

ANESTHETIC EFFECTIVENESS OF CLOVE OIL (*SYZYGIUM AROMATICUM*) ON MANFISH (*PTEROPHYLLUM SCALARE*) JUVENILE HANDLING

Efektivitas Anestesi Minyak Cengkeh (*Syzygium Aromaticum*) Pada Penanganan Benih Ikan *Manfish* (*Pterophyllum Scalare*)

Zahrah Alifia Ghaida Anrose^{1*}, Junianto², Lantun Paradhita Dewanti²,
Fittrie Meyllianawaty Pratiwy²

¹Tropical Marine Fisheries Study Program, Padjadjaran University, ²Fisheries Study Program, Padjadjaran University

Bandung Sumedang Main Street KM.21, Jatinangor, West Java 45363

*Corresponding author: zahrahanrose01@gmail.com

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ABSTRACT

Clove oil (*Syzygium aromaticum*) has a primary component in the form of eugenol compounds, ranging from 72% to 90%, along with other active ingredients such as eugenol acetate, bicornin, and β -caryophyllene, each processing anesthetic, antiseptic, antioxidant, and antimicrobial properties. These natural anesthetic components are highly effective and safe for use even at low concentrations. The research aims to determine the effective concentration of clove oil in the closed transportation of manfish (*Pterophyllum scalare*) juveniles for 5 hours. The methodology employed in this research was experimental, utilizing a completely randomized design with four treatments: treatment A 0 ml/L as the control, treatment B 0.01 ml/L, treatment C 0.015 ml/L, and treatment D 0.025 ml/L with 5 repetitions. The parameters observed were survival rate post-transportation, survival rate during rearing, and water quality. Based on the result of the research, treatment B with a concentration of 0.01 ml/L is an effective concentration for closed transportation activities and produces the highest percentage of survival rate post-transportation which is $84\% \pm 5.48$. The survival rate at the time of rearing for 7 days with the application of 0.01 ml/L clove oil also produced a high percentage of $95\% \pm 6.85$.

Key words: Antimicrobial, Antioxidant, Closed-Transportation, Rearing, Survival Rate

ABSTRAK

Minyak cengkeh (*Syzygium aromaticum*) memiliki komponen utama berupa senyawa eugenol sebesar 72-90% dan kandungan aktif lainnya yaitu eugenol asetat, bicornin, dan β -caryophyllen yang memiliki sifat anestesi, antiseptik, antioksidan, serta antimikroba. Bahan anestesi alami ini sangat efektif dan aman digunakan pada konsentrasi yang rendah. Penelitian ini dilakukan untuk menentukan konsentrasi minyak cengkeh yang efektif dalam transportasi tertutup benih ikan manfish (*Pterophyllum scalare*) selama 5 jam. Metode dalam penelitian ini adalah eksperimental dengan menggunakan Rancangan Acak Lengkap 4 perlakuan yaitu

perlakuan A 0 ml/L sebagai kontrol, perlakuan B 0,01 ml/L, perlakuan C 0,015 ml/L, dan perlakuan D 0,025 ml/L dengan pengulangan sebanyak 5 kali. Parameter yang diamati adalah tingkat kelangsungan hidup pasca transportasi dan selama pemeliharaan serta kualitas air. Berdasarkan hasil penelitian, perlakuan B dengan konsentrasi 0,01 ml/L merupakan konsentrasi yang efektif untuk kegiatan transportasi tertutup dan menghasilkan persentase kelangsungan hidup tertinggi pasca transportasi yaitu $84\% \pm 5,48$. Nilai kelangsungan hidup pada saat pemeliharaan selama 7 hari dengan pemberian 0,01 ml/L minyak cengkeh juga menghasilkan persentase yang tinggi sebesar $95\% \pm 6,85$.

Kata Kunci: Antimikroba, Antioksidan, Kelangsungan Hidup, Pemeliharaan, Transportasi Tertutup

INTRODUCTION

Manfish (*Pterophyllum scalare*) is a variety of freshwater ornamental fish originating from the Amazon River, South America and has become a mainstay export commodity that has been successfully cultivated in Indonesia with the largest production value of 33,932,993 fish in West Java Province in 2021 (DKP West Java, 2021; Wiranata et al., 2022). Based on the West Java DKP (2021), Cirebon City is recorded as producing manfish with a figure of 4,068 fish per year 2021. However, cultivators face a major obstacle, namely the difficulty of obtaining a supply of seeds because distribution activities are still limited, even though market demand is increasing (Falatehan et al., 2023). The availability of seeds is a crucial role in the success of aquaculture activities (Junita & Utami, 2019). Therefore, selecting quality seeds is a determinant in the success of healthy fish production, so transportation activities are carried out so that the seeds that will be transported from the supplier in Bekasi City to the consumers in Cirebon City with a travel time of 5 hours arrive alive.

Transportation of live fish is classified into two, namely open and closed water media. Open transportation activities are suitable for short distances and short periods of time to avoid increased mortality because they use storage areas that are in direct contact with air and are watertight (Karnila et al., 2019). Based on observations, the death rate of manfish fry in open transportation is at a percentage of 32% which is caused by a decrease in water quality. Closed transportation can transport seeds at a higher density, so it can be applied in long-distance deliveries of more than three hours (Karnila et al., 2019). However, transportation with a closed system has problems, such as water that is difficult to replace, increased mucus and fish waste which results in high levels of ammonia, and lack of oxygen diffusion which can cause death (Karnila et al., 2019; Riesma et al., 2016).

Steps that can be taken to overcome this problem are by administering natural anesthetics such as clove oil which does not leave residue if used excessively and continuously. Cloves contain the main component eugenol of 72-90% and other active ingredients, namely eugenol acetate, bicornin, and β -caryophyllen which are stimulant, anesthetic, carminative, antiemetic, antiseptic, and antispasmodic (Wael et al., 2018; Wimadani, 2020). Concentration is influenced by age, species and size of fish, activity, and seasonal conditions (Amris et al., 2020). If the dose given is too high, it will cause symptoms such as anxiety, the respiratory system and motor nerves are disturbed, the ionic balance is unstable because the K^+ cation decreases and there is an increase in the number of Na^+ , $Fe^{(3+)}$, and Ca^+ cations. (Hasibuan, 2020; Saskia et al., 2013). This happened in the research of Saskia et al. (2013) when giving clove oil with a concentration of 0.01 ml/L and death occurred in the 48th hour and caused death in the hour and doses >1 ml/L experienced death in the 24th hour due to a decrease in respiration rate due to the mixing of water with anesthetic agent. This is the basis for research to determine the effective concentration of clove oil in closed transportation of manfish (*Pterophyllum scalare*) seeds for 5 hours.

METHODS

Place and Time

The research was carried out in August-September 2023 at Ely Fish Farm 2, Taman Rahayu Regency Cileungsi, Bantargebang District, Bekasi City.

Materials

The materials used include 200 manfish seeds that have gone through a selection process with a size of 2-3 cm with a stocking density that refers to SNI 7870:2013, namely 5 fish/L (National Standardization Agency, 2013), fresh water as a living medium. test sample with a volume of 2 L, and clove oil as a natural anesthetic obtained from the marketplace.

Tools

The equipment used in this research includes an aquarium for initial rearing, harvesting and post-transportation awareness, styrofoam boxes, polyethylene (PE) plastic for packaging seeds, a fine scoop that functions to collect seeds, a syringe as a tool for collecting clove oil, pH meter as a measure of acidity, thermometer as a tool for measuring water temperature, DO meter as a tool for measuring dissolved oxygen, and cars as a means of transportation.

Methods

The method in this research was experimental using a Completely Randomized Design (CRD) with 4 treatments and 5 repetitions according to Federer's formula. The clove oil concentration used was treatment A 0 ml/L as a control, treatment B 0.01 ml/L, treatment C 0.015 ml/L, and treatment D 0.025 ml/L. Observation parameters include survival rate after transportation and during maintenance as well as water quality (dissolved oxygen, pH and temperature).

The procedure in this study consisted of preparing test animals that had been acclimatized for 7 days, then acclimated for 24 hours. The next process is preparing the media with polyethylene plastic, then giving it clove oil according to the treatment, followed by measuring the water quality, placing 10 test fish, and distributing it for 5 hours after the plastic containing the seeds is put into a styrofoam box. In the post-transportation stage, water quality measurements are carried out again.

The data obtained will be analyzed statistically using the ANOVA test with a confidence level of 95% and the Duncan test if there are real differences between treatments. Then it will be modified into tabulation.

RESULT

Survival Rate

One indicator of the success of clove oil as a post-transport anesthetic is survival. In addition, this observation was carried out to measure the percentage of seeds that were alive after 7 days of cultivation at the end of the study. The observation results are presented in Figure 1.

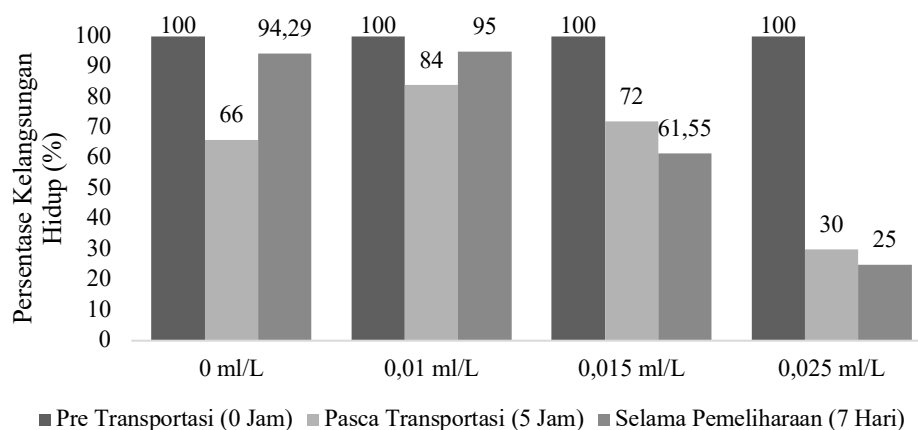


Fig 1. Graph of Manfish Survival Percentage During Research

Post-Transportation Survival Rates

The average survival value after transportation for 5 hours is seen in Table 1.

Table 1. Survival Rate of Manfish Seed Post-Transportation

Treatment (ml/L)	Average \pm StDev
A (0)	66 \pm 5,48 ^{bc}
B (0,01)	84 \pm 5,48 ^a
C (0,015)	72 \pm 8,37 ^b
D (0,025)	30 \pm 7,07 ^c

Information:

*Letters in the superscript that differ between treatments indicate significant differences according to Duncan's test (P<0.05).

Based on the test results in Table 1, it can be seen that treatment A (0 ml/L) did not show a significant difference from treatments C (0.015 ml/L) and D (0.025 ml/L). In treatment B (0.01 ml/L) there was a significant difference from treatments C (0.015 ml/L), A (0 ml/L), and D (0.025 ml/L). Meanwhile, treatment C (0.015 ml/L) was only significantly different from treatment D (0.025 ml/L). The lowest survival percentage occurred in treatment D, namely 30% \pm 7.07 with a concentration of 0.025 ml/L. This shows that the concentration of clove oil is inversely proportional to the survival rate. Survival decreased when given excessive concentrations of clove oil, so that the mortality rate of test fish seeds soared (Darmawati et al., 2021; Riesma et al., 2016). Post-transportation seed death can be seen in Figure 2.



Fig 2. Death of Manfish Seed Post-Transportation

Survival Rate During Rearing

The average survival value during 7 days of maintenance with N_0 , namely the number of samples after transportation, can be seen in:

Table 2. Survival Rate of Manfish Seeds During Rearing

Treatment (ml/L)	Average \pm StDev
A (0)	94,29 \pm 7,82 ^{ab}
B (0,01)	95 \pm 6,85 ^a
C (0,015)	61,55 \pm 8,33 ^b
D (0,025)	25 \pm 14,43 ^c

Information:
 *Letters in the superscript that differ between treatments indicate significant differences according to Duncan's test (P<0.05).

Based on the test results in Table 2, it can be seen that treatment A (0 ml/L) does not show a significant difference with treatments B (0.01 ml/L) and C (0.015 ml/L), but there is a significant difference with treatment D (0.025 ml/L). In treatment B (0.01 ml/L) there was a significant difference from treatments C (0.015 ml/L) and D (0.025 ml/L). Treatment C (0.015 ml/L) was significantly different from treatments B (0.01 ml/L) and D (0.025 ml/L). The lowest survival percentage was shown in treatment D, namely 25% \pm 14.43 and there was no significant change when compared with the average post-transportation survival of 30% \pm 7.07.

Water Quality

Water quality observations before and after transportation and during maintenance can be seen in Table 3.

Table 3. Water Quality During Research

Treatment (ml/L)	Range of Water Quality Parameter Values								
	Before Transportation			Post Transportation			During Maintenance		
	DO (mg/L)	pH	Temperature (°C)	DO (mg/L)	pH	Temperature (°C)	DO (mg/L)	pH	Temperature (°C)
A (0)	7,6-8,5	9,2-9,6	27,7-28,6	8,6-9,0	8,9-9,3	27,6-28,2	5,3-6,5	8,6-9,7	27,4-29,0
B (0,01)	7,9-8,4	9,0-9,3	27,5-28,7	9,0-9,4	9,0-9,2	27,7-28,4	5,8-6,5	8,7-9,7	27,6-29,5
C (0,015)	8,1-8,6	9,0-9,4	27,9-28,5	9,3-9,5	8,9-9,1	27,8-28,3	5,8-6,5	8,6-9,5	26,1-28,7
D (0,025)	7,9-8,3	9,0-9,5	27,9-28,7	8,9-9,5	9,0-9,2	27,9-28,5	5,7-6,5	8,7-9,5	27,5-29,3
References *	>5	6,5-7,5	25-30	>5	6,5-7,5	25-30	>5	6,5-7,5	25-30

(*) SNI 7870:2013

DISCUSSION

The low D treatment is caused by physiological changes, namely stressors arise which can trigger damage to body tissue and the ionic balance of the brain is disturbed due to the high dose of cloves and the seeds cannot tolerate the levels of eugenol compounds, and there is an increase in cortisol secretion and blood sugar levels (Firdaus et al. ., 2022; Saskia et al., 2013). Meanwhile, treatment A produced the second lowest survival value because the seeds were transported conscious and during transportation there was a shock which resulted in stress for the fish and changes in water quality.

The percentage of seed survival after transportation was not higher compared to Putra's (2016) research which used tawes fish (*Puntius javanicus*) measuring 5-7 cm with the lowest SR percentage, namely 66.6% after giving the highest concentration of 0.025 ml/L. Research by Riesma et al. (2016) produced the lowest SR value, namely 55.9% after Siamese catfish (*Pangasianodon hypophthalmus*) seeds after being given 0.02 ml/L which was the highest concentration, and Mikhsalmina et al. (2017) obtained the lowest SR, namely 3.22% after administering 0.03 ml/L clove oil to milkfish (*Chanos chanos*) seeds. The low survival value in this study was caused by the type and size of seeds used. This is confirmed by the fact that fish have a high absorption capacity for anesthetic agents due to their large body surface (Romaneli et al., 2018; Tarkhani et al., 2017). In addition, the large gill chamber size is more effective in absorbing anesthetics compared to small gill chambers (Amris et al., 2020).

In maintenance for 7 days after transportation, treatment D was the treatment with the lowest survival value. This occurs due to physiological changes caused by the condition of the test fish experiencing trauma, shock, stress, there is infection in the fish's body, the fry cannot tolerate the high eugenol content, and have difficulty adapting (Firdaus et al., 2022; Midihatama et al., 2018 ; Putra, 2016). Anesthetic agents fall into the residual and toxic category if they cause 50% mortality after anesthesia and maintenance (Ilhami et al., 2015). Based on Tarkhani et al. (2017), if excessive concentrations of clove oil are applied, hypoxaemia will occur and this will be followed by the emergence of the central nervous system. The use of clove oil in Table 2 does not have any toxic or residual effects on manfish seeds.

The survival rate of seeds during rearing was not higher than previous research conducted by Putra (2016) with the lowest percentage being 48% after tawes fish (*Puntius javanicus*) measuring 5-7 cm were given a concentration of 0.025 ml/L of clove oil and research by Midihatama et al. (2018) obtained the lowest survival value, namely 53.71% after administering a concentration of 0.006 ml/L to gourami fish seeds (*Osphronemus gourami*, Lac). The low survival in this study was caused by the high absorption capacity of eugenol on the body surface and gills (Tarkhani et al., 2017) of manfish fry.

Water quality data in Table 3 shows the lowest dissolved oxygen level was 5.3 mg/L during the post-transportation maintenance period. The low DO levels were caused because the aquarium had an aerator installed and there was increased competition when the seeds were removed from the test plastic to obtain oxygen, but the resulting DO content was lower (Wibowo et al., 2022). On the other hand, DO after transportation has a higher value, namely 9.5 mg/L because the water in the closed container experiences shock and can increase the diffusion of oxygen in the water (Nani et al., 2015). The decrease in DO occurred in previous research conducted by Putra (2016) in the range of 4-4.69 ml/L and decreased during the maintenance period to 4-4.5 mg/L.

The low value of acidity (pH) during the maintenance period, namely 8.6, is caused by the amount of carbon dioxide which tends to be absorbed by aquatic organisms, causing an H⁺ release reaction. Meanwhile, post-transportation pH had a low value due to the influence of anesthesia and increased excretion of the test fish, namely 8.9 (Riesma et al., 2016). Putra (2016) experienced a decrease in the degree of acidity. After transportation, the pH value is in

the range of 8-8.46. However, during the maintenance period the pH changes to 7-8. However, there has been an increase in research by Midihatama et al. (2018), namely post-transportation pH levels were 6.87-7 and increased to 7.63-8.04 during the maintenance period.

The maintenance temperature is 26.1°C and is the lowest temperature. This is related to the degree of acidity. According to Budiyanti & Romansyah (2016), high pH will cause a decrease in water temperature and the metabolism of the test fish. Meanwhile, the temperature in treatment A decreased significantly after transportation because the seeds were not given anesthesia, which caused an increase in ammonia and a reduction in metabolism. A decrease in water temperature occurred in Putra's research (2016). After transportation, the temperature was in the range of 26-28°C, but changed to lower during the maintenance period, namely 25-27°C. However, in research conducted by Midihatama et al. (2018) with the addition of clove oil anesthesia actually increased. After transportation, the temperature was in the range of 26.5-26.6°C and increased during the maintenance period to 26.6-29.9°C.

In accordance with SNI 7870:2013, optimum water quality for manfish cultivation and maintenance activities is DO >5 mg/L, pH 6.5-7.5, and water temperature 25-30°C (National Standardization Agency, 2013). Based on this data, dissolved oxygen and temperature can still be tolerated, while pH exceeds the threshold. However, Dwiputra et al. (2021) revealed that pH > 7 is still on the threshold of suitability and is not a bad thing for manfish because this fish can still live in extreme water temperatures and is alkaline in nature.

CONCLUSION

Providing clove oil with different concentrations had a significant effect on the survival of manfish fry after transportation and during rearing for 7 days. The effective concentration in closed transportation of manfish (*Pterophyllum scalare*) seeds for 5 hours was in treatment B with 0.01 ml/L clove oil and produced the highest survival percentage after transportation, namely 84% ± 5.48. The survival value during maintenance for 7 days with administration of 0.01 ml/L of clove oil also produced a high percentage of 95% ± 6.85.

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