The effectiveness of clove oil as an alternative to cynide in catching coral trout

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Abstract. Live reef fish are fish that have important economic value and have a very high selling value for export. Unfortunately, the process of catching reef fish still uses cyanide anesthetic, causing various problems, including damage to fish and coral reefs. As an alternative, it is recommended to use clove oil. However, it is necessary to analyze the effectiveness of clove oil as a cyanide alternative. This study aims to analyze the effectiveness of clove oil in coral trout (*Plectropomus leopardus*). This study consisted of aquarium preparation, preparation of test animals, observing fish behavior before and after exposure to clove oil and analyzing the effect of clove oil on the response of fish behavior. The experimental design used was a completely randomized design (CRD) consisting of 5 concentration treatments with 3 repetitions. Fish were put into the aquarium and then sprayed with several different concentrations of clove oil (20, 30, 40, 50, 60 ppm) until the fish passed out. Furthermore, it is analyzed based on the criteria recommended by Marking & Myer (1985). The ANOVA test results with an error rate of 5% ($\alpha = 0.05$) indicated that the time to reach the affected stage in the coral trout did not differ significantly with different clove oil concentrations ($P > 0.05$). The same results were also obtained when the equilibrium was disturbed, when it reached the fainting stage and the recovery time of the coral trout , which showed that there was no significant difference in the different clove oil concentrations ($P > 0.05$). Based on the recommendation of Marking and Meyer (1985), all concentrations of clove oil used (20–60 ppm) have met the requirements as an ideal anesthetic, where the induction time is <15 minutes and the recovery time is <5 minutes. This shows that clove oil at a concentration of 20 - 60 ppm is effectively used in anesthetics for coral trout (*Plectropomus leopardus*).

1. Introduction

The increasing demand for live reef fish for exporting has boosted the efforts of fishermen to meet this demand. One way that is usually done is by sedating the fish so that the fish they produce can be exported alive, especially since the price offered is relatively higher than the fish sold dead.

An anesthetic substance that is often used in reef fishing is cyanide. However, the use of cyanide has been banned by the government in fishing because it can cause high mortality and fish damage, so that a lot of fish is wasted due to not meeting export criteria. Rubec (2001) stated that the mortality of ornamental fish due to fishing using cyanide ranges from 30-37% [1]. Mauli (2012) also states that the mortality of ornamental fish when shipping to exporters is between 24 - 51% due to poor handling [2].

In addition, fishing using cyanide has been shown to damage the coral reef ecosystem which is the habitat of these live fish so that it can change the ecological structure of the ecosystem.

Coral reef management is needed by finding an environmentally friendly alternative to cyanide for fishing, namely clove oil. The advantages of clove oil from other anesthetics are that the price is relatively cheaper, it is safe for fish and humans so that fish is safer for consumption, easy to use, can work even in lower concentrations, is natural, and more importantly it is easy to obtain because cloves are a commodity. quite high local level in Indonesia. According to Nurdjannah (2016), the fluctuation of clove yield from year to year causes an oversupply of cloves so it is necessary to increase the diversity of clove use and its by-products [3]. Clove oil can be made from clove waste, such as clove leaves, stems and roots.

In the application of clove oil as an environmentally friendly alternative to cyanide in coral reef ecosystems, various aspects need to be studied, including the effectiveness of using clove oil in catching consumption reef fish. Coral trout is one of the consumption reef fish which has important economic value. These fish usually hide in crevices in the reef and are very difficult to catch with ordinary fishing gear, so many fishermen use cyanide in the fishing process. Therefore, it is necessary to analyze the use of clove oil concentration which is the most effective in catching the coral trout . This study aims to analyze the effectiveness of clove oil in catching coral trout .

2. Materials and Methods

2.1. Preparation of Test Animals

The test animals used in this study were 150 fish that usually hide in coral reefs, namely the coral trout (*Plectropomus leopardus*), measuring 27-30 cm in length, as many as 150 individuals. The fish samples were obtained from the catch of fishermen. The experimental fish are collected in a storage tank 1 (one) week before the experiment is carried out, so that the fish can undergo a process of adjustment (acclimatization). During the acclimatization process, the fish are given natural food 2 (two) times a day and the storage tanks are aerated and circulated water at all times. Prior to feeding, the feed is carried out so that the remaining feed and metabolic waste do not interfere with the life of the fish. Before being used as test animals, fish are fasted.

2.2. Aquarium Preparation

The experimental pool consisted of a glass aquarium measuring 1.0 x 0.5 x 1.0 m with a glass thickness of 1.5 mm which was equipped with a sufficient aerator. The aquarium is filled with seawater directly from the waters (water quality measurements were carried out in each experiment). and the water quality was adjusted to the conditions in the field (temperature 9ºC; pH 7.8; salinity 30 mg/L. Before the experiment the eugenol content in clove oil was analyzed using the HPLC ion chromatography method. The clove oil used was dissolved in 95% ethanol first. at a ratio of 1: 5 [4]. Then divided into several treatment concentrations based on the results of previous studies (20, 30, 40, 50, 60, ppm).

2.3. Experimental Design

The experimental design used was a completely randomized design (CRD) consisting of 5 concentration treatments with 3 repetitions. Two aquariums were used, one each to observe induction time (affected, balance disturbed and fainting) and recovery time. Each aquarium is filled with sea water. One aquarium is an experimental aquarium and one aquarium is a recover aquarium. Two Video Cameras were prepared each on the left and right side of the aquarium to record the movement of the fish at each stage of the behavior. The sample fish were put into the experimental aquarium. Then sprayed with several different concentrations of clove oil (20, 30, 40, 50, 60 ppm) until the fish passed out. Furthermore, observing the stages of fish behavior after spraying clove oil (modified fish behavior stages from some research [5–8]. After the fish fainted (final stage of anesthesia), the fish were removed and transferred to the recovery aquarium (clean sea water not contaminated with clove oil) to undergo the recovery process and then recorded the fish's behavior again until the fish

recovered. time to reach several behavioral stages (affected, disturbed balance, fainting and recovering) based on the video recording of the camera. Then compare the movements (behavior) of the treated fish (fish exposed to clove oil with several concentrations) with the control fish to observe there whether or not there is a difference in behavior in the sample fish.

2.4. Data analysis

Data analysis using MS Excel and SPSS software. Data normality was tested by Kolmogorov-Smirnov Test and Shapiro-Wilk Test, while variance uniformity was tested by Levene's Test. The significance level test for data differences was carried out by using single factor Anova analysis. Further analysis (Post Hoc Test) with the Tukey HSD Test. The processed data is presented in the form of tables and graphs.

3. Results and Discussion

This research has produced data in the form of the effectiveness of clove oil as an alternative to cyanide in fishing for consumption reefs. Data on induction time and recovery time for coral trout (*Plectropomus leopardus*) can be seen in Figure 1. The ANOVA test results at an error level of 5% ($\alpha = 0.05$) on the fainting time of the coral trout showed that the time to reach the fainting stage of the coral trout was not significantly different at different clove oil concentrations ($P > 0.05$). This means that there is no difference in the induction time (time to fainting stage) of coral trout (*Plectropomus leopardus*) at all clove oil concentrations tested in this study. However, in the resulting graphic image, it can be seen that there is a decrease in the induction time of clove oil concentration from a concentration of 20 ppm to a concentration of 30 ppm.

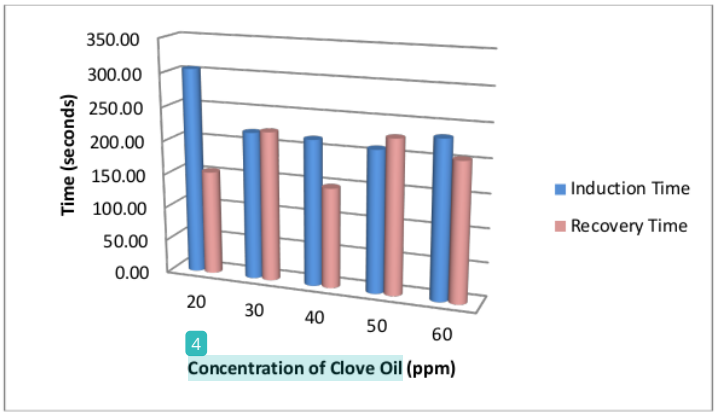

Figure 1. The mean time of induction and recovery time of coral trout after exposure to clove oil (n=3)

ANOVA test results at an error level of 5% ($\alpha = 0.05$) on the recovery time of coral trout showed that the treatment with different clove oil concentrations (20, 30, 40, 50 and 60 ppm) did not give significantly different results at recovery time. grouper fish ($P > 0.05$). The comparison of the time when the grouper began to reach the Affected Stage (TT), the Disturbed Equilibrium Stage (TKT), the Fainting Stage (TP) and the Recovery Stage (TR) can be seen in Figure 2.

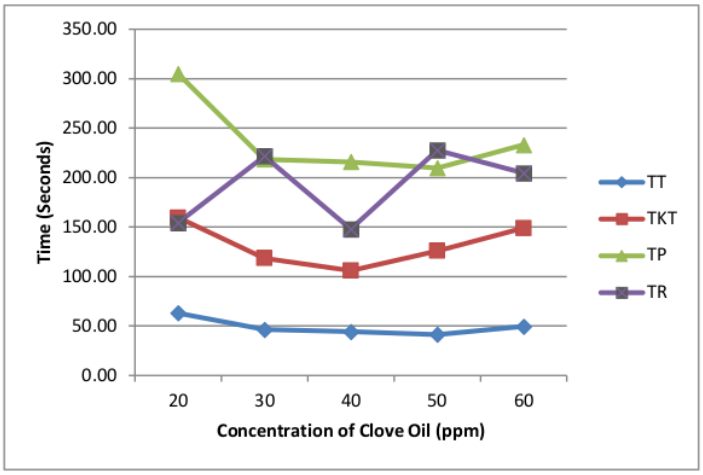

Figure 2. Comparison of the time the fish started to reach stage of affected (TT), stage of equilibrium was disturbed (TKT), stage of fainting (TP) and stage of recovery (TR) (n = 3).

The ANOVA test results with an error rate of 5% (α = 0.05) indicated that the time to reach the affected stage in the coral trout did not differ significantly with different clove oil concentrations (P> 0.05). The same results were also obtained when the equilibrium was disturbed, when it reached the fainting stage and the recovery time of the coral trout, which showed that there was no significant difference in different clove oil concentrations (P> 0.05).

Determination of the induction time of coral trout (*Plectropomus leopardus*) was carried out immediately after the fish was exposed to clove oil by spraying it until the fish could no longer move (fainted). The stages that occur before the fish faints (induced) are that the fish begin to be affected by accelerating their swimming movements but over time the swimming speed decreases (TT) then the fish will experience a balance disorder which is marked by the movement of the fish in erratic direction and the position of the body in the water is no longer normal (swimming in an upside down, vertical or tilted position), swimming speed also decreases at this stage (TKT) and in the end there is no more movement (TP). Meanwhile, the recovery time (TR) was calculated from the time the fish were introduced from the experimental aquarium to the recovery aquarium (the fish fainted) until the fish were swimming normally again.

Figure 1 shows that the higher the concentration of clove oil sprayed, the induction time for coral trout (*Plectropomus leopardus*) tends to be faster, where at a concentration of 20 ppm, the fish fainted after an average of 305 seconds, at a concentration of 30 ppm; 40 ppm and 50 ppm, the average fish fainted after 218.67 seconds, 216 seconds, 209.67 seconds. Whereas at a concentration of 60 ppm the fish fainted longer, which is an average of 233 seconds. However, based on the results of statistical tests, there was no significant difference in the time to fainting between several sprayed clove oil concentrations.

The time to reach the recovery stage for coral trout based on the results of statistical tests also showed that there was no significant difference in all concentrations of clove oil sprayed.The average recovery time was the fastest at a clove oil concentration of 40 ppm (148 seconds), while the longest recovery time was at a clove oil concentration of 50 ppm (227.67 seconds).

The higher the clove oil concentration, the faster the process of absorption of an anesthetic substance (eugenol) by the blood which then spreads to all parts of the fish's body. These substances will inhibit the formation of acetylcholinesterase thereby reducing the work of chemical mediators. As a result, the process of respiration and metabolism in fish has decreased. This will cause the fish to

pass out. Eugenol as the active substance of clove oil is a phenolic compound that has an alcohol group, which is an antiseptic substance that can weaken nerves and disrupt the nervous system [9].

According to Prasetyawati (1994), fainting conditions are unconscious conditions produced by a controlled process of the central nervous system which results in decreased sensitivity to external stimuli and low response to motion from these stimuli [10]. Fainting or numbness in fish means a poorly functioning nervous system. The process of anesthesia according to Wright and Hall (1961) [11] includes three stages:

1. Anesthetics move from the environment into the mouth of the organism's respiration.
2. Diffusion of membranes in the body which causes the absorption of anesthetics into the blood.
3. Blood circulation and tissue diffusion spread the substance throughout the body.

A decrease in induction time with increasing clove oil concentration has been observed in this study following the general pattern obtained in previous studies using clove oil or other anesthetic agents [4,8,12–18], albeit with different fish species. Cunha and Rosa (2006) has conducted research on 7 different types of coral reef fish in the tropics and showed that the induction time and recovery time of the 7 types of coral reef fish were different, but overall the induction time tended to be faster with increasing concentrations, on the contrary the recovery time [16]. tends to last longer with increasing clove oil concentration. The same thing was also reported by Griffiths who has conducted research on 8 different types of intertidal fish [15].

Induction time (fainting) and recovery time for each type of fish exposed to anesthetic agents such as clove oil were different, depending on the concentration of clove oil exposed and the fish species themselves. According to Gunn (2001), fish with large gill chambers are faster and more efficient in absorbing anesthetic substances. In addition, season, body size, activity, healthy fish, age and sex affect the induction rate of the anesthetic agent and its recovery process [19]. In this study, the factors affecting the induction speed and recovery process were negligible because only one species of the same size and the same time were used.

This study aims to determine the effectiveness of clove oil as an aid in catching coral trout s on coral reefs. Based on the ideal anesthesia recommended by Marking and Meyer (1985), the induction time is <15 minutes (750 seconds) and preferably <3 minutes (180 seconds) and recovery time <5 minutes (300 seconds) [20]. The data that has been obtained in this study indicate that all concentrations of clove oil used (20–60 ppm) have met the requirements as an ideal anesthetic, where the induction time is <15 minutes and the recovery time is <5 minutes. Research by [8] has stated that the most ideal concentration of clove oil for use on blue-yellow angelfish (*Centropyge bicolor*) is 50 ppm, where the induction time is 171.4 seconds (< 3 minutes) and recovery time 292.8 seconds (< 5 minutes).

Griffiths (2000) has analyzed the effects of clove oil on 8 species of intertidal fish in Australia and indicated that in general the most suitable concentration is 40 mg / L with induction time <180 seconds and recovery time <300 seconds [15]. Cunha and Rosa (2006) has also suggested a clove oil concentration of 20 ppm as a general guideline when anesthetizing reef fish and using higher concentrations in certain species [16]. It is also said that the lowest concentration is recommended (20 ppm) during field sampling to maximize safety and reduce mortality and stress in fish. Keene (1998) has recommended that the dose to reach stage 5 (fainting) in juvenile rainbow trout is 40 - 60 ppm of eugenol for 3 - 6 minutes [14].

These studies show that the induction time of clove oil is faster even at lower concentrations compared to other fish anesthetic agents that are known and most popularly used in several countries such as MS-222 and quinaldine [20]. Although the reported mean induction times and recovery times of clove oil were faster, they were sufficient to identify fish and record biological information [15].

The interesting thing that was observed in this study was that the movements of the fish were much calmer at each stage of anesthesia after the fish were exposed to clove oil until the fish finally passed out. In contrast to other anesthetic agents, such as cyanide, where immediately after cyanide exposure, fish will react more violently with a rebellious movement. Fish behavior after exposure to anesthetic agents will affect fish, both in the process of catching, handling and transporting fish. In this study, where the focus is on fishing objectives on coral reefs, the movement of fish after exposure to

and out of the gaps greatly affects the fishing process. If the exposed fish are calmer after coming out of the gaps, it will facilitate the catching process so that without fishing tools, the fish can be caught easily. It is different if the movements of the fish that are exposed are harder (rebelling, fluttering) after leaving the gully, making it more difficult to catch them by hand, so they need other tools in the catching process.

The same thing has also been reported by Munday and Wilson (1997) which states that fish exposed to clove oil showed a calmer movement than other anesthetic agents, such as quinaldine, MS-222, benzocaine and 2-phenoxyethanol, where anesthetic agents it is a very well known and popular anesthetic agent that is widely used to anesthetize fish [13]. However, using clove oil as a fishing aid is not only by looking at its effectiveness in inducing fish, but it is necessary to consider several factors, such as the condition of the target fish after fishing, non-target fish on coral reefs and the corals themselves.

Rahim *et al.* (2021) reported that exposure to clove oil at a concentrations of 20 ppm did not appear to have harmful effects on coral trout (*Plectropomus leopardus*) [21]. Research by Rahim *et al.* (2019) has also shown that juveniles of the clam *Tridacna derasa* did not experience a significant decline in quality (condition) after a short exposure to clove oil 20 – 70 ppm, such as might typically occur if clove oil was used in ornamental fishing on coral reefs [22]. Research on several types of corals has also shown that exposure to clove oil with certain concentrations does not cause changes in coral quality in *Porites lobata* [23], *Acropora formosa* [24], *Pocillopora damicornis* [25,26] and *Trachyphyllia geoffroy* [27].

The purpose of fishermen to perform fish anesthesia is to make the fish hiding in the gaps come out of their hiding places so that it will make it easier for fishermen in the fishing process. A number of fish will come out of the gully shortly after spraying an anesthetic agent into the gully because the fish are starting to be affected by the presence of an anesthetic agent.

According to interviews with several fishermen, they catch fish with cyanide in their actions usually using cleopatra nets installed around the target coral reefs and spraying cyanide into the coral crevices 2-4 times spraying then the fish will come out of the coral crevices. When coming out of the coral crevice, fishermen will use a fishing rod (a fishing aid) to catch fish because the fish after coming out of the reef will react strongly (revolt/float) away from the coral reef due to exposure to cyanide.

Rahim *et al.* (2015) have reported that the average time out of zebra fish from the gully is faster than the time it reaches the fainting stage and the equilibrium stage is disturbed, meaning that the zebra fish will exit the gully before reaching the Stun and Disturbed Equilibrium Stage [28]. Based on these data, it can be said that the fish will come out of the gaps when they reach the Affected Stage. These results can be used as a guide in determining the most effective concentration for removing fish from gaps. As it is known, the aim of fishermen to perform anesthesia in fishing is not to stun the fish, but to get the fish out of the gaps, so that the concentration needed to catch fish can be lower than the induction time (fainting) of the fish.

Based on the ideal anaesthesia recommended by Marking and Meyer (1985) that the induction time is <15 minutes, the ideal concentration of coral trout is 20 ppm where the average induction time at this concentration is <15 minutes [20]. Although concentrations higher than 20 ppm also have an induction time of <15 minutes, to determine the optimal (effective) time it is better to take the lowest concentration. This cannot be separated from the consideration of cost efficiency and the effect it could have on target fish, non-target and coral.

The results of this study indicated that the concentration of 20 ppm was the most effective concentration for fainting coral trout , where the induction time was <15 minutes and the recovery time <5 minutes. According to the aim of this research is to analyze the effectiveness of clove oil as a tool for fishing for consumption reef fish, so the concentration of 20 ppm is the most effective concentration, where as a fishing aid, clove oil is not an ingredient for stun fish, but only for making fish. fish out of the gaps so that they are easy to catch. However, this research has not been able to fully describe the optimal and most ideal concentration, especially in the application of clove oil as an

alternative to cyanide because this research is still on a laboratory scale. It is hoped that further research can be carried out directly in the field, especially on coral reef ecosystems.

4. Conclusions

Increasing the concentration of clove oil exposure in coral trout (*Plectropomus leopardus*) caused the induction time to tend to be faster. All concentrations of clove oil used (20–60 ppm) have met the requirements as an ideal anesthetic, where the induction time is <15 minutes and the recovery time is <5 minutes. However, the ideal/effective concentration of clove oil for use in fishing for coral trout (*Plectropomus leopardus*) is 20 ppm.

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