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5 Analysis of changes in coral trout (*Plectropomus leopardus*) morphology and tissue after exposure to clove oil

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Abstract. Clove oil has been shown to be effective as an alternative to cyanide in the catching of groupers on coral reefs. However, it is necessary to analyze the quality of fish after exposure prior to application in the field. The aim of this study was to analyze changes in morphology and tissues of the eyes, gills and liver of coral trout (*Plectropomus leopardus*) after exposure to clove oil. Experiments were conducted in an aquarium measuring 1.0 m x 0.5 m x 1.0 m filled with seawater. The fish were sprayed with several concentrations of clove oil (20, 30, 40, 50, 60 ppm) until stunned, then transferred into the tank for observation of external morphology and fish tissue. The results showed that exposure to clove oil at concentrations of 20-60 ppm had no effect on coral trout external morphology (eyes, skin, scales, fins and gill covers). Histologically, small changes were observed in the eye, gill and liver tissues starting at a concentration of 30 ppm. This study showed that exposure to clove oil at a concentrations of 20 ppm did not appear to have harmful effects on coral trout (*Plectropomus leopardus*).

1. Introduction

Live coral fish is an important economically valuable fish and has a very high selling value for export. Coral trout fish is one type of consumption fish that live on coral reefs and usually hide in crevices of coral. Because it is very difficult to catch with ordinary tools, fishermen use cyanide anesthetics in catching it to obtain live catches because the sale value of the fish is very high when sold alive. One of the requirements to meet export quality is that fish must be in a condition that lives up to the hands of consumers, there is no damage to the external morphology of fish, and is safe for human consumption. Unfortunately, the process of catching reef fish is still using cyanide anesthetics.

The use of cyanide in catching live fish has caused various problems including damage to coral reefs and damage to the fish itself. This results in many catches of fishermen being wasted because they are no longer suitable for export. Rubec (1988) state that fish affected by cyanide are quickly short of breath, even after being transferred to clean sea water, the fish caught experience physiological disorders, and as many as 80% die as a consequence [1]. Subandi N (2004) has found in fish evidence of cyanide fishing cases handled by the National Police Forensic Laboratory, experiencing abnormalities / damage to the skin, tips of the fins, and eyes [2]. To be able to meet export quality, this consumption fish must have good quality so that it can be exported alive and safe for human consumption. As an alternative to cyanide, it is recommended to use clove oil. The advantage of clove oil from other anesthetics is that the price is relatively cheaper, easy to use, can work even in lower concentrations, natural, and easily obtained because clove is a fairly high local commodity in Indonesia [3,4].

Clove oil is an ingredient that contains the active ingredient eugenol derived from the stems, flowers and leaves of the clove tree (*Eugenia aromatic*), which has been produced in Indonesia for centuries, usually used as a topical anesthetic for minor ailments such as toothaches [5]. Clove oil is also used as a highly effective fish anesthetic, known to cause rapid and calm immobilization [3,4]. In addition, fish do not require a withdrawal period after being exposed and after anesthesia, the fish will remain healthy (slimy) and not lose its appetite [6].

Previous studies have shown that clove oil is effective as an anesthetic agent in the capture of several species of fish [7–10]. However, to be able to meet export quality, this consumption fish must have good quality so that it can be exported alive and safe for public consumption. Therefore, clove oil as an alternative to cyanide still needs to be assessed, especially fish quality after capture. This study aims to observe the quality of consumption reef fish after being caught using clove oil.

2. Materials and Methods

2.1. Test Animal Preparation

The target fish used in this study were consumption fish that usually hide in coral reefs, namely the coral trout (*Plectropomus leopardus*), which is 27–30 cm long, as many as 150 individuals. Fish samples obtained from fishermen's catch. Fish samples were collected in a reservoir for one week before the experiment, so that the fish could carry out the acclimatization process. During the acclimatization process, the fish are given natural food twice 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 the remaining metabolic waste do not interfere with fish life. Before being used as a test animal, the fish were fasted for 8–10 hours.

2.2. Aquarium Preparation

The Experiment Aquarium consists of a glass aquarium measuring 1.0 m x 0.5 m x 1.0 m with a thickness of 1.5 mm, equipped with an aerator. The aquarium is filled with seawater directly from the waters and adapted to conditions in the field (temperature 9°C; pH 7.8; salinity 30 mg/L). The clove oil used is dissolved in 95% ethanol with a ratio of 1: 8 [11]. Then divided into several treatment concentrations based on the results of previous studies [7,8,12,13], namely 20, 30, 40, 50, 60 ppm. In this study, there was also a control, namely fish that were not exposed to clove oil. Before the experiment, the eugenol content in clove oil was analyzed using the HPLC ion chromatography method with a result of 83.058%. The fish sample size and water quality of the experimental container must be the same in all treatments.

2.3. Experimental Design

Experiments were conducted in an aquarium measuring 1.0 mx 0.5 m x 1.0 m filled with seawater. 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 stunned. After that it is transferred to a recovered aquarium (which contains clean seawater that was not contaminated with clove oil). After recovering, the fish were transferred to a storage tank for observation of changes in external morphology and fish tissue.

2.4. Observation of Changes in Fish External Morphological

Observation of fish external morphology was carried out after the experiment. Observation of external organs includes skin, scales, eyes, gill covers and fins. Observations were made every day, starting on day 1 to day 5 after exposure using a magnifying glass and digital camera. To find damage to the external organs of the fish, an observation was also carried out on the control fish (fish that were not exposed to clove oil) as a comparison. In addition, the external morphology of experimental fish was also compared with fish from cyanide exposure research conducted by [2], fish from cyanide fishing case evidence handled by the Indonesian Police Forensic Laboratory and fish caught by fishermen using cyanide.

2.5. Observation of Changes in Fish Tissues

Observation of fish tissue in this study was carried out after the 6th day. The fish tissues observed were eyes, gills and liver. The sample of the organs that has been taken is directly inserted into the plastic sample holder containing 10% formalin solution (the sample is totally immersed in the solution). Next, the sample holder is tightly closed, labeled and taken to the laboratory for histological analysis. In this condition, the sample can last a maximum of 1 x 24 hours and must then be processed in the laboratory. The stages of the histological analysis process in the laboratory include dehydration using methanol, paraffin infiltration, embedding, microtome incision, staining, microscopic observation. The staining used in this study was staining with hematoxylin-eosin. Then processed like other organs. This histological test was also carried out on the control fish organs. Determination of the tissue damage score in the eyes, gills and liver of the fish based on the type and extent of damage to the tissue. Changes in fish tissue due to exposure to clove oil were determined by comparing experimental fish tissue with control fish tissue.

2.6. Data analysis

Analysis of the data used is descriptive analysis, where data is presented in the form of figures and tables. To analyze the effect of clove oil on the quality of fish, by observing changes in external morphology (eyes, skin, scales, fins, and gill covers) and tissue changes (eyes, gills and liver) in fish exposed to clove oil at several concentrations, then comparing fish not exposed to clove oil (control fish).

3. Results

The quality of fish observed in this study was changes in external morphology and changes in fish tissue after exposure to clove oil. The observations showed that in all experimental fish there was no visible damage/abnormality in the external morphology, either on the skin, eyes, scales, fins or on the gill cover (Figure 1). All experimental fish were observed after exposure to clove oil until the 5th day in good condition, swimming normally and showing no signs of damage and abnormality, both of which were exposed to clove oil at concentration of 20 ppm to exposure to the highest concentration in this research, which is 60 ppm. No mortality was observed in all experimental fish until this study ended.

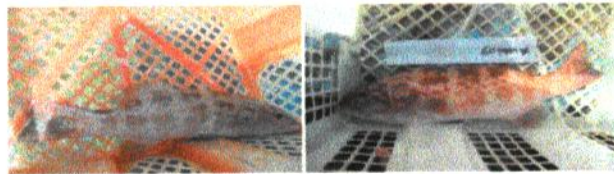


Figure 1. Photograph of coral trout after experiment (left: control fish; right: 60 ppm)

The quality of fish after exposure to clove oil observed in this study also took the form of tissue changes, including tissue changes in the eyes, gills and liver. Results of the analysis of tissues changes in coral trout after exposure (day 6 after exposure to clove oil) can be seen in Figure 2 (eye tissue), Figure 3 (gill tissue) and Figure 4 (liver tissue).

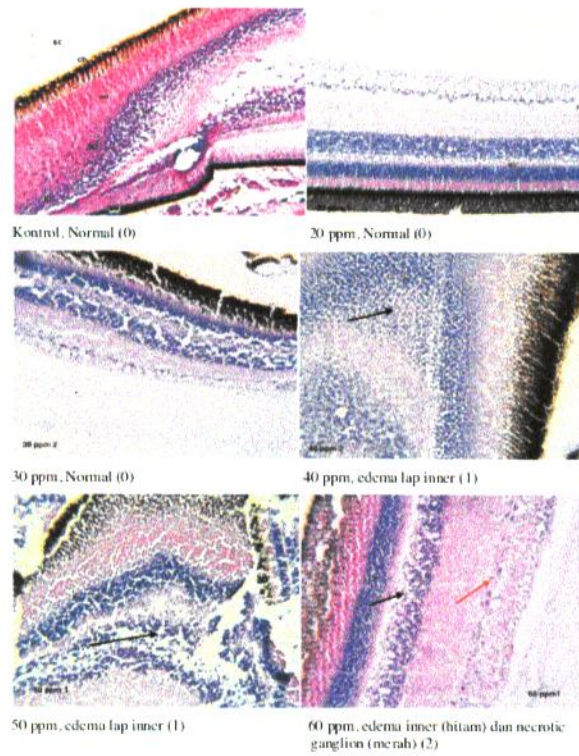


Figure 2. Changes in the tissue of a coral trout fish eye on the 6th day of observation after exposure to clove oil (Photo: 100 μm scale; arrows: abnormalities; number: damage score)

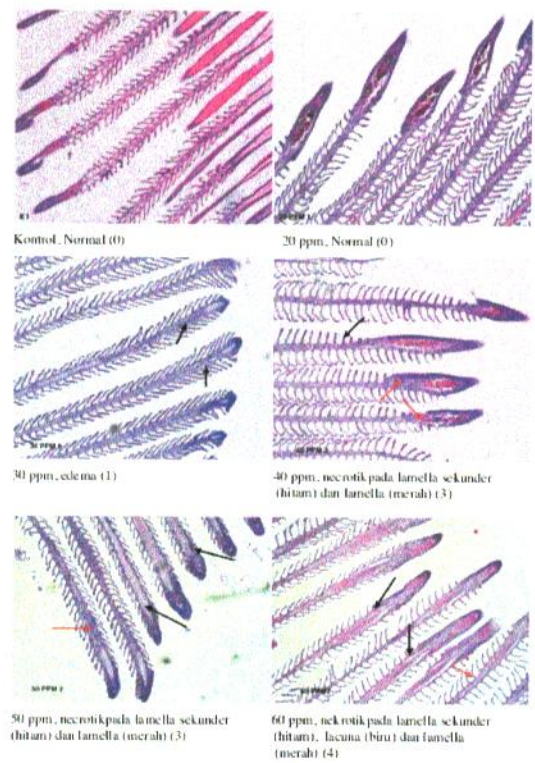


Figure 3. Changes in gill tissue of coral trout on the 6th day of observation after exposure to clove oil (Photo: 100 μ m scale; arrow: abnormalities; number: damage score)

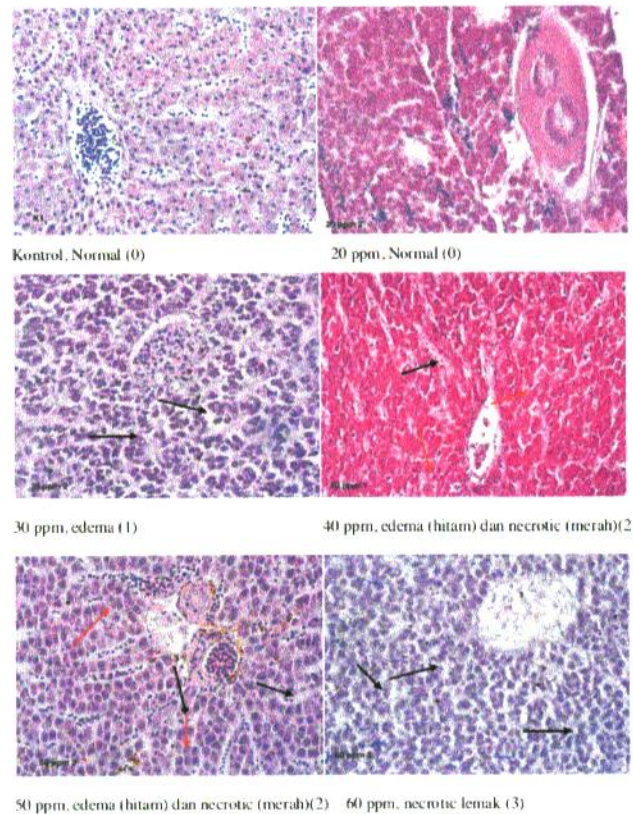


Figure 4. Changes in liver tissue of coral trout on the 6th day of observation after exposure to clove oil (Photo: $100\ \mu\text{m}$ scale; arrow: abnormalities; number: damage score)

4. Discussion

The quality of fish after exposure to clove oil can be observed by observing changes in morphology and tissue fish after exposure. This research has produced data in the form of the quality of coral trout fish after exposure to several concentrations of clove oil (20-60 ppm), namely changes in external morphology and fish tissue.

4.1. Changes in Fish External Morphological After Exposure to Clove Oil

The results of this study have shown that coral trout fish exposed to clove oil at concentrations of 20 - 60 ppm do not cause morphological changes (eyes, skin, scales, fins and gill covers) on these fish even at the highest concentrations of clove oil (60 ppm) fish still looked normal (Figure 1) and until this experiment ended no mortality was seen all fish samples observed.

Changes in the external morphology of the fish were observed every day after exposure to clove oil by observing the changes in the eyes, skin, scales, fins and gill covers. This change was observed based on the damage to the fish caught with cyanide, both in research fish, cyanide fishing case evidence handled by the Indonesian Police Forensic Laboratory as well as fish caught directly by fishermen using cyanide. Fish that were exposed to clove oil at several concentrations were then

compared with control fish (fish not exposed with clove oil). Visual observations showed that no changes were seen in fish exposed to clove oil (20 - 60 ppm), either on the skin, eyes, scales, fins or on the gill covers (Figure 1).

Observation made shortly after exposure to the 6th day, the fish still looked like the previous condition and there was no significant difference between experimental fish and control fish treated the same in the storage tank. Different results were found in the evidence of cyanide fishing cases handled by the Indonesian Police Forensic Laboratory, where the fish suffered damage such as Napoleon fish (*Chellinus undulatus*) which suffered damage to the skin and fin tips, grouper fish (*Epinephelus* spp.) who have damage to the eyes and skin and sunu fish (*Plectropomus* spp) which have damage to the skin [2]. Different results are also seen in the fish caught by fishermen who use cyanide in their catch. According to interviews with several fishermen, a lot of fish were wasted after 3-5 days of fishing because the fish had damaged the skin, some fish scales were loose, some fins were damaged, some of the fish had their gill covers off and even some fish had died. Therefore collectors do not buy them because they are not suitable for export. The fish that were damaged were finally thrown away by the fishermen and some of them were sold dead. The results of Subandi N (2004) research on grouper (*Epinephelus* spp) and sunu fish (*Plectropomus* spp) have also reported that the fish exposed to cyanide experienced a change in color on their skin to become darker shortly after exposure then a few days after that (day 3) the fish suffered damage to the skin on both sides of the body, damage to the tip of the fins, chest and tail and on the 7th day several fish died [2]. Some fish also showed clouding symptoms in the epidermal layer on the 3rd day and it got worse and on the 7th day some fish died. Symptoms of damage also occur in the gills which change color to cherry red, the gill covers appear blue shortly after exposure but eventually recover after 5 days. This shows that the use of clove oil to a concentration of 20 - 60 ppm still has not caused morphological changes (skin, eyes, scales, fins and gill cover) on coral trout fish.

4.2. Changes in Fish Tissue for Exposure to Clove Oil

Histological observations in this study were carried out to observe the effect of 1 mg clove oil on tissue changes in fish. According to Segner (1990), when the toxicant concentration is not sufficient to cause acute death, sublethal or adaptive changes can occur [14]. Histological analysis of the fish exposed to clove oil was carried out on the eyes, gills and liver. According to Vicki (2002), in fish exposed to toxic materials, external organs that are in direct contact with these materials, such as eyes, skin and gills, often experience cell damage/changes [15]. Apart from that, vital organs involved in detoxification mechanisms such as the liver, spleen and kidneys also experience the same thing. In this study, an analysis was carried out regarding the effect of sublethal clove oil exposure on the tissues and cells of vital organs of coral trout, both in direct contact with clove oil (gills and eyes), as well as the organs involved in the detoxification process (liver). Damage at the cellular level can occur in organs that are in direct contact with these toxic substances, as well as to cells of other organs that are not directly exposed but physiologically their mechanism of action is closely related to cells that are directly exposed. If the concentration of exposure to a toxic substance does not reach the lethal level of the target organism, then a cell damage/death or change which is an adjustment to the adverse effects of the toxic substance (sublethal effect) may occur [2].

Histopathological analysis of eye organs

The results of the histopathological analysis of the eye organs can be seen in Figure 2. Figure 2 shows that the eye tissue of the sunu grouper analyzed after day 6 looks normal, both in control fish and in fish exposed to clove oil with concentrations of 20 ppm and 30 ppm. This means that there are no visible signs of tissue damage at 20 and 30 ppm of clove oil exposure. However, at concentrations of 40 ppm and 50 ppm, it was seen that there was damage in the form of edema in the inner layer. Also at higher concentrations of 60 ppm there was damage in the form of necrosis. The results of this study are the same as the results of research conducted by Velišek J et al. (2005) who reported that the

exposure to clove oil at a concentration of 30 mg / L did not cause tissue changes in rainbow trout [16].

Histopathological analysis of gill organs

The results obtained in the histological analysis of gill organs in fish exposed to several concentrations of clove oil can be seen in Figure 3. The image of fish gill tissue observed on day 6 as shown in Figure 3 shows that in control fish and fish exposed to clove oil with a concentration of 20 ppm, there is no visible change in the tissue in the gills. Meanwhile, at a concentration of 30 ppm, there was a change in the form of edema, and at a concentration of 40 ppm, 50 ppm and 60 ppm, necrotic signs were observed. This means that clove oil at high concentrations can cause damage to the gill tissue of groupers. However, no damage has been seen (the fish still look normal) in the outer morphology as shown in Figure 1. This means that the fish may still be able to recover after being placed in clean water. In contrast to the results of research by Subandi N (2004) which showed eye damage that occurred in fish exposed to cyanide [2].

This research was conducted by exposing the clove oil until the fish is unconscious, while in the fishing process, the aim of the fishermen to spray an anesthetic agent is not to stun the fish, but only to get the fish out of its hiding place. Research Sri Wahyuni (2017) on kepe zebra fish that has exposed clove oil to coral reefs until the fish come out of the gaps has shown no damage to the eye tissue of the fish in all experimental samples (concentrations 20-70 ppm) [17].

Histopathological analysis of liver

The results of the analysis on the liver tissue of coral trout observed in Figure 4 showed that exposure to clove oil at a concentration of 20 ppm had not shown any changes in the liver tissue, which looked normal. Similar results were seen in control fish (which were not exposed to clove oil). Changes began to occur on exposure to clove oil at a concentration of 30 ppm, marked by edema in the inner layer of the liver tissue, but the damage was still very minor. Tissue changes were getting bigger with increasing clove oil concentration, where at a concentration of 40 ppm and 50 ppm edema and necrosis had occurred in the liver tissue of the fish. At the 60 ppm clove oil concentration, the changes that occurred were even greater, marked by the presence of fat necrosis in fish liver tissue with a moderate level of damage. The damage that occurred in the liver tissue of the fish in this study was still very mild to moderate so that the fish looked healthy and normal in external morphology and there were no signs of damage to the eyes, scales, fins, skin and gill covers based on observations that have been conducted in this study.

In this study also did not find any mortality in all fish samples. Based on this, it can be said that there is a possibility that fish with tissue damage can experience a recovery process if the level of damage is still very mild, mild and moderate. In this study, the histological analysis was limited to a recovery period of 6 days, so that in future studies it is expected to carry out a histological analysis with a longer recovery period to see whether there is a recovery process in fish exposed to clove oil after several days of experimentation.

The effect of clove oil on tissue changes in the eyes, gills and liver of fish showed that the fish exposed to clove oil at a concentration of 20 ppm had not been damaged in all experimental fish and control fish, while the eye tissue, liver and fish gills had been damaged starting in exposure to 30 ppm of clove oil. The higher the concentration of clove oil exposed, the more the level of damage. However, it is estimated that the fish can still undergo a recovery process if they are placed in clean waters for several days as evidenced by the fact that the fish look normal and there is no mortality in all fish samples.

Application of the use of clove oil as a tool in arrest

Clove oil is an anaesthetic agent used to anesthetize fish. In determining the effectiveness of using clove oil, there are several criteria that must be met based on the ideal criteria made by [18]. One of the criteria is easy handling and harmless to humans. The US Food and Drug Administration (FDA) has categorized clove oil as generally recognized as safe (GRAS) for use in dental cement and food

additives. It is also stated that clove oil is considered safe in small amounts (<1500 ppm) as a food additive (US FDA, 2007). Clove oil has also been used in several countries as a staple anaesthetic agent, including in Indonesia [36], a local analgesic and anaesthetic agent in dentistry [19].

Another criterion is that it has no persistent effect on fish physiology and behavior. Keene (1998) conducted observations of the mortality and abnormal behavior of rainbow trout and found that 2 weeks after the experimental observation period (eugenol exposure at a concentration of 20 - 140 ppm), there were no deaths and negative physiological effects on experimental fish. Clove oil is also rapidly excreted or metabolized, leaving no residue and requiring no withdrawal time. Clove oil is also known as a legal anesthetic agent that does not require a withdrawal period. The results of this study have shown that the coral trout after exposure to clove oil did not experience changes in external morphology in all treatments (concentration 20 - 60 ppm), be it on the eyes, skin, scales, gills and gill covers for 6 days of observation.

The advantages and disadvantages of clove oil as an anesthetic agent in fish have been analyzed by a number of researchers [4-6,20], and most have reported that clove oil is a safe and effective anesthetic [23]. Abdolazizi (2011) also stated that the use of clove extract at a dose of <400 ppm had no effect on sperm motility and hematological parameters in Prussian Carp (*Carassius gibelio*) [21].

This study has analyzed the histology of the eye, gill and liver organs where the results obtained have shown that the fish did not experience damage from exposure to clove oil at a concentration of 20 ppm. However, there has been damage to the tissue starting at a concentration of 30 ppm, although the level of damage is still very light to moderate. The tissue damage is still expected to recover, marked by no external morphological damage to all experimental fish and no mortality in all sample fish. The appearance of coral trout morphology on the eyes, skin, scales, fins and gill covers after exposure to clove oil gives good results (no damage) at all concentrations (20 - 60 ppm) is one of the considerations in its application. However, further research, particularly in the recovery of sample fish for histological analysis, is still needed in order to consider clove oil to be applied as an alternative to cyanide in fishing on coral reefs.

In this study, the concentration of 20 ppm is the recommended concentration for coral trout because this concentration is the lowest concentration in this study and is still effective as an anaesthetic agent in coral trout, where in determining the optimal 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, non-target fish and coral reefs.

7 Conclusion

Clove oil with a concentration of 20-60 ppm has not caused external morphological damage to the coral trout (*Plectropomus leopardus*). However, there has been a small change in the eye tissue, gills and liver of fish which begins with exposure to clove oil with a concentration of 30 ppm. Exposure to clove oil at a concentration of 20 ppm is still safe for coral trout (*Plectropomus leopardus*) and has not caused any harmful effects on the fish.

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