstable pH levels (<6 or >8) can cause suboptimal growth (Hendri *et al*., 2023). The pH stability is carried out through daily monitoring and control (Januardy *et al*., 2023). The values of DO during transportation were 4.96 ± 1.02 - 5.56 ± 0.07 mg/L, while DO during maintenance were 4.96 ± 0.58 - 5.23 ± 0.87 mg/L. The DO value is quite optimal for maintaining Bonylip barb seeds, since the DO of healthy aquaculture is above 5 mg/L (NSA 8296.1, 2016). The value of DO in the water bag is influenced by temperature. The DO concentration of 3-4 mg/L indicates symptoms of fish stress. The DO content <1 mg/L can cause lethality or death within a few hours (Marsono *et al*., 2023). The DO requirement factor is influenced by the type, stage and activity of fish during maintenance (Selanno, 2016). All results are presented in Table 4.

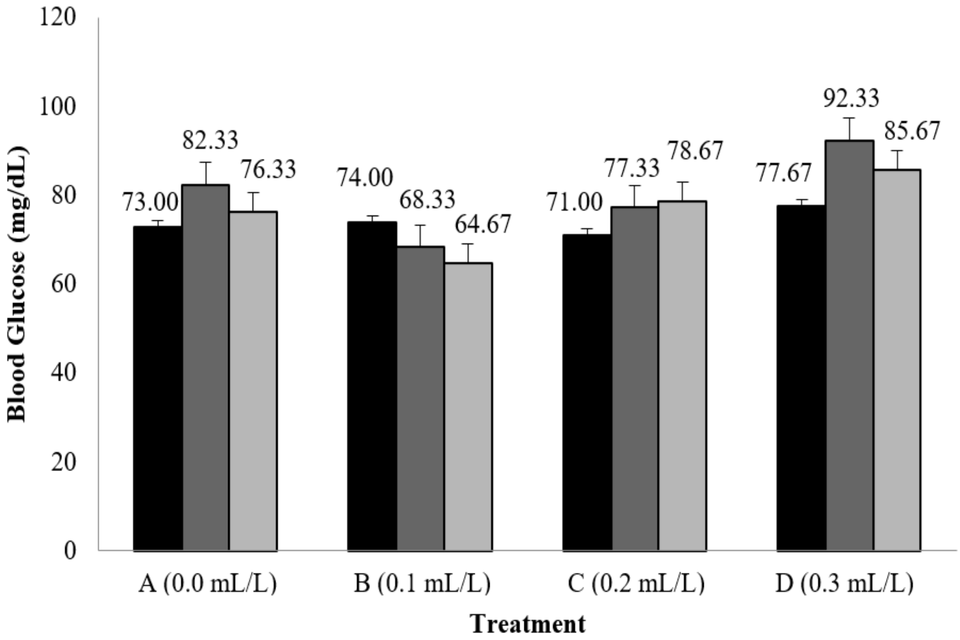

Figure 1. Blood glucose of Bonylip barb seeds before transportation (black), after transportation (dark grey), and maintenance (light grey). Treatment in additional peppermint anesthesia with different dosage: A (0 mL/L); B (0.1 mL/L); C (0.2 mL/L); and D (0.3 mL/L).

Table 4. Water quality during transportation and maintenance of Bonylip barb seeds

Variable	Treatment	Transportation	Maintenance	Eligibility
Temperature (°C)	A (0 mL/L)	28.0±0.50	28.4±0.78	28.0[a]
	B (0.1 mL/L)	27.4±0.23	27.6±0.43	
	C (0.2 mL/L)	27.7±0.35	27.4±0.35	
	D (0.3 mL/L)	28.6±0.05	28.4±0.78	
pH	A (0 mL/L)	7.0±0.05	7.2±0.23	6.5-8.5[b]
	B (0.1 mL/L)	7.2±0.00	7.2±0.05	
	C (0.2 mL/L)	7.1±0.05	7.3±0.05	
	D (0.3 mL/L)	7.0±0.10	7.2,0.23	
DO (mg/L)	A (0 mL/L)	5.25±0.73	5.23±0.87	5.0[a]
	B (0.1 mL/L)	5.57±0.07	5.09±0.45	
	C (0.2 mL/L)	5.17±0.60	4.96±0.58	
	D (0.3 mL/L)	4.96±1.02	5.23±0.66	

Note: [a)] NSA 8296.1 (2016); [b)] Dinata *et al*., (2021)

Survival Rate (SR)

The highest survival rate of Bonylip barb seeds after 8 hours of transportation was in treatment B which was 91.67±2.89% and the lowest was in treatment D which was 68.33±11.55%. The best SR of Bonylip barb seeds after 7 days of maintenance was in treatment B which was 96.30±3.21%, also the best SR overall was in treatment B which was 88.33±5.77% (Fig. 2). The good SR of Bonylip barb seeds is when it is above 80%. The SR is considered good based on the larval survival standard of 70%-80% (NSA, 1999).

The survival of Bonylip barb seeds after transportation shows that there is an effect of anesthesia on Bonylip barb seeds. The administration of peppermint oil has the effect of suppressing blood flow with menthol, menthon, iso-menthon, and methyl salicylate compounds. The use of peppermint anesthesia of 0.1 mL/L is the best dose for transporting Bonylip barb seeds in a closed system for 8 hours of travel. Fish that are not given anesthesia have higher mortality due to sudden changes and limited movement activity, so they respond more to movement in closed conditions. Indications of stress in treatment D due to high doses can indicate that there are side effects in the form of an overdose at a dose of 0.3 mL/L mixed in the transportation medium, resulting in a fairly large number of test animal deaths. Thus, the administration of peppermint oil doses to Bonylip barb seeds is recommended at a dose of 0.1 mL/L. Good stocking density management supports the ability to adapt to environmental changes during the trip (Mulyadi *et al*., 2014).

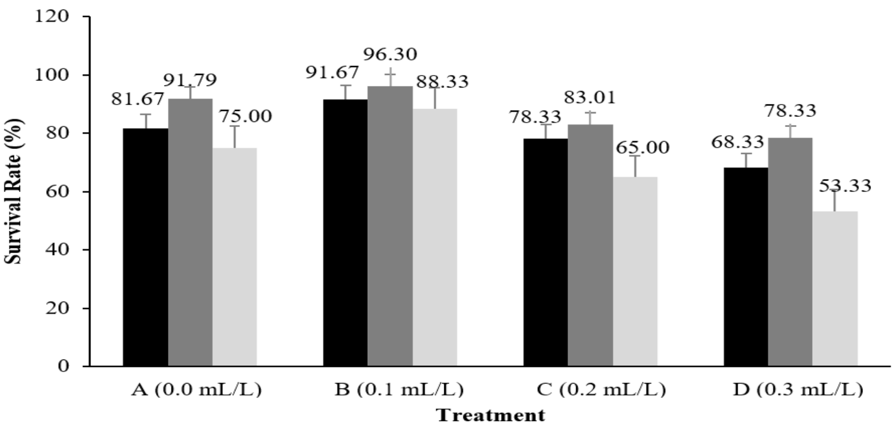

Figure 2. Survival rate (SR) of Bonylip barb seeds after transportation (black), after maintenance (dark grey), and overall (light grey).

Phytochemical Test

The results of the phytochemical test showed the composition of the active compounds in peppermint oil, namely menthol at 41.11%, menthon at 21.11%, iso-menthon at 9.65%, and methyl acetate at 4.86% (Table 5). This is confirmed by Nina *et al*. (2022), which states that mint plant extract contains 40% menthol and 10% methyl acetate. Menthol, neomenthol, menthol stereoisomers are cyclic monoterpenes and work with menthone, iso-menthone, and other compounds to provide a cooling effect as a sedative that suppresses the rate of fish respiration (Zhao *et al*., 2022).

The composition of peppermint compounds helps prevent a decrease in glucose when fish are traveling. The active ingredients of peppermint extract have a calming and stress-reducing effect (Adamiah *et al*., 2023). The use of peppermint anesthesia on Bonylip barb seeds is effective in preventing hypoxia so that the fish can be calmer during transportation (Guo and Brian, 2021).

Table 5. Phytochemical test of peppermint oil (*Mentha piperita* L.)

Compound Components	Composition (%)
Menthol	41.11
Menthone	21.11
Iso Menthone	9.65
Methyl Acetate	4.86

Conclusion

The conclusions obtained based on this study are (1) the best dose for blood glucose levels of Bonylip barb seeds in treatment B was 68.33±3.06 mg/dL after transportation and 64.67±3.06 mg/dL after maintenance, and (2) the best survival rate was in treatment B at 91.67±2.89% after transportation, 96.30±3.21% after maintenance, and 88.33±5.77% overall. This study is expected to provide basic information for aquaculture practitioners when transporting Bonylip barb, to ensure the fish remain safe. Further studies are needed to examine the effects of the peppermint oil on the fish's responsive organs.

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