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Effectiveness of Papaya Leaf Extract (*Carica papaya* L.) for the Treatment of Koi (*Cyprinus rubrofuscus*) Infected with *Aeromonas hydrophila* Bacteria

Kiki Widiyasari Husain
Student, Department of Pendidikan Teknologi Pertanian,
Universitas Negeri Makassar, Indonesia
Patang
Lecturer, Department of Pendidikan Teknologi Pertanian,
Universitas Negeri Makassar, Indonesia
Andi Alamsyah Rivai
Lecturer, Department of Pendidikan Teknologi Pertanian,
Universitas Negeri Makassar, Indonesia

Abstract:
The purpose of this study was to analyze the effect of giving papaya leaf extract (Carica papaya) and the most effective dose in inhibiting Aeromonas hydrophila bacterial infection in Koi (Cyprinus rubrofuscus). This research is an experimental study with four treatments: Control Treatment (K): 0 ppm, Treatment A: 1000 ppm, Treatment B, and Treatment C: 1400 ppm. Data were processed using SPSS Version 24 with Analysis (ANOVA) and Kruskal-Wallis H. The results showed that giving papaya leaf extract had an effect on inhibiting Aeromonas hydrophila bacterial infection in koi. The condition of the fish improved on the 7th day after immersion; it was shown that the way they swam began to return to normal, and the clinical symptoms reduced due to infection. The lowest inhibition level was in the control treatment, the condition of the fish worsened every day, and the highest survival was in treatment B, namely 86.67%, the average behavior scale is 5.00, and the average morphological scale is 5.00. The average water quality temperature is 27.2-28.1˚C, the average pH is 7.1-7.2, the average dissolved oxygen is 5.7-5.8 ppm, and the ammonia is 0.25 ppm.

Keywords: *Papaya leaves, treatment, koi, Aeromonas hydrophila*

1. Introduction

Koi cultivation is a business that has good prospects in the ornamental fish business. However, cultivation cannot be separated from various diseases, and one of the causes is a disease caused by bacteria. When a fish is infected with bacteria, it has a great potential to infect other fish and can cause death and losses to farmers. There are various types of bacteria that can cause disease in fish.

One disease that can attack freshwater fish, both ornamental and consumption fish, and can kill fish up to 80-100% within 1-2 weeks is MAS disease (*Motile Aeromonas Septicaemia*) caused by the bacterium *Aeromonas hydrophila* (Haryani *et al*., 2012). *Aeromonas hydrophila* bacteria is a type of bacteria that is pathogenic and can cause systematic disease and result in mass death (Tanjung *et al*., 2011).

Handling of diseases caused by Aeromonas hydrophila bacteria can be done using chemicals and natural ingredients. Natural ingredients are thought to be good at dealing with A. hydrophila bacteria because they do not contain residue. The use of chemicals in large quantities and continuously will have negative impacts, such as bacterial resistance to chemicals, accumulation of residues for fishery commodities, and environmental pollution (Tarigan, 2014).

One of the active ingredients that are easy to obtain for treating diseases caused by Aeromonas hydrophila infection, which is quite efficient and environmentally friendly, is papaya leaves (*Carica papaya* L.) which is one of the most widely cultivated plants in Indonesia. Papaya leaves contain chemicals that can inhibit microbial growth.

The use of papaya leaves as a treatment for fish has been carried out by several previous researchers. Research on the effectiveness of papaya leaf extract (*Carica papaya* L.) for the treatment of goldfish (*Cyprinus carpio* L) seeds infected with Aeromonas hydrophila bacteria has been carried out by Rahmat (2014), and the effectiveness of papaya leaf (*Carica papaya* L.) soaking water in the treatment of catfish masamo (*Carias* sp.) wounds has been carried out by Adli and Saputra (2020). Based on this, this research has conducted research on the utilization of papaya leaf extract in the treatment of koi fish infected with *Aeromonas hydrophila*.

2. Research Purposes

The purpose of this study was to analyze the effect of giving papaya (*Carica papaya*) leaf extract in inhibiting *Aeromonas hydrophila* bacterial infection in koi (*Cyprinus rubrofuscus*) and to determine the most effective dose of papaya leaf extract to inhibit *Aeromonas hydrophila* bacterial infection in koi (*Cyprinus rubrofuscus*).

3. Method

This type of research is experimental, using a completely randomized design with 4 treatments and 3 replications. The treatments being tested were:

- Treatment K, which was without giving papaya leaf extract,
- Treatment A was soaking koi fish infected with *Aeromonas hydrophila* bacteria by giving papaya leaf extract as much as 1000 ppm,
- Treatment B is soaking koi fish infected with *Aeromonas hydrophila* bacteria by giving papaya leaf extract as much as 1200 ppm, and
- Treatment C is soaking koi fish infected with *Aeromonas hydrophila* bacteria by giving papaya leaf extract as much as 1400 ppm

The research procedures included providing media and test animals, preparing bacterial isolates (*Aeromonas hydrophila*), making papaya leaf extract, infecting fish, soaking, raising fish, and observing. Data collection techniques used in this study were: observation, testing of research variables, including water quality, and observation of fish health. The analysis technique used in this study was ANOVA analysis of variance, which previously carried out an assumption test based on the normality test to find out whether the data used in the study were normally distributed or not. If the data were normally distributed, then a homogeneity test and One Way Anova analysis of variance test was carried out to find out whether there were significant differences which were processed using the SPSS Version 24 program, and if there were significant differences followed by the Duncan test with a 95% confidence level. If the data are not normally distributed, then data analysis will use the Kruskal-Wallis H statistical test.

4. Results and Discussion

4.1. Effect of Papaya Leaf Extract in Inhibiting Aeromonas hydrophila Bacterial Infection

4.1.1. Observation of Fish Behavior

Based on the results of research that had been conducted for 16 days, it was found that there were differences in the percentage of changes in behavior in each treatment after soaking with papaya leaf extract (*Carica papaya*). Treatment B showed the best changes in fish behavior at the end of the study (day 16) with an average behavior scale of 5.00, followed by treatment A with an average behavior scale value of 4.67, treatment C with an average value average 4.33 and Treatment K showed very low behavior change with a value of 0.33. Based on the results of the Kruskal-Wallis H test, it was shown that treatment with papaya leaf extract affected fish behavior on the 12th to the last day (16th day) with a significant value ($P<0.05$). On the last day, the behavior change showed significantly different values ($0.038 <0.05$). Further tests were carried out in the form of a PostHoc test, with the results of treatment K (control) significantly different from treatment B.

Observation of clinical symptoms was carried out by observing the behavior of fish after being infected. After showing initial symptoms due to infection, the fish were then soaked in papaya leaf extract for 48 hours. The results showed that at the beginning of immersion, koi fish showed symptoms, namely swimming at the bottom of the pond, being around aeration, then the fish swam to the surface, and visible stress, and these symptoms continued on the 2nd day after immersion.

After soaking, the fish were transferred to the maintenance aquarium. On the first day of observation, untreated fish (control) swam in a tilted body position due to reduced body balance due to infection with *Aeromonas hydrophila* bacteria. Lestari (2006) states that *Aeromonas* hydrophila bacteria are capable of disrupting swimming balance so that fish become abnormal in swimming, such as fish swimming slowly or vertically and decreasing the response to eating fish due to disturbed body metabolic processes.

In treatments A and B, the fish were kept quiet at the bottom of the aquarium approaching aeration and swimming slowly, whereas in treatment C with 1400 ppm extract, there were fish that died due to the administration of too high a dose. In papaya leaves, there are saponin compounds that cause foam in the water, so fish have difficulty getting oxygen. This statement is in line with the opinion of Zebua *et al*. (2019) that saponin compounds in high doses that cross the tolerance limit of the fish body can cause poisoning and often even kill. Saponins enter the blood circulation through the gills. When oxygen is taken from the water, saponin compounds enter the body and bind to hemoglobin, causing fish to lack blood and cause death (Lukistyowati, 2012).

On day 4 the fish still showed the same symptoms, and more fish stayed at the bottom of the aquarium. Then on the 7th day, the fish in treatments A and B began to actively swim, and in treatment C, the fish moved slowly, while in the K treatment (control), there were several fish that swam upside down and swam slowly. On the 10th day, the fish in treatment B started to move normally and were agile, followed by treatments A and C on the 11th day, while in the control treatment, changes in fish behavior decreased due to the control treatment not being treated with papaya leaf extract, in which papaya leaves contain antibacterial compounds.

4.1.2. Observation of Fish Morphology

Based on the results of research that had been carried out for 16 days on changes in fish morphology parameters, it was found that there were changes in the treatment with papaya leaf extract (*Carica papaya*) and without treatment. The results of observations on the last day showed that the fastest morphological changes were found in treatment B, with an average of 5.00 on the morphological scale, then followed by treatment A, with an average morphological scale of 5.00, treatment C with an average of 4.33 and treatment K with an average of 1.00 indicating that the changes in fish morphology were the slowest without papaya leaf extract treatment.

In figure 1, it can be seen that there are morphological changes that occur in koi fish after infection and immersion. On the 5th day, the morphological conditions of the fish showed exophthalmia, loose scales, and black body color. This happened because for several days, and on the 13th day in the K treatment, the condition of the fish got worse with lots of loose scales on the fish and death. In treatment A, the fish experienced *Cloudy Eye*s (appearance of white membranes on the eyes). In treatment B, some of the fish were no longer injured, and in treatment C, the color of the fish's body blackened.

The results of the Kruskal-Wallis H test showed that the treatment had an effect from day 12 to the last day (day 16) with a significant value ($P<0.05$). On the last day, the morphological changes showed that the treatment had an effect ($0.023 <0.05$). Further tests were carried out in the form of a Post Hoc test, with the results of treatment K (control) significantly different from treatment B and treatment A.

Treatments	Fish Observation Results (day)	
	5th day	13th day
Control (No Extract)	 Exophatalmia	 Fish scales out
A : Papaya leaf extract 1000 ppm	 Fish scales out	 Cloudy Eye
B : Papaya leaf extract 1200 ppm	 Fish scales out	 Healthy Fish
C : Papaya leaf extract 1400 ppm	 Fish scales out & black body color	 Black body color

Figure 1: Observation of Fish Morphology

Changes that occur in fish infected with A. hydrophila bacteria are:

- Fish secrete excessive mucus,
- Inflammation,
- Pale body color,
- Scaly fins,
- Scales fall out,
- Exophthalmia, and
- Distended abdomen

The first clinical symptoms to appear were inflammation (inflammation), and then the fish was soaked with papaya leaf extract for 48 hours at different doses. On the first day of immersion, the clinical symptoms continued, and on the second day of immersion, other symptoms occurred, namely excessive mucus production. This is in accordance with the statement of Haryani *et* al. (2012) that the clinical symptoms of fish are characterized by inflammation (inflammation) which is characterized by swelling and redness at the injection site. This symptom is seen in all artificially infected koi fish.

4.1.3. Observation of Fish Anatomical (gill)

Based on the results of observations on the last day (16th day), the slowest changes in fish organs occurred in the control treatment (without papaya leaf extract) with pale and damaged gills. While organ changes that are relatively fast occurred in treatment B with fresh red gill color by giving papaya leaf extract (*Carica papaya*). The results of fish observations can be seen in figure 2.

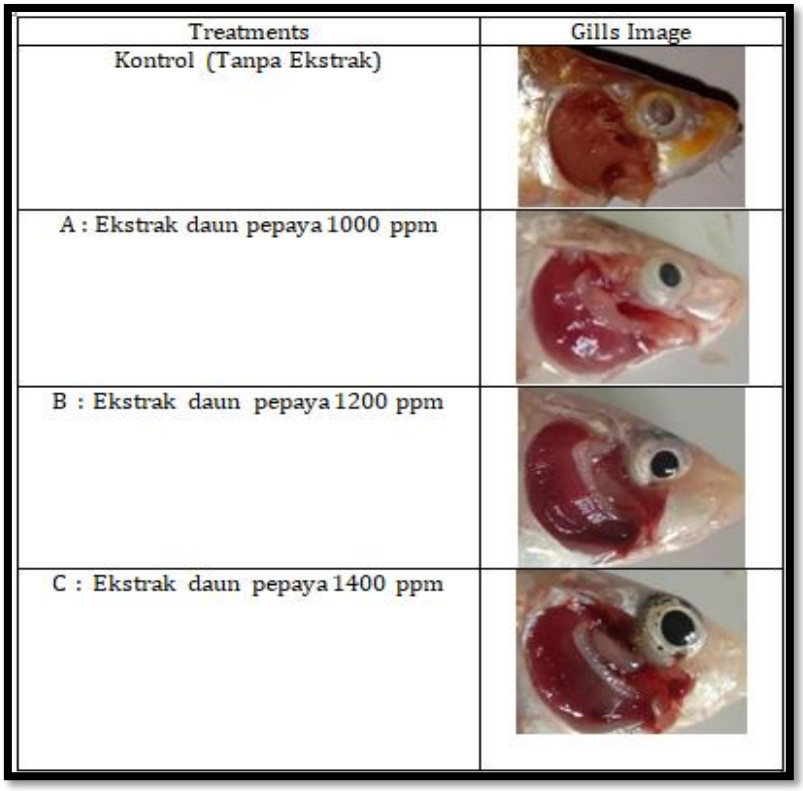

Figure 2: Observations on Fish Gills

On the last day, observations were made of fish gills. The control treatment showed clinical symptoms, namely pale, brown, and damaged gills, whereas, in the C treatment, the gills showed white spots on the gills. The best conditions were shown in treatment B with fresh red gills, followed by treatment A with fresh red gills.

If the disease is chronic, the color of the gills will turn brown, and the gill filters will clot, so they don't function properly (Wirawan *et al*., 2018). Diseased gill organs can also experience telangiectasia, which is the dilation of capillaries that can make it difficult for fish to breathe (Noviyanto *et al*., 2022). Diseased fish gills will show changes in the color of the gills to pale red or pink.

4.1.4. Fish Growth Rate

4.1.4.1. Absolute Weight Growth

Observation of absolute weight growth parameters was carried out at the beginning and end of the study. From the results of the study, it was shown that there was a change in weight in the fish treated, namely treatment A showed a result of 0.31 g, treatment B showed the best results and was higher than the other treatments, namely 0.44 g, treatment C showed a result of 0.20 and treatment K (control) showed a negative result of -0.12 g. The results of observing the absolute weight growth parameter can be seen in figure 3.

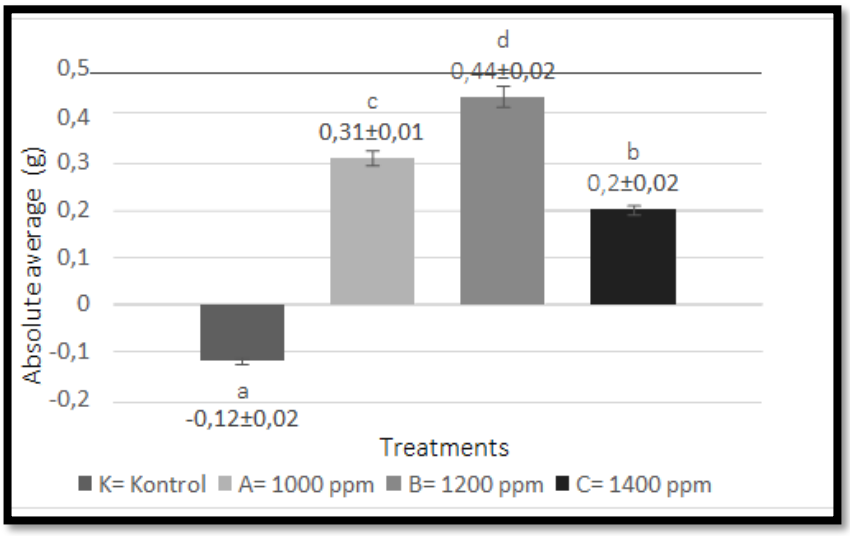

Figure 3: Absolute Weight Average Value (g)

Based on the test results (ANOVA) showed that the treatment had an effect on the absolute weight growth rate with a sig value (0.000 <0.05). Furthermore, Duncan's test was carried out with the results of all treatments significantly different between the K treatment (control) and the other treatments.

4.1.4.1.1. Absolute Length Growth

Observation of absolute length growth was carried out by measuring the difference in length at the start and end of the study. The length of the fish is measured from the tip of the head to the tip of the tail of the fish seeds. The results of the study showed an increase in the length of the fish treated, including treatment B which had the highest yield with an average length of 0.28 cm, followed by treatment A, with an average length of 0.23, and treatment C, with an average length of 0.21 cm, and finally, treatment K (control) with a very low yield of 0.03 cm. The results of observing the absolute length growth parameters of fish can be seen in figure 4.

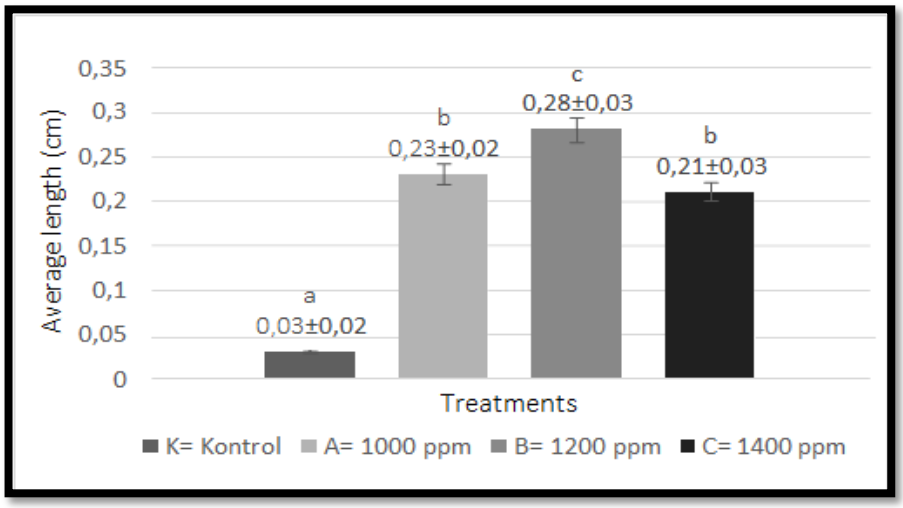

Figure 4: Average Absolute Length of Fish

The test results (ANOVA) showed that the treatment had an effect on the absolute length growth rate with a sig. value (0.000 <0.05). Furthermore, Duncan's test was carried out with the results of treatment K (control) significantly different from treatments A, B, and C, whereas treatment A was not significantly different from treatment C but significantly different from treatments B and K (control) and treatment B was significantly different from treatments K, A and C.

4.1.4.1.2. Specific Weight Growth

Hasil penelitian menunjukkan bahwa adanya peningkatan berat spesifik pada ikan yang diberi perlakuan ekstrak daun pepaya, the treatment that experienced a rapid increase in weight was in treatment B with an average yield of 0.28%, then followed by treatment A with a specific weight of 0.22%, treatment C which was 15% and treatment K (control) decreased with results - 0.09%. The results of observing the specific weight growth parameters can be seen in figure 5.

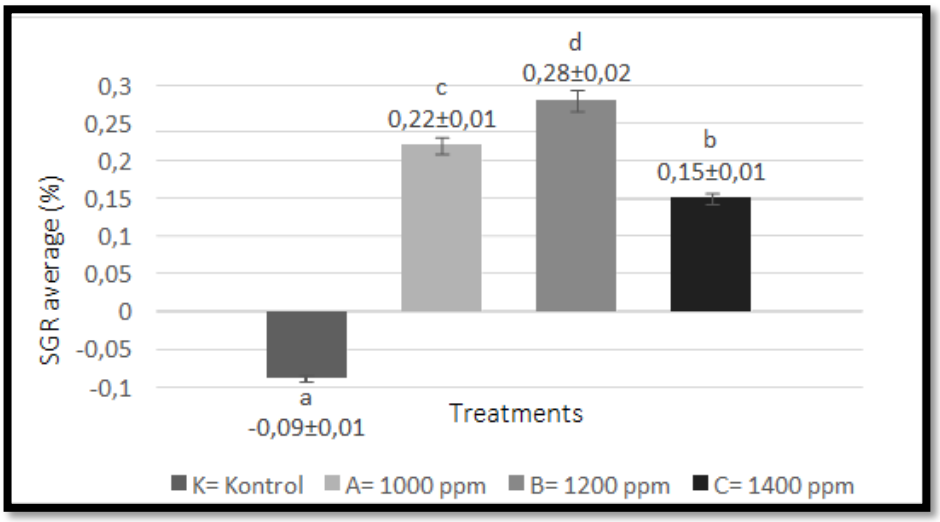

Figure 5: Average Specific Weight of Fish

The test results (ANOVA) showed that the treatment affected the growth rate of specific gravity with a sig. value (0.000 <0.05). Furthermore, Duncan's further test was carried out with the results of all treatments significantly different between treatment K (control) and other treatments.

4.1.4.1.3. Survival Rate

The results of observations on the survival parameters (survival rate) for 16 days, starting from 2 days of immersion and 14 days of observation, showed that there were changes in the survival rate of fish from several treatments to maintain koi fish life. The results of the study showed differences in survival between fish that were given papaya leaf extract (*Carica papaya*) and the control treatment that was not given papaya leaf extract. Based on the results of observations, it showed that the highest koi fish survival was in treatment B, with a total percentage of 86.36%, which was then followed by treatment A, with a percentage of 66.66%, and treatment C, with a percentage of 53.33%, while the lowest survival rate was in the control treatment, namely 33.33%. The results of observations on survival parameters can be seen in figure 6.

Berdasarkan hasil Uji Kruskal-Wallis H menujukkan bahwa perlakuan dengan pemberian ekstrak daun pepaya memberikan pengaruh terhadap kelangsungan hidup ikan koi dengan nilai sig (0,022<0,05). Selanjutnya dilakukan uji lanjut PostHoc dengan hasil perlakuan K (kontrol) berbeda nyata denganperlakuan B.

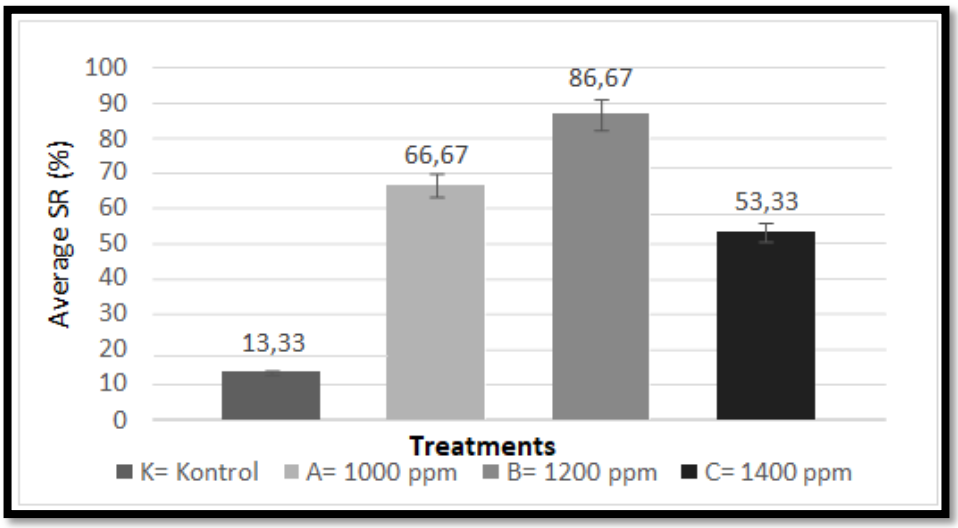

Figure 6: Average Survival Rate

4.2. Effective Dosages of Papaya Leaf Extract

The results of the study showed that the most effective dose in inhibiting *Aeromonas hydrophila* bacterial infection was a dose of 1200 ppm, at which dose the recovery rate for infected fish was relatively fast on day 5 after 48 hours of immersion both in terms of behavior, morphology (eyes and scales) and anatomy (gills). Whereas at doses of 1000 and 1400 ppm, the results obtained after soaking the fish recovery rate were relatively slower, where changes occurred on the 7th day of observation. In the control treatment (without extract), the condition of the fish worsened every day.

4.3. Water Quality

4.3.1. Water Temperature (°C)

Based on the observations of water quality parameters that have been carried out, it was found that the average temperature for each treatment ranged from 27.2°C-28.1°C. This shows that the quality of the temperature in the aquarium is still in the range of good conditions for koi fish life. Changes in temperature for each treatment are quite important things that can affect fish life. Normal temperature for koi fish ranges from 25°C–30°C.

4.3.2. Water pH

Based on the pH observations that have been made, the results show that the average for each treatment ranges from 7.1 to 7.2, and this shows that the quality of the pH in the aquarium is good for the survival of fish and the optimal pH for the survival of koi fish ranges from 6.5–8.

4.3.3. Dissolved Oxygen (ppm)

Based on the dissolved oxygen observations that have been carried out, the results show that the average for each treatment ranges from 5.7–5.8 ppm. This indicates that the quality of dissolved oxygen in the aquarium is good for the survival and growth of koi fish. Dissolved oxygen is an important thing in the survival of koi fish with optimal dissolved oxygen levels, namely > 5 ppm.

4.3.4. Ammonia (ppm)

Based on the ammonia observations that have been made, the same results were obtained in each treatment. Ammonia measurements were carried out twice, namely on the 7th day and 16th day. The results showed that the average level of ammonia at the time of the study was 0.25 ppm. This indicated that the quality of ammonia in the aquarium was in the range of concentrations that were good for the survival and growth of koi fish.

5. Conclusion

Giving papaya leaf extract (*Carica papaya*) has an effect on inhibiting Aeromonas hydrophila bacterial infection in koi fish with the best treatment of 1200 ppm, the condition of the fish is getting better from day to day both in terms of behavior, morphology, anatomy, growth and survival of fish. A Papaya leaf extract dose of 1200 ppm is the most optimal dose for controlling *Aeromonas hydrophila* bacteria in Koi fish (*Cyprinus rubrofuscus*).

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