

# Application of Rosmery (*Rosmarinus officinalis*) Solution to Reduce Marine Leeches in “Cantang” Hybrid Grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*)

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**Abstract.** One common disease that attacks hybrid grouper is marine leech (*Zeylanicobdella* sp.). Therefore, it is necessary to take safe and environmentally friendly treatment steps, namely bathing fish infected with marine leeches with rosemary solution. This research examined the application of rosemary solution with different concentrations and bathing times to decrease the intensity of marine leech infections in "cantang" hybrid grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*). The experimental design of this research was a randomized factorial design (rosemary solution concentration factor and bathing time factor) with three replications. This experimental's treatments were a combination of rosemary solution concentrations (0, 7.5, and 15 mL/L) and bathing times (20, 40, and 60 minutes). The experiment showed that the interaction of concentration and bathing time of rosemary significantly eliminate the number of leeches in "cantang" hybrid grouper. The rosemary solution at concentrations of 15 mL/L and 7.5 mL/L significantly differed from the control; 15 mL/L was the best concentration for reducing leeches intensity, with an average intensity reduction of 29.91%. The best duration of bathing rosemary to decrease the intensity of leeches in "cantang" hybrid grouper is 60 minutes, with an average reduction in intensity of 19.21%. However, the concentration and duration of bathing in rosemary solution could not decrease the prevalence of leeches in "cantang" hybrid grouper. Bathing grouper fish in rosemary solution at a dose of 15 mL/L for 60 minutes is the best treatment to decrease the intensity of leech attacks on grouper fish by 44.85%. Bathing hybrid grouper with rosemary solution is one solution to decrease the intensity of marine leech attacks on fish farming.

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## 1 Introduction

The "Cantang" hybrid grouper is a fish that is a hybrid between a tiger grouper and a giant grouper and is a source of marine animal protein. Grouper hybrids have great cultivation potential because they grow faster than their relatives, namely the tiger and giant grouper. Grouper has high economic value and significant market demand. Grouper cultivation is increasingly intensive to meet market demand. Increasingly intensive aquaculture activities can increase the spread of disease in the aquaculture environment. This disease is caused by bacterial, viral, fungal or parasitic infections, which can be transmitted to hybrid grouper fish.

One common disease that attacks hybrid grouper hybrid grouper (*E. fuscoguttatus* x *E. lanceolatus*) is the Hirudinea marine leech (*Zeylanicobdella* sp.) which is an ectoparasite [1]. The Hirudinea marine leech attaches itself very tightly using suckers to its host's body and draws blood from its host (fish) as a source of nutrition. Fish attacked by marine leeches show clinical symptoms, namely slow movements and weak swimming on the surface or water column. Infection from this ectoparasite causes the fish's fins to dry and reddish, and wounds occur on the fish's skin [2]. The wound on the fish skin can be an entry point for secondary bacterial infections such as *Vibrio alginolyticus* bacteria [3], which can cause fish death. This infection can also cause fish to lose their appetite, so fish growth decreases. The infection rate of hybrid grouper (*E. fuscoguttatus* x *E. lanceolatus*), which infects hirudinea leeches in aquaculture locations, can reach 100% [4]. This leech infection can reduce consumers' interest in buying it.

This infection must be handled so that the leech infection does not spread to the aquaculture environment. Chemicals such as formalin and H<sub>2</sub>O<sub>2</sub> are commonly used to treat parasitic infections in fish [5]. However, using the formalin and H<sub>2</sub>O<sub>2</sub> will leave residue on the fish, so it is unsafe for fish eaters, and these chemicals are also dangerous for the farming environment. We have to find a solution to use ingredients that do not leave residue and are environmentally friendly, namely, herbal ingredients.

A herbal ingredient that has the potential to act as an antiparasitic in fish is rosemary. Bathing in a solution of rosemary leaves at a dose of 50 g/L is effective in reducing the intensity and prevalence of *Dactylogyrus* infecting carps and has also been proven to be safer than soaking in a solution of ethanol extract of rosemary leaves [6]. The compounds 1,8 Cineole,  $\beta$ -Pinene,  $\alpha$ -Pinene, and camphor in rosemary leaves have antihelminthic activity [6]. The potential of rosemary to control leeches in grouper infected with leeches has never been studied. This research was conducted to test the potential of rosemary as an anti-leech through immersion. Concentration and bathing time are things that need to be taken into account in the process of soaking fish, so it is necessary to test the concentration and soaking time of rosemary to control infection of marine leeches (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*).

## 2 Materials and Methods

### 2.1 Making rosemary solution

Rosemary are washed thoroughly with running water. After that, the rosemary are mixed with distilled water (1:1) and then blended. The blended solution was then filtered using filter paper. The rosemary solution is ready to be used for treatments.

## 2.2 Preparation of test fish

The test fish used in this study were “cantang” hybrid grouper infected by marine leeches (*Zeylanicobdella* sp.). The test fish were 10-13 cm in size and kept in floating net cages in Tembeling, Bintan.

## 2.3 Preparation of the test container

Fish infected by leeches in floating net cages were transferred to test containers. The test containers used were 27 round buckets with a capacity of 12 litres, which were then filled with 5 litres of seawater.

## 2.4 Immersion the test fish with rosemary solution

“Cantang” hybrid grouper infected by marine leeches are bathed in a rosemary solution with the concentration and duration of bathing depending on the treatment. The research design was a randomized factorial design with three replications, the factors being the differences concentrations of rosemary solution (0, 7.5, and 15 mL/L) and bathing times (20, 40, and 60 minutes).

## 2.5 Observation of the intensity and prevalence of leeches

Observation of leech intensity was carried out before and after immersion using the formula.

$$Int = \frac{\sum p}{N}$$

where:

Int = Intensity of leech attacks (leeches/fish)

$\sum p$  = Number of leeches that attack (leeches)

N = Number of test fish infected with leeches (fish)

Observation of leech prevalence was carried out after immersion using the formula.

$$Prev = \frac{n}{N} \times 100$$

where:

Prev = Prevalence of leech in fish (%)

n = Number of fish infected by leeches (fish)

N = Number of fish observed (fish)

## 2.6 Data analysis

Data on parasite intensity and leech prevalence in fish were analyzed using Two-Way ANOVA (Analysis of Variance) to see the effect of treatment. The Tukey test is carried out if the treatment has a significant impact. The analysis uses the SPSS IBM 25 application.

### 3 Results and Discussions

#### 3.1 Intensity of Leech

The effect of the concentration and bathing time of the rosemary solution on the percentage reduction in the intensity of sea leeches is shown in Table 1. After the analysis, it was found that there was an interaction between the concentration of the rosemary solution and the bathing time used in providing an influence on the percentage reduction in the number of the *Zeylanicobdella* sp. leech attack hybrid grouper. The best concentration of rosemary solution to decrease the percentage intensity of *Zeylanicobdella* sp. in hybrid grouper fish, namely 15 mL/L sea water, and this concentration was significantly different ( $P < 0.05$ ) with a concentration of 7.5 mL/L sea water and the control (without bathing in rosemary solution). Apart from that, the best bathing time for rosemary solution is to decrease the intensity of *Zeylanicobdella* sp. in the hybrid grouper; it was 60 minutes, which was significantly different ( $P < 0.05$ ) from the bathing time of 20 minutes and 40 minutes. Combining a rosemary solution concentration of 15 mL/L and a bathing time of 60 minutes decreased the leech intensity by 44.85% in the hybrid grouper and was the best treatment.

**Table 1.** Percentage reduction in leech intensity after immersion fish in rosemary solution.

| Bathing Time (minutes)                                 | Concentration of rosemary leaves (mL/L) |                   |                    | Average of percentage reduction in leech intensity (%) |
|--------------------------------------------------------|-----------------------------------------|-------------------|--------------------|--------------------------------------------------------|
|                                                        | 0                                       | 7.5               | 15                 |                                                        |
| 20                                                     | 0.00                                    | 4.08              | 11.25              | 5.11 <sup>a</sup>                                      |
| 40                                                     | 0.00                                    | 9.90              | 33.65              | 14.51 <sup>b</sup>                                     |
| 60                                                     | 0.00                                    | 12.79             | 44.85              | 19.21 <sup>c</sup>                                     |
| Average of percentage reduction in leech intensity (%) | 0.00 <sup>a</sup>                       | 8.92 <sup>b</sup> | 29.91 <sup>c</sup> | (+)                                                    |

Note: Different letter in the superscript showed the significant different between the treatment ( $P < 0.05$ ).

(+) : significant different between concentration and bathing time ( $P > 0.05$ ).

The rosemary solution can decrease the intensity of marine leeches infecting the "cantang" hybrid grouper because the rosemary solution contains ingredients that are toxic to marine leeches. Rosemary extract contains active components such as terpenes like 1,8-Cineole and  $\beta$ -Pinene, which have anti-monogenic properties that infect fish [6]. However, the active ingredient with anti-leech properties in the rosemary solution is unknown. Further research must be conducted on the active anti-leech ingredients in rosemary solution.

Rosemary solution has the potential as an anti-leech. Soaking fish infected with leeches in rosemary solution at a concentration of 15 mL/L for 60 minutes can decrease the intensity of marine leeches by up to 44.85%. The higher the concentration and the longer the immersion time in the research, the better it is for reducing the intensity of marine leeches. Therefore, it is necessary to conduct further research regarding adding a more optimal concentration and immersion time to decrease the intensity of marine leeches on fish.

In previous research, grouper infected with marine leeches soaked in seawater with the addition of garlic flour at a concentration of 0.75 g/L for 60 minutes was only able to decrease the intensity of marine leeches by 18.45% [6]. Aqueous rosemary extract at a dose of 100 g/L for 30 minutes decreased the intensity of *Dactylogyrus minutus* in carps compared to water immersion [6]. Apart from its anti-leech properties, rosemary can also increase immunity in fish; the essential oil in rosemary used in feed can improve haematological parameters and increase the young beluga's immunity [7]. Bathing rosemary to control sea leech infections in grouper fish can be considered.

### 3.2 Prevalence of Leech in Fish

The effect of bathing in rosemary solution on the leech prevalence in fish is shown in Table 2. The interaction between rosemary concentration and bathing time did not affect the prevalence of marine leeches in hybrid grouper. The prevalence of leeches in hybrid grouper was still 100% in all treatments after bathing with or without rosemary solution.

**Table 2.** Prevalence of leeches in fish after bathing fish in rosemary solution.

| Bathing Time (minutes)             | Concentration of rosemary leaves (mL/L) |     |     | Average of prevalence of leech (%) |
|------------------------------------|-----------------------------------------|-----|-----|------------------------------------|
|                                    | 0                                       | 7.5 | 15  |                                    |
| 20                                 | 100                                     | 100 | 100 | 100                                |
| 40                                 | 100                                     | 100 | 100 | 100                                |
| 60                                 | 100                                     | 100 | 100 | 100                                |
| Average of prevalence of leech (%) | 100                                     | 100 | 100 | 100                                |

Note: (-) : no significant different between concentration and bathing Time ( $P < 0.05$ ).

Prevalence is the percentage of fish infected with leeches to the number of all fish in the research tank. The prevalence of fish infected with leeches was still 100% after soaking the fish in rosemary solution. The rosemary solution can decrease the intensity of leech attacks but was not able to decrease it 100%, so there were still leeches attached to the cantang grouper, so the prevalence was still 100%. The concentration and duration of administration of herbal concoctions at optimal levels must be considered. The dose of rosemary solution and bathing time in the rosemary solution in the study could be more optimal so that the immersion treatment could be more effective in reducing prevalence[8].

## 4 Conclusion

Rosemary solution has the potential as an anti-leech. Soaking grouper infected by leeches in a rosemary solution with a concentration of 7.5 mL/L for 60 minutes reduced the intensity by 44.85%. However, bathing grouper infected by leeches in rosemary solution has not been able to reduce the prevalence of leeches in fish. Fish farmers can use rosemary to control leech infections in cantang grouper fish.

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