JURNAL PERIKANAN DAN KELAUTAN
Volume 30 No. 1, February 2025: 136-143

e-issn : 2721-8902
p-issn : 0853-7607

The Use of Turmeric Extract (*Curcuma domestica*) for the Treatment and Survival of Comet Fish (*Carassius auratus auratus*) Infected with *Aeromonas hydrophila* Bacteria

Penggunaan Ekstrak Kunyit (Curcuma domestica) untuk Pengobatan dan Kelangsungan Hidup Ikan Komet (Carassius auratus auratus) yang Diinfeksi Bakteri Aeromonas hydrophila

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Received
5 January 2025

Accepted
31 January 2025

Abstract

Comet fish (*Carassius auratus auratus*) is one type of freshwater ornamental fish that is highly demanded by the ornamental fish hobbyist community because it has a variety of colors and attractive physical forms. However, comet fish cultivation has decreased due to disease attacks that lead to death caused by *Aeromonas hydrophila* bacteria. Therefore, farmers generally use chemical drugs such as antibiotics to treat diseases in comet fish, yet for antibiotics, and it is necessary to research herbal medicines that contain antibacterial content, such as turmeric (*Curcuma domestica*). This research aims to determine the effect of immersion time using turmeric extract solution for treatment and survival rate of comet fish seeds infected with *A.hydrophila* bacteria. This is experimental research with a Completely Randomized Design (CRD) consisting of four treatments and three replicates, namely treatment A (control), B (50 minutes immersion), C (60 minutes immersion), and D (70 minutes immersion). The results showed that using turmeric extract affected treating comet fish seeds infected with *A.hydrophila* bacteria. As for the survival rate, the results of analysis of variance (ANOVA) showed that the use of turmeric extract had no significant effect, with the percentage value of the survival rate of comet fish seeds of treatment A (63.30%), B (90.00%), C (80.00%), and D (86.60%).

Keywords: *Aeromonas hydrophila*, Aquaculture, Diseases

Abstrak

Ikan komet (*Carassius auratus auratus)* merupakan salah satu jenis ikan hias air tawar yang banyak digemari oleh masyarakat penghobi ikan hias, karena ikan ini mempunyai warna yang beragam dan bentuk fisiknya yang menarik, tetapi akibat serangan penyakit yang disebabkan oleh bakteri *Aeromonas hydrophila* menjadikan ikan komet mengalami kematian, sehingga budidaya ikan komet menurun. Oleh karena itu, pembudidaya pada umumnya menggunakan obat-obatan berbahan kimia seperti antibiotk untuk mengobati penyakit pada ikan komet, tapi penggunaan antibiotik kurang efisien, tidak ekonomis dan tidak ramah lingkungan. Tindakan yang perlu dilakukan untuk mengurangi penggunaan antibiotik, maka perlu dilakukan penelitian penggunaan obat-obatan dari herbal yang memilki kandungan antibakteri yaitu kunyit (*Curcuma domestica*). Penelitian ini bertujuan untuk mengetahui pengaruh lama perendaman menggunakan larutan ekstrak kunyit untuk pengobatan dan tingkat kelangsungan hidup benih ikan komet yang diinfeksi

bakteri *A. hydrophila*. Penelitian ini menggunakan metode eksperimen dengan Rancangan Acak Lengkap (RAL) 4 perlakuan dan 3 ulangan, yaitu perlakuan A (kontrol), B (perendaman 50 menit), C (perendaman 60 menit), dan D (perendaman 70 menit). Hasil penelitian menunjukan bahwa penggunaan ekstrak kunyit berpengaruh nyata untuk pengobatan benih ikan komet yang diinfeksi bakteri *A.hydrophila*. Sedangkan untuk tingkat kelangsungan hidup, hasil analisis sidik ragam (ANOVA) menunjukan bahawa penggunaan ekstrak kunyit tidak berpengaruh nyata, dengan nilai presentase tingkat kelangsungan hidup benih ikan komet yaitu perlakuan A (63.30%), B (90.00%), C (80.00%) dan D (86.60%).

Kata kunci: *Aeromonas hydrophila*, Akuakultur, Penyakit

1. Introduction

Comet fish (*Carassius auratus auratus*) is one of the many types of freshwater ornamental fish that are very famous among ornamental fish enthusiasts because this fish has a variety of colors with a combination of yellow, orange, white, golden black, and its attractive physical shape resembles a koi fish. Unlike koi fish, which are visually seen to have a body shape that tends to be large, comet fish have a body shape that tends to be smaller than koi fish. In addition, comet fish are one of the choices to be kept or cultivated because comet fish are a type of ornamental fish that can be cared for in ponds and aquariums.

In the fish farming system, the comet fish always experiences obstacles due to environmental and other factors. One factor that often becomes an obstacle in fish farming is disease, which can cause death in fish. Factors that cause fish disease come from parasites, fungi, bacteria, and viruses. The disease that often attacks freshwater ornamental fish or comet fish is MAS (*Motil Aeromonas Septicemia*), which is caused by the bacteria *Aeromonas hydrophila* (Simalango & Sinaga, 2018).

Eradication of disease-causing bacteria can currently be done using drugs such as antibiotics. However, the use of antibiotics on a large scale is less efficient because, apart from being uneconomical, the impact is an increase in the types of bacteria that are resistant to antibiotics and can pollute the environment (Maisyaroh et al., 2018). Therefore, using antibacterials from herbal plant extracts is an alternative for safer, more effective, and more efficient control (Utomo et al., 2015), one of which is herbal plant extract, namely turmeric. Turmeric (*Curcuma domestica*) is a medicinal plant known to have many benefits, especially from its rhizome. Compounds that have antibacterial activity in turmeric rhizomes include curcuminoids, essential oils, resins (including terpenoids, triterpenoids, and phenylpropene), flavonoids, tannins, saponins, alkaloids, fats, carbohydrates, vitamins, and others.

Curcumin is believed to have the ability as an antibacterial agent that can be an option to treat infections and reduce the possibility of resistance to antibiotic use. Therefore, this study aims to determine the effect of immersion time using turmeric extract solution for treatment and the survival rate of comet fish seeds infected with *A.hydrophila* bacteria.

2. Material and Method

2.1. Time and Place

This research was implemented in August - September 2024, located at the Gorontalo City Fish Seed Center (BBI), Jl. Madura No. 08, Paguyaman, Kota Tengah District, Gorontalo Regency.

2.2. Methods

The design used in this study was a completely randomized design (CRD) with four treatments, each repeated three times. The treatments given were different immersion times using turmeric extract, namely treatment A (control/no immersion), B (50-minute immersion), C (60-minute immersion), and D (70-minute immersion).

2.3. Procedure

2.3.1. Making Turmeric Extract

Extract from turmeric is made by immersion (maceration). Turmeric is cleaned well and then left to dry naturally. After the turmeric is dry, remove the turmeric skin and thinly slice it, following the size of the turmeric circle, using a knife. This is done to speed up the drying process. Drying in an open room exposed to light sunlight lasts three days until the turmeric is completely dry. After drying, the next step is to grind the turmeric pieces using copper until they form a powder, then sift them until you get a fine turmeric powder.

The turmeric powder that has been obtained is put into a 2 L glass jar, then add 95% ethanol, as much as 3x500 ml, and stir briefly using a small spatula until evenly mixed, then shake using a stirrer for 3 days then let stand for

24 hours. After 24 hours of standing, we get the filtrate to separate the filtrate from the solvent, namely using a satin cloth until no more solvent drips. The resulting thick extract is poured into a small glass jar and labeled. It can be used to control MAS disease caused by *A.hydrophila* bacteria attacking comet fish seeds.

2.3.2. Making Growth Media for A. hydrophila Bacteria.

The media used to grow bacterial inoculants is TSB (Tryptic Soy Broth) with a ratio of 0.8 g TSB dissolved in 20 ml of distilled water. The mixture is poured into an Erlenmeyer flask, and then a magnetic stirrer is used to mix the media evenly. Do this process for about 5 minutes, then remove the magnetic stirrer. After that, place it on a hot plate to be heated until boiling. After boiling, transfer it to an autoclave for the sterilization process with a sterilization time of 30 minutes at a temperature of 121 ℃ with a pressure of 2 atm. After sterilization, place it on a laminar flow workbench to add *A.hydrophila* bacteria samples.

2.3.3. Provision of A. hydrophila Isolates

Aeromonas hydrophila bacteria samples were rejuvenated in TSB media and then incubated using a Shaker incubator for 24 hours at a temperature of 28℃. After incubation for 24 hours, the test bacterial suspension was measured for absorbance using a spectrophotometer, which showed that the bacterial suspension had a turbidity of around 1.5 nm, then reduced using physiological NaCl to a turbidity level according to the Mc Farland standard, namely 0.5 with a bacterial density of 10^8 CFU/mL. After the *A.hydrophila* bacterial isolate was ready, the bacteria were infected with comet fish seeds at 0.1 mL/fish. After the comet fish seeds showed clinical symptoms, the seeds were soaked in a turmeric extract solution and stored for 14 days.

2.3.4. Preparation of Tools and Materials

The containers used in this study were aquariums measuring 30 cm x 25 cm x 20 cm, totaling 21, with 9 for immersion comet fish with turmeric extract and 12 for maintaining comet fish seeds. Before the aquarium is used, it must be cleaned using dishwashing liquid and then rinsed with clean water. The aquarium is filled with 10 L of water per container using a measuring cup and continuously aerated. This study used fish seeds measuring 6-8 cm. The number of comet fish seeds used was 120, with a stocking density of 10 fish/container.

2.3.5. Making Turmeric Extract Solution

The dose of turmeric extract used in the immersion solution for treating diseases caused by *A. hydrophila* bacteria is 0.6 g/L. The immersion solution using turmeric extract first prepares nine aquariums cleaned and filled with clean water with an initial volume of 10 L. Furthermore, the turmeric extract is weighed as much as 6 g per container using an analytical scale, dissolved in 1 L of water from each treatment, and poured into the immersion aquarium slowly through the container wall. After the immersion solution is prepared, each container is filled with ten test fish.

2.4. Research Parameters

2.4.1. Symptoms of A.hydrophila Bacterial Infection

Aeromonas hydrophila bacterial infection symptoms are clinical symptoms seen from changes in physical condition and behavior in comet fish seeds. Observations of these clinical signs are carried out after comet fish seeds are infected by *A.hydrophila* bacteria, where monitoring is carried out for three days after the bacterial infection occurs.

2.4.2. Healing of Aeromonas hydrophila Bacterial Infection

Healing of *A.hydrophila* bacterial infection is a clinical symptom seen from changes in physical condition and behavior in comet fish seeds infected with *A.hydrophila* bacteria after being soaked or treated with turmeric extract solution, such as changes in morphology and behavior.

2.4.3. Survival Rate

Survival can be calculated using the formula as follows:

$$SR = \frac{Nt}{No} \times 100\ \%$$

Information :

SR : Survival (%)
No : Number of test fish at the start of the study (fish)
Nt : Number of test fish at the end of the study (fish)

2.4.4. Water Quality

Water is a living medium for aquatic organisms and is an important factor to consider in its quality to support the life of organisms. Temperature (C), dissolved oxygen (DO), and acidity (pH) are the main parameters that can

affect water conditions. Water quality measurements during the maintenance period are carried out at the beginning, every week, and at the end. Water quality measurement activities are carried out in the afternoon.

2.5. Data Analysis

The parameters observed in this study were the survival rate of comet fish seeds and the water quality observation stages. To determine whether immersion using turmeric extract can affect the survival of comet fish seeds infected with *A.hydrophila* bacteria, the data obtained were analyzed using analysis of variance (ANOVA) with a 95% confidence level and continued with Duncan's further test to determine the differences between treatments.

3. Result and Discussion

3.1. Symptoms of A.hydrophila Bacteria Infection

Symptoms of *A.hydrophila* bacterial infection are clinical symptoms seen from changes in physical condition and behavior in fish. These clinical symptoms are observed after the fish are infected with *A.hydrophila* bacteria; observations are carried out for 3 days after bacterial infection in comet fish seeds.

Table 1. Clinical symptoms of comet fish

Day	Clinical symptoms	
	Morphology	Behavior
I.	Observations on the 1st day after the comet fish seeds were infected with *A.hydrophila* bacteria, some wounds had discharged pus at the injection site, the stomach was enlarged and swollen, and some scales fell off. This did not happen to all fish in each treatment.	The behavior shown by comet fish seeds on the 1st day after being infected with *A.hydrophila* bacteria, namely swimming slowly or even staying still at the bottom of the aquarium, no response to food when fed so that the water in the aquarium is dirty.
II.	On the 2nd day after bacterial infection, the comet fish fry showed symptoms of red spots around the injection wound up to the pectoral fins and the eyes of the fish fry, which looked protruding or swollen.	On the 2nd day after being infected with Aeromonas hydrophila bacteria, the comet fish fry showed abnormal movements as seen from the haphazard swimming of the comet fish fry.
III.	On the 3rd day after bacterial infection, the comet fish fry experienced increasingly severe damage to their bodies, so their back muscles had holes, and the tested fish died, indicating serious injuries.	The 3rd day of observation of the behavioral symptoms of comet fish fry after being infected with *A.hydrophila* bacteria showed the movement of the fish fry, which seemed to swim irregularly following the rotation of the air bubbles.

Based on observations for 3 (three) days after being infected with *A.hydrophila* bacteria, the comet fish fry showed morphological changes such as clinical symptoms and behavior, as shown in Table 1. Morphological changes demonstrated by the comet fish fry after being infected with *A.hydrophila* bacteria, including purulent wounds, an enlarged/swollen stomach, and some scales falling off on the 1st day, the presence of red spots around the injection wound to the pectoral fins, protruding/swollen eyes, and on the 3rd day the symptoms or morphological changes shown by the comet fish fry were wounds from the infection so that the fish's back looked perforated.

Clinical symptoms or morphological changes that attack comet fish seeds after being infected with *A.hydrophila* bacteria show symptoms of disease caused by *A.hydrophila* bacteria, according to Wulan (2022), where fish infected with MAS (*Motil Aeromonas Septicemia*) have reddish spots on the body, peeling scales, pale and brittle fins and reddish wounds on the fish's body. Saputra & Indaryanto (2018) also stated that fish infected with MAS (*Motile Aeromonas Septicemia*) have signs such as loss of appetite, skin lesions, bleeding in the gills, a swollen stomach due to fluid, detached scales, and detached tail fins.

The clinical symptoms in the comet fish seeds are also following research by Pratama et al. (2022), where these symptoms occur in Rajadanu goldfish that have been attacked by a disease caused by the bacteria *A.hydrophila*, namely the appearance of minor wounds to wounds that enlarge and open and discharge pus, exophthalmia or protruding eyes and the appearance of ulcers, matters is supported by the statement (Yuhana et al., 2008), fish attacked by *A.hydrophila* bacteria will experience bleeding in parts of the body, especially in the chest, stomach and base of the fins.

The behavioral changes shown by the comet fish fry after being infected with *A.hydrophila* bacteria include swimming slowly and some even staying at the bottom of the aquarium on the 1st day. Abnormal movements can be seen from how the fish fry swim haphazardly and irregularly following the air bubbles and the lack of appetite so that the water quickly becomes dirty due to unfinished food. The behavioral changes shown by the comet fish fry are the same as the statement by Damayanti et al. (2024). In his research, fish showed behavioral changes within 24 hours after infection, where they tended to swim less actively or spend more time at the bottom of the aquarium and experienced a decrease in appetite.

Figure 1. Physical condition of comet fish seeds after bacterial infection
Description: (a). Purulent wounds at the injection site and enlarged stomach, (b). Scales falling out, (c) reddish spots, (d) bulging eyes, (e) widening wounds

3.2. Healing of A. hydrophila Bacterial Infection

Comet fish seeds infected with *A.hydrophila* bacteria were treated using turmeric extract at a dose of 0.6 g/L by immersion in turmeric extract solution with a immersion time according to treatment A (control/without giving turmeric extract), B (50-minute immersion), C (60-minute immersion) and D (70-minute immersion) which were then observed for the healing process of clinical symptoms and behavior every day for 14 days.

Table 2. Healing of *A.hydrophila* bacterial infections

P	Day	Clinical symptoms	
		Morphology	Behavior
A.	I-XIV	Comet fish seeds in treatment A for days 1 to 14 did not show any healing of wounds caused by Aeromonas hydrophila bacteria, and even some comet fish seeds died; the cause of the lack of healing in comet fish seeds was that they were not soaked using turmeric extract.	In treatment A, from observations on days 1 to 14, there was no change or healing; the comet fish seeds were still swimming abnormally, and there was no response to feed.
B.	I.	Observations on the 1st day after the comet fish seeds were soaked using turmeric extract solution did not show any changes or healing of the clinical symptoms demonstrated by the comet fish seeds in treatment B, namely protruding eyes and wounds that were still wide open at the injection site.	The behavior shown by comet fish seeds on the 1st day after being soaked using turmeric extract solution is that they still swim slowly and stay at the bottom of the aquarium. There is no response to food when fed, making the water in the aquarium dirty.
	III.	Observations on the 3rd day after the seeds were treated still did not show any healing of clinical symptoms; namely, the eyes were still swollen, some fish seeds even went blind, and the wounds remained the same size.	The 3rd day of observation of the behavioral symptoms of comet fish fry showed the movement of the fish fry, which seemed to swim irregularly following the rotation of the air bubbles.
	V.	On the 5th day, the wound from the injection site on the back of the comet fish fry began to show signs of flesh regeneration, and the size of the wound started to shrink.	On the 5th day of observation, the comet fish seeds began to swim normally and responded to the feed.
	X.	On the 10th day, the wound on the back of the comet fish seed had shrunk, whereas on the 5th day, it was still significant.	On the 10th day of observation, the comet fish fry began to swim normally and responded to the feed.
	XIV.	On the 14th day, after immersion them using turmeric extract solution, the comet fish seeds get better, with their eyes and stomachs improving and their wounds healing.	On the 14th day of observation, the comet fish fry began to swim normally and responded to the feed.
C.	I.	Observations on the 1st day after the comet fish seeds were soaked using turmeric extract solution did not show any changes or healing of the clinical symptoms of the comet fish seeds in treatment C, namely protruding eyes and wounds that were still wide open at the injection site.	The behavior shown by comet fish seeds on the 1st day after being soaked using turmeric extract solution is that they still swim slowly and stay at the bottom of the aquarium. There is no response to food when fed, making the water in the aquarium dirty.
	III.	Observations on the 3rd day after the fish seeds were soaked in turmeric extract solution showed healing, namely, the red spots around the pectoral fins had disappeared, although the wound at the injection site had not shown any changes.	The 3rd day of observation of the behavioral symptoms of comet fish fry showed the movement of the fish fry, which seemed to swim irregularly following the rotation of the air bubbles.
	VII.	On the 7th day, the wound on the back of the comet fish seed had shrunk because of the regeneration of the flesh, whereas on the 5th day, it was still large.	On the 7th day of observation, the comet fish fry began to swim normally and responded to the feed.
	X.	On the 10th day, the wound on the back of the comet fish seed had closed.	On the 10th day of observation, the comet fish fry began to swim normally and responded to the feed.
	XIV.	On the 14th day, after immersion using turmeric extract solution, the wounds of the comet fish fry improved and closed on the 10th day.	On the 14th day of observation, the comet fish fry began to swim normally and responded to the feed.
D.	I.	Observations on the 1st day after the comet fish seeds were soaked using turmeric extract solution did not show any changes or healing of the clinical symptoms of the comet fish seeds in treatment D, namely protruding eyes and wounds that were still wide open at the injection site.	The behavior shown by comet fish seeds on the 1st day after being soaked using turmeric extract solution is that they still swim slowly and stay at the bottom of the aquarium. There is no response to food when fed, making the water in the aquarium dirty.

P	Day	Clinical symptoms	
		Morphology	Behavior
	V.	On the 5th day, the wound from the injection site on the back of the comet fish seed showed signs of flesh regeneration, and the wound size began to shrink.	On the 5th day of observation, the comet fish seeds began to swim normally and responded to the feed.
	X.	On the 10th day, the wound on the back of the comet fish seed had closed.	On the 10th day of observation, the comet fish fry began to swim normally and responded to the feed.
	XIV.	On the 14th day, after immersion using turmeric extract solution, the comet fish seeds improved, with the wounds closing on the 10th day.	On the 14th day of observation, the comet fish fry began to swim normally and responded to the feed.

Based on observations for 14 (fourteen) days after the comet fish seeds showed clinical symptoms in morphology and abnormal behavioral symptoms due to *A.hydrophila* bacteria soaked using turmeric extract solution according to treatment B (immersion for 50 minutes), C (immersion for 60 minutes) and D (immersion for 70 minutes), showed healing of wounds or other clinical symptoms that attacked the comet fish seeds caused by *A.hydrophila* bacteria, as well as behavior which showed changes, namely the fish seeds swam normally and actively and there was a response to the feed given. The healing experienced by comet fish seeds infected with bacteria after treatment using turmeric extract solution was due to the ability of the turmeric plant to cure *Motile Aeromonas Septicemia* (MAS) disease caused by *A.hydrophila* bacteria derived from the active compounds contained in turmeric extract. Turmeric extract is known to contain curcuminoids (7.798%) (Suprihatin et al., 2020), essential oil (2.8%) (Rivai et al., 2019), flavonoids (4.8%) (Effendi, 2019), alkaloids (1.9%) (Rivai et al., 2019), tannins, and saponins. These compounds have antiseptic and antibacterial properties comparable to chloramphenicol, effectively treating MAS disease in fish.

In treatment A (control) or without immersion with turmeric extract solution, the wounds on the comet fish seeds were still large without any changes in shrinking or closing, in addition to the absence of healing of wounds on the body of the comet fish seeds, the behavior shown was still abnormal swimming such as swimming in circles following the rotation of air bubbles, no response to feeding so that the water quickly became dirty because the feed was not eaten. This is what caused some comet fish seeds in treatment A to die.

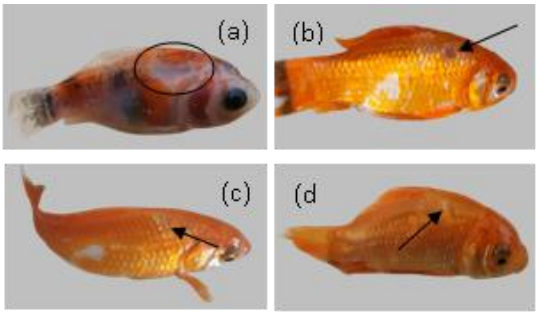

Figure 2. Clinical symptoms at the end of maintenance
Note: (a). Treatment A, (b). Treatment B, (c). Treatment C, and (d). Treatment D.

3.3. Survival Rate

Survival rate compares the number of individuals at the end of maintenance with those stocked at the beginning. Based on Figure 3, it can be seen that the survival rate of comet fish seeds in each treatment is different. Following the calculation formula, the survival rate of comet fish seeds during 14 days of maintenance, namely in treatment B (50-minute immersion), had the highest survival rate of 90%, followed by treatment D (70-minute immersion) with a survival rate of 86.60%, treatment C (60-minute immersion) with a survival rate of 80%, while treatment A (without immersion) showed the lowest survival rate of 63.30%.

The high and low survival rate of comet fish seeds during the study was thought to be influenced by the environment, water quality, and the number of unfinished food leftovers so that the water in the aquarium looked cloudy and the fish experienced stress and, resulted in death. This follows (Fernando et al., 2019) that the large amount of feed given and not consumed by the fish will cause the water to become cloudy and the fish to experience stress. Meanwhile, according to Prakoso et al. (2018), water quality is an essential factor that influences the survival rate of fish.

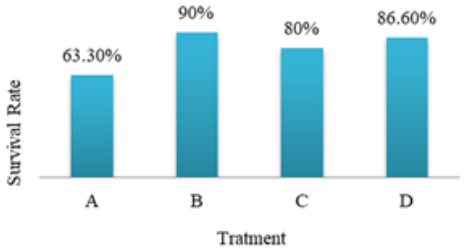

Figure 3. Survival Rate

3.4. Water Quality

Water quality is one of the crucial elements in fisheries management. In the context of fish, water quality can be interpreted as water suitable to support fish survival and growth. The water quality that has the most influence on fish life is temperature, pH, and DO.

Table 3. Water quality during maintenance

Treatment	Temperature (℃)	DO (mg/L)	pH
A	26.85	4.9	8.1
B	26.88	4.5	8.4
C	27.00	4.8	8.2
D	26.95	4.9	8.5
Standard	20 – 30 (Gunawan & Suraya, 2019)	> 4 (Wihardi et al., 2014)	6.5 – 8.5 (Wihardi et al., 2014)

Based on the table above, it can be seen that the water quality of each treatment does not have much difference, whereas in treatment A (control), the temperature is 26.85℃, DO 4.9 mg/L, and pH 8.1. In treatment B (50 minutes), the temperature is 26.88℃, DO 4.5 mg/L, and pH 8.4. Treatment C (60 minutes), the temperature is 27.00℃, DO 4.8 mg/L, and pH 8.2. In treatment D (70 minutes), the temperature is 26.95℃, DO 4.9 mg/L and pH 8.5. The water quality values during maintenance are still within the optimal range for comet fish cultivation because they are still within the standard water quality values that are good for fish cultivation.

4. Conclusions

Based on the results and discussion, it can be concluded that the administration of turmeric extract with different immersion times can treat comet fish seeds infected with *A.hydrophila* bacteria, where at the end of maintenance, the clinical symptoms of comet fish seeds showed healing and the immersion time using turmeric extract solution did not significantly affect the survival rate of comet fish seeds infected with *A.hydrophila* bacteria with the highest comet fish seed survival value obtained in treatment B (50 minutes) which was 90.00%.

5. Suggestion

Further research is needed by increasing the immersion time using turmeric extract solution to find the immersion time that can affect the survival of comet fish seeds infected with *A.hydrophila* bacteria.

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