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EFFECTIVENESS OF PAPAYA LEAF EXTRACT (*Carica Papaya* L.) IN THE TREATMENT OF KOI FISH (*Cyprinus Rubrofuscus*) SEEDS INFECTED WITH *AEROMONAS HYDROPHILA* BACTERIA

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Abstract

One of the most popular freshwater fish species to farm, both domestically and internationally, is the koi fish (Cyprinus Rubrofuscus). However, koi fish farming is often attacked by bacterial diseases caused by the Aeromonas hyrophila bacterium, so it becomes a concern because this bacterium can cause death in koi fish. Papaya leaves were chosen as an alternative medicine in the treatment of koi fish infected with bacteria, considering that papaya leaves contain many compounds that are antibacterial and have no side effects when used. As for this study, the papaya leaves used were young papaya leaves which were extracted using the maceration method. This study was aimed to determine the effectiveness in treating koi fish infected with bacteria and what concentration of papaya leaf extract is effective as an antibacterial to treat koi fish seeds that are attacked by Aeromonas hydrophila bacteria. Papaya leaf extract was administered at different concentrations, namely 0 ml (control), 5 ml, 10 ml, 15 ml within 30 days before and after the koi fish seeds were attacked by Aeromonas hydrophila. The result revealed that papaya leaf extract has effectiveness as an antibacterial which can slow down the growth of Aeromonas hydrophila bacteria at a concentration of 15 ml with a survival rate of 95%. Therefore, the dose of papaya leaf extract is directly proportional to its effectiveness as an antibacterial. In other words, the greater the dose of papaya leaf extract, the more effective it will be in treating koi fish seeds that are infected with bacteria.

Keywords: *Aeromonas Hydrophila Bacteria, Effectiveness, Fish Farming, Koi Fish, Papaya Leaf Extract*

1. INTRODUCTION

The use of plants has now been converted into antibacterial functions because they contain compounds that are antibacterial. The use of this plant is believed to have a function that has been used for generations by the community. Some of the advantages of using plants as an alternative are that medicinal plants are relatively easy to obtain because there are still many of them in nature making it easier for people who want to use them, do not cause resistance, and do not have a harmful impact on water (Kardinan, 2003). One of the plants that can be used as an alternative antibacterial medicine is the papaya plant, especially the leaves which contain a lot of antibacterial compounds (Amalia, 2021).

Papaya is a plant that has been widely studied because almost all parts of papaya have health benefits, especially the leaves (Paramesti, 2014). The chemical compounds

contained in papaya leaves are tannins, alkoids, flavonoids l, terponoids, and saponins, so they are widely used because they are antiseptic, anti-inflammatory, and antibacterial (Tuntun, 2016). In this study, papaya leaves were used that met the criteria for young leaves that were still fresh and still green in color. Papaya leaves will then be extracted using ethanol through maceration.

Papaya leaves that have been extracted will be used to treat koi fish infected by *Aeromonas hydrophila* bacteria. This bacterium is one of the bacteria that can attack freshwater fish populations and cause fish death (Nurjannah et al., 2013). Poor freshwater fish conditions can cause fish to become stressed and susceptible to disease (Azmi et al., 2013).

Based on the discussion or background above, the purpose of this research was to find out the effectiveness of treating koi fish infected with bacteria and what concentration of papaya leaf extract is effective as an antibacterial to treat koi fish seeds that are attacked by *Aeromonas hydrophila* bacteria.

2. RESEARCH METHODS

In this study, the design used was a Completely Randomized Design (CRD) with 1 treatment with 4 different concentrations and 3 controls in the morning, afternoon, evening. To reduce errors, two repetitions were made for each. The method used in this study is an experimental method, where this method can be used scientifically (valid), because it is done by strictly controlling the variables that can interfere with the experiment from outside (Anggito & Setiawan, 2018). This research will be conducted with 4 concentrations of papaya leaf extract which are detailed as follows
The treatment below:
Treatment K: The dose of papaya leaf extract is 0 ml.
Treatment A: The dose of papaya leaf extract is 5 ml.
Treatment B: The dose of papaya leaf extract is 10 ml.
Treatment C: The dose of papaya leaf extract is 15 ml.

2.1. Container Preparation

Provide 8 containers and label each container. Then fill each of these containers with 5 liters of water in each container. Before each container is given fish, first turn on the aerator as a source of oxygen for the fish.

2.2. Koi Fish Adaptations

The koi fish used are fish that are 2-3 cm in size. Before being tested, koi fish were adapted so that they could easily adapt to the environment in the container and stabilize the condition of the fish so that they were not infected with other bacteria before being tested. Koi fish were put into containers of 10 fish each into the 8 containers provided and maintained for 2 days. Fish were fed Pf500 every 2 times a day. The fish were reared for 2 days until their condition was completely stable with a high appetite and no death occurred.

2.3. Making Papaya Leaf Extract

Papaya leaf extract is made by using young, fresh, and perfect papaya leaves. Before the extraction process is carried out, the papaya leaves are washed thoroughly in running

water and to facilitate the extraction process the papaya leaves are mashed by cutting them. After that, the papaya leaves are blended until smooth. Preparation of papaya leaf extract was carried out by maceration method using 98% ethanol. Where the papaya leaves that have been blended are divided into two parts of 250 grams each and soaked in a 98% ethanol solution covered with plastic wrap so that it is airtight for 3 days and 3 nights at room temperature. During the soaking process the mixture of papaya leaves and 98% ethanol is stirred for 10 minutes every day. The thick papaya leaf extract was then separated by 98% ethanol and filtered through filter paper. To get a thick extract with a concentration of 100%, a distillation process is carried out where the thick extract of papaya leaves is put into the distillatory.

2.4. Making Bacterial Culture Media

Prepare tools and materials to be used in the manufacturing process. Wrap the petri dish using paper for sterilization in the sterilizer cupboard. Calculating the media requirement for 200 ml of aquadest using a measuring cup. Weighing nutrient agar using digital scales as much as 4 gr. Measure as much as 200 ml of aquadest then put nutrient agar and aquadest into the Erlenmeyer flask. Dissolving nutrient agar by heating on a hotplate stirrer. As well as ensuring the media dissolves perfectly. After that, put all the tools that will be used into the autoclave to be sterilized again for 30 minutes at 121oC. Carry out the process of making bacterial culture media in a room that already has an air flow laminator (LAF) cabinet available to keep it sterile.

2.5. Bacterial Breeding

Take samples using a micropipette and put them into an Erlenmeyer flask which already contains a physiological solution and stir until homogeneous. The aim is to use a 0,85 physiological solution for diluting the sample solution so that the resulting bacterial colonies are not too dense (Arfianty et al., 2017). Diluting the sample solution was carried out 6 times and each dilution was put into a test tube. After that, the results of the dilution were transferred to a petri dish. Pour the nutrient agar solution that has warmed into the petri dish which already contains the bacterial sample. Leave the media at room temperature until it solidifies. If the media has solidified, the petri dish is tightly closed using plastic wrap. Insert the petri dish into the incubator cupboard and do the incubation process within 24 hours. After the bacterial colonies were formed, the number of bacterial colonies was counted using the colony center tool. Then dilute the bacteria using Nutrien Broth so that the resulting bacteria are in liquid form. Take each of the 2 bacterial colonies using a sterile loop and put them into the Nutrien Broth which has been dissolved with distilled water to make a bacterial suspension. Then homogenized and incubated for 24 hours until the bacterial suspension looks cloudy. The bacterial suspension is ready to use.

2.6. Koi Fish Seed Infection

Ensuring that the koi fish seeds that will be infected are healthy, 10 fish each/container. Koi fish seeds were infected by means of a bacterial suspension that had been made and poured into each container as much as 2 L. After 15 minutes, the fish were

transferred to a new container. Left for 3 days to observe the changes that occur in the fish.

2.7. Papaya Leaf Extract Effectiveness Test

After that, the fish were left for 3 days without treatment and the researchers observed the changes that occurred in the fish. Then the fish were given the results of papaya leaf extract with different doses and left for 7 days and the researchers observed the changes that occurred in the fish. Furthermore, to take data on the effectiveness of papaya leaf extract in the treatment of koi fish seeds infected by Aeromonas bacteria.

2.8. Data analysis

The data analysis technique in this study was carried out qualitatively descriptively based on theory which focused on observations made by researchers on the effectiveness of papaya leaf extract doses as an antibacterial and explained the interrelationships between variables. Fish response data to papaya leaf extract, clinical symptoms, and water quality were analyzed descriptively to see the differences in each treatment.

3. RESULTS AND DISCUSSION

This research was conducted to test the effectiveness of papaya leaf extract in the treatment of bacterial infected koi fish. For each treatment, different doses of papaya leaf extract were given. For treatment A, a dose of 5 ml of papaya leaf extract was given. For treatment B, a dose of 10 ml of papaya leaf extract was given. And for treatment C, a dose of 15 ml of papaya leaf extract was given. For treatment D (Control) a dose of 0 ml of papaya leaf extract was given. Giving different doses produces the main data in the form of recovery in fish and survival rates in fish.

3.1. Symptoms that Occur in Fish

Observations made on koi fish aim to find out how the condition of the fish differs after being infected with Aeromonas bacteria and how the condition of the fish is after being treated. The method used to infect fish in this study was by incorporating the test fish into the bacteria *Aeromonas hydrophila* for 1 minute. After that, observations were made of the changes that occurred in the fish after the koi fish seeds were infected with bacteria and after being given papaya leaf extract with different doses as an antibacterial for 30 days can be seen in the following table:

Table 1. Observations of changes that occur in fish

T	After Infected	After Treatment
K	On the body of the fish there is redness and bleeding, the fish doesn't move much, the fish doesn't respond when given food and even some fish don't want to eat.	The bleeding on the fish's body is still very clearly visible, some fish still look stressed, the fish have not responded when given food.
A	On the body of the fish there is redness and bleeding, the fish doesn't move much, the fish doesn't respond when given food and even some fish don't want to eat.	The wounds on the fish's body still bleed and the fish are still lacking in responding when given food.

B	On the body of the fish there is redness and bleeding, the fish doesn't move much, the fish doesn't respond when given food and even some fish don't want to eat.	The wounds on the fish's body began to look better, but they were still lacking in responding to food, the fish began to be seen moving actively.
C	On the body of the fish there is redness and bleeding, the fish doesn't move much, the fish doesn't respond when given food and even some fish don't want to eat.	The wound on the fish's body has healed, the fish is swimming normally, when it is given food, the fish has responded very well.

Observation of the characteristics of fish infected with the bacteria was observed within 3 days after being infected with *Aeromonas hydrophila* bacteria. *Aeromonas hydrophila* bacteria is a bacterium that has opportunistic pathogenic properties which can cause illness and death in fish. This disease occurs due to attack by *Aeromonas hydrophila* bacteria when fish are under stress. Conditions that can cause fish to experience stress include overcrowding, poor water quality, and inadequate nutrition (Novita, 2015). Circumstances that might cause fish to experience stress are too many fish in one container, poor water quality, too much leftover feed that contaminates the water, or insufficient food availability.

Figure 1. Koi fish that are free of *Aeromonas hydrophila* bacteria

Figure 2. Koi Fish Seeds that have been Infected with *Aeromonas hydrophila* Bacteria

Figure 3. Koi Fish Seeds after being Treated with Papaya Leaf Extract

3.2. Administration of Papaya Leaf Extract

Based on the research that has been done, it was found that there was a significant difference between treatment K (0 mL), treatment A (5 ml), treatment B (10 ml), and treatment C (15 ml). Treatment of fish by administering papaya leaf extract had the highest survival rate in treatment C (15 ml), namely 95%. This proves that the ability of papaya leaf extract to treat koi fish seeds attacked by Aeromonas bacteria is very effective. The alkoid karpain compound contained in papaya leaves is one of the active ingredients that functions as an antibacterial (Kalie, 2006). The content or active ingredients of flavonoids and tocophenols in papaya leaves are compounds that have hydroxyl groups and are the most abundant in plants. The two active ingredients have anti-inflammatory properties so they can reduce wounds or bleeding in the fish's body.

3.3. Fish Survival (SR)

From the research that has been done, it can be seen that there is antibacterial activity in koi fish seeds that have been infected with *Aeromonas hydrophila* bacteria for 30 days showed the best survival results of koi fish seeds, namely 95% in treatment C (15 mL). Data on the average survival of koi fish seeds can be seen in the following diagram:

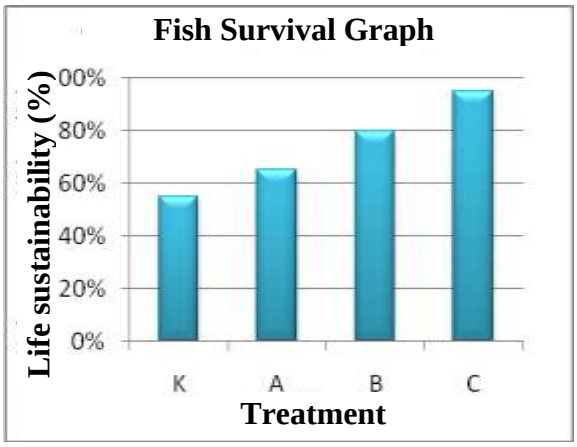

Figure 4. Graph of Fish Survival

The diagram shows that the higher the dose of papaya leaf extract, the higher the survival rate of the koi fish obtained. It can be seen that the lowest survival of koi fish seeds is 55% in the K treatment (control). Hence, with the administration of antibacterial

to fish that have been infected with *Aeromonas hydrophilla* bacteria can increase the survival rate in fish. Sainah et al. (2016) stated that "the ability of fish to adapt can affect differences in the survival rate of these fish. Attacks by *Aeromonas hydrophilla* bacteria present in fish can cause fish to experience stress". Fish that have a weak immune system will die, because these fish are unable to survive against attacks from bacteria. This proves that fish do not experience the process of immunologic stimulation so that bacteria easily attack the host in fish and infection develops. Meanwhile, fish that have a good immune system will be able to survive and this shows that non-specific defenses increase in fish (Lukistyowati & Kurniasih, 2011).

3.4. Water Quality

Table 2. Water Quality Before Treatment

Treatment	Parameter		
	pH	Temperature	DO
A1	7,37	27,6	7,1
A2	7,37	27,6	7,1
B1	7,37	27,6	7,1
B2	7,37	27,6	7,1
C1	7,37	27,6	7,1
C2	7,37	27,6	7,1
K1	7,37	27,6	7,1
K2	7,37	27,6	7,1

Table 3. Water Quality After Treatment

Treatment	Parameter		
	pH	Temperature	DO
A1	7,89	28,9	6,4
A2	7,82	28,6	6,2
B1	7,82	28,7	6,5
B2	7,96	28,1	6,3
C1	7,73	28,8	6,6
C2	7,84	29,0	6,9
K1	7,91	28,6	6,7
K2	7,86	28,7	6,5

The results of the degree of acidity (pH) obtained in this study were around 3,37-7,91 which pH is still included in normal water conditions for the survival of koi fish seeds. According to the National Standardization Agency (2009) a good degree of acidity (pH) for koi fish is around 6,5-8,5. The pH value on the survival of fish has a very large influence. For the results of measuring the water temperature in this study, it was found that it was 27,6-29,0 ^{o}C, which this temperature is still included in normal water conditions for the survival of koi fish seeds. According to the National Standardization Agency (2009) a good water temperature for koi fish is around 25-32 ^{o}C. Then the measurement of oxygen levels (DO) in this study obtained results around 6,1-7,1 which shows that this water condition is still normal for the survival of koi fish. Normal oxygen

levels for fish survival are above 5ppm. If the oxygen level in the water is low, the supply of oxygen in the water will decrease which will disrupt the breathing process in fish and can even cause stress to the fish.

4. CONCLUSION

In conclusion, the use of papaya leaf extract as an antibacterial has effectiveness in inhibiting the growth of the Aeromonas hyrophila bacteria which attacks koi fish seeds and can increase the survival of koi fish seeds. Besides, concentration of 15 ml of papaya leaf extract is the best concentration to inhibit the growth of *Aeromonas hydrophila* bacteria with a survival rate of 95%. Where the higher the concentration of papaya leaf extract given, the higher its effectiveness against *Aeromonas hydrophila* bacteria.

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