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Efficacy of Clove Oil (*Syzygium aromaticum*) as a Natural Anaesthetic at Various Doses on the Survival Rate of Fighting Fish (*Betta sp.*) During Closed Transport

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ABSTRACT

Fighting fish (*Betta sp.*) is a popular ornamental fish with high economic value, requiring special handling during maintenance, transportation, or medical procedures to prevent stress and injury. However, concerns about their negative impacts on human health, the environment, and potential residues in fishery products have led regulatory bodies to restrict or prohibit their use. In Indonesia, the Ministry of Marine Affairs and Fisheries through Regulation No. 1/PERMEN-KP/2019 has officially categorised MS-222 as a restricted veterinary pharmaceutical substance, thereby driving the aquaculture industry to seek safer and more sustainable alternatives. Therefore, a safe natural anaesthetic alternative is needed, such as clove oil containing eugenol. This research is important to study the effectiveness of clove oil as a natural anaesthetic for fighting fish, to provide a safe, effective, and environmentally friendly guide for cultivators and entrepreneurs. This research used a Completely Randomized Design (CRD) method with 4 different clove oil doses (0.1 ml, 0.2 ml, 0.3 ml, and 0.4 ml) and 6 replications, where each plastic contained 100 ml of water, 1 fighting fish, and oxygen, was sealed with a rubber band, placed in a styrofoam box, and simulated for an 8-hour journey. The results showed that the administration of different clove oil doses significantly affected the survival rate of fighting fish (*Betta sp.*) in closed transport ($p < 0.05$). The optimal dose identified was 0.1 ml per 100 ml of water, with the highest survival rate reaching 96.67%. This dose resulted in an induction time of 29.83 minutes, an anaesthesia duration of 265.00 minutes, and a recovery time of 25.17 minutes, and showed no negative impact on water quality parameters during transport.

INTRODUCTION

Indonesia, as an archipelagic nation with abundant biodiversity, possesses significant potential in the aquaculture sector, particularly in ornamental fish. According to the latest report from the Ministry of Marine Affairs and Fisheries (KKP), Indonesia's ornamental fish export value reached a two-decade record of USD 42.47 million in 2025 (KKP, 2025). This achievement solidifies Indonesia's position as the world's second-largest exporter of ornamental fish. One freshwater ornamental species with high economic value and enduring global popularity is the betta fish (*Betta sp.*). Data from Statistics Indonesia (BPS) indicates that betta fish rank as the third-largest cultivated ornamental fish commodity, with production volumes reaching 273.38 million head (BPS, 2025). Their unique morphology, stunning color variations, and a hobbyist ecosystem reaching 2.5 million people make this fish a primary pillar of both domestic and international markets (Hidayat, 2021; Priandini, 2019). The ever-increasing demand is driving an industrial transformation toward digitalization and strict standardization in breeding, cultivation, and medical handling to meet global market requirements.

In the context of maintenance and commercialisation, fighting fish often require special handling to minimise stress and prevent injury. One crucial technique, especially for long-distance transport, is anaesthesia or sedation. Sedation aims to calm the fish, reduce metabolic rate, and minimise oxygen consumption and metabolic waste production, thereby increasing survival during transport (Hidayah, 2017). The use of effective, safe, and environmentally friendly anaesthetics is a priority in the modern aquaculture industry.

Historically, various synthetic chemicals such as MS-222 (tricaine methanesulfonate) and quinaldine sulfate have been widely used as fish anaesthetics. However, concerns about their negative impacts on human health, the environment, and potential residues in fishery products have driven the search for safer and more sustainable alternatives (Ministry of Marine Affairs and Fisheries, 2019). Clove oil (Syzygium aromaticum), rich in the active compound eugenol, has emerged as a promising natural anaesthetic candidate. Eugenol is known to have local anaesthetic properties and can suppress metabolic rate and provide a sedative effect on fish (Midihatama et al., 2018; Mustafa, 2021). Several previous studies have shown the effectiveness of clove oil on various fish species, both for consumption and ornamental purposes (Mbuilima et al., 2024; Tanbiyaskur et al., 2024).

Despite its acknowledged potential, the application of clove oil as an anaesthetic for fighting fish, especially in the context of closed transport, requires further in-depth study. Closed transport, which involves packing fish in airtight containers with a limited oxygen supply, requires an anaesthetic that can maintain the fish's condition for a certain period without causing toxic effects or high mortality. Fluctuations in water quality parameters such as pH, temperature, dissolved oxygen, and ammonia are also crucial factors to consider during this transport process

The fighting fish (*Betta sp.*) is a high-economic-value aquaculture commodity that is highly susceptible to stress and mortality during long-distance transport. Conventional anaesthesia techniques using synthetic compounds such as MS-222 have been banned due to their toxicity to humans and the environment (Ministry of Marine Affairs and Fisheries, 2019), creating an urgent need for safe and sustainable anaesthetic alternatives. Previous studies have examined the potential of clove oil (Syzygium aromaticum) as a natural anaesthetic in various fish species such as tilapia and catfish (Mbuilima et al., 2024; Tanbiyaskur et al., 2024), with the main focus on induction and recovery efficacy. However, comprehensive studies on the optimal dose of clove oil specifically for fighting fish in closed transport systems—which involve unique water-quality dynamics such as fluctuations in dissolved oxygen and ammonia accumulation—remain very limited.

This study offers an integrative approach by examining not only conventional anaesthetic parameters (induction time, sedation duration, recovery), but also long-term physiological impacts through survival-rate analysis and water-quality changes during an 8-hour transport simulation. The novelty of the research lies in its holistic approach that combines pharmacological aspects of anaesthesia with the eco-physiology of closed-system transport for sensitive ornamental fish, as well as the determination of a species-specific dose that has not been optimised before. Specifically, this study aims to: (1) analyse the effect of different clove-oil doses on the survival rate of fighting fish; (2) determine the optimal dose for closed transport; (3) investigate its impact on water-quality parameters; and (4) provide evidence-based practical recommendations for industry stakeholders.

METHOD

Research Location and Time

This research was conducted in Amansari Village, Rengasdengklok District, Karawang Regency, West Java Province. The research was conducted from October 18 to October 25, 2025, with the main experimental phase (transport simulation) carried out on October 25, 2025. The preparation phase, including procurement and acclimatisation of fish for seven days prior to the experiment, was conducted from October 11 to October 17, 2025, ensuring that all fish were in healthy and standardised condition before treatment began.

Research Materials

a. Test Animals

The test animals used were fighting fish (*Betta sp.*) with an average length of 4 cm. Each fish was placed in a plastic bag containing 100 ml of water. The total number of fighting fish used in this research was 240 individuals, divided into 4 treatments with 6 replications each, resulting in a total of 24 experimental units.

b. Tools and Materials

The tools used in this research included plastic bags, styrofoam boxes, rubber bands, a thermometer, a stopwatch, a DO meter, a camera, writing instruments, adhesive tape, a syringe, and a ruler. The materials used included fighting fish, freshwater, and clove oil (Syzygium aromaticum).

Table 1. Tools Used

No	Tool Name	Purpose
1	Plastic bag	Container for fish packaging
2	Styrofoam box	Container for packaged fish shipment
3	Rubber band	To tie the plastic bag
4	Thermometer	Measure water temperature
5	Stopwatch	Measure induction, anaesthesia, and recovery times
6	DO Meter	Measure dissolved oxygen (DO) level
7	Camera	Research documentation tool
8	Writing tools	Record research results
9	Adhesive tape	Seal the styrofoam
10	Syringe	Measure clove oil dose
11	Ruler	Measure fish length

Table 2. Materials Used

No	Material Name	Purpose
1	Fighting Fish	Research subject
2	Water	Solvent medium and fish container
3	Clove Oil	Anaesthetic material

Research Design

This research used an experimental method with a Completely Randomized Design (CRD). There were 4 clove oil dose treatments and each treatment was repeated 6 times, resulting in a total of 24 experimental units. The minimum number of 6 replications was chosen based on Federer's calculation to ensure statistical validity ([$n \geq 6$]).

The applied treatments were as follows:

a. Treatment A (Dose 1): Clove oil 0.1 ml per 100 ml water.
b. Treatment B (Dose 2): Clove oil 0.2 ml per 100 ml water.
c. Treatment C (Dose 3): Clove oil 0.3 ml per 100 ml water.
d. Treatment D (Dose 4): Clove oil 0.4 ml per 100 ml water.

Research Procedure

a. Research Preparation

Preparation included:

1) Provision of styrofoam: Purchasing, cleaning, and drying styrofoam boxes.

2) Provision of fighting fish: Purchasing fighting fish from breeders, sorting by uniform size, and quarantining fish before packaging.
3) Preparation of clove oil solution: Purchasing clove oil, measuring the dose accurately using a syringe, and dissolving it in the packaging water medium.

b. Research Implementation

1) Preparing 240 plastic bags for packaging.
2) Preparing water media with clove oil doses according to the treatment, then measuring initial water quality (pH, temperature, DO).
3) Preparing styrofoam boxes as shipping containers.
4) Placing 1 fighting fish into each plastic bag containing 100 ml of treated anaesthetic water medium.
5) Adding oxygen to the plastic bag, then sealing it with a rubber band.
6) Placing the plastic bag containing the fish into the styrofoam box.
7) Simulating a closed journey for 8 hours.
8) After 8 hours, re-measuring water quality parameters (pH, temperature, DO, ammonia) and observing behaviour and calculating the fish survival rate.

Research Parameters

a. Effectiveness of Clove Oil: Assessing the effect of clove oil dose (0.1, 0.2, 0.3, 0.4 ml/100 ml water) on:
   1) Anaesthetic induction time.
   2) Duration of fish in anaesthetised state.
   3) Fish recovery time.
   4) Survival Rate (SR).
b. Water Quality: Measuring water quality parameters at the beginning and end of transport:
   1) pH (acidity).
   2) Temperature (°C).
   3) Dissolved Oxygen (DO, mg/L).
   4) Ammonia Level (ppm).

Calculation of Survival Rate

The survival rate was calculated using the following formula (Effendi, 1997):

$$SR = (Nt / No)\ \ 100\%$$

where:
SR = Survival Rate
Nt = Total number of fish alive at the end of the research
No = Total number of fish at the start of the research

Data Analysis

The obtained data were first tested for the assumptions required for parametric analysis. Normality of data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variance between treatment groups was evaluated using Levene's test. Data that met both assumptions ($p > 0.05$ for normality and homogeneity) were subsequently analysed using one-way analysis of variance (ANOVA) to determine the effect of treatments on the observed variables. If the ANOVA results showed a significant ($p < 0.05$) or very significant ($p < 0.01$) difference, it was followed by Tukey's HSD (Honestly Significant Difference) post-hoc test to determine which specific treatment groups differed significantly from each other. All statistical analyses were performed using IBM SPSS Statistics 26 software.

RESULT AND DISCUSSION

Behavioural Response of Fighting Fish After Transport

Table 3 presents an overview of the behavioural response of fighting fish after simulated closed transport with different clove oil doses. In general, a clear gradation of response is seen with increasing anaesthetic dose.

Table 3. Behavioural Response of Fighting Fish After Transport with Various Clove Oil Anaesthetic Doses

Behaviour	Dose 0.1 ml	Dose 0.2 ml	Dose 0.3 ml	Dose 0.4 ml
Swimming Pattern	Active swimming with normal orientation, able to move to the bottom and surface of the container.	Slow swimming with normal orientation, tending to stay at the bottom of the container.	Unstable swimming (listing), occasionally floating upside down towards the surface.	Passive floating on the surface with upside-down body orientation, showing no swimming effort.
Fin Condition & Movement	All fins (dorsal, anal, caudal, ventral) fully expanded and moving actively.	Fins expanded but movement is slow and limited, especially the ventral fin.	Most fins are clenched (not expanded), only pectoral fins move weakly.	All fins are tightly clenched to the body, no fin movement observed.
Gill Condition & Movement	Operculum opens and closes regularly (60-80 times/minute), gill colour bright red.	Operculum opens and closes slowly (40-50 times/minute), gill colour slightly pale.	Operculum movement is very infrequent and irregular (<20 times/minute), gill colour pale.	No operculum movement observed, gills tightly closed and whitish-pale in colour.
Visual Response	Quick response to movement outside the container, body colour bright and contrasting.	Slow response to visual stimuli, body colour slightly faded.	No response to visual stimuli, body colour very pale and unattractive.	No response to any stimuli, body colour dark/dull and excess mucus present.

Source: Researcher's observation (2026)

Based on the observations, the behavioral responses of fighting fish after transport showed a clear gradation corresponding to the increase in clove oil anaesthetic dose. In the low-dose treatments (0.1 and 0.2 ml), the fish exhibited the best condition with active swimming patterns and normal orientation, fully expanded fins, and regular opercular movement with bright gill colouration. Conversely, at high doses (0.3 and 0.4 ml), It is important to note that the interpretations of stress level and nervous system depression presented in this section are based exclusively on direct behavioural observations, as no physiological biomarkers (such as plasma cortisol, blood glucose, or haematocrit) were measured in this study. This represents a limitation of the current research design. Behavioural indicators — including swimming pattern, fin posture, opercular movement frequency, and visual response — were used as proxies for physiological status, an approach consistent with established ethological assessment methods for fish welfare (Braithwaite & Boulcott, 2007; Conte, 2004). The observed progression from active, normal behaviour at 0.1 ml to complete unresponsiveness and excess mucus production at 0.4 ml is consistent with the known dose-dependent CNS depression profile of eugenol reported by Inoue et al., (2003) and Anderson et al., (1997). Nevertheless, future studies should incorporate physiological measurements — particularly plasma cortisol and blood glucose as the primary stress biomarkers — to provide a more complete and mechanistically validated picture of the stress response. The recent study by Sintuprom et al., (2024), which measured blood chemistry and stress-related gene expression in Betta splendens during clove oil anaesthesia, provides a complementary physiological framework that supports the behavioural patterns observed here..

These findings are consistent with existing literature. In accordance with the study by Suratno et al., (2023), optimal anaesthetic recovery conditions were observed in the low-dose groups, where fish showed gradual recovery through body movement, active opercular activity, and response to external stimuli. In contrast, in the high-dose treatments (C: 0.3 ml and D: 0.4 ml), the fish exhibited severe physiological stress symptoms, characterized by unstable swimming patterns (upside-down floating), clenched fin posture, absence of gill movement, and pale gill colouration. According to Wibowo et al., (2022), these behavioural and physiological manifestations represent stress responses aimed at adapting to extreme environmental changes and restoring body homeostasis, which in the context of this study appeared to be significantly disrupted by the excessive anaesthetic effects of clove oil.

Anaesthetic Induction Time

Analysis of variance (ANOVA) showed that administering different clove oil doses had a very significant effect on the anaesthetic induction time in fighting fish (F = 89.794, p = 0.000). This means that different doses significantly affect the time required for the fish to reach an anaesthetised state.

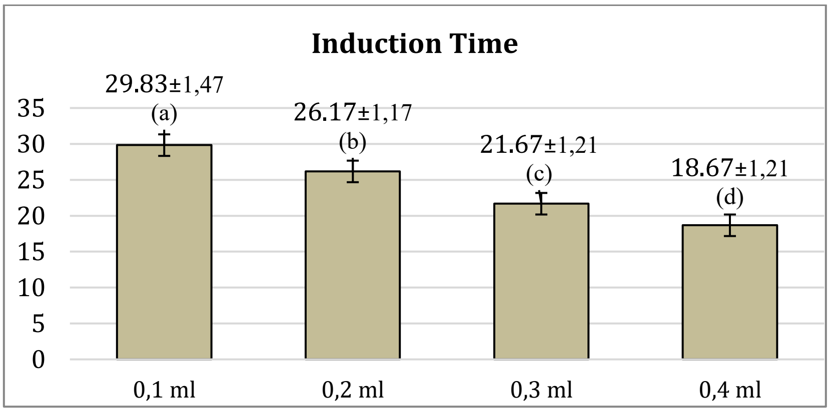

Figure 1. Advanced Test of Fighting Fish for Induction Time
Source: Processed data (2026)

Note: Different alphabetic notations (a, b, c, d) indicate significant differences between doses used at α = 5%

The Tukey HSD post-hoc test confirmed significant differences among all treatment pairs. The fastest induction time occurred at the 0.4 ml dose (average 18.67 minutes), followed by 0.3 ml (21.67 minutes), 0.2 ml (26.17 minutes), and longest at the 0.1 ml dose (29.83 minutes). The Tukey HSD post-hoc test confirmed significant differences among all treatment pairs. The fastest induction time occurred at the 0.4 ml dose (mean 18.67 ± 1.03 minutes), followed by 0.3 ml (21.67 ± 1.03 minutes), 0.2 ml (26.17 ± 1.47 minutes), and longest at the 0.1 ml dose (29.83 ± 1.33 minutes). This represents a dose-dependent reduction in induction time of approximately 37.5% from the lowest (0.1 ml) to the highest dose (0.4 ml). Specifically, each incremental doubling of dose reduced induction time by 11.6–17.4%, confirming a clear pharmacological dose–response relationship. This shows an inverse relationship between clove oil concentration and induction time: the higher the dose, the faster the fish reaches the anaesthetised condition (r = −0.97, indicating a strong negative correlation between dose and induction time).

The principle of anesthetic action indicates that higher concentrations of anesthetic agents enhance their penetration and distribution in the nervous system, thereby accelerating the onset of effect. Different doses of clove oil significantly affected induction time, where higher concentrations resulted in faster anesthesia in fish. This aligns with the study by Amris et al., (2020) stating that lower concentrations of clove oil lead to longer induction times, and is reinforced by the findings of Vercellini et al., (2023) regarding the efficacy of eugenol in achieving rapid anesthesia in fish. The lipophilic mechanism of clove oil enables rapid absorption through the gills and systemic distribution within the fish's body (Javahery et al., 2012), allowing doses of 0.3 ml and 0.4 ml to achieve induction times under 30 minutes.

Although eugenol's induction time at lower dosages (e.g., 0.1 ml) may not meet the preferred standard of achieving anesthesia in less than 3 minutes for certain aquaculture scenarios (Weber et al., 2009), studies indicate a consistent reduction in induction time with higher dosages, affirming the compound's utility as an efficient anesthetic (Silva et al., 2023). This consistent pattern of decreasing induction time with increasing dose aligns with findings in various other fish species, demonstrating that dose addition effectively accelerates anesthetic onset ((Fitri, 2020). Thus, while the very fast induction standard remains a challenge at lower doses, increasing the concentration of eugenol ensures its effectiveness as an anesthetic in aquaculture practices (Silva et al., 2023; Vercellini et al., 2023).

Anaesthesia Duration

ANOVA showed that clove oil dose had a very significant effect on the duration fighting fish remained anaesthetised (F = 161.792, p = 0.000).

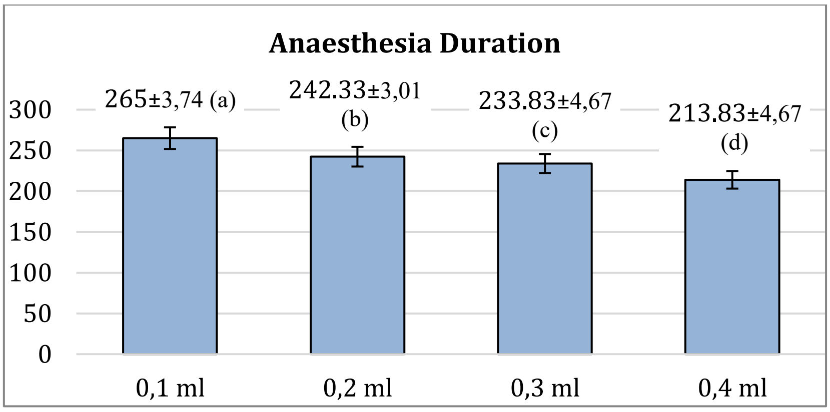

Figure 2. Advanced Test of Fighting Fish for Anaesthesia Duration
Source: Processed data (2026)

Note: Different alphabetic notations (a, b, c, d) indicate significant differences between doses used at α = 5%

The Tukey HSD post-hoc test showed that each dose resulted in a statistically different anaesthesia duration. The 0.4 ml dose resulted in the shortest anaesthesia duration (213.83 minutes), while the 0.1 ml dose resulted in the longest anaesthesia duration (265.00 minutes).

This observation indicates that administering higher doses of anesthetics not only accelerates the induction of anesthesia but may also shorten its overall duration. This behavior can be attributed to the physiological responses in fish at elevated anesthetic concentrations. Higher doses can stimulate a stress response that enhances metabolic activity, leading to the more rapid clearance of the anesthetic compounds from the system, although this acceleration may come with increased risks of toxicity (Rorig et al., 2023). Conversely, optimal doses, such as 0.1 ml and 0.2 ml, may yield a more controlled and prolonged anesthetic duration, reducing the likelihood of excessive physiological stress that could compromise the fish's survival (Ganjoor, 2021). Findings from studies emphasize the necessity of balancing dosage to minimize stress while maximizing anesthetic efficacy, supporting the rationale for utilizing moderate concentrations to ensure both optimal anesthesia and fish welfare in aquaculture practices (Ganjoor, 2021; Rorig et al., 2023).

Recovery Time

ANOVA results showed that administering different clove oil doses did not have a significant effect on the recovery time of fighting fish (F = 0.000, p = 1.000).

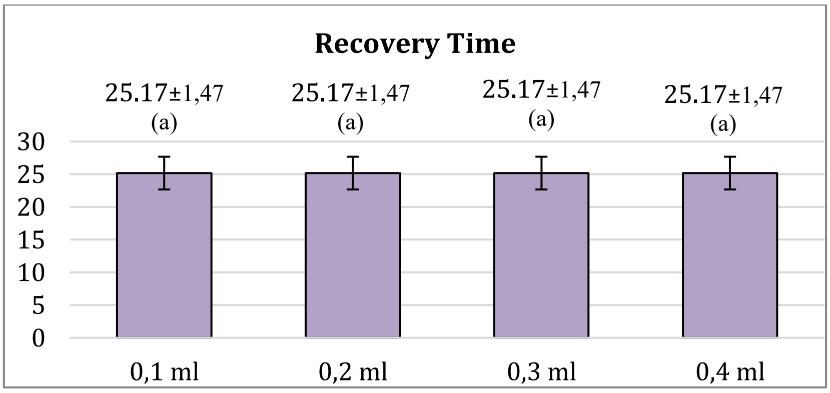

Figure 3. Advanced Test of Fighting Fish for Recovery Time
Source: Processed data (2026)

Note: The same alphabetic notation (a) indicates no significant difference between the doses used at α = 5%

The Tukey HSD post-hoc test confirmed the ANOVA findings, where all dose treatments were grouped into the same homogeneous subset. The mean recovery time for all treatments was identical, at 25.17 minutes, with a significance value of 1.000. These results indicate that variations in clove oil dose within the tested range (0.1 - 0.4 ml) did not produce statistical differences in the duration of fish recovery after the anesthetized period ended.

This consistent recovery capacity can be attributed to the pharmacokinetic properties of eugenol, the active compound in clove oil, which appears to facilitate similar elimination and metabolism processes across various doses (Wang et al., 2024)(Wang et al., 2024). Higher concentrations of eugenol did not significantly alter recovery time, indicating that the fish's metabolic response to the anesthetic remains stable, thus providing predictability in handling practices and protocols for aquaculture applications (Sintuprom et al., 2024). This predictability is further reinforced by recent findings emphasizing the beneficial balance that clove oil offers between effective anesthesia and optimal recovery, enhancing fish welfare during transport and handling procedures (Sintuprom et al., 2024; Wang et al., 2024).

Survival Rate (SR)

ANOVA showed that the administration of clove oil dose had a very significant effect on the survival rate of fighting fish (F = 166.263, p = 0.000).

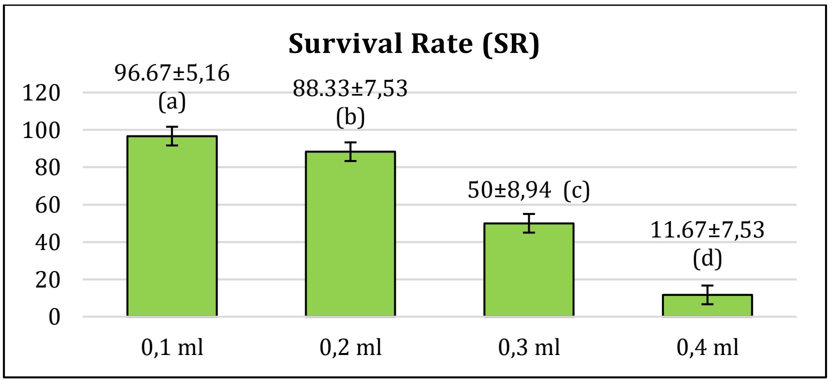

Figure 4. Advanced Test of Fighting Fish for Survival Rate (SR)
Source: Processed data (2026)

Note: Different alphabetic notations (a, b, c, d) indicate significant differences between doses used at α = 5%

The Tukey HSD post-hoc test showed that the 0.1 ml dose (96.67% SR) and the 0.2 ml dose (88.33% SR) were not significantly different from each other, but both were significantly different from the 0.3 ml dose (50.00% SR) and the 0.4 ml dose (11.67% SR). The 0.3 ml dose was also significantly different from the 0.4 ml dose. This pattern clearly shows that increasing the clove oil dose drastically reduces the survival rate, especially at doses of 0.3 ml and above, with the 0.1 ml dose producing the highest survival rate (96.67%; standard deviation 0.516), followed by the 0.2 ml dose (88.33%; standard deviation 0.753), the 0.3 ml dose (50.00%; standard deviation 0.894), and the lowest at the 0.4 ml dose (11.67%; standard deviation 0.753). These results are consistent with the study by Pellu et al., (2018) stating that the optimal dose of clove oil for fish transport is around 0.15%.

This inverse relationship between anesthetic dose and survival rate is a direct consequence of excessive physiological depression effects at high doses, supported by the pharmacological mechanism of eugenol as the main active component of clove oil. Although low doses (0.1-0.2 ml) provided adequate anesthetic effect with induction times of 26.17-29.83 minutes and anesthesia durations of 242.33-265.00 minutes without compromising survival, high doses (0.3-0.4 ml) exceeded the tolerance threshold, causing excessive physiological stress and high mortality. These results are consistent with studies by Nurkholifah et al., (2022) and Darmawati et al., (2021) showing the optimal limits of clove oil use in fish transport. The selection of the 0.1 ml dose proved most optimal in maintaining the balance between adequate anesthetic effect and high fish survival

Water Quality

a. pH

ANOVA showed that clove oil dose had a very significant effect on water pH at initial (F = 57.143, p = 0.000) and final (F = 82.733, p = 0.000) measurements.

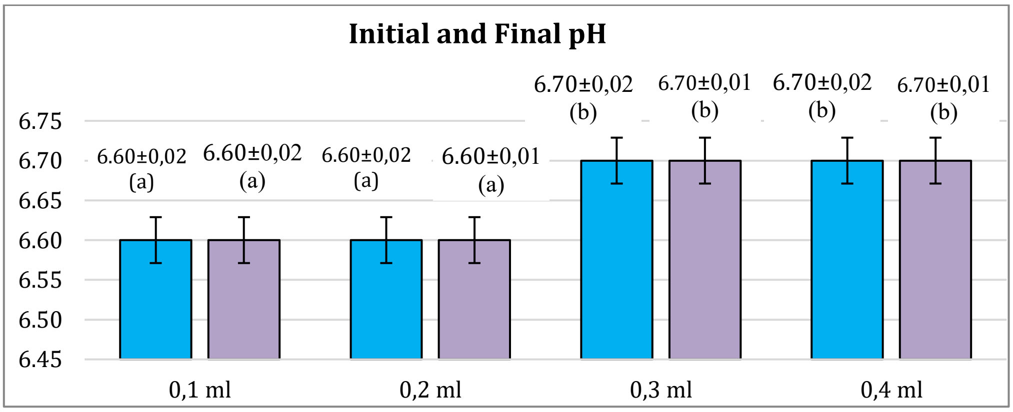

Figure 5. Advanced Test of Fighting Fish for Initial and Final pH
Source: Processed data (2026)

Note: Different alphabetic notations (a, b) indicate significant differences between doses used at α = 5%

The Tukey HSD post-hoc test revealed a consistent grouping pattern for initial and final pH. For initial pH, treatments were grouped into two homogeneous subsets: the first subset contained low doses (0.1 ml and 0.2 ml with mean pH 6.605) and the second subset contained high doses (0.3 ml and 0.4 ml with mean pH 6.705). The same pattern was observed for final pH, where low doses (0.1 ml and 0.2 ml) formed a subset with mean pH 6.595-6.598, while high doses (0.3 ml and 0.4 ml) formed a separate subset with mean pH 6.698. The results showed that water pH values during the study ranged from 6.595 to 6.705, which are still within the optimal tolerance limits for fish life according to Susanto (1986), namely pH 6.5-7.5, and Rofiq & Görgülü (2014) who stated that these values remain within the acceptable tolerance limits for fish. Although statistically significant differences existed between low and high dose groups, the absolute differences in pH values were very small (approximately 0.1 units) and remained within the normal range for fish maintenance.

According to Amri & Khairuman (2003), tilapia can live normally at water pH 6-7, so the small fluctuations observed in this study did not cause detrimental physiological effects and are unlikely to negatively impact fish health, which is crucial for ensuring their survival and welfare during anesthetic procedures (Uthirapathy, 2023). This pH stability indicates that clove oil at the various doses tested did not cause drastic changes in water acidity, unlike other organic materials that can lower pH through decomposition processes as described by Rismunandar (1986). This reinforces the advantage of clove oil as an anesthetic that is relatively stable with respect to essential water quality parameters during transport, as it does not cause drastic changes in water acidity, thereby maintaining the stability of the aquatic environment critical for fish during transport and handling (Sintuprom et al., 2024; Uthirapathy, 2023).

Temperature

ANOVA results showed that clove oil dose did not significantly affect initial temperature (F = 0.000, p = 1.000), but very significantly affected final temperature (F = 42.891, p = 0.000).

The Tukey HSD post-hoc test confirmed the ANOVA findings. For initial temperature, all dose treatments (0.1 ml to 0.4 ml) were grouped into one homogeneous subset with an identical mean of 27.05°C, indicating that initial temperatures were relatively uniform across all treatment groups. Conversely, for final temperature, treatments were grouped into two

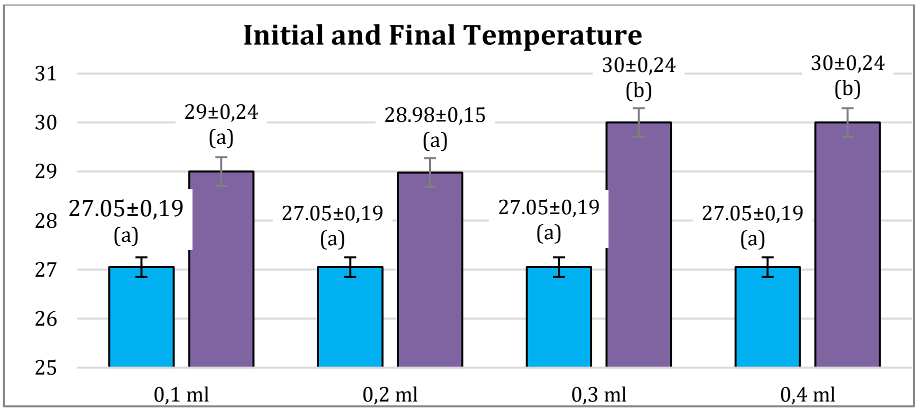

Figure 6. Advanced Test of Fighting Fish for Initial and Final Temperature
Source: Processed data (2026)

Note: Different alphabetic notations (a, b) indicate significant differences between doses used at α = 5%

significantly different subsets: the first subset contained low doses (0.1 ml and 0.2 ml with mean temperatures of 28.98-29.00°C) and the second subset contained high doses (0.3 ml and 0.4 ml with a mean temperature of 30.00°C), with a difference in final mean temperatures of approximately 1°C. Although the temperature increase at high doses was statistically significant, the temperature increase across all treatments remained within the optimal tolerance range for fish according to Effendi (2003), namely 25-32°C, and Hassan et al., (2021) who stated that these temperature variations remain within tolerable limits for fish.

As poikilothermic animals as described by Taufik et al. (2008), fish metabolism and endurance are highly dependent on environmental temperature, so fluctuations still within these tolerance limits do not cause significant negative impacts. The increase in final temperature, especially at high doses, is more likely caused by external factors such as sunlight intensity during the study which can increase the metabolic rate of anesthetized fish (Cunha et al., 2015), rather than the direct effect of clove oil itself, given the absence of a consistent relationship between anesthetic dose and changes in water temperature. Nevertheless, it is important to emphasize that significant temperature fluctuations can induce stress in fish, thereby impacting their overall welfare during handling and transport (Hassan et al., 2021). Therefore, careful monitoring of water temperature in conjunction with anesthetic administration is crucial to mitigate potential stress responses in fish. This indicates that clove oil as an anesthetic does not directly affect water temperature dynamics within the tested dose range, but external factors still need to be controlled to maintain aquatic environment stability.

Dissolved Oxygen (DO)

ANOVA results showed a very significant effect of clove oil dose on initial ($F = 157.143$, $p = 0.000$) and final ($F = 40.429$, $p = 0.000$) dissolved oxygen levels.

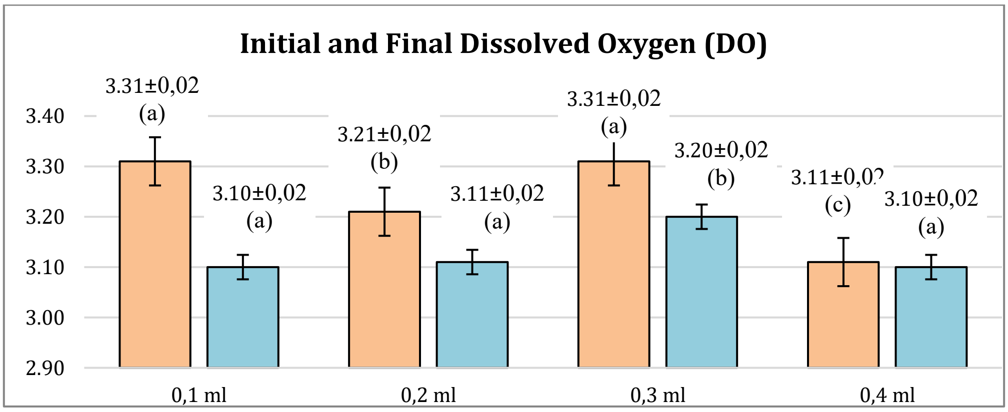

Figure 7. Advanced Test of Fighting Fish for Initial and Final Dissolved Oxygen (DO)
Source: Processed data (2026)

Note: Different alphabetic notations (a, b, c) indicate significant differences between doses used at α = 5%

The Tukey HSD post-hoc test revealed a specific grouping pattern. For initial DO, treatments were divided into three significantly different homogeneous subsets: the first subset contained the 0.4 ml dose (mean DO 3.105 mg/L), the second subset contained the 0.2 ml dose (mean DO 3.205 mg/L), and the third subset contained the 0.1 ml and 0.3 ml doses (mean DO 3.305 mg/L). For final DO, treatments were divided into two subsets: the first subset contained the 0.1 ml, 0.4 ml, and 0.2 ml doses (mean DO 3.095-3.105 mg/L) which were not significantly different, and the second subset contained the 0.3 ml dose (mean DO 3.195 mg/L) which was significantly different from the first subset.

A notable decline in DO was observed across all treatments following the transport phase, indicating oxygen consumption by the fish, as well as potential metabolic and decomposition processes (Sari et al., 2025). The dissolved oxygen concentrations observed across all treatments ranged from 3.095 to 3.305 mg/L throughout the 8-hour transport simulation. While these values are below the generally recommended threshold of ≥ 5 mg/L for optimal fish health in open aquaculture systems (Boyd, 1990; Effendi, 2003), they must be interpreted within the specific context of anaesthetised closed transport. Eugenol, the active compound in clove oil, is known to suppress metabolic rate and reduce oxygen consumption in anaesthetised fish (Javahery et al., 2012; Ross & Ross, 2008).

As a consequence, anaesthetised fish have significantly lower oxygen demand compared to non-anaesthetised fish under the same conditions. Based on the metabolic suppression reported by Midihatama et al., (2018) for eugenol-anaesthetised fish, the effective oxygen demand at these DO concentrations may be adequate to maintain the aerobic metabolism of sedated fish for the 8-hour duration. This is supported by the relatively high survival rates observed at lower doses (96.67% at 0.1 ml), suggesting that dissolved oxygen levels, although below standard open-system recommendations, did not constitute a lethal stressor within the experimental timeframe. However, these concentrations should be considered borderline, and supplementary oxygen addition is strongly recommended for transport exceeding 8 hours or in warmer ambient conditions (>30°C) to prevent hypoxia-related mortality.

Particularly, the most substantial drop in DO occurred in the 0.1 ml and 0.4 ml treatments, aligning with findings that emphasize increased oxygen consumption during anesthesia, especially as fish begin to recover in a closed environment (Malkawi et al., 2025). As expressed by Barus (2004), dissolved oxygen is a critical factor in aquatic ecosystems and is influenced by various factors including temperature. The decrease in DO in most treatments, especially at doses of 0.1 ml and 0.4 ml (from initial means of 3.305 mg/L and 3.105 mg/L to a final mean of 3.095 mg/L), is thought to be related to increased oxygen consumption by fish during the anesthetic process leading to anesthesia, as explained by (Aini et al., 2014).

However, the inconsistent pattern where the highest dose (0.4 ml) showed a relatively small decrease in DO, while the 0.3 ml dose actually maintained higher DO, indicates that the relationship between anesthetic dose and oxygen consumption is complex and may be influenced by other factors such as stress levels and metabolic activity of fish which differ at each dose. It is important to note that although the measured DO concentrations remained within acceptable limits for fish survival during the 8-hour transport period (ranging from 3.095 mg/L to 3.305 mg/L), longer transport durations would require oxygen supplementation or a reduction in fish density to mitigate potential stressors associated with low oxygen levels (Malkawi et al., 2025; Provenzani et al., 2025).

Ammonia

ANOVA results showed that clove oil dose did not have a significant effect on ammonia levels in the water medium ($F = 0.000$, $p = 1.000$).

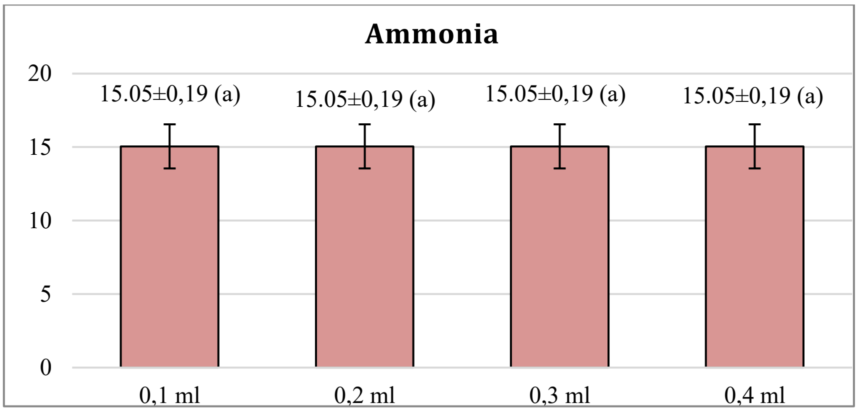

Figure 8. Advanced Test of Fighting Fish for Ammonia Level
Source: Processed data (2026)

Note: The same alphabetic notation (a) indicates no significant difference between the doses used at α = 5%

The Tukey HSD post-hoc test confirmed the findings from the ANOVA analysis, where all dose treatments (0.1 ml, 0.2 ml, 0.3 ml, and 0.4 ml) were grouped into the same homogeneous subset. The average total ammonia (NH_3 + NH_4^+) level for all treatments was 15.05 ppm. While this total ammonia value appears high, the toxic un-ionised ammonia fraction (NH_3) is strongly influenced by water pH and temperature (Boyd, 1990). At the measured water pH of 6.6–6.7 and temperature of 27–30°C, the proportion of un-ionised NH_3 is approximately 0.4–0.5% of total ammonia (Emerson et al., 1975). This yields an effective NH_3 concentration of approximately 0.06–0.08 mg/L, which is well below the threshold of 0.5 mg/L NH_3 considered harmful to most freshwater fish (EPA, 1999). Therefore, while total ammonia concentrations were elevated, the actual toxic fraction remained within acceptable limits for fish welfare during the 8-hour transport simulation. These results indicate that variations in clove oil dose within the tested range did not cause significant differences in ammonia accumulation in the water medium during the study, indicating that clove oil does not directly contribute to increased ammonia production (Meng et al., 2025).

The stable ammonia levels across all treatments demonstrate that the addition of clove oil at various doses did not trigger significant increases in ammonia production in the water medium during the study period. Although the measured ammonia value was relatively high (15.05 ppm) and above the optimal threshold, this increase in ammonia levels is likely the result of accumulated metabolic waste from the fish during the 8-hour transport period, compounded by the limited water volume (Zaimah & Zaman, 2024). According to Boyd's criteria in Lubis & Nasution (2002) stating that ammonia content of 0.62-2 ppm is still good for fish life, the values obtained in this study were above the optimal threshold.

However, based on the statement by Leagler in Rosyadi & Rasidi (2015) that ammonia levels up to 1.5 ppm can still be tolerated for fish farming, it should be noted that the conditions of closed transport system research have different dynamics from farming systems. The consistent ammonia concentrations indicate that clove oil does not significantly disrupt nitrification or decomposition processes within the experimental timeframe. The stability of ammonia levels across all treatments shows that clove oil does not contribute to water quality degradation through increased nitrogen load, although in its application, adequate water quality management systems need to be considered to maintain ammonia levels within safe limits for fish. However, for longer transport scenarios, effective water quality management, especially ammonia control, is vital to ensure the health and welfare of fish during transit (Alqarawy, 2019). Therefore, implementing strategies to mitigate ammonia accumulation, such as water aeration or reducing fish density, becomes essential for longer durations (Alqarawy, 2019).

CONCLUSION

This study demonstrates that different doses of clove oil (*Syzygium aromaticum*) have a significant effect on the survival rate of fighting fish (*Betta sp.)* during closed transport. Higher doses (0.3 ml and 0.4 ml per 100 ml water) caused a dramatic decline in survival rate to below 50% (50.00% and 11.67%, respectively), indicating that these concentrations exceed the physiological tolerance threshold of the species. In contrast, lower doses (0.1 ml and 0.2 ml) maintained high survival rates of 96.67% and 88.33%, respectively, demonstrating adequate anaesthetic efficacy without compromising fish welfare.

The optimal dose of clove oil for closed transport of fighting fish is 0.1 ml per 100 ml of water. This dose produced the highest survival rate (96.67%) with an adequate induction time of 29.83 minutes, a sufficient anaesthesia duration of 265.00 minutes, and a consistent recovery time of 25.17 minutes. Water quality parameters (pH, temperature, dissolved oxygen) remained within acceptable physiological tolerance ranges throughout the 8-hour transport simulation. Overall, clove oil at a dose of 0.1 ml/100 ml represents a safe, effective, and environmentally friendly anaesthetic alternative for the closed transport of fighting fish, with practical relevance for aquaculture practitioners and ornamental fish traders seeking to reduce transport-related mortality.

Future research should address the limitations identified in this study by incorporating direct physiological biomarkers — particularly plasma cortisol, blood glucose, and haematocrit — to more comprehensively characterise the stress response of fighting fish at different clove oil doses. Additionally, studies examining the optimal oxygen-to-water volume ratio in closed transport packaging, the effect of fish density on water quality dynamics, and the longevity of clove oil anaesthetic efficacy beyond the 8-hour window would provide more robust practical guidelines for the ornamental fish industry. The influence of fish age, body weight, and reproductive status on anaesthetic sensitivity also warrants further investigation, as the current study used fish of uniform size (approximately 4 cm) without distinguishing sex or physiological condition. Expanding this research to other commercially valuable *Betta sp.* variants (e.g., *Betta imbellis, Betta mahachaiensis*) and validating findings under actual commercial transport conditions would further strengthen the evidence base for clove oil as the anaesthetic of choice for fighting fish.

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