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Utilization of *Ageratum conyzoides* Leaf Extract as a Natural Anesthetic for Sustainable Closed-System Transportation of Tinfoil Barb (*Barbonymus schwanenfeldii*) Fingerlings

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Abstract: The transportation of tinfoil barb (*Barbonymus schwanenfeldii*) fingerlings is a critical stage in aquaculture production because transportation stress can increase metabolic activity, deteriorate water quality, and reduce fish survival. This study evaluated the effectiveness of billygoat-weed (*Ageratum conyzoides*) leaf extract as a natural anesthetic agent and determined its optimal concentration for closed-system transportation of tinfoil barb fingerlings. A Completely Randomized Design (CRD) consisting of five treatments, namely 0, 2, 3, 4, and 5 mg L^{-1} extract concentrations with three replications, was applied. Transportation was conducted for 12 h under closed-system conditions. The observed parameters included behavioral responses, induction time, recovery time, hematocrit, blood glucose, survival rate, and water quality. The results showed that increasing extract concentrations accelerated induction time but prolonged recovery time among anesthetized treatments. The 5 mg L^{-1} treatment produced the fastest induction time (19.25 ± 1.87 min), whereas the 2 mg L^{-1} treatment resulted in the shortest recovery time (9.06 ± 0.45 min). Fish treated with 4 mg L^{-1} extract exhibited the lowest post-transport blood glucose concentration (136.0 ± 16.3 mg dL^{-1}), the highest post-transport survival rate (93.3%), and retained 90.0% survival after a seven-day recovery period. Therefore, 4 mg L^{-1} was identified as the optimal concentration for maintaining physiological stability and improving the survival of tinfoil barb fingerlings during closed-system transportation.

Keywords: *Ageratum conyzoides*; Closed-system transportation; Natural anesthetic; Sustainable aquaculture; Tinfoil barb (*Barbonymus schwanenfeldii*)

Introduction

The tinfoil barb (*Barbonymus schwanenfeldii*) is an indigenous Indonesian freshwater fish with considerable economic value and promising prospects for aquaculture development, particularly in Kalimantan and Sumatra. Despite its commercial potential, the distribution of tinfoil barb fingerlings remains constrained by the high susceptibility of the species to transportation-induced stress. Physiological stress during transport elevates cortisol secretion and respiratory activity, leading to increased oxygen consumption and the accumulation of metabolic wastes, especially ammonia, which subsequently deteriorate water quality and compromise fish survival (Iwama, 1998). Closed-system transportation is widely adopted in aquaculture practices because it is practical, cost-effective, and suitable for long-distance shipment. Nevertheless, the limited water volume and restricted gas exchange in this system often result in declining dissolved oxygen levels, ammonia accumulation, and fluctuations in temperature and pH, thereby increasing the risk of mortality during transport. Transportation success is therefore strongly influenced by factors such

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as stocking density, travel duration, water quality management, and the application of anesthetic agents (Luz & Favero, 2024). Prolonged transportation duration has also been reported to exacerbate water quality deterioration and increase mortality rates in tinfoil barb fingerlings (Wibowo et al., 2022).

One practical strategy to minimize transportation stress is the application of anesthetic agents that suppress fish metabolic activity during transit. Conventional synthetic anesthetics have demonstrated high effectiveness in reducing stress responses; however, their application is frequently associated with several limitations, including chemical residues in fish tissues, relatively high costs, and potential environmental impacts (Cunha et al., 2018). Consequently, increasing attention has been directed toward the development of plant-derived anesthetics as safer and more environmentally sustainable alternatives. Natural anesthetics reduce metabolic rates, oxygen consumption, and ammonia excretion, thereby maintaining water quality throughout transportation. Previous studies have demonstrated that stress mitigation through natural compounds contributes significantly to improved physiological stability and adaptive capacity in fish exposed to environmental changes (Karnan, 2022). Furthermore, Rawung et al. (2023) highlighted the promising role of plant-based anesthetic agents in supporting sustainable aquaculture transportation systems.

Among the potential botanical anesthetics, billygoat-weed (*Ageratum conyzoides*) leaves contain several bioactive compounds, including flavonoids, alkaloids, saponins, tannins, and essential oils, which exhibit sedative and anesthetic properties in aquatic organisms (Nuge et al., 2021). The biological activity of *A. conyzoides* leaf extract has been confirmed through phytochemical evaluations showing the presence of active metabolites with potential applications as biological agents (Yuningsih & Putra, 2025). Several studies have reported successful use of *A. conyzoides* extracts in fish transportation; however, direct comparison among studies remains difficult because different extract formulations and dosage units have been employed. For example, Saputra et al. (2020) utilized ethanol extract in a mass-based dosage of 2.5 mg L^{-1} during the transportation of freshwater pomfret (*Colossoma macropomum*) fingerlings and achieved a survival rate of 97.8% (Farida et al., 2017). In contrast, other studies used liquid extract formulations expressed in volume-based units, including 4 mL L^{-1} for jelawat (*Leptobarbus hoevenii*) fingerlings with a survival rate of 73.33%, 3.5 mL L^{-1} for giant gourami (*Osphronemus goramy*) (Firdaus et al., 2022), 3 mL L^{-1} for milkfish (*Chanos chanos*) prospective broodstock (Mukminin et al., 2018), 3 mL L^{-1} for common carp (*Cyprinus carpio*) (Syamsiah et al., 2023), and 4.5 mL L^{-1} for comet fish (*Carassius auratus*) fingerlings (Lendra et al., 2025). These differences indicate that the effective concentration of *A. conyzoides* extract is highly species-specific and may depend on fish physiology, body size, and extract formulation (Lendra et al., 2025).

To date, no studies have evaluated the effectiveness of *A. conyzoides* leaf extract as a natural anesthetic for tinfoil barb (*B. schwanenfeldii*) fingerlings during closed-system transportation. This represents an important research gap considering the increasing demand for high-quality fingerlings and the absence of species-specific recommendations for anesthetic application in this commodity. The novelty of the present study lies in the evaluation of *A. conyzoides* leaf extract as a natural anesthetic specifically for tinfoil barb fingerlings under closed transportation conditions and in the determination of the optimal concentration capable of maintaining physiological stability, water quality, and post-transport survival. The findings are expected to contribute not only to the development of environmentally friendly fish transportation technology but also to improve the efficiency of seed distribution systems and support the sustainable expansion of Indonesia's freshwater aquaculture sector.

Methods

The experiment was conducted for 15 days at the Laboratory of the Faculty of Fisheries and Marine Sciences, Universitas Muhammadiyah Pontianak, Indonesia. The closed-system transportation trial was carried out along the Pontianak–Singkawang route, representing approximately 12 hours of transportation under commercial distribution conditions. A total of 200 tinfoil barb (*Barbonymus schwanenfeldii*) fingerlings with a total length of 8–12 cm were obtained from the Central Fish Hatchery Center (BBIS) Anjungan, West Kalimantan. The use of 200 fish was intended to provide sufficient reserves during acclimatization and fasting periods and to avoid undersampling caused by mortality before the experiment. Following a seven-day acclimatization period, during which fish were fed commercial pellets twice daily, 150 healthy and uniformly sized fingerlings were selected and randomly assigned to the experimental units. Feed was withheld for 24 h before transportation to minimize metabolic waste production during shipment.

Experimental Design

The study employed an experimental method using a Completely Randomized Design (CRD) consisting of five treatments and three replications, resulting in a total of 15 experimental units. Each experimental unit consisted of one sealed transportation bag containing 10

fish, resulting in a total experimental population of 150 fingerlings (Ardilla et al., 2023). The treatments consisted of different concentrations of *Ageratum conyzoides* leaf extract as follows:
P0 = 0 mg L^{-1} (control, without extract)
P1 = 1 mg L^{-1} extract
P2 = 2 mg L^{-1} extract
P3 = 3 mg L^{-1} extract
P4 = 4 mg L^{-1} extract

The concentration range was determined based on previous studies reporting the effectiveness of *A. conyzoides* extract as a natural anesthetic in fish transportation and preliminary observations conducted prior to the experiment.

Preparation of Ageratum conyzoides Leaf Extract

Fresh billygoat-weed leaves were collected from local agricultural areas, washed with running water, and air-dried under shade conditions for approximately seven days until a constant weight was obtained. The dried leaves were then ground into powder using a laboratory grinder (Erida et al., 2023). Approximately 500 g of dried leaf powder were macerated in 80% ethanol using a solvent-to-material ratio of 1:10 (w/v) for 72 h at room temperature with periodic stirring every 24 h. The filtrate was subsequently separated using filter paper and concentrated using a rotary evaporator at 40–45°C under reduced pressure until a viscous crude extract was obtained. The concentrated extract was stored at 4°C until use. The use of 500 g of plant material ensured sufficient extract yield for all experimental treatments and improved the reproducibility of the extraction procedure.

Transportation Procedure

Each transportation bag (20 × 40 cm polyethylene bag) was filled with 5 L of clean freshwater and stocked with ten fingerlings. The required amount of *A. conyzoides* extract was weighed according to the treatment concentration and dissolved uniformly into the transportation water before fish introduction. After fish stocking, the bags were supplied with medical-grade oxygen using a water-to-oxygen ratio of 1:3, resulting in approximately 5 L of water and 15 L of oxygen per bag (Obirikorang et al., 2022). The bags were tightly sealed using rubber bands and placed inside styrofoam boxes to minimize temperature fluctuations during transportation. The transportation simulation was conducted for approximately 12 h along the Pontianak–Singkawang route without opening the bags during transit, thereby maintaining a true closed transportation system.

Observation Parameters

The observed parameters included: Induction time to anesthesia (minutes); Recovery time after transportation (minutes); Survival rate after transportation (%); Water quality parameters, including temperature, dissolved oxygen (DO), pH, and total ammonia concentration; Blood glucose concentration; and Hematocrit value.

Survival rate was determined immediately after the completion of the 12-h transportation period, when the bags were opened and fish were transferred to recovery aquaria. Additional observations were conducted during a seven-day post-transport rearing period to evaluate delayed mortality.

Blood Sampling Procedure

Blood sampling was conducted using a destructive sampling approach to avoid repeated handling stress and physiological bias associated with multiple blood collections from the same individual fish. For each observation period, separate fish were randomly selected from reserve stocks maintained outside the transportation units: baseline measurements before transportation, measurements immediately after transportation, measurements after seven days of recovery (Simide et al., 2017). Blood samples were collected from the caudal vein using heparinized syringes for hematocrit and glucose analysis. This procedure ensured that the number of fish within each transportation unit remained constant throughout the transportation period and that physiological measurements were not confounded by repeated blood sampling stress.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) at a 95% confidence level. When significant differences among treatments were detected ($P < 0.05$), Duncan's Multiple Range Test (DMRT) was performed to identify differences between treatment means (Wahid et al., 2017). The experiment consisted of five treatment groups representing different concentrations of *Ageratum conyzoides* leaf extract used as a natural anesthetic agent during the transportation of tinfoil barb (*Barbonymus schwanenfeldii*) fingerlings. Treatment A served as the control group and received no billygoat-weed leaf extract (0 mg L^{-1}). Treatment B involved the administration of *A. conyzoides* leaf extract at a concentration of 2 mg L^{-1}, while Treatment C received 3 mg L^{-1} of the extract. Treatment D was administered 4 mg L^{-1}, whereas Treatment E received the highest concentration of 5 mg L^{-1}. The selected concentration range was established based on findings from previous studies and preliminary observations aimed at identifying an effective and safe dosage for reducing transportation stress in tinfoil barb fingerlings under closed-system transportation conditions.

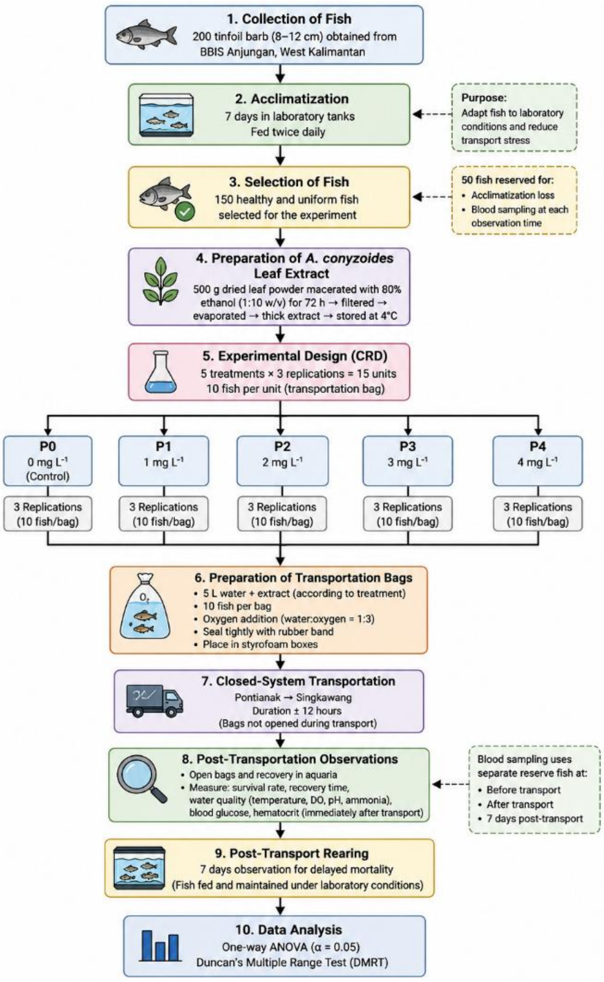

Figure 1. Experimental procedure of closed-system transportation using *A. conyzoides* leaf extract

Research Procedures

The implementation of this study began with the preparation of 15 aquarium units measuring 30 × 30 × 30 cm, which were cleaned, dried, and arranged randomly. Each aquarium was filled with water to a height of 15 cm. Furthermore, the preparation of billygoat-weed leaf extract (*Ageratum conyzoides*) was carried out using the method of Mentari et al. (2020) with mature billygoat-weed leaf simplicia that had been wet-sorted, washed, dried, cut into small pieces, and dry-sorted. Subsequently, 5 grams of simplicia were placed into a container and added with 80% ethanol solution until the simplicia were completely submerged. The sample was soaked for 3 to 5 days, filtered, and then concentrated using a rotary evaporator. Afterward, the extract was dried using a heating device to obtain a thick extract with a herbal aroma. The resulting billygoat-weed leaf extract was then prepared for each treatment and replication.

Subsequently, tinfoil barb (*Barbonymus schwanenfeldii*) fingerlings measuring 8–12 cm were selected and acclimatized for 7 days in the laboratory. During the acclimatization period, the fish were fed commercial feed at 3% of their body biomass twice daily and fasted for 24 hours before use. After the fasting period, billygoat-weed leaf extract was added according to the five treatments with three replications. The concentration of billygoat-weed leaf extract was calculated based on the volume of water in the container. Thus, if the experiment used 1 liter of water, the concentration of billygoat-weed leaf extract was multiplied by the volume of water in the container (Farida et al., 2015).

Afterward, the experimental fish were placed into double-layer plastic packing bags measuring 20 × 40 cm. The prepared billygoat-weed leaf extract was added according to the treatment dosage into the plastic packing bags containing water and 10 experimental fish per unit. The plastic bags were then filled with oxygen at a water-to-oxygen volume ratio of 1:3, tied securely, and placed into styrofoam boxes. Each box contained three plastic packing bags arranged according to the predetermined placement design.

After the packaging process was completed, a transportation trial was conducted for 12 hours using a car along the Pontianak–Singkawang–Pontianak route. After transportation, the fish were acclimatized by floating the plastic bags in the aquarium for approximately 15 minutes before releasing the fish into the rearing medium. The tinfoil barb fingerlings were reared for 7 days in aquariums equipped with aeration. Feeding was carried out twice daily. Observations of survival rate were conducted every 2 hours, while observations of water quality and fish behavior were carried out before and after transportation.

Induction Time and Sedative Duration

Induction time is the period required for tinfoil barb (*Barbonymus schwanenfeldii*) fingerlings to reach an unconscious condition after the administration of billygoat-weed leaf extract (*Ageratum conyzoides*). Meanwhile, sedative duration is the time required for the fish to regain consciousness after the anesthesia and transportation processes were completed. Measurements were conducted using a stopwatch and expressed in minutes. The induction time was measured from the moment the anesthetic substance was added to the transportation medium until the fish lost consciousness, indicated by slowed operculum movement and the fish remaining at the bottom of the container. Observations of sedative duration were conducted from the time the fish were transferred to the recovery medium until they resumed active swimming and responded normally to stimuli.

Hematocrit Levels

Hematocrit levels were observed to determine the physiological response of tinfoil barb fingerlings to stress during the transportation process. Measurements were conducted before transportation, after transportation, and on the seventh day of the rearing period. Blood samples were collected using microhematocrit tubes and centrifuged at 3500 rpm for 15 minutes. Hematocrit values were calculated based on the ratio of erythrocyte volume to total blood volume and expressed as a percentage (%).

Blood Glucose Levels

Blood glucose levels were measured to determine the stress level of fish resulting from anesthetic treatment and transportation. Blood samples were collected from the base of the fish tail using a 1 mL syringe, and the blood was then dropped onto a digital glucometer strip. Observations were conducted before transportation, after transportation, and on the seventh day of the rearing period. The measurement results were expressed in mg/dL.

Survival Rate

Survival rate was observed to determine the survival success of tinfoil barb fingerlings during the transportation process and post-transportation rearing period. Observations were conducted by calculating the number of surviving fish at the end of the study compared to the initial number of fish. Survival rate values were expressed as percentages (%).

Water Quality

Water quality observations were conducted to determine the condition of the transportation and rearing media throughout the study. The observed water quality parameters included temperature, degree of acidity (pH), dissolved oxygen (DO), and ammonia. Measurements were conducted before and after transportation as well as during the rearing period using a thermometer, pH meter, DO meter, and ammonia test kit. Water quality data were analyzed descriptively as supporting data for the study.

Data Analysis

Behavioral response and water quality data were analyzed descriptively. Meanwhile, induction time, sedative duration, hematocrit levels, blood glucose levels, and survival rate data were analyzed using analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test to determine differences among treatments.

Results and Discussion

Behavioral Response During Anesthesia

The administration of billygoat-weed leaf extract (*Ageratum conyzoides*) showed that the extract was capable of reducing the activity of tinfoil barb (*Barbonymus schwanenfeldii*) fingerlings until they reached an unconscious condition. These changes differed among treatments with different concentrations of billygoat-weed leaf extract, whereas no behavioral changes were observed in the control treatment during the 30-minute observation period. The behavioral responses of tinfoil barb fingerlings during anesthesia can be seen in Table 1.

The observation results showed that Treatment E (5 mg/L) produced the fastest induction time, namely 19.25 minutes. Treatment D with a concentration of 4 mg/L had an average induction time of 21.56 minutes, which was longer than Treatment E. Treatment C with a concentration of 3 mg/L had an average induction time of 23.06 minutes, which was longer than Treatments D and E. Meanwhile, Treatment B with a concentration of 2 mg/L had an average induction time of 26.73 minutes, which was longer than Treatments C, D, and E. This pattern indicates a relationship between extract concentration and induction time duration. These findings are consistent with the principles of fish anesthesia, in which anesthetic agents are used to suppress activity, metabolism, stress responses, and the risk of injury during handling or transportation. Minaz et al. (2024) explained that the effectiveness of fish anesthesia is strongly influenced by the type of anesthetic agent, concentration, fish species, fish size, and environmental conditions of the rearing medium. Therefore, billygoat-weed leaf extract has the potential to be used as a natural anesthetic for the transportation of tinfoil barb fingerlings.

Although the concentration of 5 mg/L resulted in the fastest induction time, this treatment may not necessarily represent the most optimal concentration. High concentrations may accelerate unconsciousness, but they can also increase the risk of stress, delayed recovery, and even mortality. Meanwhile, concentrations of 3–4 mg/L may be considered a more moderate range for further studies, particularly when combined with observations of recovery time, survival rate, dissolved oxygen, pH, temperature, and ammonia levels. This finding is consistent with the study of Mukminin et al. (2018) on milkfish (*Chanos chanos*), which reported that billygoat-weed leaf extract could be used as an anesthetic in fish transportation and that certain concentrations were able to improve fish survival. Based on the results of this study, the administration of billygoat-weed leaf extract anesthesia at different concentrations in tinfoil barb fingerlings was descriptively acceptable because there was a consistent tendency that increasing concentrations accelerated fish immobilization.

Table 1. Behavior of tinfoil barb fish seeds during the anesthesia process

Fainting Time (minutes)	Bandotan Leaf Extract Concentration				
	A (0 mg/L)	B (2 mg/L)	C (3 mg/L)	D (4 mg/L)	E (5 mg/L)
0–8	Operculum movement is normal and fish movement is active	The movement of the operulum is normal and the fish movements are active	Normal operculum movement and active fish movement, some fish look panicked	The response decreases, some fish start to panic	Fish panic and move unbalanced, fast operculum movement
9–16	Operculum movement is normal and fish movement is active	The fish start to look panicked, the fish often appear on the surface, the fish's response decreases.	Fish response decreases, fish movement is unbalanced, some fish are in an upside down position	The movement of the operulum is fast, the fish's movements start to become unbalanced, some fish start to faint	The fish's movements slowed down, the body position of some fish was upside down and some fish began to faint.
17–23	Operculum movement is normal and fish movement is active	The movement of the operulum slows down, the fish's movement decreases and it loses balance, slowly the fish begins to faint.	Fainted fish (23.06)*	Fainted fish (21.56)*	Fainted fish (19.25)*
24–30	Operculum movement is normal and fish movement is active	Fainted fish (26.73)*	-	-	-

Behavioral Response During Recovery

The recovery process of tinfoil barb fingerlings occurred gradually after the fish were transferred into clean water media supplied with aeration. According to Gunawan (2013), dissolved oxygen entering through the gills helps remove residual anesthetic substances from the bloodstream and excrete them through the excretory system. Therefore, aeration is an important component in the recovery process. In the initial phase, the fish were still under the influence of anesthesia, characterized by tilted or motionless body positions, weak responses, and the gradual appearance of mouth, fin, and operculum movements. The behavioral responses of tinfoil barb fingerlings during the recovery process after anesthesia using billygoat-weed leaf extract are presented in Table 2.

Table 2. Behavior of tinfoil barb fish seed during the resuscitation process

Consciousness Time (minutes)	Bandotan Leaf Extract Concentration				
	A (0 mg/L)	B (2 mg/L)	C (3 mg/L)	D (4 mg/L)	E (5 mg/L)
0–5	The fish moved normally because it did not faint.	The mouth, fins and operculum begin to move, some fish begin to move but are still weak.	The mouth, fins and operculum begin to move, the fish's position is still tilted.	The mouth, fins and operculum begin to move, the fish's position is still tilted.	The mouth, fins and operculum begin to move, the fish's position is still tilted.
6–10	The fish moved normally because it did not faint.	Some fish are starting to swim actively (normally) and some fish are still adapting (9.06)*	Some fish start to swim actively (normally) and the rest start to move but weakly.	The fish started to appear to be moving but weakly, at the 10th minute some of the fish started to swim actively.	Some fish started to respond, the rest were still lying down, at the 12th minute the fish started to move but weakly.
11–15	The fish moved normally because it did not faint.	-	Fish swimming actively (11.59)*	Fish swimming actively (11.71)*	Some fish are starting to move, but it's weak. (14.88)*

The results showed that Treatment B (2 mg/L) produced the fastest recovery time with an average of 9.06 minutes, whereas Treatment E (5 mg/L) resulted in the longest recovery time of 14.88 minutes. Treatments

C (3 mg/L) and D (4 mg/L) had average recovery times of 11.59 minutes and 11.71 minutes, respectively. In the control treatment (0 mg/L), the fish did not undergo a recovery process because no anesthetic was administered. Differences in recovery time among treatments may have been caused by the amount of active compounds absorbed, the physiological adaptability of the fish, fish size, duration of exposure, temperature, pH, dissolved oxygen, and stress conditions during packaging.

The concentration of 5 mg/L was indeed effective in inducing anesthesia; however, it produced the longest recovery time. In contrast, the concentration of 2 mg/L resulted in the fastest recovery, although it should be considered whether this concentration was sufficiently effective in suppressing fish activity during transportation. These findings are consistent with the study of Syamsiyah et al. (2022), which reported that increasing concentrations of billygoat-weed leaf extract prolonged the sedative and recovery times of common carp (*Cyprinus carpio*). In addition, the study of Firdaus et al. (2022) on giant gourami (*Osphronemus gourami*) also demonstrated that high anesthetic concentrations caused slower recovery due to the high residual anesthetic compounds absorbed into the fish body.

At a concentration of 4 mg/L, a relatively balanced effect was achieved between induction speed and the fish's recovery ability. This concentration was considered sufficiently effective in suppressing fish activity during transportation without causing excessively long recovery times. This finding is in line with the study of Karim et al. (2024), which stated that the use of natural anesthetic agents at moderate doses is more effective in maintaining a balance between anesthetic efficiency and fish recovery speed. Excessively high anesthetic concentrations may accelerate induction, but they tend to prolong recovery time and increase physiological stress in fish during transportation.

Induction Time and Sedative Duration

Induction time is the duration from the moment the fish are in a conscious condition until they become unconscious or immobilized. The unconscious condition occurs when the fish lose consciousness or when the central nervous system functions inadequately, resulting in reduced sensitivity to external stimuli and a decreased motor response to such stimuli (Pellu & Rebhung, 2018). Sedative duration is the recovery period from the unconscious condition until the fish regain consciousness and resume normal activities. Recovery time was calculated from the moment the experimental fish were placed into the recovery container supplied with aeration until the fish fully regained consciousness. The results of anesthesia using billygoat-weed leaf extract (*Ageratum conyzoides*) at concentrations of 0 mg/L, 2 mg/L, 3 mg/L, 4 mg/L, and 5 mg/L, along with the average induction times, are presented in Table 3.

Table 3. Induction period and sedative period (Mean ± Standard Deviation) of tinfoil barb fish fry during anesthesia

Treatments	Induction Period (minutes)	Sedative Period (minutes)
A (0 mg/L)	-	-
B (2 mg/L)	26.73 ± 0.58[a]	9.06 ± 0.45[a]
C (3 mg/L)	23.06 ± 2.71[b]	11.59 ± 1.40[b]
D (4 mg/L)	21.56 ± 1.06[b]	11.71 ± 0.46[b]
E (5 mg/L)	19.25 ± 1.87[c]	14.88 ± 1.01[c]

The observation results showed that Treatment E with a concentration of 5 mg/L produced the fastest induction time, namely 19.25 ± 1.87 minutes, whereas Treatment B (2 mg/L) showed the longest induction time of 26.73 ± 0.58 minutes. In the control treatment (0 mg/L), the fish did not become unconscious during the observation period. This finding indicates that increasing the concentration of billygoat-weed leaf extract accelerated the entry of active compounds into the fish body, resulting in a more rapid reduction in movement activity and consciousness. Thus, the higher the concentration of billygoat-weed leaf extract administered, the shorter the induction time obtained. These results are consistent with the study of Pratama et al. (2017), which reported that the essential oil of billygoat-weed leaves had a significant effect on the induction and recovery times of koi fish, with faster induction times observed in certain treatments compared to others.

Differences in letters in the post hoc test results indicated that Treatments C and D were not significantly different, whereas Treatments B and E belonged to different groups. This means that increasing the concentration from 3 mg/L to 4 mg/L did not produce a strong effect on induction time, but the concentration of 5 mg/L demonstrated a more pronounced anesthetic effect. This finding is also consistent with Firdaus et al. (2022), who found that billygoat-weed leaf extract significantly affected induction time, sedative duration, blood glucose levels, and the survival of giant gourami (*Osphronemus gourami*) during transportation.

Billygoat-weed leaves are classified as a natural anesthetic material because they contain active compounds. According to Barelrina et al. (2021), billygoat-weed leaves contain alkaloids such as flavonoids, which possess analgesic, antibacterial, anti-inflammatory, antiallergic, and anticancer properties. The anesthetic substances absorbed into the blood vessels are transported to the central nervous system, namely the brain and spinal cord, where they block synaptic dopamine receptors, inhibit dopamine release, and suppress the central nervous system. This mechanism produces sedative effects, muscle relaxation, reduced spontaneous activity in response to external stimuli, and decreased metabolic and respiratory activity, thereby causing the fish to become unconscious.

The results for sedative duration showed that Treatment B (2 mg/L) produced the fastest recovery time, namely 9.06 ± 0.45 minutes, whereas Treatment E (5 mg/L) showed the longest sedative duration, namely 14.88 ± 1.13 minutes. These results indicate that the higher the concentration of billygoat-weed leaf extract administered, the longer the time required for the fish to regain consciousness. This condition occurred because, in Treatment B, the lower concentration absorbed into the fish body enabled the fish to metabolize and eliminate the anesthetic compounds more easily. This finding is consistent with Palmi et al. (2018), who stated that the anesthesia process occurs through the diffusion of anesthetic substances from the environment into the respiratory organs, resulting in the absorption of anesthetic compounds into the bloodstream and their circulation throughout the fish body.

The post hoc test results showed that Treatment B (2 mg/L) was significantly different from Treatment C (3 mg/L), whereas Treatments C and D (4 mg/L) were not significantly different. Treatment E (5 mg/L) produced the longest sedative duration and differed significantly from the other treatments.

During the recovery process, the fish demonstrated the ability to readapt to clean water supplied with aeration. Aeration helped increase dissolved oxygen availability, thereby supporting respiration and accelerating the elimination of anesthetic substances from the fish body through the gills. This condition is consistent with the principles of fish anesthesia, which state that an ideal anesthetic agent should effectively induce unconsciousness while also being rapidly eliminated from the body so that the fish can recover within a relatively short period after being transferred to the recovery medium (Schroeder et al., 2021). The recovery time of tinfoil barb (*Barbonymus schwanenfeldii*) fingerlings ranged from 9 to 15 minutes, which is categorized as successful but not yet ideal because recovery occurred within or slightly above 10 minutes and had not yet reached approximately ±5 minutes. Park et al. (2018) stated that an appropriate anesthetic concentration generally produces a rapid induction time and a recovery time within 10 minutes. As the fish begin to regain consciousness, their metabolic processes increase, along with the demand for readily available oxygen for respiration. This occurs because higher concentrations of billygoat-weed leaf extract cause the fish to recover more slowly due to the absorption of greater amounts of antimetabolic compounds, such as alkaloids and aromatic substances, contained in the billygoat-weed leaves.

Hematocrit Levels

Hematocrit, defined as the ratio of blood cell volume to blood plasma and expressed as a percentage, is influenced by various factors such as stress, environmental conditions, and physiological changes. Hematocrit values below 20% indicate anemia, whereas values above 45% indicate polycythemia, which may be caused by erythrocyte swelling, stress, or hypoxia. Infection may also cause a decrease in hematocrit levels (Arlanda et al., 2018; Palmi et al., 2018). In addition, decreased hematocrit values indicate that the fish are experiencing infection. The results of hematocrit measurements of tinfoil barb (Barbonymus schwanenfeldii) fingerlings during the study are presented in Table 4.

Table 4. Hematocrit levels (Mean ± Standard Deviation) of tinfoil barb fish seed before transportation, post-transportation, and maintenance period

Treatments	Average Hematocrit Level of Tinfoil barb Fish Seeds (%)		
	Before Transportation	Post Transportation	Maintenance Period ± 7 days
A (0 mg/L)	35.8 ± 0.6	21.1 ± 0.8^{a}	25.5 ± 2.1^{a}
B (2 mg/L)	37.2 ± 0.7	19.3 ± 2.8^{b}	24.0 ± 3.5^{a}
C (3 mg/L)	30.7 ± 2.7	18.7 ± 2.2^{c}	24.4 ± 1.0^{a}
D (4 mg/L)	38.3 ± 1.8	16.6 ± 0.9^{d}	26.6 ± 2.1^{a}
E (5 mg/L)	35.8 ± 1.4	20.2 ± 3.1^{e}	25.6 ± 3.7^{a}

Based on the hematocrit levels of tinfoil barb fingerlings before transportation, the values ranged from 30.7–38.3%. Meanwhile, the hematocrit measurements after transportation showed that Treatment A (0 mg/L) had a hematocrit value of 21.1%, followed by Treatment E (5 mg/L) with an average hematocrit value of 20.0%, Treatment B (2 mg/L) with a hematocrit value of 19.3%, Treatment C (3 mg/L) with a hematocrit value of 18.7%, and Treatment D (4 mg/L) with a hematocrit value of 16.6%.

Changes in hematocrit values may indicate physiological stress in fish or alterations in the oxygen-carrying capacity of the blood. The decrease in hematocrit levels of tinfoil barb fingerlings after transportation was caused by the entry of various compounds into the fish body, resulting in a decrease in red blood cells and an increase in white blood cells, thereby causing anemia in the fish (Arlanda et al., 2018). Normal hematocrit levels in teleost fish range from 20–30%, while marine fish generally have hematocrit values of approximately 42%. Fish experiencing anemia have a minimum hematocrit percentage of 10% (Saragih et al., 2015).

Meanwhile, the hematocrit measurements of tinfoil barb fingerlings during the ±7-day rearing period returned to normal, ranging from 24.0–26.6%. The highest hematocrit value was observed in Treatment D (4 mg/L) with a hematocrit level of 26.6%, whereas the lowest value was observed in Treatment B (2 mg/L) with a hematocrit level of 24.0%. Compared with the post-transportation condition, the hematocrit measurements during the rearing period were within the normal range. This occurred because, during the rearing period, the hematocrit levels of the tinfoil barb fingerlings had returned to normal as the fish adapted to the environment. In addition, healthy fish conditions and appropriate feeding intensity were able to increase fish appetite, thereby improving the hematocrit values of the tinfoil barb fingerlings.

The mechanism of hematocrit percentage changes during fish stress begins with the reception of stress-causing factors by receptor organs, after which the information is transmitted to the hypothalamus in the brain through the nervous system. The hypothalamus then stimulates chromaffin cells to secrete catecholamines through sympathetic nerve fibers. The presence of catecholamines activates lipopolysaccharides that attack blood components, which function to reduce hematocrit levels in fish (Madyowati & Muhajir, 2018).

Blood Glucose Levels

Blood glucose functions as an important energy source for cellular metabolism, particularly in the brain. The results of blood glucose observations in tinfoil barb (Barbonymus schwanenfeldii) fingerlings during the study are presented in Table 5.

Table 5. Blood glucose levels (Mean ± Standard Deviation) of tinfoil barb fish seed before transportation, post-transportation, and maintenance period

Treatments	Average Blood Glucose Levels of Tinfoil barb Fish Seeds (mg/dl)		
	Before Transportation (mg/dl)	Post Transportation (mg/dl)	Maintenance Period ±7 days (mg/dl)
A (0 mg/L)	83.3 ± 11.5	190.7 ± 11.0[a]	87.3 ± 6.0[a]
B (2 mg/L)	77.3 ± 15.5	164.0 ± 25.2[b]	68.7 ± 10.7[a]
C (3 mg/L)	82.3 ± 7.51	145.7 ± 7.2[c]	81.0 ± 6.6[a]
D (4 mg/L)	78.7 ± 14.7	136.0 ± 16.3[d]	72.3 ± 4.7[a]
E (5 mg/L)	85.0 ± 5.0	142.3 ± 11.0[d]	76.3 ± 3.2[a]

Based on the measurement results, the blood glucose levels of tinfoil barb fingerlings before transportation ranged from 77.3–85.0 mg/dL and remained within the normal physiological range for fish. After transportation, blood glucose levels increased in all treatments. The measurement results showed that the highest blood glucose level was observed in Treatment A (control; 0 mg/L) at 190.7 mg/dL, followed by Treatment B (2 mg/L) at 164.0 mg/dL, Treatment C (3 mg/L) at 145.7 mg/dL, Treatment E (5 mg/L) at 142.3 mg/dL, and Treatment D (4 mg/L) at 136.0 mg/dL. Normal blood glucose levels in fish generally range from 40–90 mg/dL. Therefore, the increase in blood glucose levels after transportation indicates that the tinfoil barb fingerlings experienced physiological stress responses due to the transportation process. The elevated blood glucose levels during and after transportation were presumably influenced by stress caused by anesthetic treatment and environmental conditions during transportation, which affected fish biochemical parameters, including glucose metabolism (Aydin et al., 2020).

An increase in blood glucose level is one of the main physiological indicators of stress response in fish. When fish experience stress due to handling, transportation, high stocking density, or anesthetic exposure, the body responds through activation of the neuroendocrine system, which triggers the release of stress hormones such as cortisol and glucocorticoids. The activation of these hormones stimulates glycogenolysis and gluconeogenesis processes, resulting in increased mobilization of glucose into the bloodstream as an energy source to maintain physiological functions under stress conditions.

Fish experiencing stress require greater energy to maintain osmotic balance, respiration, and metabolic activity. These conditions cause blood glucose levels to increase as an adaptive response to environmental changes. The results of this study are consistent with the findings of Akbar et al. (2021), which stated that increased stress during transportation causes elevated blood glucose levels due to increased metabolic energy demands in fish. According to Iwama (1998), stress in fish activates the neuroendocrine system, which triggers cortisol secretion as the primary stress hormone. Cortisol subsequently increases glucose mobilization into the bloodstream to provide energy in response to unstable environmental conditions.

Although blood glucose levels increased in all treatments, fish treated with billygoat-weed leaf extract (*Ageratum conyzoides*) showed lower blood glucose levels compared to the control. This finding indicates that billygoat-weed leaf extract, as a natural anesthetic, was able to help suppress the physiological stress response of fish during transportation by reducing metabolic activity and energy requirements. Therefore, the use of billygoat-weed leaf extract has the potential to improve the physiological condition and resilience of tinfoil barb fingerlings during live transportation.

The results of blood glucose measurements in tinfoil barb fingerlings during the rearing period showed a decrease in glucose levels in all treatments. The highest blood glucose level was found in Treatment A (control; 0 mg/L) at 87.3 mg/dL, followed by Treatment C (3 mg/L) at 81.0 mg/dL, Treatment E (5 mg/L) at 76.4 mg/dL, Treatment D (4 mg/L) at 72.3 mg/dL, and the lowest value was observed in Treatment B (2 mg/L) at 68.7 mg/dL. The decrease in blood glucose levels during the rearing period indicates that the tinfoil barb fingerlings had been able to readapt to the rearing environment. Stable environmental conditions without sudden changes or additional stress-inducing substances allowed the fish to return to a more normal physiological condition. Furthermore, fish that had passed the stress phase caused by transportation began utilizing glucose-derived energy for metabolic processes and physiological recovery.

The results of this study are consistent with Syamsiyah et al. (2022), who stated that fish blood glucose levels decrease after the transportation phase and during the rearing phase. This decrease indicates the physiological adaptation process of fish to a more stable environment. Similar findings were also reported by Firdaus et al. (2022), who found that the blood glucose levels of giant gourami (*Osphronemus gourami*) decreased after the recovery phase due to reduced stress levels during rearing.

Physiologically, stress in fish triggers increased cortisol and glucocorticoid hormone levels, resulting in the mobilization of glucose into the bloodstream as an energy source. However, when environmental conditions return to stability, stress hormone activity decreases, causing blood glucose levels to gradually return to normal conditions. This is consistent with the statement of Barton (2020), who explained that blood glucose is a secondary stress response that increases during stress phases and decreases again once fish have adapted to their environment.

Post-Transportation Survival Rate

The survival rate of tinfoil barb (Barbonymus schwanenfeldii) fingerlings is one of the main indicators of success in live fish transportation. Survival rate is influenced by the ability of the fish to adapt to stress conditions during the transportation process. The results of the study showed varying survival rates at different treatment concentrations using billygoat-weed leaf extract (Ageratum conyzoides): Treatment A (0 mg/L) was 53.3%, Treatment B (2 mg/L) was 60.3%, Treatment C (3 mg/L) was 80.0%, Treatment D (4 mg/L) was 93.3%, and Treatment E (5 mg/L) was 60.0% (Table 6).

Based on Table 6, it can be seen that the administration of billygoat-weed leaf extract affected the post-transportation survival rate of tinfoil barb fingerlings. Treatment A without billygoat-weed leaf extract at a concentration of 0 mg/L resulted in the lowest survival rate of 53.3%, whereas Treatment D with a concentration of 4 mg/L produced the highest survival rate of 93.3%.

Table 6. Survival (Mean ± Standard Deviation) of tinfoil barb fish seeds post-transportation

Treatments	Average Post-Transportation Survival Rate (%)		
	Initial	Post-Transportation	Survival Rate (%) ± SD
A (0 mg/L)	10	5	53.3 ± 5.8[a]
B (2 mg/L)	10	6	63.3 ± 11.5[b]
C (3 mg/L)	10	8	80.0 ± 10.0[c]
D (4 mg/L)	10	9	93.3 ± 11.5[d]
E (5 mg/L)	10	6	60.0 ± 10.0[e]

The results showed that a billygoat-weed leaf extract concentration of 4 mg/L was the optimal concentration tolerated by tinfoil barb fingerlings. At this concentration, the fish were calmer and exhibited lower movement responses compared to the other treatments. This condition was presumably due to the ability of billygoat-weed leaf extract to suppress fish metabolic activity, thereby reducing oxygen consumption and stress levels during transportation. These findings are consistent with the study of Saputra et al. (2020) on the closed transportation of pomfret (*Colossoma Macropomum*), which showed that the addition of billygoat-weed leaf extract improved survival rate and helped maintain water quality and fish physiological responses. The similarity in the anesthetic material used strengthens the assumption that billygoat-weed leaves have potential as an additive for live fish transportation.

In Treatment E with a concentration of 5 mg/L, the survival rate decreased to 60.0%. This decrease was presumably caused by the high concentration of active compounds in the billygoat-weed leaf extract, which began to produce toxic effects such as respiratory disturbances, delayed recovery, and physiological stress in fish. This finding is consistent with Tanbiyasakur et al. (2018), who stated that if the concentration of anesthetic substances is too high and cannot be tolerated by fish, the compounds become toxic and cause fish mortality. Residual anesthetic substances accumulate in the fish body, damaging internal organ function and causing breathing difficulties that may ultimately lead to death. This is supported by Yudhistira et al. (2020), who stated that the interaction of anesthetic substances within the nervous system decreases brain function due to reduced oxygen intake. This decrease may result from excessive inhibition of oxygen binding in the blood vessels. Consequently, the fish experience excessive loss of consciousness and require greater effort to eliminate the metabolites of the anesthetic compounds from their bodies. Fitria et al. (2019) stated that impaired oxygen binding in the blood affects oxygen transport to body tissues, thereby disrupting metabolic processes in fish. Billygoat-weed leaves are known to contain various bioactive compounds such as flavonoids, alkaloids, saponins, tannins, terpenoids, and essential oils. These compounds may produce biological effects, but at high concentrations they have the potential to disrupt the physiological balance of fish (Adelya et al., 2022).

Survival Rate During the Rearing Period

The survival rate of tinfoil barb fingerlings during the post-transportation rearing period is an important parameter for evaluating the ability of the fish to undergo physiological recovery after experiencing transportation stress. The rearing period was conducted for approximately 7 days after transportation to observe the endurance and adaptability of the fish to the new rearing environment.

The results showed that Treatment D with a concentration of 4 mg/L achieved the highest survival rate of 90.0%, whereas Treatment A (0 mg/L) achieved 50.0%, Treatment B (2 mg/L) achieved 60.0%, Treatment C (3 mg/L) achieved 56.7%, and Treatment E (5 mg/L) achieved 53.3%, indicating lower survival rates (Table 7).

Table 7. Survival (Mean ± Standard Deviation) of tinfoil barb fish seeds during the rearing period

Treatments	Average Survival Rate (%)		
	Initial	Maintenance Period	Survival Rate (%) ± SD
A (0 mg/L)	10	5	50.0 ± 10.0[a]
B (2 mg/L)	10	6	60.0 ± 10.0[a]
C (3 mg/L)	10	5	56.7 ± 15.3[a]
D (4 mg/L)	10	9	90.0 ± 10.0[b]
E (5 mg/L)	10	5	53.3 ± 5.8[c]

The analysis results indicated that the administration of billygoat-weed leaf extract at a concentration of 4 mg/L produced the highest survival rate during the post-transportation rearing period, namely 90.0%. The high survival rate in this treatment indicates that the fish were able to recover physiologically after experiencing transportation stress. This condition was presumably due to the optimal concentration of billygoat-weed leaf extract, which was able to suppress metabolic activity and reduce fish stress levels during transportation, thereby maintaining stable physiological conditions after transfer to the rearing medium. These findings are consistent with the study of Nurkomaria et al. (2021), which stated that the use of natural anesthetics in fish transportation can improve survival rates and maintain fish physiological conditions during the rearing period. The study explained that natural anesthetics can suppress fish movement activity, thereby reducing energy expenditure during transportation.

However, the results of this study differ from those of Maryani et al. (2019) on catfish fingerlings, which demonstrated consistently high survival rates under various plant-based anesthetic treatments. These differences may have been influenced by differences in fish species, fingerling size, transportation duration, water quality, and fish sensitivity to natural anesthetic compounds. Tinfoil barb fingerlings were presumed to have lower physiological tolerance to high concentrations of billygoat-weed leaf extract compared to other fish species.

In Treatments A, B, C, and E, mortality still occurred during the rearing period. This condition was presumably caused by weakened physiological conditions after transportation and the adaptation process to the new environment. In addition, at high concentrations such as Treatment E (5 mg/L), the active compounds in billygoat-weed leaf extract were presumed to begin exerting toxic effects on fish organs. This study is also consistent with the opinion of Husen & Sharma (2014), who stated that stress in fish during transportation and handling can cause physiological changes such as increased cortisol levels, elevated glucose levels, body ion imbalance, and mortality. The use of anesthetics at optimal doses can reduce stress, whereas excessive doses may produce negative effects. Firdaus et al. (2022) also stated that higher concentrations of billygoat-weed leaf extract contain higher levels of secondary metabolite compounds, which may affect fish survival rates.

Live fish transportation is one of the critical stages in aquaculture activities because it can cause stress due to environmental changes, limited oxygen availability, increased metabolic activity, and ammonia accumulation. The use of natural anesthetic agents aims to suppress fish movement activity and metabolic rate so that the energy expended during transportation becomes lower.

Water Quality

Water quality observations were conducted at three different times, namely before transportation, after transportation, and after the rearing period. The observed parameters included temperature, pH, dissolved oxygen (DO), and ammonia (Table 8).

The observation results showed that transportation caused changes in water quality; however, these changes remained within ranges that supported the survival of tinfoil barb (Barbonymus schwanenfeldii) fingerlings. Water temperature during the study ranged from 27.1–28.2°C and remained relatively stable before transportation, after transportation, and after the rearing period. This temperature stability indicates that the transportation medium remained in conditions suitable for the survival of tinfoil barb fingerlings. Temperature is one of the water quality parameters that strongly affects metabolic activity, oxygen consumption, and fish stress levels during transportation. Excessively high or low temperature changes may increase fish physiological activity, thereby accelerating energy and oxygen consumption. Temperatures ranging from 27–28°C were identified as important for fish transportation, consistent with the optimal range of 25–32°C reported by Darwis et al. (2019). Higher temperatures increase metabolic rates, which subsequently increase oxygen consumption and may become critical because oxygen availability decreases. Neubauer & Andersen (2019) also emphasized that temperature fluctuations affect fish metabolism, particularly during transportation conducted in the morning, which may subsequently influence temperature changes.

Table 8. Water quality observations during research

Treatments	Parameter	Before Transportation	Post-Transportation	After Transportation
A (0 mg/L)	Temperature (°C)	27.3	27.5	27.3
	pH	6.8	6.5	6.5
	DO (mg/L)	6.0	4.3	6.1
	Ammonia	0.0	0.1	0.1
B (2 mg/L)	Temperature (°C)	27.1	27.5	27.4
	pH	6.3	6.2	6.3
	DO (mg/L)	6.4	4.3	6.2
	Ammonia	0.0	0.1	0.1
C (3 mg/L)	Temperature (°C)	27.5	28.1	27.4
	pH	6.3	6.3	6.4
	DO (mg/L)	6.9	4.1	5.8
	Ammonia	0.0	0.3	0.2
D (4 mg/L)	Temperature (°C)	28.0	28.2	27.9
	pH	6.3	6.5	6.6
	DO (mg/L)	6.2	4.3	6.3
	Ammonia	0.0	0.3	0.1
E (5 mg/L)	Temperature (°C)	28.0	28.1	27.9
	pH	6.4	6.3	6.5
	DO (mg/L)	6.2	4.3	6.7
	Ammonia	0.0	0.1	0.2

The pH values ranged from 6.2–6.8. The pH values tended to decrease slightly after transportation, particularly in Treatments B, C, and E. Nevertheless, these pH values remained within the tolerance range for freshwater fish. This condition is consistent with the Indonesian National Standard (SNI) guidelines for fish fingerling rearing, which recommend pH levels between 6.5 and 8.5. The decrease in pH after transportation was presumably caused by increased fish metabolic activity within the closed transportation system, resulting in the accumulation of carbon dioxide (CO_2) and metabolic waste that made the transport medium more acidic. These findings are consistent with the study of Midihatama et al. (2018), which reported that the transportation of giant gourami (Osphronemus gourami) fingerlings in a closed system caused a decline in water quality, including changes in pH due to fish respiration and metabolic activity. The study explained that CO_2 accumulation during transportation could reduce water pH levels. This finding is also supported by Hong et al. (2019), who reported that fish density during the transportation of golden pompano affected water pH due to fish metabolic activity during transport.

The DO values before transportation ranged from 6.0–6.9 mg/L and then decreased after transportation to 4.1–4.3 mg/L. The decrease in DO indicates oxygen consumption by fish during transportation. Oxygen levels may decline significantly due to increased temperature, high stocking density, excessive feeding, and increased organic matter. According to Farida et al. (2015), a minimum dissolved oxygen concentration of 2 mg/L is required for transportation, whereas the SNI recommends maintaining DO levels above 5 mg/L for fish fingerling rearing.

Ammonia levels during the study ranged from 0.0–0.3 mg/L. Ammonia was not detected before transportation but increased after transportation, particularly in Treatments C and D, which reached 0.3 mg/L. This increase was presumably derived from fish metabolic waste during transportation. The identified ammonia values were still suitable for tinfoil barb aquaculture, supporting the allowable limit of 0.5 mg/L established by Fazil et al. (2017). Excessive ammonia may cause stress and gill damage in fish, thereby increasing mortality risk, as up to 90% of nitrogen waste is excreted as ammonia through the gills. Overall, this study highlights the importance of monitoring these water quality parameters to maintain optimal fish health and survival during transportation and rearing. After the rearing period, ammonia levels tended to decrease or remain low, ranging from 0.1–0.2 mg/L.

In general, the water quality conditions in this study remained suitable to support the survival of tinfoil barb fingerlings. Although decreases in DO and increases in ammonia occurred after transportation, these changes were still within the tolerance limits of the fish.

Conclusion

The results of this study demonstrated that the administration of *Ageratum conyzoides* leaf extract significantly influenced the anesthetic response, physiological condition, water quality characteristics, and survival of tinfoil barb (*Barbonymus schwanenfeldii*) fingerlings during closed-system transportation. Increasing extract concentrations accelerated induction time, whereas recovery time tended to increase with increasing anesthetic concentration among the anesthetized treatments tested (2–5 mg L^{-1}). The 5 mg L^{-1} treatment produced the fastest induction time, while the lowest concentration tested, namely 2 mg L^{-1}, resulted in the shortest recovery time. Physiological indicators showed that fish receiving *A. conyzoides* extract experienced lower post-transport blood glucose levels than the control treatment, indicating reduced transportation stress, while hematocrit values returned to normal ranges after the recovery period. Furthermore, the administration of 4 mg L^{-1} extract produced the highest post-transport survival rate (93.3%) and post-rearing survival rate (90.0%), indicating that this concentration provided the most favorable balance between anesthetic effectiveness, physiological stability, and fish survival. Therefore, the research hypothesis was accepted, and *A. conyzoides* leaf extract can be considered a promising natural anesthetic agent for live transportation of tinfoil barb fingerlings, offering a more environmentally friendly and potentially economical alternative to synthetic anesthetics in aquaculture practices.

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Author Contributions

Research conceptualization, methodology development, experimental implementation, data collection, data analysis, and preparation of the initial manuscript draft, N.A.; research supervision, methodology validation, interpretation of research findings, and critical revision of the manuscript substance, E.D.; data processing and visualization, reference checking, manuscript editing, and manuscript finalization for publication, R.A. All authors have read and approved the final version of the manuscript and are responsible for the content of the article regarding the utilization of billygoat-weed leaf extract (Ageratum conyzoides) as an anesthetic agent for tinfoil barb (Barbonymus schwanenfeldii) fingerlings in a closed transportation system.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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