Original Research Article

THE EFFECT OF AQUEOUS CATTAPA LEAF EXTRACT ON THE TREATMENT OF GOURAMY JUVENILE (Osphronemus goramy) INFECTED WITH SAPROLEGNIA sp.

ABSTRACT

The purpose of this research was to determine the effect of cattapa leaf solution (*Terminalia goramy*) on the treatment of juvenile goramy (*Osphronemus goramy*) infected with the fungal pathogen *Saprolegnia* sp. This study used a completely randomized design method with 5 treatments and 3 replications. The treatments given consisted of treatment A (control, without cattapa leaf solution), while treatment B, C, D and E were 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm consecutively. The parameters observed were survival rate, scale of fungal growth intensity (scaled from 1 to 5) and water quality. The scale of fungal growth and administration of cattapa leaf solution with different doses did not significantly affect the survival of goramy ($p > 0.05$). However, the highest survival rate of goramy was found in treatment B (500 ppm) which was 59% ± 38.31 and the lowest was found in treatment E (2000 ppm) at 21% ± 4.61. Increase of goramy leaves concentration was thought to cause stress of fish hence response to feeding was lower affecting the survival rate. Infections of *Saprolegnia* were still found from fish in all treatments although treated with cattapa leaf solution. In treatment D (1500 ppm), *T. cattapa* had a slight effect in the reduction of *Saprolegnia* found on the body of fish where decrease in the scale of fungal growth from scale 5 (highy infected stage) to scale 3 (medium infected stage) were found in several fish. It is possible that the majority of Gouramy fish in this research was unable to resist and recover from the fungal infection *Saprolegnia* sp. Water quality parameters in this study such as temperature, pH, DO and ammonia were still in a suitable condition for the maintenance of gourami juvenile.

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Keywords: Cattapa leaf, Gouramy, *Saprolegnia* sp., resistance, fungal growth.

1. INTRODUCTION

Indonesia has considerable potential in the development of fish farming, one of which is Giant Goramy (*Osphronemus goramy*). It is a freshwater fish native to Indonesia which belongs to the Labyrinthici family and possessing a high increase demand [1]. Goramy cultivation has obstacles in its development. These obstacles are in the form of declining production such as high mortality reaching 55-95% due to disease, lack of feed nutrition and poor water quality environment [2].

One of the pathogen that infects fish and freshwater fish eggs is *Saprolegnia* sp. The characteristics of fish and fish eggs attacked by the fungus *Saprolegnia* sp. can be identified through the external organs of fish. The affected part of the fish organ will be overgrown by a group of fungal mycelium that resembles a lump of fine thread (*hype*) that looks like cotton so it is called a white cottony growth [3].

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The continuous use of chemicals and antibiotics with inappropriate concentrations can cause new problems, namely increasing parasite resistance to these synthetic compounds and the problems they cause to the environment [4]. Therefore, there is a need for safer alternative drugs to be used in controlling diseases caused by the fungus *Saprolegnia* sp [5]. One of the traditional plants that has the potential to treat diseases caused by the fungus *Saprolegnia* sp. is cattapa leaf (*Terminalia catappa*) [6].

Research on the use of cattapa leaf solution for the treatment of *Saprolegnia* sp. in goramy juvenile was still not well known. So far, research on the use of cattapa leaf solution especially using aqueous extract has focused more on treating fish infected with *Aeromonas hydrophila* bacteria or for treating *Saprolegnia* sp. on

fish eggs. Tilapia soaked in a solution of 200 ppm of cattapa leaves had the lowest prevalence of parasites [7]. In addition, the solution of cattapa leaves has an effect on increasing innate immune responses and disease resistance in tilapia hence effective to protect fish against bacterial pathogens [8]. Soaking cattapa leaf extract was also known to provide an effective rearing medium for larval gourami [9]. Hence, in general the use of plant extract solutions was known to be able to overcome *Saprolegnia* sp. effectively through the immersion method on fish[10] but the use of cattapa leaf extract to treat *Saprolegnia* in juvenile fish has not been studied. Through this research, it is hoped that information regarding cattapa leaf extract on the handling of *Saprolegnia* sp. can be obtained considering the efficacy of cattapa leaves which are very good for fish health.

This research was conducted using an experimental method by testing various doses of cattapa leaf solution prepared with aqueous extraction on goramy juvenile infected with *Saprolegnia* sp. The dosing of the solution is designed so as not to cause side effects such as toxicity. Determination of the dose of cattapa leaf solution refers to previous studies using papaya leaf solution for the treatment of fish that are attacked by *Aeromonas hydrophila*. In a study conducted by Maftuch et al (2016) it was found that a solution of 730 ppm of cattapa leaves was quite effective on goldfish juvenile infected with *Aeromonas hydrophila* although survival at the time of treatment was only 53% [11]. In this study, the doses of the cattapa leaf solution tested were control (without solution), 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm. The dose determined in this study refers to research that uses herbal solutions (ketapang leaves) to treat parasitic attacks on tilapia (Oreochromis niloticus)[12]. The determination of the dose in this study has also taken into account the toxicity aspect of the cattapa leaf solution. Given the experimental nature of this study, it is estimated that the administration of cattapa leaf solution can inhibit the growth of fungi on gourami juvenile compared to without the use of cattapa leaf solution.

2. MATERIALS AND METHODS

The research was carried out from September to October 2020 starting with maintenance activities at Ciparanje Fish Farm, Universitas Padjadjaran. As much as 200 juvenile gourami with the size of 5-7 cm was transported from Purwakarta Regency, West Java Indonesia. Fish were then then acclimatized for one week to make the fish adapt to their environment and monitor the condition of the fish before testing. Fish were kept densely in cement ponds filled with water originating from irrigation source. This method rearing increased the occurrence of saprolegnia infection in fish. After the induction process with *Saprolegnia*, The temperature used in the aquariums ranged from 28-30^{o}C. 13 fish were added each for each aquarium, then feeding was carried out every morning at 07.00 am and 04.00 pm in the afternoon. Every morning siphoning is carried out to remove metabolic waste and feed residue in the aquarium before feeding.The rearing container and the fish cement ponds were washed and then dried for one day. Goramy juvenile was induced with fungi after conditioning newly transported in cement ponds. Afterwards treatment was carried out using the desired aqueous extract solution from cattapa leaves for 7 days. The equipment used in the research included aquarium, aerators, liquid disinfectant, fish scoop net, pH meter, thermometer, DO meter, surgical instrument, measuring cup, wieight scale, aquarium heater, object glass, microscope and stationery. Materials used included: stock cattapa leaf aqueous solution, fish pellets and gurame fish juveli (5-7 cm) that have been infected with fungi.

The aquarium was arranged randomly. The aquarium was filled with 40 liters of water per aquarium then a heater and aeration are installed to maintain water quality. The fiber container as fish seed stock was filled with water and deposited for 2 days before being used. The process of observation were made on goramy which has white spots on the fish skin such as cotton. Then observations were made and it was found that the goramy had been infected with the fungus *Saprolegnia* sp. Goramy juvenile infected with fungus have clinical symptoms such as loss of balance, tend to stay in the corner of the aquarium, loss of appetite, there are white spots on the fish's external organs, such as the head, gill cover, fins and fish skin. If the scale of fungal growth increases, there is a secondary infection, namely bacteria that grow with the structure of the fungus *Saprolegnia* sp. which is characterized by a change in the color of the fungal filaments to brown. The process of treatment was carried out using a solution of cattapa leaves according to treatment, namely control, 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm. The treatment was carried out for 7 days to observe the scale of fungal growth on the body of the fish and the effect of treatment using cattapa leaves on the growth of fungus on the body of the goramy. Observations were repeated 3 times with feeding twice a day. Then, siphoning was carried out in the morning after soaking using a solution of cattapa leaves for two days. After the treatment was completed, the aquarium media was cleaned by soaking in chlorine for 1 day then rinsed with clean water and dried.

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This research used an experimental method consisting of 5 treatments: without katapang leaf solution, addition of katapang leaf solution with a concentration of 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm. The parameters observed in this research were scale of fungal growth, fish survival and water quality.

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3. RESULTS AND DISCUSSION

3.1 Survival Rate

Table 1. Survival Rate of Goramy Juvenile

Treatment	Replicant			Average (%)
	1	2	3	
A	15	15	62	31[a]
B	77	85	15	59[a]
C	31	15	23	23[a]
D	85	23	46	51[a]
E	23	23	15	21[a]

Based on the graph above, it can be seen that the highest average survival of goramy juvenile was found in treatment B (500 ppm) which was 59% and the lowest was found in treatment E (2000 ppm) at 21%. The average survival of goramy juvenile in A (control) was 31%, treatment C (1000 ppm) was 23%, and treatment D (1500 ppm) was 51%. Survival rate (Natality or SR) is the percentage value of the number of fish that live during the maintenance period [13].

Based on the results of the analysis of variance, it was found that the addition of cattapa leaf solution had no effect on the survival of goramy juvenile. Survival between treatments was not significantly different considering that the variation between replicates was quite high, although the numbers showed that in treatment B the average was quite good. The addition of cattapa leaf solution concentration in treatment D (1500 ppm) resulted in increased survival, while the addition of a solution of 2000 ppm in treatment E resulted in decreased survival. The decrease in survival rate in treatment E was thought to be due to the poisoning of compounds contained in the solution of cattapa leaves, namely saponins. Harborne (1987) in Ariyani *et.al* (2016) states that high concentrations of saponin compounds that exceed the body's tolerance limit can cause poisoning and often even turn off. Based on observations during maintenance, the death of goramy juvenile was thought to be due to fungal infection. Fish infected with *Saprolegnia* showed difficulty in breathing but physiological responses such as electrolyte conditions and fish osmoregulation system were not studied further. Clinical abnormalities that can be observed are the appearance of white cotton on the body of the goramy juvenile turning brown[14].

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3.2 Scale of Fungal Growth

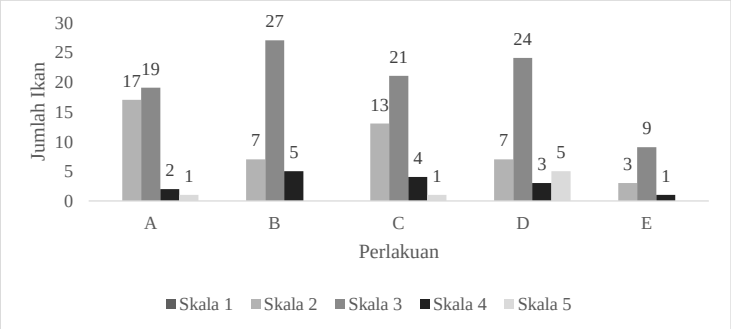

Figure 1. First Day of Scale of Fungal Growth

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Based on observations on the first day (Figure 1), the fungal growth was attempted to be evenly distributed in each treatment, but when statistically observed, it turned out that the diversity of fungal growth was

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quite varied. The scale of fungal growth is an indication to show the conditions of fungal growth in the test fish from good conditions to the most severe conditions. The growth of the fungal on the first day on a scale of 3 was the most abundant fungal growth found in fish, as many as 100 fish. A scale of 3 was found in each treatment and the most found in treatment B as many as 27 individuals. Fungal growth on a scale of 2 was found in 47 fish, the most found in treatment A as many as 17 fish. Fungal growth on a scale of 4 was found in 15 fish, the most found in treatment B was 5 fish. The fungal growth on a scale of 5 was the most severe fungal growth found in 7 fish, the most found in treatment D as many as 5 fish.

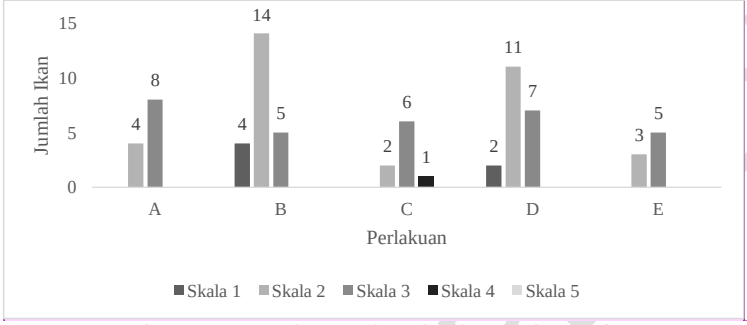

Figure 2. Seventh Day of Scale of Fungal Growth

Based on the graph above (Figure 2), the seventh day of fungal growth on a scale of 2 was the most mushroom growth found in 34 fish. Fungal growth on a scale of 2 was mostly found in treatment B as many as 14 fish. The growth of scale 1 fungus was found in 6 fish, the most was found in treatment B as many as 4 fish. Fungal growth on a scale of 3 was found in 31 individuals, the most was found in treatment A as many as 8 individuals. Fungal growth on a scale of 4 was only found in treatment C as many as 1 fish.

Observation of goramy juvenile on the first day to the seventh day showed a change in behavior. On the first day the fish are still active swimming and normal appetite. However, on the second day to the seventh day the changes were marked by the behavior of the fish which started to move slowly, the balance in swimming began to be disturbed, and the appetite began to decrease. The results of macroscopic observations, it was seen that the *Saprolegnia* fungus covered the bodies of some of the test fish and the color changed to brown until finally the fish died due to increasingly severe infections. In some other test fish, the fungus attached to the fish body was gradually released from the fish body until the scale of fungal growth in the test fish decreased.

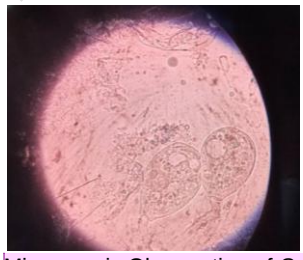

Figure 3. Microscopic Observation of *Saprolegnia*

Observations of fungal growth on the first and seventh days showed differences. The data obtained showed that soaking using a solution of ketapang leaves reduced the growth of the fungus Saprolegnia sp. This is presumably due to the phytochemical content in ketapang leaves which function as antimicrobials. Ketapang leaves contain flavonoids, saponins, triterpenes, diterpenes, phenolic compounds, and tannins[15]. The flavonoid content functions as an antioxidant, antimicrobial and also anti-inflammatory[16]. The content of saponins has the ability as a cleanser or antiseptic to kill or prevent the growth of microorganisms that arise in the wound so that the wound does not experience severe infection[17]. The content of tannins accelerates wound healing by several cellular mechanisms, namely scavenging free radicals and reactive oxygen,

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increasing wound grafting and increasing the formation of capillaries and fibroblasts[18].

The effectiveness of cattapa leaf solution in reducing fungal growth was seen on the 7th day while on the first day, the test fish had already died from fungal infection. This was presumably because the condition of the test fish was weak after administration of the cattapa leaf solution. So that his body is unable to withstand the attack of the fungal infection *Saprolegnia* sp. In addition, fungal infections that attack fish and the effect of treatment at the time of initial administration which requires an adaptation process makes the fish stressed and the response to food decreases so that the body's resistance decreases. Research by Juniati *et.al* (2015) on tilapia infected with the fungus *Saprolegnia*, the first day to the seventh day (first week) fish behavior still looks normal. However, in the second week, the condition of the test fish began to look abnormal. Movement becomes rather slow, inactive, and balance in swimming begins to be disturbed. The results of macroscopic observations, it was seen that the wounds on some of the test fish looked slightly reddish in color with a white membrane around the wound. The results of microscopic observations of the tissue of the injured test fish showed that the *Saprolegnia* hyphae had grown in these tissues which already had spore sacs that were round and some were slightly oval[19].

The condition of the fish in the second week of the research by Juniati *et.al* (2015) was in accordance with the condition of goramy juvenile which had been observed for 7 days. The mortality that occurred in goramy juvenile was thought to be due to infection with the fungus *Saprolegnia* which had previously attacked goramy juvenile. The growth of the *Saprolegnia* fungus on goramy juvenile has gone through a 7-day incubation period[19]. Fish infected with the fungus *Saprolegnia* look lethargic, lose balance and are generally prone to death [20].

Figure 4. Goramy Juvenile Affected by *Saprolegnia*

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Changes in behavior in test fish that were attacked by the fungus *Saprolegnia* were characterized by reduced appetite, white cotton-like spots attached to the fish's external organs, such as gill covers, skin and fins, and frequent swimming in the corner of the aquarium or on the surface of the water. This is in line with the statement of Susanto *et.al* (2014) that fish infected with the *Saprolegnia* fungus are characterized by the presence of a collection of fine threads (*hyphae*) that look like cotton at the base of the tail, changes in skin color and the growth of a grayish-white fungus which is getting progressively worse. widened and abnormal fish behavior such as slow movement and frequent swimming on the surface of the water and decreased appetite[21].

3.3 Water Quality

Table 2. Water Quality

Parameter	Treatment				
	A	B	C	D	E
Temperature (^{0}C)	28-34	30-35	30-32	30-35	26-32
pH	6.9-7.41	7.25-7.34	6.9-7.3	6.85-7.52	6.8-7.44
DO (mg/l)	6.2-8.3	6-7.7	4.5-7.5	6.8-7.7	5-7.6
Amoniak (mg/l)	0.03-0.09	0.03-0.09	0.03-0.09	0.03-0.09	0.03-0.09

Water quality is a limiting factor in the growth of cultured fish, including goramy, so that the quantity and quality of water used in fish farming activities must be optimal to meet the needs of fish life. The survival of fish is largely determined by water quality. The state of the water quality of the experimental research media shows the ranges that allow goramy to live well [22].

In this study, the highest maintenance medium temperature reached 35^{0}C. This condition contradicts the statement that the appropriate temperature for the survival of goramy is 29^{0}C-30^{0}C, while temperatures

above <24^{0}C and >30^{0}C are not suitable for the survival of goramy [12]. The degree of acidity (pH) that is suitable for the survival of goramy is 7-8 [23]. Dissolved oxygen (DO) which is suitable for the survival of goramy is 4-9 mg/l. Ammonia levels that can be tolerated by gouramy are 0.0 – 0.12 mg/l [24].

The content of tannins and flavonoids in cattapa leaves is marked to maintain water quality in fish maintenance. In addition, humic acid content also plays a role, one of which can lower pH [25].

CONCLUSION

Based on the research results, it was found that the highest survival rate of goramy juvenile was found in treatment B (500 ppm) which was 59% and the lowest was in treatment E (2000 ppm) at 21%. Based on the data on the fungal growth scale on the first and seventh days, the best response to soaking ketapang leaf solution for the treatment of Saprolegnia infection was in treatment D (1500 ppm). Soaking using a solution of cattapa leaves can reduce the growth of the fungus *Saprolegnia* sp. The effectiveness of cattapa leaf solution in reducing fungal growth was seen on the 7th day while on the first day, the test fish had already died from fungal infection. This was presumably because the condition of the test fish was weak after administration of the cattapa leaf solution. So that his body is unable to withstand the attack of the fungal infection *Saprolegnia* sp. Water quality parameters in this study such as temperature, pH, DO and ammonia are still in a suitable condition for the maintenance of goramy juvenile.

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