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Effect of Papaya Leaf Solution (*Carica papaya* Linn) on the Hatching Percentage and Survival Rate of Dumbo Catfish Larvae (*Clarias* sp.)

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Abstract

Eggs of dumbo catfish are known to stick to the substrate due to the presence of a mucus layer, which causes suboptimal oxygen supply and low hatching percentage. This indicates that the mucus layer must be lowered while eliminating the need for a substrate during hatching. One of the natural ingredients that can be used for this purpose is papaya leaf, which contains the proteolytic enzyme papain. Therefore, this study aims to determine the effect of papaya leaf solution on the percentage of live embryos after soaking, hatching percentage, hatching time, survival rate, and abnormality percentage of dumbo catfish larvae. This research was carried out at the Basic Fisheries Laboratory, Aquaculture Study Program, Fisheries Department, Faculty of Agriculture, Sriwijaya University in August-October 2022. A Completely Randomized Design (CRD) was used, which consisted of four treatments and three repetitions. The treatment given was the soaking of eggs in papaya leaf solution with different concentrations of (P0/control), 2 g/0.96 L (P1), 4 g/0.96 L (P2), and 6 g/0.96 L (P3). The results showed that P2 was the best treatment with 100% live embryos after soaking, 94.06% hatching percentage, 20.24 hours hatching time, 92.78% survival rate, and 0.93% abnormality percentage. Based on these results, the soaking of dumbo catfish eggs in papaya leaf solution could increase the hatching percentage and survival rate.

INTRODUCTION

Statistical data from the Ministry of Maritime Affairs and Fisheries (2021) showed that the total catfish production in Indonesia in 2019 was 981.623,80 tonnes, but this volume decreased drastically to 347.511,48 tonnes in 2020. Furthermore, Hasan *et al*. (2016) stated that frequent disease attacks in the hatchery and rearing phases constrained catfish farming activities in this country. Another notable constraint is the low hatching of eggs, which is usually caused by the presence of a mucus layer Yustiati *et al*. (2016). The hatching of dumbo catfish without the provision of a substrate has been reported to cause clustering due to the presence of a mucus layer, leading to hatching failure. Eggs that fail to hatch can become a breeding ground for diseases,

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thereby infecting other fish eggs. This indicates that it is crucial to remove the mucus layer to prevent clumping and optimize the oxygen supply to increase the hatching percentage.

Antibiotics, malachite green, and methylene blue are commonly used to prevent disease attacks in fish, but their long-term use can lead to bacterial resistance and affect the environment and humans (Hasan *et al*., 2016). Therefore, the use of natural materials is recommended because they are easier to obtain, have abundant stocks, and do not cause adverse effects (Fanni *et al*., 2018). One such material is papaya leaf, which has been reported to prevent disease-causing bacteria in fish due to their karpain alkaloids, polyphenols, and flavonoid content (Haryani *et al.*, 2012). Fitri *et al*. (2018) stated flavonoid compounds can damage the cell layer of bacteria, thereby leading to death. The sap, fruit, stem, and leaf of papaya have also been shown to contain proteolytic enzymes, such as papain (Permata *et al*., 2016). Saputra *et al*. (2014) stated that proteolytic enzymes can remove the mucus layer, leading to optimal oxygen supply to eggs and prevention of fungus proliferation.

Several studies have been carried out to investigate the effect of papaya solution on the hatching percentage of fish eggs. For example, soaking siamese catfish eggs at a dose of 1.5 g/L for 1 minute produced the highest hatching percentage of 84.25% (Eka, 2014). The treatment of Carp in 4 g/L solution for 10 minutes gave a value of 95.33% (Rifai, 2015), while local catfish at four g/L for 20 minutes produced 82.67% (Rachman, 2016). Based on previous reports, there are no studies on the impact of papaya solution on dumbo catfish. Therefore, this study aims to determine the effect of papaya leaf on the hatching percentage and survival rate of dumbo catfish larvae.

METHODOLOGY

Ethical Approval

The fish used in this study were treated properly, and surgery was carried out only on dead male dumbo catfish broodstock.

Place and Time

This study was carried out at the Fisheries Basic Laboratory, Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University, from August to October 2022.

Research Materials

The materials used consisted of dumbo catfish broodstock weighing 1.74 kg (male) and 1.82 kg (female), catfish eggs, papaya leaf, potassium permanganate, commercial pellets (30% protein), gonadotropin hormones, tissue, filter paper, ammonia measuring material (standard solution, benzene, and Clorox), and 0.9% NaCl. Meanwhile, the tools included an aquarium measuring 30x30x30 cm, a concrete tub measuring 2x1x1 m, a 4 L jar volume, microscope type binocular, fine drain, blower, scales, sieve mesh 60, heater, knife, surgical scissors, blender, stove, pan, basin, plastic measuring cup, cloth, injecting syringe, chicken feather, pH meter, DO meter, and spectrophotometer.

Research Design

This study was carried out using a completely randomized design (CRD), which consisted of four treatments and three replications. The treatment given was the soaking of dumbo catfish eggs in papaya leaf solution with different concentrations of control (P0), 2 g/0.96 L (P1), 4 g/0.96 L (P2), and 6 g/0.96 L (P3).

Work Procedure

Study Container Preparation

The soaking container used in this study was a 4 L jar, which was filled with papaya leaf solution. The jars were cleaned with clean water, and the papaya leaf solution was prepared based on the treatment method and poured into each container.

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The hatchery container used was an aquarium measuring 30x30x30 cm, which was cleaned with clean water and allowed to dry. Subsequently, it was filled with clean water up to a height of 20 cm (water volume 18 L). A total of 20 mg/L of potassium permanganate was added for sterilization, and the mixture was left for one day. The water in the container was removed and refilled with 18 L of water (Pamula *et al.*, 2019), followed by the addition of aeration and heater installations. The container used for rearing the broodstock was a concrete pond measuring 2x1x1 m, which had been cleaned and filled with water.

Papaya Leaf Solution Making

The papaya leaf solution was prepared using young papaya leaves obtained from private plantations in Tanjung Batu, Ogan Ilir, South Sumatra, which were washed under running water. The leaf was then cut into pieces, dried in the sun for five days, and ground in a blender. Subsequently, the sample was sifted using a sieve with a mesh of 60 to obtain flour. A total of 150 g of flour was obtained using 350 g of papaya leaf. To prepare the leaf solution with a concentration of 2 g/0.96 L (P1), 6 g of powder was added to 3 L of water and boiled. The solution was allowed to cool and then filtered to separate the dregs. The process yielded 2.9 L, which was placed in the soaking vessel. The same procedure was also used to prepare the solution for P2 and P3, and the concentration was adjusted based on the treatment.

Broodstock Preparation

The broodstock of dumbo catfish was obtained from farmers in the Gandus area, Palembang. The male and female breeders used had a weight of 1.74 kg and 1.82 kg, respectively. Furthermore, brood maintenance and selection were first carried out before spawning (Dewanggani *et al.*, 2021). The dumbo catfish broodstock was reared separately for 30 days by being fed 3% of their body weight twice a day in the morning and evening. The samples were first fasted a day before the injection (Samara *et al.*, 2019). The injection was then administered 0.3 mL/kg ovaprim to both breeders (Sinjal, 2014).

Spawning was carried out artificially with a ratio of males to females of 1:1 (Dewanggani *et al.*, 2021). Sampling of sperm and eggs from each broodstock was then performed for 10 hours after the injection. The sperm sacs of male dumbo catfish broodstock were dissected, and the attached blood was removed with a tissue. Subsequently, the sac was cut into small pieces and placed in 0.9% NaCl in a ratio of 1:4. Eggs were then collected from the female Dumbo catfish broodstock by gently massaging the abdomen to the urogenital opening. The sperm and eggs were mixed in a dry container with a chicken feather, followed by clean water to start the fertilization process.

Soaking, Hatching Eggs, and Rearing Larvae

The sample used in this study consisted of fertilized eggs placed in the hatching medium with a stocking density of 78 eggs/L. Furthermore, soaking was carried out in the prepared jars for 20 minutes based on the time frame proposed by Rachman (2016). Eggs that die after soaking, during hatching, or have become larvae, according to Yonarta *et al.* (2021), must be disposed of immediately after dead eggs and larvae to prevent the development of fungi. Meanwhile, the eggs that successfully hatch into larvae are maintained for three days. Eggs were then stocked in the aquarium and allowed to hatch into larvae, followed by maintenance for three days.

The formula for calculating the percentage of live embryos after soaking can be calculated using the following formula:

$$\frac{\text{total of eggs after soaking}}{\text{total of eggs before soaking}} \text{x} 100\%$$

The formula for hatching percentage can be calculated using the formula of Suquet *et al.* (2005) as follows:

$$\frac{\text{total of hatched larvae}}{\text{total of eggs incubated}} \text{x} 100\%$$

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The formula for hatching time can be calculated using the following formula of (Muslim and Yonarta, 2017):

Egg hatch time 70% - Egg fertilization time

The survival rate formula can be calculated using the Effendie (2002) formula as follows:

$$\frac{\text{Total of fish at the finish of rearing}}{\text{Total of fish at the start of rearing}} \text{x}100\%$$

The abnormality percentage formula can be calculated using the formula of Nirmala *et al*. (2006) as follows:

$$\frac{\text{Total of larvae abnormal}}{\text{Total larvae}} \text{x}100\%$$

Data Analysis

The observed parameters included the percentage of live embryos after soaking, hatching percentage, hatching time, survival rate, abnormality percentage, and water quality measurements. All data obtained apart from water quality data is analyzed using Analysis of Variance (ANOVA). If it shows a real effect, then it is continued with the Least Significant Difference (LSD) test at a 95% confidence interval. Meanwhile, water quality data was analyzed descriptively.

RESULTS AND DISCUSSIONS

Growth, Feed Efficiency, and Protein Efficiency Ratio

Papaya leaf solution contains ingredients that influence the percentage of live embryos, the percentage of hatching, and the length of hatching time for dumbo catfish eggs. Ingredients such as the papain enzyme have been proven to increase especially the hatching percentage and hatching time, as shown in Table 1 below.

Table 1. Percentage of live embryos after soaking, hatching percentage, and time of dumbo catfish.

Treatment	Percentage of live embryos after soaking $LSD_{\alpha 0,05} = 0.46$	Hatching percentage $LSD_{\alpha 0,05} = 3.47$	Hatching time (hours) $LSD_{\alpha 0,05} = 0.42$
P0	100 ± 0.00^{b}	80.14 ± 1.44^{a}	24.35 ± 0.17^{d}
P1	100 ± 0.00^{b}	90.43 ± 1.42^{b}	21.23 ± 0.27^{c}
P2	100 ± 0.00^{b}	94.06 ± 1.60^{c}	20.24 ± 0.14^{b}
P3	98.57 ± 0.40^{a}	87.39 ± 1.55^{b}	19.70 ± 0.12^{a}

Description: Numbers in the same column followed by different superscript letters show significantly different results on the LSD test with a 95% confidence interval.

The average survival of three-day-old dumbo catfish larvae in papaya leaf solution with different concentrations is shown in Figure 1.

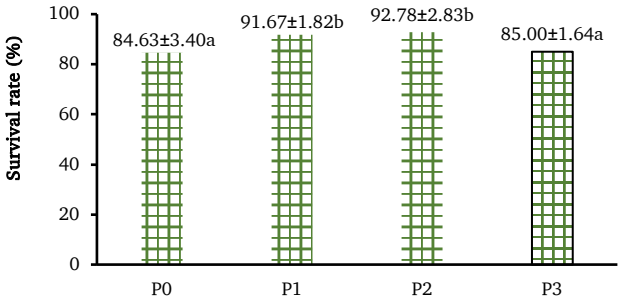

Figure 1. The survival rate of dumbo catfish larvae.

Description: Different superscript letters show significantly different results on the LSD test with a 95% confidence interval (5.83).

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The average abnormality percentage of dumbo catfish larvae in papaya leaf solution with different concentrations is presented in Figure 2.

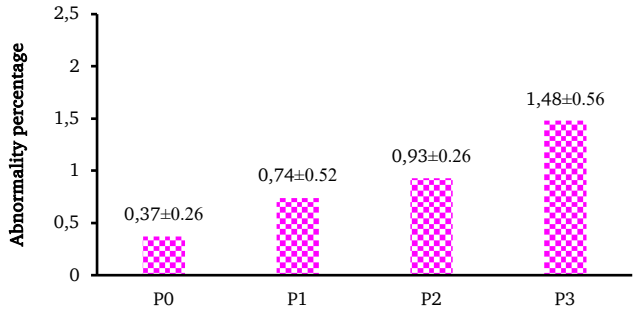

Figure 2. Abnormality percentage of dumbo catfish larvae.

Water quality data measured during this study included pH, DO, and ammonia, while the temperature was set within the range of 30±0.5°C.

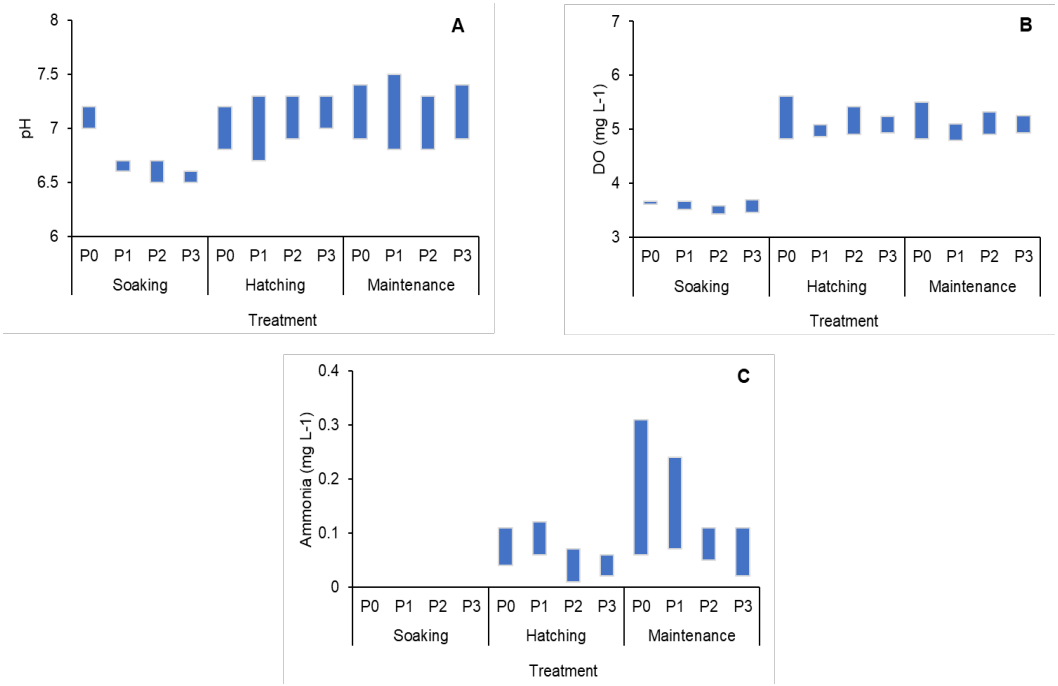

Figure 3. Water quality for hatching eggs and rearing larvae of dumbo. (A) pH, (B) Dissolved oxygen, and (C) Ammonia.

The analysis of variance results showed that papaya leaf solution had a significant effect on the percentage of live embryos after soaking, the hatching percentage, and the hatching time of dumbo catfish. Table 1 shows that the hatching percentage in (P2) papaya leaf solution concentration 4 g/0.96 L significantly differed from the other treatments. However, the hatching time at P3 was significantly different and faster compared to others. The provision of papaya leaf solution increased the hatching percentage and time in dumbo catfish eggs. This was because papaya leaf contained the enzyme papain (Yonarta *et al*., 2021) and tannins (Jati *et al*., 2019), which reduced the mucus layer, leading to the optimal supply of oxygen to eggs. Saputra *et al*. (2014) stated that papain was a proteolytic enzyme that

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reduced the mucus layer (glycoprotein), thereby increasing oxygen supply and preventing fungus development. According to Badarullah *et al*. (2020), tannins had similar effects, which prevented eggs from sticking together and protected them from being attacked by fungi and foreign objects. According to Mukti *et al*. (2020), aquatic organisms, including eggs and fish, require oxygen for metabolic processes. Papaya leaf also contains saponins (Jati *et al*., 2019), making the shells thinner and facilitating hatching (Saenal *et al*., 2020).

Although papaya leaf solution 6 g/0.96 L (P3) had the fastest hatching time, the hatching percentage obtained was lower than P1 and P2. According to Mulyani and Johan (2020), increased or excessive doses of proteolytic enzyme can thin the egg layer (chorion), thereby inhibiting hatching. Fadilah *et al*. (2017) stated that papaya leaf contained 0.30% saponins and 11.34% tannins. Furthermore, Zubaidah *et al*. (2021) reported that excessive levels of tannins can cause the fish eggs to erode and shrink, leading to the inability to hatch. According to Sulmartiwi *et al*. (2013), the administration of excess saponins can cause toxic properties in fish

The control showed the lowest hatching percentage and time compared to other treatments due to the thick mucus layer on eggs. The thick mucus layer made it difficult for the embryos in fish eggs to obtain oxygen supply, leading to hatching failure (Mulyani and Johan, 2020), (Patricius *et al*., 2019). Furthermore, the samples in P0 were attacked by the fungus *Saprolegnia* sp., which caused failed hatching. Saputra *et al*. (2014) stated that the mucus layer found in fish eggs was an ideal location for developing diseases, such as fungi. According to Hasan *et al*. (2016), *Saprolegnia* sp. could cause weakening and shrinking of the shell, thereby causing death.

The analysis of variance showed that the papaya leaf solution had a significant effect on the survival of dumbo catfish larvae. The $LSD_{\alpha 0.05}$ test results revealed that the survival rate of treatment P2 was significantly higher compared to P0 and P3 but not substantially different from P1, as shown in Figure 1. Furthermore, this high rate was due to protection from active compounds, such as tannins and steroids in papaya leaf (Jati *et al*., 2019), preventing disease attacks.

According to Alamsjah *et al*. (2011), tannins had antibacterial properties by damaging membranes and cell walls in bacteria, thereby causing growth inhibition and death. Steroids also exhibited similar properties by causing damage to the structure of cell membranes in microbes (Suliani *et al*., 2016). Treatment P0 (control) gave the lowest survival rate compared to others. The low survival rate of the larvae was due to the large number of eggs that failed to hatch, leading to the proliferation of the fungus *Saprolegnia* sp. According to Kusdarwati *et al*. (2017), *Saprolegnia* sp. has a cotton-thread-shaped hypa, which has a faster development rate than other fungi. Diana *et al*. (2017) stated that this fungus infected tawes eggs within one hour. Fanitalya *et al*. (2012) reported that it continued to develop after infecting fish eggs and later attacked the larvae, thereby causing death.

The analysis of variance showed that the results had no significant effect. Still, the level of abnormality of the larvae increased along with the concentration of the papaya leaf solution, as shown in Figure 2. This was because the solution lowered the hatching time, which caused eggs to hatch prematurely due to incomplete development. Furthermore, the abnormality observed in dumbo catfish larvae was the development of a crooked tail. Similar findings were also obtained by Mulyani and Johan (2020), where an increased dose of cherry leaf extract made the shells thinner and break more quickly, leading to abnormal larvae.

Water quality plays a crucial role in aquaculture activities, and its instability causes diseases and death in cultivated fish (Riza *et al*., 2014). Temperature control was

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carried out in all treatment groups during hatching and larval rearing using a heater set at 30±0.5°C. The pH, dissolved oxygen, and ammonia used in this study ranged from 6.5-7.5, 4.80-5.61 mg/L, and 0.01-0.031 mg/L, respectively. The results showed that these parameters were in the acceptable range for the hatching and rearing of dumbo catfish larvae. Based on the recommendation of the National Standardization Agency of Indonesia (2014), good water quality for dumbo catfish larvae as temperature, pH, minimum dissolved oxygen, and maximum ammonia of 25-30°C, 6.5-8, 3 mg/L, and 0.01 mg/L, respectively (Prayogo *et al*., 2018). Although the ammonia obtained during the larval rearing phase was 0.31 mg/L, Mulyani and Johan (2020) stated that dumbo catfish larvae can tolerate up to 0.97 mg/L.

CONCLUSION

Papaya leaf solution treatment with a concentration of 4 g/0.96 L (P2) is the best treatment and is recommended in the process of hatching eggs from dumbo catfish because it has been proven to speed up the hatching time process and increase the hatching percentage.

CONFLICT OF INTEREST

There was no conflict of interest.

AUTHOR CONTRIBUTION

The three authors of this publication each contributed to the execution of the research as well as its composition.

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