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Absolute Growth and Survival Rate of Koi Fish Infected with *Aeromonas hydrophila* bacteria with the Treatment of Telang Leaf Extract

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ABSTRACT

This study aims to determine the best concentration of Telang leaf extract in an effort to treat the infection of *Aeromonas hydrophila* bacteria that attacks koi fish so as to produce the highest survival rate and the greatest absolute growth. The method used in this research was the experimental method using Completely Randomized Design (CRD) with five treatments and three replications. The treatment given was soaking koi fish infected with *Aeromonas hydrophila* in telang leaf extract for 48 hours with concentrations of 0, 150, 300, 450 and 600 ppm. Koi fish is infected with *Aeromonas hydrophila* bacteria by injecting intramuscularly as much as 0.1 ml/head with a bacterial density of 10^8 cfu/mL. The parameters observed were survival rates and weight growth. The results showed that the use of telang leaf extract with a concentration of 300 ppm was the best and most effective treatment for treating koi fish infected with *Aeromonas hydrophil*a because it produced the highest survival rate and the highest weight growth compared to other treatments.

Keywords: *Aeromonas hydrophila*, absolute growth, koi fish seeds, survival rate, telang leaf extract

INTRODUCTION

Koi fish (*Cyprinus carpio*) is known to have the beauty of its shape and color, therefore koi fish is one of the freshwater ornamental fish that is in great demand and is also believed to bring luck. In the process of carrying out fish farming activities, one of the inhibitor in the production process is fish disease caused by bacteria which can cause disability and even death. This will cause a big loss for the koi fish farmers because the beauty of their bodies decreases if they are attacked by the disease and will reduce the aesthetic value of the koi fish.

Aeromonas hydrophila is one of the bacteria that attacks koi and causes Motil Aeromonas Septicemia (MAS). *Aeromonas hydrophila* infects fish through the surface of the body or injured gills then enters blood vessels and other internal organs[1]. In general, farmers provide antibiotics for the healing process, but lately, treatment using antibiotics is not recommended because it is not easily biodegradable and can pollute the environment[2].

There are other alternatives in the treatment of the fish, namely the telang plant (*Clitoria ternatea*) because the leaves have antibacterial activity such as steroid group compounds, flavonoids, triterpenoids and saponins which are known to have fungicidal effects. There are several ways that can be done in the treatment of fish attacked by *A. hydrophila*, namely through injection, immersion, through feed/oral, and applying. However, treatment by immersion is the most applicable method compared to others because it is easy to do[3]. The purpose of this research is to determine the best concentration of telang leaf extract in the treatment of *Aeromonas hydrophila* bacterial infections that attack koi fish so as to produce the highest survival rate and the greatest absolute growth. The usefulness of this research is to provide some information about telang leaves as a treatment for infection with *Aeromonas hydrophila* bacteria in aquaculture activities.

METODOLOGY

Time and Place

This research was conducted from November to April 2019 and carried out three stages, namely the preparation of telang leaf material at the Experimental Garden of the Faculty of Agriculture, Padjadjaran University, then a preliminary study consisting of making telang leaf extract carried out at Unpad Central Laboratory, LC_{50} test was conducted at Building 4 Education Laboratory and the inhibitory power test (*in vitro*) carried out at the Building 3 Biotechnology Laboratory, followed by the main research, namely fish infection, treatment and observations carried out at the Education Laboratory of Building 4, Faculty of Fisheries and Marine Sciences, Padjadjaran University.

Tools and Materials

The tools used were blenders, kitchen scales, digital scales, plastic measuring cups, filters, closed dark containers, spatulas / stirrers, rotatory evaporators, aquariums, aerators, autoclaves, hot plates, stir plates, magnetic stirrers, petridisks, osseous needles, L .glass, 6mm paper disc, parafilm, bunsen burner, incubator, digital caliper, laminar flow, thermometer, DO meter and pH meter. The materials used were koi fish, *A. hydrophila* isolates, Telang leaves, Sodium Agar (NA), Sodium Broth (NB), distilled water, physiological NaCl 0.9%, technical methanol and commercial PF 800 feed.

Research Method

The method used in this research was the experimental method using Completely Randomized Design (CRD) with five treatments and three replications. The treatment given was soaking koi fish infected with *A. hydrophila* in telang leaf extract with concentrations based on the results of *in vitro* and in vivo tests as follows :

- Treatment A : Control treatment without additional of telang leaf extract
- Treatment B : Soaking with 150 ppm Telang leaf extract
- Treatment C : Soaking with 300 ppm Telang leaf extract
- Treatment D : Soaking with telang 450 ppm leaf extract
- Treatment E : Soaking with 600 ppm Telang leaf extract

RESEARCH PROSEDURE

Preliminary Research

Extract Making

The making of Telang leaf extract was carried out using maceration method. Telang leaves as much as 5 kg, cleaned with flowing and drained water, cut into small pieces and dried for ± 7 days, mashed using a blender then macerated in a closed dark container, given a 10-liter technical methanol solution until submerged, stirred and left to stand for 2 days at room temperature. The results of maceration were filtered using Whattman filter paper No.42, remaserated 3 times, the filtering results were evaporated in rotatory evaporation at a temperature of 60^{o}C with a speed of 65 rpm to form a paste of ± 650 grams.

LC_{50} Test

This LC50 test refers to US-EPA (2002), carried out to determine the ability of a test chemical in terms of turning off test animals[4] and to obtain concentrations with 24-hour koi mortality by 50%. The initial step is 10 aquariums, washed and dried, filled with 10 liters of water and aerated. After that, the fish are put in an aquarium of 10 fish each. Aquariums are labeled according to treatment (10 ppm, 100 ppm, 500 ppm, 1000 ppm and 5000 ppm). Telang leaf extract is weighed and put in an aquarium according to the treatment. Observed the

survival of these fish for 24 hours to 48 hours. Fish remain fed twice a day on adlibitum. Fish mortality observations were analyzed using EPA probit to obtain LC50 values, and determine the relative toxicity of a chemical against living organisms[4].

***In vitro* Test**

The in vitro test is a test method that is carried out to see the antimicrobial action of telang leaves. The method used in this test is the diffusion method or the Kirby-Bauer antibiogram paper disc method[5]. The parameters observed were measuring the diameter of the clear zone around the well with the calipers[6]. The initial procedure for carrying out in vitro tests is sterilization of tools and materials using autoclave. The next step is to make TSA media, TSA that has been made, sterilized using autoclave, the TSA is waited for a few minutes until the temperature is not too high, then poured enough TSA into the petridisk, leave it to dry. After drying, 1 ml of culture of *A.hydrophila* with a density of 10^8 CFU/ml was put in a petridish containing TSA, flattened using L glass slowly and rotating, after being evenly distributed, a paper dish which had been immersed with telang leaf extract was added and incubated for 24 hours with 30^{o}C incubator temperature. After 24 hours, the inhibitory zone is formed and measured using a caliper. All activities are carried out in laminar air flow so that the tools and materials remain sterile.

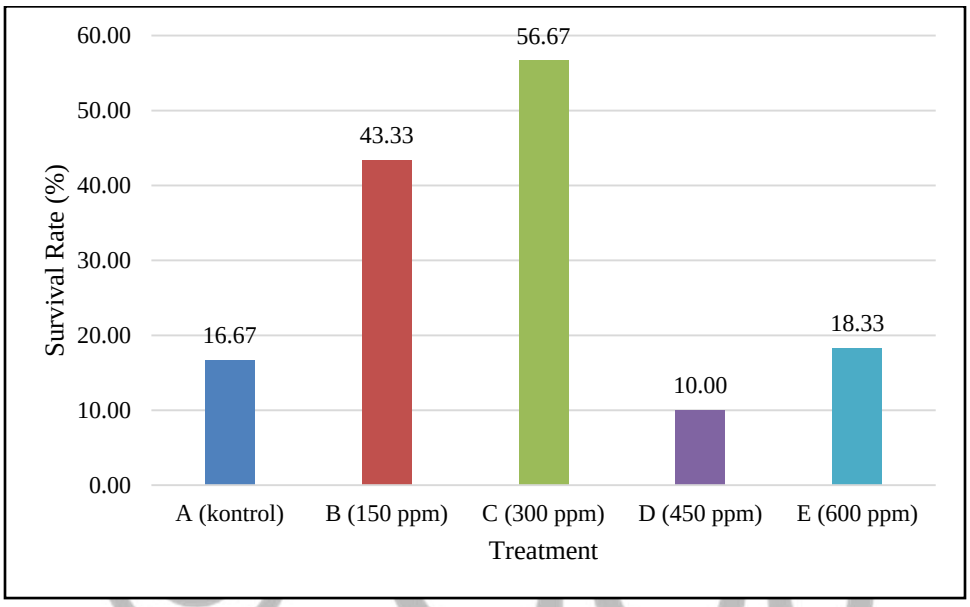
MAIN RESEARCH

Fifteen aquariums filled with 20 liters of water, koi fish are inserted into the aquarium with a density of 1 tail/liter, weighing the weight of the initial biomass of each aquarium. Then, koi fish were infected by the bacteria *A.hydrophila* through an intramuscular injection of fish body with a bacterial density of 10^8 CFU/ml as much as 0.1 ml. From each aquarium water was taken using the dipper as a sample for observing the initial water quality. After the injection process, clinical symptoms are observed, if the clinical symptoms appear as fish gather near aeration and there is inflammation in the injection site, then immersion of the test fish with different concentrations of telang leaf extract namely control, 150 ppm, 300 ppm, 450 ppm, 600 ppm, the amount of telang leaf extract needs is weighed according to treatment and then put in an aquarium according to treatment and immersion for 48 hours, also observed is the number of fish deaths. After 48 hours, water changes are made with new water and changes in the body of the fish are observed. Fish are maintained and observed for 14 (the first 5 days of treatment and 9 days after the maintenance period)[7] because fish can be said to be completely cured after 14 days after treatment. During maintenance, fish are fed twice a day and routinely used every day to squeeze the water quality. After 14 days, the fish was observed by the body and its survival, then another sampling of water quality was carried out and the weight of the final biomass was weighed.

RESULT AND DISCUSSION

Survival Rate

Based on the results of observations carried out for 14 days of maintenance, after soaking with telang leaf extract, koi fish infected with *A. hydrophila* produced different mortality in each treatment. The highest amount of mortality was found in treatment D (450 ppm) and the lowest in treatment C (300 ppm). Because it has a different mortality rate for each treatment, the survival rate also varies for each treatment (Picture 1).



Picture 1. Survival rate of koi fish

On the graph of the survival rate (Picture 1), it can be seen that the highest survival produced by treatment C. This shows that the content of antibacterial compounds in telang leaves can work optimally because it can inhibit the growth of *A. hydrophila* bacteria and prevent the release of toxin substances by these bacteria. Damage to microbial cells causes the transport of compounds and ions to occur in bacterial cells, so that bacteria experience a lack of nutrients needed for growth and eventually die[8], and can also be seen that survival in treatment C is quite high compared to other treatments, 56.67% .

Treatment D has the lowest survival rate compared to the other treatments even lower than treatment A which is not given immersion treatment which is 10%, this is presumed because in addition to fish not strong against the attack of *A. hydrophila* bacteria, fishes are also not strong with concentrations that given because it is too high to experience death. In telang leaves there are antibacterial saponin compounds which are toxic compounds if used in excessive doses which can inhibit the exchange of oxygen to breathe and eventually suffocate[9]. Saponins inhibit oxygen exchange through the osmoregulation process, enter the bloodstream which results in damage to the gill tissue and disrupts the respiratory process because the gills become swollen.

Based on the results of analysis of variance, giving Telang leaf extract can be said to have a significantly differ-

ent effect. Duncan's test results showed that there were significant differences between treatment A (control), D (450 ppm) and E (600 ppm) with treatment C (300 ppm), but there was no significant difference between treatment B (150 ppm) and treatment C (300 ppm) which means that treatment C (300 ppm) is the best treatment (Table 1).

Table 1. Koi fish survival rate

Treatment	Survival Rate	The Result of Arcsin Transformation	Notation
A (control)	16,67	22,28	A
B (150 ppm)	43,33	41,14	Ab
C (300 ppm)	56,67	48,83	B
D (450 ppm)	10,00	11,07	A
E (600 ppm)	18,33	20,94	A

Note : The value followed by the same letter is not significantly different based on the Duncan test at the 95% confidence level

It can be seen in Table 1, treatment A (control), treatment D (450 ppm), and treatment E (600 ppm) give results that are not significantly different because survival is relatively the same, it is assumed that the active ingredient in the leaves of telang is less effective for treatment at these concentrations, besides the concentration used in treatments D and E too high and being toxic for fish, it can also be seen from the results of inhibitory tests that have been carried out in preliminary studies that the ability to inhibit telang leaf extract against *A. hydrophila* is moderate, because at a concentration of 100 ppm it produces an average clear zone of 6.41 mm, and the largest clear zone average is at a concentration of 5000 ppm which is 13.41 mm. Inhibition with a diameter of 20 mm or more is classified as very strong, the diameter of the inhibitory power of 10–20 mm is classified as strong, the diameter of the inhibition zone of 5-10 mm is moderate and the inhibition diameter of less than 5 mm is classified as weak[6].

The Duncan test results (Table 1) also showed that treatment B (150 ppm) had no significant difference with treatment C (300 ppm), but treatment C (300 ppm) produced the greatest survival compared to treatment B (150 ppm) which was 56, 67% or half percent of the total fish, then treatment C is the best treatment. The percentage of survival is relatively small in a small scale of cultivation, but it can help large farmers because it can save half of its production affected by disease, this shows that the content of compounds in telang leaves namely triterpenoids, flavonoids and saponins is effective as an antibacterial in concentration certain. In the study [6] using ketapang leaf extract, the best dose for the treatment of carp infected with A. hydrophila was 1500 ppm and resulted in a survival of 66.67%, when compared to the use of telang leaves, telang leaves could

be said to be more effective because at a concentration of 300 ppm it can produce the best survival of 56.67%.

Absolute Growth

Based on the results of a 14-days observation of koi fish infected with *A. hydrophila* after immersion treatment with telang leaf extract it was seen that the average increase in weight on koi fish was different for each treatment (Table 2). The average growth of absolute weights ranges from 0.75-0.55%.

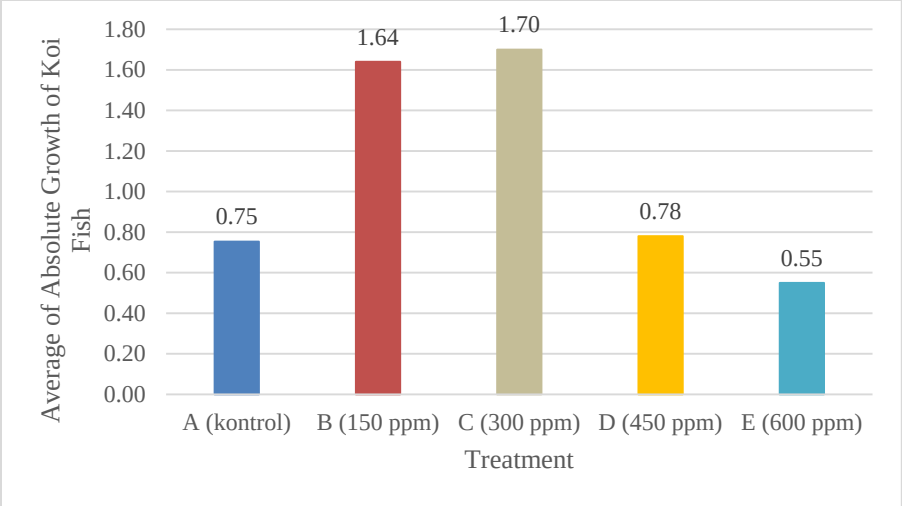
Table 2. Average of Absolute weight growth of koi fish

Treatment	Average of Absolute weight growth (gram)
A (control)	0,75±0,62[a]
B (150 ppm)	1,64±0,53[a]
C (300 ppm)	1,70±0,26[a]
D (450 ppm)	0,78±0,99[a]
E (600 ppm)	0,55±0,43[a]

Note : The value followed by the same letter is not significantly different based on the Duncan test at the 95% confidence level

During the 14 days of observation, there was an increase in the weight of koi fish but only a slight increase in weight occurred, at least this increase in weight occurred due to various factors, in the study[10] it was stated that the factors affecting fish growth were fish size, temperature, feed, and environment. In Mulqan's (2017) study, it was also stated that fish growth is influenced by several factors, namely internal factors and external factors, as well as internal factors including heredity, disease resistance and ability to use food, while factors from outside includes physical, chemical and aquatic biology.

Based on the results of analysis of variance, immersion of telang leaf extract in fish infected with *A. hydrophila* showed no significant difference between treatments on the growth of absolute weight of koi fish which means the data was not significantly different at the 95% test level. The absence of real differences is influenced by several factors, one of which is the loss of appetite for fish because the fish is attacked by bacteria *A. hydrophila* which causes fish losses its appetite. This is in accordance with the statement[11] that fish infected with the bacterium *A.hydrophila* will decrease their appetite.



Picture 2. The Absolute Growth Rate of Koi Fish

In the graph of Picture 2, the results obtained from statistical calculations do not show significant differences between treatments even though the average values obtained differ between treatments. During the 14-days observation, treatment C with 300 ppm soaking of telang leaf extract produced the highest absolute weight average among the other treatments which was 1.70 g, then there was treatment B with 150 ppm soaking of telang leaf extract resulting in an average absolute weight of 1.64 g, and the lowest absolute weighting average is at treatment E which is 0.55 g. The average value in treatment E shows lower results compared to treatment A (control), this is presumed because in treatment E, fish are stressed due to injection activities and the fish becomes diseased due to bacterial attack, the fish attacked by bacteria A.hydrophila will growth is hampered because there are toxins produced by extracellular production. These bacterias will disrupt the balance of the system in the body[12], then the concentration used in treatment E is too high because telang leaves contain saponins which can be toxic at high doses causing disturbed fish metabolism, while in treatment A (control) there is no poison from the outside that affects its metabolism so the energy used by fish in treatment A is only for self-defense from bacterial attacks and the remaining energy is absorbed through feed even though it is still used for growth.

Another factor that makes the observation of absolute weight is not significantly different is that the energy factor in the body of the koi fish is more used for self-defense because the fish is diseased, according to[13] growth occurs due to excess energy derived from feed after reduced by the energy of metabolism and energy contained in feces, so that the energy absorbed and used less then does not significantly affect the process of fish growth, this statement is reinforced by an explanation from the study[14], that apart from the amount of energy in feed, fish growth can be affected also by the condition of the fish when stressed, diseased or a change in the environment of the place his life.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that telang leaf extract can inhibit the growth of *A. hydrophila* bacteria. The best concentration in the use of telang leaf extract to treat koi fish and produce the highest survival rate of 56.67% and produce the largest absolute growth of 1.70 g is 300 ppm.

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