



**The Influence of Binahong (*Anredera cordifolia*) Leaf Powder Addition to Feed on the Prevalence and Survival Rate of Red Tilapia (*Oreochromis niloticus*) seeds Infected by *Aeromonas hydrophila* Bacteria**

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Received 20 November 2019; Accepted 1 October 2020; Available online 31 December 2020

### ABSTRACT

Feed is one main factor in aquaculture activities which influences the quantity and quality of aquaculture commodities. Feed can also increase the cultivated fish survivability to prevent from fish diseases. Binahong leaf powder is one material added in the fish feed making which contains active compounds functioning as antibacterial agent. This study did aim at examining the influence of Binahong leaf powder addition to feed on the prevalence and survival rate of Red Tilapia seeds infected by *Aeromonas hydrophila* bacteria. This research employed an experimental method using a randomized complete design with four treatments. Each treatment consisted of different Binahong leaf powder dosage used as the material in feed making with treatment A (5%), treatment B (7%), treatment C (9%), and treatment D (control). The test animals were  $\pm$  5 cm red tilapia seeds infected by *Aeromonas hydrophila* bacteria. Feeding was given twice, in the morning and evening with the feeding rate of 10% per day of the total body weight. The research results showed that the addition of Binahong leaf powder did not influence the prevalence and survival rate of red tilapia seeds infected by *Aeromonas hydrophila* bacteria.

**Keywords:** *Aeromonas hydrophila*, Bacteria, Binahong, Disease, Feed

### ABSTRAK

Pakan merupakan salah satu faktor utama dalam kegiatan akuakultur yang mempengaruhi kuantitas dan kualitas komoditas akuakultur. Pakan juga dapat meningkatkan kelangsungan hidup ikan budidaya untuk mencegah penyakit ikan. Bubuk daun binahong adalah salah satu bahan yang ditambahkan dalam pembuatan pakan ikan yang mengandung senyawa aktif yang berfungsi sebagai agen antibakteri. Penelitian ini bertujuan untuk menguji pengaruh penambahan bubuk daun Binahong pada pakan dengan prevalensi dan tingkat kelangsungan hidup biji Tilapia Merah yang terinfeksi oleh bakteri *Aeromonas hydrophila*. Penelitian ini menggunakan metode eksperimental dengan menggunakan rancangan acak lengkap dengan empat perlakuan. Setiap perlakuan terdiri dari dosis bubuk daun Binahong berbeda yang digunakan sebagai bahan dalam pembuatan pakan dengan perlakuan A (5%), pengobatan B (7%), pengobatan C (9%), dan pengobatan D (kontrol). Hewan uji adalah  $\pm$  5 cm biji nila merah yang terinfeksi oleh bakteri *Aeromonas hydrophila*. Pemberian pakan diberikan dua kali, pada pagi dan sore hari dengan tingkat pemberian makan 10% per hari dari total berat badan. Hasil penelitian menunjukkan bahwa penambahan bubuk daun Binahong tidak mempengaruhi prevalensi dan tingkat kelangsungan hidup benih nila merah yang terinfeksi oleh bakteri *Aeromonas hydrophila*.

**Kata kunci:** *Aeromonas hydrophila*, bakteri, Binahong, penyakit, pakan

## 1. Introduction

Freshwater fish farming is one business developed in many Indonesian territorial areas which is influenced by various factors including environmental quality, cultivation techniques, quantity and quality of feeds, as well as diseases caused by many bacteria and viruses (Salikin *et al.*, 2014). Diseases either caused by bacteria or viruses in freshwater fish farming are considered as one serious problem faced by the fish farmers as having the potential to cause great losses in the form of increasing fish mortality rate. Those diseases were experienced by the fish as the result of interaction between three components in aquatic ecosystems: weak host (fish), the existence of pathogenic organisms, and poor environmental quality. Fish diseases are caused by parasites, bacteria, or fungi.

Tilapia (*Oreochromis niloticus*) is one widely cultivated species due to its fairly good economic value. In general, Tilapia is very popular and used as one widely consumed animal protein source. The main problems influencing the success of freshwater fish farming are feed quality and disease attacks. freshwater fish diseases come from various sources including viruses, bacteria, parasites and water quality (Raka, Kartika, & Andayani, 2016). The diseases attacking the freshwater fish may result in stunted fish growth and even the cultivated fish death. One of bacteria attacking the freshwater fish cultivation is *Aeromonas hydrophila* by infecting the fish irritating the fish skin, triggering the excessive mucus production, pale gills, gasping for air so that the fish frequently hang on the water surface or the edge of the pond, decreasing the fish appetite, weakening the fish movements, as well as damaging and making a reddish tail due to the rupturing fish fins' capillary blood vessels. Besides, freshwater fish become weak with a dull and pale body color and stunted growth.

The medication and prevention against the fish diseases may be performed in various ways, including by administering antibiotics or other chemical compounds functioning as the antibacterial agents. The administration of antibiotics to the freshwater fish may result in the decreasing water quality which may have negative impacts on the cultivated freshwater fish. One natural material which potentially becomes an antibacterial agent is Binahong leaf. Binahong plants grow very quickly, easily to cultivate, do not need a large area as the growing media and may be planted in the yard. Binahong plants have round leaves, propagating vines, as

well as generative and vegetative reproduction system. The pharmacological tests, found that Binahong plants had a number of active compounds functioning as antibacterial, anti-obesity, anti-hyperglycemic, anti-mutagenic, antiviral, anti-ulcer and anti-inflammatory agents (Kurniawan *et al.*, 2014).

Further analysis on antimicrobial substances found that in Binahong leaves contained saponins, alkaloids, polyphenols, terpenoids, essential oils, tannins, and flavonoids (Salikin *et al.*, 2014). Binahong plants are believed to have a variety of medicinal properties ranging from minor to severe illnesses, including those caused by microorganisms. Thus, a research on the use of Binahong leaf powder in fish feed making functioning as an antibacterial agent for freshwater fish farming is greatly necessary to conduct.

## 2. Materials and Methods

This experimental research used a completely randomized research design. The study was conducted in March 2019. The obtained data was analyzed using a descriptive and quantitative method. This study employed four treatments due to the percentage differences of Binahong leaf powder addition for fish feed making with three replications. The treatments consisted of treatment A with the dosage of 5% per kg of feed, treatment B with the dosage of 7% per kg of feed, treatment C with the dosage of 9% per kg of feed and treatment D as the control (without Binahong leaf addition). Feed is made by grinding the artificial feed of F800 until smooth and then mixed with Binahong leaf powder in accordance with the predetermined dosages in those four treatments reshaped similarly into artificial feed used in this study. Feed was administered twice a day with the feed rate of 10% per day. The tested animals were Tilapia (*Oreochromis niloticus*) seeds with the size of  $\pm 5$  cm kept in aquarium containers with the density of 1 fish/liter. Fish seeds were first infected with *Aeromonas hydrophila* bacteria. The infected fish seeds were then cultivated for 24-48 hours or until the clinical symptoms occurred due to the bacterial infection of *A. hydrophila*. The fish which had the clinical symptoms were then administered with the artificial feed based on the predetermined dosage treatments for two weeks. The measured parameters were the fish prevalence and its survival rate. If the result of Analysis of Variance shows significant differences between treatments, further Least Significant Difference

(LSD) test will be performed. The data was processed using the SPSS program. Data was then analyzed using Analysis of Variance at the 95% confidence level.

### 3. Results and Discussion

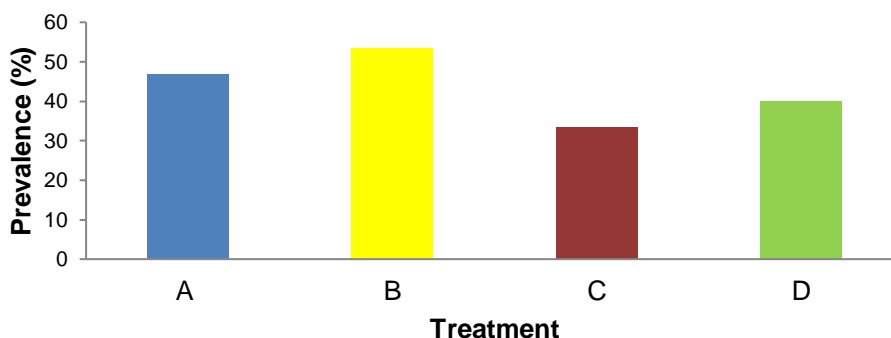
#### 3.1. Prevalence

Tilapia (*Oreochromis niloticus*) seeds infected with *Aeromonas hydrophila* bacteria will show the clinical symptoms, such as peeling scales, cuts on the skin and disturbance to the fish body's balance. The fish seeds infected by bacteria will experience metabolic disorders and result in slow growth, and even death. The treatments performed using natural materials containing active compounds functioning as antibacterial agent is one best alternative to be use. This study used Binahong leaf powder in the artificial feed making to determine its influence on the prevalence and survival rate of Tilapia seeds infected by *A. hydrophila* bacteria. The research results on the use of Binahong leaf powder in the artificial feed making on the prevalence and survival rate of Tilapia (*Oreochromis niloticus*) seeds infected by *A. hydrophila* bacteria were presented in Figure 1.

The results showed that the Binahong leaf powder addition to the artificial feed making produced a different prevalence in the Tilapia seeds infected by *A. hydrophila*. The lowest prevalence value was those in treatment C, while

the highest was those in treatment B. Prevalence is the number of fish still infected by the bacteria after treatments. These results indicated that treatment with the dosage of 9% per kg of feed gave the lowest prevalence value, meaning that the number of Tilapia (*Oreochromis niloticus*) seeds infected by bacteria at the end of this study was the least. These results were then tested using analysis of variance at a 95% confidence level to determine the influence on Binahong leaf powder addition on the prevalence of Tilapia (*Oreochromis niloticus*) seeds infected by *A. hydrophila* bacteria. The results of analysis of variance in details were presented in Table 1.

The results of analysis of variance at a 95% confidence level showed that the Binahong leaf powder addition at different dosages did not influence the prevalence of tilapia seeds infected by *A. hydrophila* bacteria. The influence of Binahong leaf powder addition in the fish feed making administered to the Tilapia seeds infected by the bacteria was not only influenced by the content of owned active compounds but also various factors. Factors which might also influence the function of active compounds found in Binahong leaves including the amount of addition, feed quality, and environmental quality when cultivating the Tilapia seeds. The amount of Binahong leaf powder addition may influence the active compound concentrations. The previous research stated that the higher the amount of Binahong leaf addition, the higher the



**Figure 1.** Prevalence of *Aeromonas hydrophila* infection on *Oreochromis niloticus* treated with Binahong powder

**Table 1.** Results of Analysis of Variance in Prevalence of *Aeromonas hydrophila* infection on *Oreochromis niloticus* treated with Binahong powder

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	666.67	3	222.22	.123	.944
Within Groups	14400.00	8	1800.00		
Total	15066.67	11			

of Binahong leaf powder concentration. This will also influence the height of anti-bacterial compound content which is able to inhibit the bacteria infecting the fish seeds (Raka, Kartika, & Andayani, 2016).

### 3.2. Survival rate

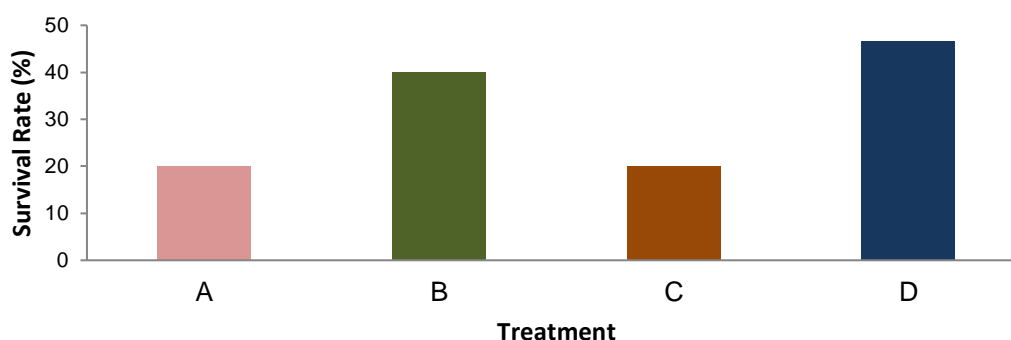
The parameter which was also observed in this study was that of Tilapia fish infected by *A. hydrophila* bacteria. The research results were presented in detail in **Figure 2**. The results were then analyzed using analysis of variance to determine its influence on the survival rate of Tilapia seeds infected by the bacteria (**Table 2**). The graph above showed that the highest survival rate was obtained in treatment D, while the lowest one was obtained in treatment A and treatment C. The results of analysis of variance showed that the Binahong leaf powder addition to feed with different dosages did not have an influence (significant value  $0.588 > 0.05$ ) on the survival rate of Tilapia seeds infected by *A. hydrophila* bacteria.

The prevalence and survival rate of fish seeds infected by the bacteria might be influenced by various factors. The content of active compounds belonging to Binahong leaves in this study did not influence the prevalence and survival rate of fish seeds infected by the bacteria. Meanwhile, the results of previous studies suggested that the fish might give a specific response to bacterial infections. The ability to respond specifically was also applicable

with its ability to respond the given immunostimulant.

The immunostimulants derived from plants have the function to increase the fish's resistance against the diseases by increasing its specific defense mechanisms (Sharma, Deo, Tandel Riteshkumar, Chanu, & Das, 2010). This differently influenced the infected fish's responses when given the antibacterial compounds derived from plants. The use of active compounds functioning as antibacterial agents in aquaculture activities is very necessary. This is due to the long-term period of antibiotic use which may result in adverse reactions to the cultivated fish. The influence of antibiotic use for a long period may result in antibiotic resistance against bacteria or antibiotic residues in the environment and fish. The use of antibacterial agents derived from plants may increase the immunostimulants and stimulate the immune response and enhance the fish' existing immune mechanisms (Villegas and Hosokawa, 2004). Immunostimulants also have additional benefits, such as increasing growth and survival rates for fish experiencing stress. Many immunostimulants have been proven effective in carps (Abasali & Mohamad, 2010)

The results of phytochemical analysis stated that Binahong plants contained active compounds, such as alkaloids, saponins, quinones, phenolics and flavonoids. These active compounds have the potential to become antibacterial agents. The working mechanisms of flavonoid active compounds start by binding



**Figure 2.** Survival Rate of Tilapia (*Oreochromis niloticus*)

**Table 2.** Results of Analysis of Variance on the Survival Rate of Tilapia (*Oreochromis niloticus*)

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1700.00	3	566.67	.680	.589
Within Groups	6666.67	8	833.33		
Total	8366.67	11			

themselves into proteins through the hydrogen bonds which result in the damaged protein structures, cell wall stability and disrupted plasma membrane that the bacteria undergo lysis. Meanwhile, the alkaloid compounds may disturb the constituent components of peptidoglycan in bacterial cells that the cell wall layer is not completely formed and result in the cell death. Polyphenols have the antibacterial properties with the working mechanisms by damaging the bacterial cell membranes. Saponin is one antiseptic functioning to kill or inhibit the microorganisms growing in the wounds caused by infection that it does not spread the infection wider. Saponins can also form the complex compounds with nucleophilic amino acids in proteins which may result in proteins losing their functions (Salikin et al., 2014).

Several studies have been conducted on the content of active compounds belonging to various plants functioning as antibacterial agents. The research conducted on wheat extracts stated that the cultivation potential value is good in terms of growth improvement, immune response, and resistance to *A. hydrophila* when the diet is added with 10 g kg<sup>-1</sup> of wheat extract. The oat extract potential in fish and other pathogens as immunostimulants for aquaculture use is highly recommended (Baba, Acar, Öntaş, Kesbiç, & Yilmaz, 2016). Garlic extract may also inhibit the growth of pathogenic bacteria in fish, especially for carps. Garlic extract has an antibacterial impact that it may be used as one effective alternative material to use in preventing from the bacterial attacks to the freshwater fish. The use of antibacterial agents derived from the natural materials is considered excellent due to their positive impacts on the environment (Farahani, et al., 2012).

#### 4. Conclusion

Based on the research results, it can be concluded that Binahong (*Anredera cordifolia*) leaf powder addition to feed did not influence the prevalence and survival rate of tilapia (*Oreochromis niloticus*) seeds infected by *Aeromonas hydrophila* bacteria.

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