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# Control of marine leeches infecting hybrid grouper *Epinephelus fuscoguttatus* >< *E. lanceolatus* with garlic *Allium sativum* powder

# Pengendalian lintah laut yang menginfeksi ikan kerapu hibrida *Epinephelus fuscoguttatus* >< *E. lanceolatus* dengan bubuk bawang putih *Allium sativum*

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## ABSTRACT

Grouper has a large market demand and to meet this, the intensification of grouper cultivation is being carried out more massively. This situation increases the spread of diseases, one of which is sea leeches. This study aimed to obtain the concentration and duration of immersion garlic powder for the control of leeches that infects hybrid grouper (*Epinephelus fuscoguttatus* >< *E. lanceolatus*). This study used a factorial randomized design (concentration factor and garlic soaking time factor) with nine treatments and three replications. The treatment in this study was a combination of 0 g/L 0.375 g/L, and 0.750 g/L garlic powder concentration and immersion times of 20 minutes, 40 minutes, and 60 minutes. The results showed that concentrations of 0.375 g/L and 0.750 g/L were able to significantly reduce the intensity of leeches infecting hybrid grouper, namely 7.43% and 13.29%, respectively. The interaction between concentration and duration of immersion garlic powder on the percentage decrease in intensity was not significantly different. Immersion of garlic powder for 60 minutes was able to reduce the intensity of the leech by 9.56% and the results were significantly different with the immersion time of 20 and 40 minutes. However, the concentration and duration of soaking garlic powder were not able to reduce the prevalence of hybrid grouper infected with leeches. The R2 value of 0.748 means that there is a relationship between garlic powder concentration and the decrease in leech intensity in hybrid grouper. Powder garlic concentration of 0.750 g/L and immersion time of 60 minutes was able to reduce the intensity of leeches in hybrid grouper, but it was not effective in reducing the prevalence of fish infected with leeches.

Keywords: garlic, hybrid grouper, sea leech

## ABSTRAK

Ikan kerapu memiliki permintaan pasar yang besar dan untuk memenuhi hal tersebut, maka intensifikasi budidaya ikan kerapu perlu dilakukan secara lebih masif. Keadaan ini meningkatkan penyebaran penyakit, salah satunya lintah laut. Penelitian ini bertujuan untuk menentukan konsentrasi dan lama perendaman tepung bawang putih untuk pengendalian lintah yang menginfeksi ikan kerapu hibrida (*Epinephelus fuscoguttatus* >< *E. lanceolatus*). Penelitian ini menggunakan rancangan acak faktorial (faktor konsentrasi dan faktor lama perendaman bawang putih) dengan sembilan perlakuan dan tiga ulangan. Perlakuan di dalam penelitian ini adalah kombinasi antara konsentrasi tepung bawang putih 0%, 3%, 6%, dan lama perendaman 20 menit, 40 menit, dan 60 menit. Hasil penelitian menunjukkan bahwa konsentrasi 0,375 mg/L dan 0,750 mg/L mampu menurunkan intensitas lintah yang menginfeksi ikan kerapu cantang secara signifikan, yaitu sebesar 7,43% dan 13,29%. Interaksi antara konsentrasi dan lama perendaman bubuk bawang putih tidak berbeda signifikan terhadap penurunan intensitas lintah. Perendaman bawang putih selama 60 menit mampu menurunkan intensitas lintah sebesar 9,56% dan hasilnya berbeda signifikan dengan lama perendaman 20 dan 40 menit. Namun konsentrasi dan lama perendaman bawang putih tidak mampu menurunkan angka prevalensi kerapu cantang yang terinfeksi lintah. Nilai R2 sebesar 0,748 menunjukkan hubungan antara konsentrasi bubuk bawang putih dan penurunan intensitas lintah pada ikan kerapu cantang. Konsentrasi bawang putih 0,750 g/L dan waktu perendaman 60 menit mampu menurunkan intensitas serangan lintah pada kerapu hibrida, namun tidak efektif menurunkan prevalensi ikan terinfeksi lintah.

Kata kunci: bawang putih, ikan kerapu cantang, lintah laut

## INTRODUCTION

A hybrid of tiger grouper and kertang grouper is one source of marine animal protein that has a large market. Hybrid grouper called "cantang" (*Epinephelus fuscoguttatus* >< *E. lanceolatus*) is more potential to be cultivated because it has faster growth than its pure brood fish, namely tiger grouper and kertang grouper. Grouper has a large market demand and to meet this, the intensification of grouper cultivation is being carried out more massively. This situation increases the spread of the disease. Infective diseases in "cantang" grouper are caused by bacteria, viruses, fungi, and parasites. Parasitic infections that often infect cantang grouper are sea leeches (Palm *et al.*, 2015).

One of the diseases that often infects "cantang" hybrid grouper is ectoparasite infection of the hirudinea leech (*Zeylanicobdella* sp.). Sea leeches infect several organs, such as the mouth, eyes, body skin surface, gill cover, and fins. Hirudinea sea leeches attach to their host using a sucker that serves to suck the host's blood as a source of nutrition. The leech attaches very strongly to the body of its host. The prevalence of hybrid grouper infected with Hirudinea leeches in cultivation sites can reach 100% (Nofasari *et al*., 2019). The affected grouper will be pale in color, there will be sores and bleeding in the affected area. The wound area can be an entry point for secondary bacterial infection. Infection with this disease also causes slow growth in fish and decreases consumer interest in consuming the fish.

To overcome these problems, it is necessary to take control measures. The control of leeches that infect hybrid grouper is often done by using chemicals treatment such as formalin and $H_2O_2$. But the use of these chemicals will leave residues for fish, so it is not safe for consumers of these fish and these chemicals are also not safe for the environment. A solution is needed to use materials that leave no residue and are safe for the environment, namely by using natural ingredients.

A potential natural ingredient for controlling leeches is garlic powder (Bahmani & Kopaei, 2014) because garlic contains alkaloids and thiosulfinates which are capable of killing leeches (Eftekhari *et al*., 2012). Garlic extract proved to be effective as an anti-leech, with a dose of 600 g/ml capable of immobilizing leeches within 68 minutes (Bahmani *et al*., 2013). The effectiveness test of garlic in vitro at a concentration of 3.12% within 60 minutes effectively kills sea leeches (Zafran *et al*., 2021). In vivo research in the field has never been done, so it is necessary to test the effectiveness of garlic (*Allium sativum*) powder as an anti-leech in "cantang" hybrid grouper. This study aimed to obtain the concentration and duration of immersion garlic for the control of leeches that infect "cantang" hybrid grouper.

## MATERIALS AND METHODS

The experimental design was a factorial randomized design with nine treatments and three replications with a factor of garlic concentration and immersion time. The treatments in this study are as follows.

Treatment K20: Immersion without garlic (0 g/L) with a immersion time of 20 minutes.
Treatment K40: Immersion without garlic (0 g/L) with a immersion time of 40 minutes.
Treatment K60: Immersion without garlic (0 g/L) with a immersion time of 60 minutes.
Treatment A20: Immersion with a concentration of 0.375 g/L garlic with a immersion time of 20 minutes.
Treatment A40: Immersion with a concentration of 0.375 g/L garlic with a immersion time of 40 minutes.
Treatment A60: Immersion with a concentration of 0.375 g/L garlic with a immersion time of 60 minutes.
Treatment B20: Immersion with a concentration of 0.750 g/L garlic with a immersion time of 20 minutes.
Treatment B40: Immersion with a concentration of 0.750 g/L garlic with a immersion time of 40 minutes.
Treatment B60: Immersion with a concentration of 0.750 g/L garlic with a immersion time of 60 minutes.

### Making garlic powder (*Allium sativum*)

Garlic (*Allium sativum*) peeled and then washed with running water. Garlic is sliced thinly and then dried in the oven at 60°C until dry. After that, the garlic is blended until it forms a powder, then sieved with a 75 μm (Fauziah *et al*., 2015).

### Preparation of test fish

The test fish used were "cantang" hybrid grouper (body length: 10-13 cm) infected with sea leeches (*Zeylanicobdella* sp.) which was kept in floating net cages in Tembeling, Bintan.

**Preparation of the test container**

The test container used was a round bucket with a capacity of 12 liters as many as 27 buckets, then filled with eight liters of seawater.

**Immersion the test fish with garlic**

Garlic powder according to the treatment dose was put into an immersion container and then eight liters of sea water was added and stirred until homogeneous. A total of five hybrid grouper in each container that have been infected with sea leeches were immersed in water containing garlic powder according to the concentration dose and duration of immersion in the treatments.

**Observation of the intensity and prevalence of leeches**

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

$$Int = \left(\frac{\sum p}{N}\right)$$

Note:

Int = Intensity of parasite attack expressed (parasites /individual)

$\sum p$ = Number of invading parasites (parasites)

N = Number of samples infected with parasites (individual)

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

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

Note:

Prev = Prevalence or incidence (%)

N = Number of samples infected with parasites (individuals)

N = Number of observed samples (individuals)

## RESULTS AND DISCUSSION

**Result**

*Intensity of Leech*

The intensity of leeches in "cantang" hybrid grouper before and after soaking in the seawater containing garlic powder is presented in Table 1. The intensity of leeches in hybrid grouper after immersion in garlic decreased.

The effect of concentration and duration of immersion garlic on the percentage decrease in intensity of leech present in Table 2. The interaction between concentration and duration of immersion garlic on the percentage decrease in intensity was not significantly different (P> 0.05). The best concentration of garlic to decrease the percentage of leech intensity in hybrid grouper was 0.750 g/L, and the treatment was significantly different (P<0.05) with concentrations at 0.375 g/L and control without garlic immersion. Meanwhile, the best duration of soaking garlic to reduce the

Table 1. Intensity after and before immersion with garlic.

| Treatments | Intensity before immersion (individuals/fish) | Intensity after immersion (individuals/fish) | Percentage of Decrease Intensity (%) |
|---|---|---|---|
| K20 | 11.47 | 11.47 | 0.00 |
| K40 | 11.47 | 11.47 | 0.00 |
| K60 | 11.47 | 11.47 | 0.00 |
| A20 | 11.07 | 10.47 | 5.42 |
| A40 | 11.07 | 10.33 | 6.63 |
| A60 | 11.07 | 9.93 | 10.24 |
| B20 | 11.20 | 10.20 | 8.93 |
| B40 | 11.20 | 9.80 | 12.50 |
| B60 | 11.20 | 9.13 | 18.45 |

Note: K20: Immersion without garlic (0 g/L) with a immersion time of 20 minutes; K40: Immersion without garlic (0 g/L) with a immersion time of 40 minutes; K60: Immersion without garlic (0 g/L) with a immersion time of 60 minutes; A20: Immersion with a concentration of 0.375 g/L garlic with a immersion time of 20 minutes; A40: Immersion with a concentration of 0.375 g/L garlic with a immersion time of 40 minutes; A60: Immersion with a concentration of 0.375 g/L garlic with a immersion time of 60 minutes; B20: Immersion with a concentration of 0.750 g/L garlic with a immersion time of 20 minutes; B40: Immersion with a concentration of 0.750 g/L garlic with a immersion time of 40 minutes; B60: Immersion with a concentration of 0.750 g/L garlic with a immersion time of 60 minutes.

intensity of leeches in hybrid grouper was 60 minutes, where the treatment was significantly different (P<0.05) from the immersion time of 20 minutes and 40 minutes.

*Prevalence of leech*

The prevalence of fish infected with leeches after immersion in the seawater containing garlic powder is presented in Table 3. Immersion of hybrid grouper with garlic concentrations of 0.375 g/L and 0.750 g/L soaked for 20 minutes, 40 minutes, and 60 minutes did not have a significant effect on the prevalence of the incidence of hybrid grouper infected with leeches.

*Regression Analysis Garlic concentration and decreased intensity of leeches*

The graph of the relationship between garlic concentration and the percentage decrease in leech intensity in hybrid grouper is presented in Figure 1. The relationship between the decrease in the intensity of leeches infecting hybrid grouper (Y) and the concentration of garlic (X) with the regression equation Y= 19.11 X=0.1619. A negative b value of 19.11 indicates that the higher the concentration of garlic, the higher the decrease in the intensity of leeches infecting hybrid grouper. Increasing the concentration

Table 2. The effect of concentration and duration of immersion garlic on the percentage decrease in intensity of leech.

| Duration of immersion (minutes) | Concentration of garlic (g/L) | | | Average |
|---|---|---|---|---|
| | 0 | 0.375 | 0.750 | |
| 20 | 0.00 | 5.42 | 8.93 | 4.78[a] |
| 40 | 0.00 | 6.63 | 12.50 | 6.38[a] |
| 60 | 0.00 | 10.24 | 18.45 | 9.56[b] |
| Average | 0.00[a] | 7.43[b] | 13.29[c] | (-) |

Note: Different superscript letter in the average showed the significant different among the treatments (P<0.05).
(-): no interaction between concentration and duration of immersion (P>0.05).

Table 3. Prevalence of leech.

| Duration of immersion (minutes) | Concentration of garlic (g/L) | | | Average |
|---|---|---|---|---|
| | 0 | 0.375 | 0.750 | |
| 20 | 100 | 100 | 100 | 100 |
| 40 | 100 | 100 | 100 | 100 |
| 60 | 100 | 100 | 100 | 100 |
| Average | 100 | 100 | 100 | (-) |

Note: Different superscript letter showed the significant different between the treatment (P<0.05).
(-): no interaction between concentration and duration of immersion (P>0.05).

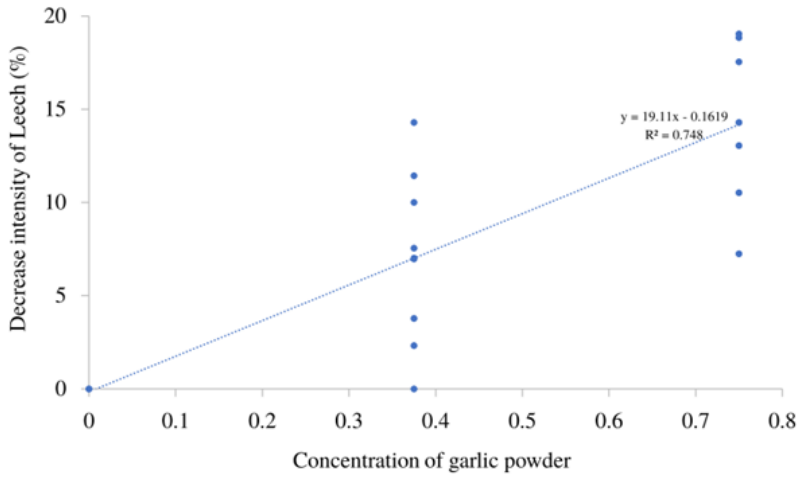


Figure 1. Graph of the relationship between garlic concentration and the percentage decrease in leech intensity in "cantang" hybrid grouper (*Epinephelus fuscoguttatus* >< *E. lanceolatus*).

of garlic by 1 g/L will decrease the intensity by 19.11%. The $R^2$ value of 0.748 means that relationship between garlic concentration and the decrease in leech intensity in hybrid grouper.

**Discussion**

Natural ingredients that have the potential to control leeches are garlic (Bahmani & Kopaei, 2014) because garlic contains alkaloids and thiosulfinates which are capable of killing leeches (Eftekhari *et al*., 2012). Garlic application to hybrid grouper infected with leeches can be given as powder. Garlic powder is expected to be able to control leeches, making garlic powder that is very simple to apply in the field by cultivators. Giving garlic powder requires a lower cost than using garlic extract, where the manufacture of garlic extract requires costs for dissolving and the extraction process is more complex (Moulia *et al*., 2018) than garlic powder. The composition of garlic powder and raw garlic is similar (Gutiérrez *et al*., 2021). Using crushed garlic cloves is better than using oil from garlic extract because crushed garlic cloves give a better effect in a longer time to control of trichodinosis and gyrodactylosis in hatchery reared *Oreochromis niloticus* seed (Abdelgalil & Aboelhadid, 2012).

Intensity is the number of parasites that infect each infected host. The intensity of leeches in hybrid grouper (*Epinephelus fuscoguttatus* >< *E. lanceolatus*) decreased after soaking in garlic at concentrations of 0.375 g/L and 0.750 g/L, and the best concentration to reduce the intensity of leeches was 0.750 g/L. This is thought to be due to an organosulfur compound in the form of allicin in garlic (Moulia *et al*., 2018) which has a cytotoxic effect on leeches (Bahmani & Kopaei, 2014). The duration of soaking garlic in fish infected with leeches also affected decreasing the intensity of leeches, with the best soaking time being 60 minutes. This is presumably because the longer the leech is exposed to the allicin compound in garlic, the more toxic it is to the leech. This is following research Zafran *et al*. (2021) that garlic extract is effective in killing sea leeches at a concentration of 3.12% within 60 minutes on a laboratory scale. Garlic powders as much as 10% and 20% mixed in the feed was able to reduce the intensity of monogenean parasites that infect guppies compared to control (Fridman *et al.*, 2014).

Prevalence is the ratio of the number of infected and uninfected fish by the parasites. The prevalence of leech-infected fish after immersion in garlic was not significantly different from that without immersion in garlic. Whole fish immersed in garlic remains infected by leeches. This is presumably because the concentration is still too low, so the allicin content in garlic is not too toxic to all leeches, so it is not able to kill all leeches attached to the hybrid grouper (*Epinephelus fuscoguttatus* >< *E. lanceolatus*). While the prevalence of tilapia infected with *Trichodina* and *Gyrodactylus* soaked in crushed garlic cloves for 1 hour at a concentration of 300 mg/L was 69% (Abdelgalil & Aboelhadid, 2012), where this prevalence was still relatively high.

The regression test obtained an R2 value is 0.748, which means the concentration of garlic affects the decrease in leech intensity in fish that infect hybrid grouper (*Epinephelus fuscoguttatus* >< *E.lanceolatus*) as much as 74.8%. This shows that there is a significant effect between the concentration of garlic and the intensity of leeches that infect "cantang" hybrid grouper, it is possible that increasing the concentration of garlic optimally will have a more significant effect on decreasing the intensity of leeches in hybrid grouper. This is following Militz *et al*. (2013) that garlic extract administered as a therapeutic bath was shown to have antiparasitic properties towards *Neobenedenia* sp. infecting farmed barramundi. Garlic contains allicin which is the main active compound that has antiparasitic properties used for veterinary purposes (Gutiérrez *et al*., 2021).

## CONCLUSION

Garlic concentration of 0.750 g/L and immersion time of 60 minutes was able to reduce the intensity of leeches in hybrid grouper (*Epinephelus fuscoguttatus* >< *Epinephelus lanceolatus*), but it was not effective in reducing the prevalence of fish infected with leeches.

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