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The clove oil effect on faunting time and survival rate of *Oreochromis niloticus*

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ABSTRACT

To overcome excess residues and fish mortality rates during transportation, natural ingredients, namely clove oil, can be used. Compounds in clove oil are considered safe because they use natural ingredients. The purpose of this study was to determine the effectiveness of using clove oil as an anesthetic agent with different doses on the length of time of unconsciousness and survival rate of tilapia. This research was conducted from May to June 2023. This research was carried out at the Bungus Fish Seed Center, Padang, and West Sumatera. The method used in this study is the experimental method. The design used in this study was a completely randomized design (CRD) consisting of 4 treatments and 3 replications. The treatment used in this study is use of clove oil with different doses. Treatment A1 = 0.5 ml L^{-1} water, A2 = 1 ml L^{-1} water, A3 = 1.5 ml L^{-1} water and A4 = 2 ml L^{-1} water. Observations of changes in tilapia behavior and water quality were analyzed descriptively, while the length of time of stupor and survival of tilapia obtained during the study were analyzed using analysis of variance (ANOVA). To see the differences between treatments, the Duncant Multiple Range Test (DMNRT) was performed. Data is processed using the SPSS 26 program. The conclusion that can be considered in this study is that the administration of clove oil with different doses as an anesthetic has a significant effect on the longest induction time, on the length of time the fish were unconscious, the length of time the fish regained consciousness and survival rate of tilapia. In treatment A2 with a dose of 1 mL L^{-1} of water, it was optimal to stun tilapia for 244 minutes with a survival of 88.89%.

Introduction

Tilapia fish (*Oreochromis niloticus*) which are of consumption size are widely demanded by consumers to be raised or re-cultivated as parents or for consumption, the distinctive taste and better texture of the meat make many people like it. The demand for tilapia in a live state is widely demanded by the public, one of the important reasons for trading live fish is to avoid the issue of using preservatives such as formalin which can endanger health. As well as for traders the price of live fish is higher than the price of dead fish (Palibu, 2019). According to Khalil *et al.*, (2019), the price of live fish is four times higher than the price of dead fish.

However, the obstacle that occurs in the process of transporting tilapia to reach consumers is the high mortality rate of fish. Especially the transportation of fish from the fish parlor to the cultivation location, and the harvest from the cultivation location to the consumer or buyer. Where the cause of death is stress and fish activity during handling and transportation (Cahyono, 2012). Tilapia consumption size with a weight of 150-300 g/head, already has a sharp and sharp dorsal fin, which is feared to injure each other and damage the transportation bag if the activity is excessive during transportation.

To prevent damage and decrease in the quality of the fish reaching the consumer, an immotilization technique (stunning) is carried out, which causes the fish to fall asleep and cannot feel. So that the level of activity and metabolism of fish decreases, and facilitates the process of transporting fish for a long time with low mortality. From previous research, information has been obtained that the

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transportation of fish in a live state can be carried out using both natural and artificial anesthetics (Khalil *et al*., 2013).

So far, fishery entrepreneurs use synthetic anesthetic MS-222 and several other anesthetic drugs which are relatively expensive and difficult to obtain. As well as the side effects of using chemicals that can be toxic, leaving residues and toxic substances that can harm fish and people who eat these fish and the environment (Wimadani, 2020). To overcome excess residues and fish mortality rates during transportation, natural ingredients, namely clove oil, can be used.

Plants contain a wide range of natural chemical compounds, such as flavonoids, alkaloids, polyphenols, polysaccharides, quinonoids, and saponins. Plant natural compounds typically exhibit a wide range of activities against insects, fungi, bacteria, and viruses (Akbar *et al*., 2023). Compounds in clove oil are considered safe because they use natural ingredients (Rahman *et al*., 2013). Apart from being cheap, clove oil is easily available in the market and the use of natural herbal ingredients is believed to be safer than artificial anesthetics. Clove oil (*Syzygium aromaticum*) contains essential oils both in flowers (10-20%), stalks (5-10%) and leaves (1-4%). It was further explained that clove oil has a large eugenol content which is antiseptic and anesthetic in quantity (70-80%) (Rahman *et al*., 2013).

The purpose of this study was to determine the effectiveness of using clove oil as an anesthetic agent with different doses on the length of time of unconsciousness and survival rate of tilapia.

Materials and Methods

Location and time of research

This research was conducted from May to June 2023. This research was carried out at the Bungus Fish Seed Center, Padang, and West Sumatera.

Methods and Research Design

The method used in this study is the experimental method. The design used in this study was a completely randomized design (CRD) consisting of 4 treatments and 3 replications. The treatment used in this study is use of clove oil with different doses. Treatment A1 = 0.5 ml L^{-1} water, A2 = 1 ml L^{-1} water, A3 = 1.5 ml L^{-1} water and A4 = 2 ml L^{-1} water.

Parameters observed

Fish Induction Time (minutes)

The length of time induction of fish was observed starting when the fish began to show anxiety, swimming to the surface, the movements began to slow down, the fish swam sideways, the operculum weakened, the fish began to calm down (less agile movements) and looked slow in responding to stimuli, until the fish began to look faint (no response). stimulation) is calculated in minutes (Darmawati *et al*., 2021).

Length of Time Fish Stun (minutes)

The length of time the fish fainted was observed starting when the fish began to faint showing the fish looked still, floating on the surface of the water, there was no response to external stimuli, the mouth and operculum moved very slowly until the fish began to appear conscious, counted in minutes (Khalil *et al*., 2013).

Length of Time for Fish to Reconsume (minutes)

The length of time the fish regained consciousness was observed starting when the fish had started moving again, starting to swim again with normal movements, namely the balance was stable, the direction of swimming was regular and it could respond to stimuli given quickly, calculated in minutes (Rahman *et al*, 2013).

Fish Survival Rate (%)

The percentage of survival (Survival Rate/SR) of fish was observed during the anesthetic process and after being anesthetized (maintained) for 14 days, observations were made every day, with the formula according to (Sari *et al*., 2017).

$$\mathbf{SR\ (\%) = \frac{Nt}{No} \times 100\ \%}$$

Notes :
Nt = Number of live fish at the end of rearing (tails)
No = Number of live fish at the start of rearing (tails).

Water Quality Parameters

Water quality parameters were measured before and after the addition of clove oil on the same day, which was done after the fish were fully aware, the observed were pH, DO, ammonia, temperature and turbidity.

Data analysis

Observations of changes in tilapia behavior and water quality were analyzed descriptively, while the length of time of stupor and survival of tilapia obtained during the study were analyzed using analysis of variance (ANOVA). To see the differences between treatments, the Duncant Multiple Range Test (DMNRT) was performed. Data is processed using the SPSS 26 program.

Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Volume 12, Number 3, Page 354-360

Results

The length of time the tilapia fainted was observed starting from the time the fish was put into a container containing clove oil until the fish lost consciousness; it was called the induction time. The results of the One Way Anova analysis show that the use of clove oil at different doses has a significant effect on the longest induction time. Duncan's further test showed that treatment A1 (0.5 mL L^{-1}) was significantly different from the other treatments and treatment A4 (2 mL L^{-1} was not significantly different from treatment C (1.5 mL L^{-1}).

The length of time for fish to faint (induction) during the study showed that in treatment A1 (0.5 mL L^{-1}) the average fish fainted within 2 minutes, this was slightly longer than treatment B (1mL L^{-1}) which was 1 minute 55 seconds, and in treatment A3 (1.5 mL L-1) the average induction time of fish was 1 minute 35 seconds, followed by treatment A4 (2 mL L^{-1}) which was 1 minute 25 seconds. From the results of research conducted on tilapia size ± 200 g/head which had previously been fasted for 24 hours, anesthetized using clove oil with different doses can be seen in Table 1.

Table1. Length of Fish Induction (minutes)

Treatments	Length of Induction Time (minutes)
A1	2.00 ± 0.006[a]
A2	1,55 ± 0.006[b]
A3	1,35 ± 0.127[c]
A4	1,25 ± 0.006[c]

Note: Different superscript letters in the same column indicate significantly different

The length of time the fish fainted was observed since the tilapia began to faint and was transferred to a concrete tub with water without clove oil, then left in the net until the fish appeared to be conscious, then the time was counted when the fish appeared to regain consciousness. The longest time the fish fainted was in treatment A2 with a dose (1 mL L^{-1}) which resulted in a fainting time of 244 minutes. Followed by treatment A3 (1.5 mL L^{-1}) 220 minutes, treatment A1 (0.5 mL L^{-1}) 150 minutes and treatment A4 (2 mL L^{-1}) tilapia fainted only 11 minutes. Based on the One Way Anova analysis, it was shown that the use of clove oil at different doses had a significant effect ($P<0.05$) on the length of time the fish were unconscious. Treatment A2 (1 mL L^{-1}) was significantly different from the other treatments, while treatment A4 (2 mL L^{-1}) was significantly different from treatment A3 (1.5 mL L^{-1}) and treatment A1 (0.5 mL L^{-1}). The results of the study of the length of time the fish fainted are presented in Table 2.

Table 2. Length of Time for Fish to Fain (minutes)

Treatments	Length of Time for Fish to Fain (minutes)
A1	150.33 ± 0.577[a]
A2	244.67 ± 0.577[b]
A3	220.33 ± 0.577[c]
A4	11.00 ± 1.732[d]

Note: Different superscript letters in the same column indicate significantly different

The length of time the fish regained consciousness was observed starting when the fish had started moving again until they quickly responded to the stimulus given (total awareness), by adding aeration. The results of observations on the length of time for tilapia to regain consciousness showed that in the lowest treatment, namely treatment A1 (0.5 mL L^{-1}), the average tilapia returned to consciousness with a length of time of 4.52 minutes, while for treatment A2 (1 mL L^{-1}) tilapia took an average of 7.45 minutes to regain consciousness, while treatment A3 (1.5 mL L^{-1}) tilapia returned to consciousness at an average time of 10.13 minutes.

The results of the One Way Anova test showed that the use of clove oil had a significant effect on the length of time the fish regained consciousness. Based on Duncan's further test, treatment A3 (1.5 mL L^{-1}) was significantly different from the other treatments and treatment A1 (0.5 mL L^{-1}) was significantly different from treatment A2 (1 mL L^{-1}). The length of time the fish regain consciousness is presented in Table 3.

Table 3. Length of time for fish to regain consciousness (minutes)

Treatments	Length of time for fish to regain consciousness (minutes)
A1	4.52 ± 0.017[a]
A2	7.45 ± 0.006[b]
A3	10.13 ± 0.006[c]
A4	0.00 ± 0.000[d]

Note: Different superscript letters in the same column indicate significantly different

The highest survival rate of fish was in treatment A2 (1 mL L^{-1}) with 88.89% survival followed by treatment A3 (1.5 mL L^{-1}) with 77.78% survival while in treatment A1 (0.5 mL L^{-1}) survival was 77.77% and

treatment A4 (2 mL L^{-1}) had the lowest survival. Based on the One Way Anova analysis, it showed that the use of clove oil at different doses had a significant effect on survival ($P<0.05$). The dose of clove oil given to treatment A4 (2 mL L^{-1}) was significantly different from treatment A2 (1.5 mL L^{-1}), A3 (1.5 mL L^{-1}) and treatment A1 (0.5 mL L^{-1}). Survival is the number of fish that can survive during the study. Fish survival was observed during the anesthetic process and after being anesthetized or kept under observation for 14 days. Table 4 follows the survival of tilapia during the anesthetic process using clove oil.

Table 4. Fish Survival (%)

Treatments	Length of Time for Fish to Fain (minutes)
A1	77.77 ± 19.249^{a}
A2	88.89 ± 19.249^{a}
A3	77.78 ± 38.429^{a}
A4	0.00 ± 0.000^{b}

Note: Different superscript letters in the same column indicate significantly different

Based on the test results, the water quality before and after the addition of clove oil was still within the acceptable threshold for the survival of tilapia, but the ammonia and turbidity tests increased.

The water quality observed included pH, DO, temperature, ammonia and turbidity. The results of water quality observations can be seen in table 5. Where the results of water quality showed that the average for each treatment before adding clove oil was pH around 7, DO reached around 5.42 mL L^{-1}, temperature was 25^{0}C, ammonia was around 0.020 and turbidity was around 1.12 NTUs. Meanwhile, the average water quality for each treatment after adding clove oil was pH around 6.9, DO 4.49 mg/L, temperature around 25^{0}C, ammonia reaching around 0.122 ppm and turbidity around 1.93 NTU.

Discussion

The fast induction time experienced by fish is because high doses of clove oil contain high anesthetic ingredients, causing more and more absorption into the respiratory tissues and resulting in the nervous system not functioning properly (Cahyono and Mulyani, 2012). The results of observations made at the time of induction of tilapia, the higher the dose of clove oil given, the faster the induction time.

The behavior of the fish due to the anesthetic effect that arises when the tilapia stun, namely the fish looks panicked, anxious, swims irregularly to the surface, the fish swims sideways, there is no response from external stimuli, and some fish stay in the container. This statement is in accordance with the opinion of Arlanda *et al.*, (2018) who said that the behavior of fish during stunning is decreased fish movement, decreased fish response to stimulation, slowed operculum movements and metabolic work systems and decreased respiration in the fish's body.

In the opinion of Rahayu and Supriyatna, (2012), the mechanism of stunning fish is through the gills, it enters the bloodstream quickly, the anesthetic agent works by inhibiting the performance of cytochrome enzymes, resulting in reduced supply of glucose and oxygen to the brain. With reduced oxygen supply to the brain that causes the fish to lose consciousness or faint.

The effect of clove oil doses on fish behavior varies, this depends on the fish's resistance to clove oil doses. Not all fish lose consciousness, balance and movement activity. The fish doesn't look like it's dead, but the fish is just calm on the bottom of the water, if given a shock in the form of tapping the surface water containing fish, the fish will swim but unbalance and return to stay at the bottom of the water. This is in accordance with the statement of Rahman *et al.*, (2013) which said that in each experimental treatment there was a difference in terms of the effect received by each fish, not all fish lost consciousness, there were some fish that remained silent and if given a stimulus the fish would move erratically and return to rest.

The use of clove oil in treatment A4 with a dose of 2 mL L^{-1} of water, the fish only had time to faint with an average time of 11 minutes, as seen from the movement of the fish before being put into the net, the fish operculum was still seen moving slowly, the mouth and fins moved weakly and at on average in the 11th minute the fish experience mass death, it is suspected that the fish are not able to withstand too high levels of clove oil. Edison *et al.*, (2017) stated that the length of time a fish faints depends on the resistance of the fish's body itself. The stronger the body's resistance of a fish, the faster the anesthetic can be neutralized or broken down from its body. This shows that tilapia in treatment D (2mL L^{-1}) were unable to withstand the dose of clove oil given, so they experienced mass death.

In contrast to treatment A3 with a dose of 1.5 mL L^{-1}, the fish were still able to withstand a dose of clove oil and could stun fish for 220 minutes, as well as the dose in treatment A2 (1 mL L^{-1}) could stun fish for 244 minutes, in contrast to treatment A1 at a lower dose of 0.5 mL L^{-1} water can only stun fish for 150 minutes. This study of the length of time of fainting was almost the same as the study conducted

by Khalil *et al.*, (2013) where the best dose of nutmeg oil was 1.1 ppm which could stun fish for 253 minutes, and this was not much different from the dose in treatment A2 of 1 ml. /L stupor time is 244 minutes.

At the highest dose, namely treatment A4 (2 mL L^{-1}), tilapia experienced mass death, where the operculum and fins of the fish had no movement. In a study by Edison *et al.*, (2017), said that the difference in the conscious recovery of fish depends on the immune system of the fish itself, the stronger the immune system of the fish, the faster the fish will recover consciously. From the results of the study it was found that the fastest time for the fish to wake up was in treatment A with a dose of 0.5 mL L^{-1} which only took 4.52 minutes, this was because the dose of clove oil used was less so that the substances that entered the fish's body can be neutralized quickly, in the opinion of Edison *et al.*, (2017), the higher the concentration of metabolites of the anesthetic extract that enters the fish's body, the longer it takes for the fish to neutralize these substances. At a dose of 1.5 mL L^{-1} it showed that the fish were awake in an average of 10.13 minutes, this took a long time compared to treatment A2 with a dose of 1 mL L^{-1} of water which required an average of 7.45 minutes. Whereas in treatment A4 with a dose of 2 mL L^{-1} of water, the tilapia fainted only 11 minutes and then experienced mass death.

The behavior of fish when sober was seen in treatment A2 (1 mL L^{-1}) where fish that were still alive showed signs of awareness, such as movements that although slow and irregular from the fins, operculum and base of the fish's tail, and began to show movement body swim slowly then active again. In treatments A3 (1.5 mL L^{-1}) and A (0.5 mL L^{-1}), the fish looked more panicked when they came to their senses when the water was clapped suddenly, where some of the fish looked restless swimming irregularly and some of the fish slowly showed move slowly. In treatment A4 (2 mL L^{-1}) all fish were collapsed, some fell to the bottom of the water and floated to the surface of the water. Visually, as seen in treatment A4, the fish's body looked still without the slightest movement.

The process of the fish returning to their senses with the addition of aeration aims to make the water contain enough oxygen for the total resuscitation process of the fish, because oxygen plays an important role in the process of resuscitation of fish. Farida, *et al.*, (2012) in Firdaus *et al.*, (2022) states that the conscious mechanism for fish after being anesthetized using an anesthetic is by giving water containing oxygen which will enter the fish's body through the gills, then into the bloodstream. The oxygen-containing water will clean the anesthetic material which will be released through the fish's sewer, so the fish that are starting to become conscious will be followed by increasing the fish's metabolism and increasing the need for oxygen.

The survival of tilapia in treatment A1 (0.5 mL L^{-1}) and treatment A3 (1.5 mL L^{-1}) was not different from treatment A2 (1 mL L^{-1}). Where treatment A2 survival was 88.89%, this was relatively good for survival in fish stunning compared to treatments A1 and A3 sequentially, namely in treatment A1 fish survival was only 77.77% and in treatment A3 77.78%. It is suspected that in treatment A1 the fish when stunned and awakened show excessive panic, so that the anesthetic effect when the fish recovers begins to disappear, whereas in treatment A3 with higher doses and the fish experience panic during stunning, resulting in excessive stress and the fish's body is unable to tolerate incoming anesthetic effect.

The survival rate of tilapia in treatment A4 (2 mL L^{-1}) was 0.00% or experienced mass death after being anesthetized and could only survive for approximately 11 minutes, this can be seen in the length of time the fish fainted, where the use of higher doses could caused the weak condition of the fish when stunning with clove oil. Tanbiyaskur *et al.*, (2018) stated that tuba root extract which is an anesthetic substance containing eugenol which remains in the fish's body will damage the performance of the organs so that the fish experience difficulty breathing and there is a balance in swimming patterns and eventually death. This is also related to the level of endurance of each fish. In this study the researchers used pure clove oil distilled from clove leaves, where clove leaves contain 1-4% essential oil, according to the statement of Rahman *et al.*, (2013) clove oil contains a large amount of eugenol (70-80%) which Has anesthetic and antiseptic properties, clove oil leaves contain 1-4% essential oil.

The survival of tilapia in treatment A1, A2, and A3 after anesthesia, after being reared for 14 days after anesthesia, did not experience death, this is presumably because the effect of clove oil has slightly reduced on the tilapia body, the reduced effect of clove oil on the tilapia body caused by water that continues to flow in the concrete tub where the maintenance is, so that oxygen and water continue to enter the maintenance container.

The quality of the water before and after the addition of clove oil did not change much and was still at the proper threshold for the survival of tilapia based on the quality standards of Government

Regulation Number 82 of 2001. After adding clove oil, the fish environment was different from usual, this caused the fish to experience discomfort and a little stress. which is characterized by frantic movements of swimming to the surface, thus requiring excess oxygen to survive, then there is a decrease in DO in each treatment compared to the previous one.

In a state of fish that is experiencing stress, the fish suddenly releases feces, which causes an increase in ammonia in the water, the increase in ammonia that occurs is still within the quality standard. With the feces excreted by fish into the water, it automatically increases turbidity, but is still within the quality standard limits. The temperature and pH in the water are still within normal limits for tilapia, this is still in accordance with the recommended quality standards. When the water temperature decreases, carbon dioxide will increase, so the pH will decrease, and vice versa.

Conclusion

The conclusion that can be considered in this study is that the administration of clove oil with different doses as an anesthetic has a significant effect on the longest induction time, on the length of time the fish were unconscious, the length of time the fish regained consciousness and survival rate of tilapia. In treatment B with a dose of 1 mL L^{-1} of water, it was optimal to stun tilapia for 244 minutes with a survival of 88.89%.

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