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Optimization of Java Barb (*Barbonymus Gonionotus*) Egg Hatching by Immersion in *Jatropha Curcas* L. Leaf Extract

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A B S T R A C T

This study aimed to evaluate the effect of Jatropha curcas leaf extract on the hatching rate and survival rate of Java barb (Barbonymus gonionotus) larvae. Fish eggs were subjected to immersion treatments using Jatropha curcas leaf extract at a concentration of 4 g/L. The control group received no extract treatment. The results showed a significant increase in hatching rate and larval survival in the treatment group compared to the control. The hatching rate improved from 64.40±2.97% in the control to 84.00±3.16% in the treatment group, while the survival rate increased from 65.82±1.50% to 85.24±1.00%. Water quality parameters (temperature, pH, and dissolved oxygen) remained within the optimal range during the 15-day observation period. The presence of tannins and flavonoids in the leaf extract is believed to reduce egg adhesiveness and fungal infections, leading to improved oxygen exchange and overall larval viability. These findings suggest that Jatropha curcas leaf extract can be used as an effective natural agent to enhance the reproductive success of Java barb.

Keywords: *Jatropha curcas*, Java barb, Hatching rate, Survival rate, Natural extract

1. Introduction

The *Jatropha curcas* L. leaves have shown potential as antifungal agents (Made et al., 2017; Nuria et al., 2009) and are therefore suspected to be effective against *Saprolegnia* sp. This antifungal activity is attributed to the presence of various bioactive compounds such as saponins, tannins, terpenoids, alkaloids, steroids, glycosides, phenols, and flavonoids, which have been proven to inhibit fungal growth (Sharma et al., 2012). A common issue encountered in hatchery practices is the high mortality rate during egg incubation. One of the main factors contributing to reduced hatching success is the presence of *Saprolegnia* sp. infections on the eggs.

Java barb (*Barbonymus gonionotus*) is a freshwater fish species with high popularity among the public and is considered a significant economic aquaculture commodity (Hastuti et al., 2024; Subandiyono et al., 2018; Sabrina et al., 2018; Yuniarti et al., 2023). This species holds promising potential for the development of freshwater aquaculture enterprises. According to Diana and Safutra (2018) and Ageng et al. (2023), Java barb has strong cultivation potential due to its adaptability and minimal land requirements for rearing. As an endemic species of Indonesia, Java barb can be cultured year-round, supported by the country's tropical climate and favorable environmental conditions. Consequently, the availability and production of high-quality Java barb seed is a key factor in ensuring the success of its aquaculture development.

In Java barb (*Barbonymus gonionotus*) hatchery practices, the egg hatching phase is a critical stage, with hatching success serving as a key indicator of aquaculture performance. One of the main challenges during this stage is the high rate of embryonic mortality. Agustin and Rahardja (2013) reported that seed production of Java barb remains relatively low, with hatching rates reaching only 22% from a total of 10,000 eggs. Therefore, efforts to improve fertilization rates and hatching success are essential. One contributing factor to low hatching rates is fungal infection. The presence of fungi in the Java barb egg incubation medium can inhibit the development of both eggs and larvae (Triwardani et al., 2022). The most commonly encountered fungus in such conditions is *Saprolegnia* sp. (Hastuti & Subandiyono, 2016; Velichkova et al., 2021). Optimizing the hatching process of Java barb eggs through immersion in *Jatropha curcas* L. leaf extract represents an innovative approach in aquaculture. This method aims to enhance hatching success and improve larval quality. *Jatropha curcas* leaves are known for their antimicrobial and antioxidant properties, which may protect eggs from pathogenic infections and oxidative stress during incubation. Moreover, a comparative analysis between this method and conventional hatching techniques is necessary to evaluate its effectiveness and efficiency on a larger production scale. The results of such optimization are expected to contribute to increased Java barb aquaculture productivity and the advancement of more sustainable aquaculture practices.

Considering the antifungal properties of *Jatropha curcas* L. leaves (Sharma et al., 2012), this study employed a *Jatropha curcas* L. leaf extract solution in the incubation of Java barb (*Barbonymus gonionotus*) eggs to improve hatching success. This research was to examine the role of *Jatropha curcas* L. leaf extract in enhancing egg hatchability and larval survival of Java barb.

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The objective of this study was to evaluate the effect of *Jatropha curcas* L. leaf extract on the hatching success and larval survival of Java barb (*Barbonymus gonionotus*). Specifically, the study aimed to determine whether the antifungal properties of *Jatropha curcas* L. could improve egg viability and reduce mortality during the incubation phase, thereby contributing to increased seed production and overall hatchery performance.

2. Materials and methods

This research was conducted from June to July 2024 at the Freshwater Aquaculture Center in Muntilan, located in Muntilan District, Magelang Regency, Central Java Province, with the postal code 56411. The experiment was arranged using a completely randomized design (CRD), consisting of two treatments with five replications each. Treatment A served as the control group without the addition of *Jatropha curcas* L. leaf extract (0 g/L), while Treatment B involved the application of the extract at a concentration of 4 g/L. Each experimental unit consisted of a container filled with 10 liters of water and stocked with 100 fertilized Java barb (*Barbonymus gonionotus*) eggs. The experimental period lasted for 15 days, during which observations were conducted to evaluate hatching rate, larval survival rate, and water quality parameters.

2.1. Experimental Fish and Rearing Protocols

The test specimens used in this study were sexually mature Java barb (*Barbonymus gonionotus*) broodstock that were ready for spawning, from which fertilized eggs were collected. The broodstock were obtained from the Freshwater Aquaculture Center (Balai Budidaya Ikan Air Tawar) in Muntilan, Magelang, Central Java. The eggs used in this study were fertilized, healthy, and high-quality eggs of Java barb (*Barbonymus gonionotus*). A total of 1,000 eggs were required for the experiment and were evenly distributed across the experimental containers. Each experimental unit contained 100 eggs, ensuring uniform density to support the validity of the observational results.

Selected test eggs were immersed for 4 minutes in two treatment solutions of *Jatropha curcas* L. leaf extract: treatment A (control) without extract (0 g/L) and treatment B with a concentration of 4 g/L. After immersion, the eggs were transferred to hatching containers filled with 10 liters of clean water equipped with an aeration system to maintain optimal dissolved oxygen levels. The incubation period lasted between 13 and 20 hours, or until all eggs had fully hatched.

Following hatching, the larvae produced from each treatment group were counted and recorded for data analysis. The hatched larvae were then reared until they reached the first nursery phase (Nursery I), which lasted for 15 days. The rearing procedure was based on the Indonesian National Standard (SNI, 1999) for common carp (Cyprinus carpio), a species within the same family (Cyprinidae) as the Java barb (*Barbonymus gonionotus*). According to the SNI guidelines, the nursery I phase for common carp lasts 15 days, during which the larvae typically reach a length of 1–3 cm. During this rearing period, Java barb larvae were initially fed with an adequate amount of boiled egg yolk. Furthermore, Starting from day 4 until day 15, the larvae were subsequently fed *Artemia* twice daily, in the morning and afternoon.

2.2. Variables and Measurement Methods

2.2.1. Phytochemical Test

Phytochemical testing was conducted based on the method developed by Andriyanto et al. (2016). The phytochemically analyzed compounds from Jatropha curcas L. leaves included alkaloids, flavonoids, saponins, steroids, terpenoids, phenols, and tannins. The identification of these compounds aims to determine the bioactive potential contained in the Jatropha leaf extract, which may play a role in biological activity and applications in the field of aquaculture.

2.2.2. Hatching Rate (HR)

The quantity of larvae produced from a spawning event is largely determined by the hatching rate, which is defined as the percentage of fertilized eggs that successfully hatch. The hatching rate was assessed using a sampling method throughout the study. For *Barbonymus gonionotus* (Java barb), the hatching rate was calculated according to the formula proposed by Hastuti et al. (2024) and Triwardani et al. (2022), with calculations performed at the conclusion of the experiment. The formula is expressed as follows:

$$HR(\%) = \frac{\text{Number of hatched eggs}}{\text{Number of fertilized eggs}} X\ 100$$

This metric serves as an indicator of spawning success and is essential for evaluating the effectiveness of each treatment applied during the study.

2.2.3. Survival Rate (SR)

The survival rate of Java barb (Barbonymus gonionotus) larvae was assessed 15 days post-hatching. The calculation followed the method described by Subandiyono & Hastuti 2016), and was conducted at the end of the experimental period using the following formula:

SR = (Nt / N0) × 100%

Where:

SR = Larval survival rate (%)

Nt = Number of larvae at the end of the 15-day rearing period (individuals)

N0 = Number of larvae stocked at the beginning of the experiment (individuals)

2.2.4. Water quality parameters

Water quality was assessed at the beginning of the experiment, both before and after the addition of Jatropha curcas L. leaf extract to the water. The parameters measured during the study included pH, dissolved oxygen (DO), and temperature, following the procedures described by APHA, 2005.

2.2.5. Statistical Analysis

Statistical analysis was performed on hatching rate and survival rate data. An independent t-test was conducted using SPSS software version 22.0, with a confidence level of 95%. Water quality data were analyzed descriptively and compared against standard water quality parameters for the incubation and rearing of Java barb (*Barbonymus gonionotus*) eggs and larvae.

3. Results and Discussion

3.1. Phytochemical Screening

The *Jatropha curcas* leaf solution used (4 g/L) contained flavonoids, phenols, and tannins at concentrations of 0.009%, 0.060%, and 0.065%, respectively. Phytochemical screening was conducted as a preliminary quantitative analysis to identify the presence of secondary metabolites in the *Jatropha curcas* L. leaves. These leaves contain compounds that influence the developmental process of Java barb eggs. A high flavonoid content can degrade the egg membrane or wall, facilitating early larval emergence. This aligns with Inaya et al. (2015), who stated that flavonoid compounds can disrupt cell membranes by denaturing membrane proteins, thereby increasing membrane permeability and causing leakage of cellular contents, including from within egg cells. However, this effect may also lead to the death of the eggs if the cells inside have not fully developed and are not yet ready to hatch. Additionally, if hatching does occur, the larvae may be premature and have a significantly reduced lifespan. Therefore, in this study, a *Jatropha curcas* leaf extract concentration of 4 g/L was used, which appeared to effectively increase the hatching rate of Java barb eggs. The hatching rate (HR) reached 84.00 ± 3.16%, which was higher than the control group without immersion in *Jatropha curcas* leaf extract (64.40 ± 2.97%).

The phenolic constituents in the *Jatropha curcas* leaf extract also contribute to protein denaturation and disruption of cell membranes. Baharudin et al. (2016) explain that phenolics compromise membrane integrity and denature proteins by solubilizing the lipids in the cell wall; at elevated extract concentrations, this activity can extend to the chorion layer, rendering it fragile and prone to rupture, which in turn leads to premature larval emergence.

Tannins further facilitate chorion softening by activating the enzyme chorionase (Baharudin et al., 2016). Because chorionase exhibits optimal activity under acidic conditions—and tannins are inherently acidic—the presence of tannins accelerates the enzymatic degradation of the chorion, promoting its rapid loosening.

Immersing eggs in a solution of Jatropha curcas leaf extract effectively reduces their adhesive properties, preventing them from clumping together and facilitating optimal oxygen exchange during embryogenesis. This improvement in egg handling conditions contributes to a higher hatching success rate. These results are consistent with the findings of Woynarovich and Horvath (1980), who reported that tannin concentrations between 3.0 and 4.8 g/L are effective in significantly reducing the stickiness of fish eggs.

3.2. Hatching Rate (HR)

The percentage of hatching rate is presented in Figure 1. Based on the hatching rate (HR) calculations, immersion of Java barb eggs in a 4 g/L *Jatropha curcas* leaf extract solution had a positive effect on increasing the hatching rate. The control group showed a hatching rate of 64.40±2.97%, while the treatment group, which received the 4 g/L *Jatropha curcas* extract immersion, demonstrated a significantly higher hatching rate of 84.00±3.16%.

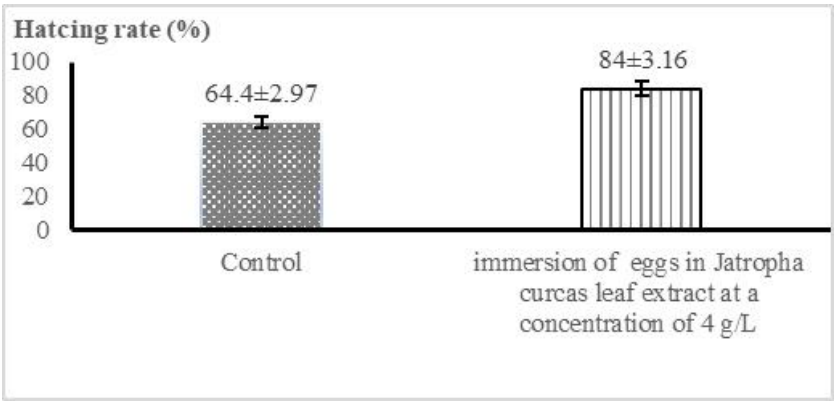

Figure 1. Percentage of egg hatchability in Java barb treated with a Jatropha curcas leaf extract immersion.

The tannin and flavonoid compounds present in the *Jatropha curcas* leaf extract solution play a significant role in enhancing the hatching success of Java barb eggs. These bioactive compounds help inhibit fungal growth on the eggs and reduce adhesiveness between them (Malik & Inriyani, 2015; Mahyuddin et al., 2020). As a result, the risk of fungal infection is minimized, and oxygen exchange between the eggs and their environment becomes more efficient. Consequently, the hatching rate of Java barb eggs treated with *Jatropha curcas* leaf extract is notably higher (Figure1). A similar result was reported by Mahyuddin et al. (2020) in common carp (Cyprinus carpio L) eggs immersed in Jatropha curcas leaf extract solution, where the highest hatching rate of 86.60% was observed at a concentration of 4.0 g/L.

3.3. Survival Rate (SR)

The survival rate of Java barb larvae originating from eggs immersed in Jatropha curcas leaf extract reached 85.24±1.00%, while the control group showed a lower survival rate of 65.82±1.50%. According to Gusrina (2008), the number of larvae surviving until the end of the rearing period is closely related to the hatching rate, with egg quality being a key factor in determining larval viability. Haser et al. (2018) further stated that improved immunity in fish, both at the larval and broodstock stages, is strongly influenced by the presence of antimicrobial compounds, which contribute to higher hatching success and reduce the incidence of abnormalities in larvae. Survival rate was monitored over a 15-day period to assess whether the hatched larvae developed normally, exhibited abnormalities, or experienced mortality.

Based on the results of the study, the survival rate of Java barb larvae was considered relatively high. Diana and Safutra (2018) reported that the use of natural feed resulted in a larval survival rate of 64.40% in Java barb. The high survival rate observed in this study is presumed to be influenced by the appropriate and intensive use of natural feed throughout the rearing period. In this experiment, the larvae were initially fed with boiled chicken egg yolk, followed by Artemia starting on the fourth day. Maleko et al. (2014) also stated that the type and quality of feed provided have a significant impact on the survival rate of larvae.

3.4. Water quality

Water quality plays a crucial role in supporting the survival of aquatic organisms. Water quality management during the study aimed to reduce the risk of mortality that could lead to failure in the rearing process (Triwardani et al., 2022). The results of water quality measurements, including temperature, pH, and dissolved oxygen (DO), over a 15-day period are presented in Table 1.

Table 1. Water Quality Parameters During the Experimental

Treatment	Range of Values		
	Temperature (°C)	pH	DO (mg/L)
Control	21.0 – 25.9	7.0 – 7.9	5.6 – 8.5
Immersion in 4 g/L Jatropha curcas leaf extract	21.2 – 27.0	7.0 – 7.8	6.1 – 8.5
	22 - 28	6,5 - 8,5	≥ 3

Table 1 indicates that the water quality in the hatching and larval rearing containers for Java barb was within acceptable ranges to support larval survival and development. This indicates that the differences in hatching rate and survival rate were influenced by the treatment involving immersion in Jatropha curcas leaf extract.

4. Conclusion

The immersion of Java barb (*Barbonymus gonionotus*) eggs in *Jatropha curcas* leaf extract at a concentration of 4 g/L significantly improved both hatching and survival rates compared to the control group. The presence of bioactive compounds such as tannins and flavonoids in the extract helped

reduce egg adhesiveness and fungal infections, thus enhancing oxygen exchange and overall larval viability. Additionally, water quality parameters remained within optimal ranges during the experiment, supporting the success of larval rearing. These findings suggest that *Jatropha curcas* leaf extract is a promising natural alternative to improve reproductive outcomes in freshwater aquaculture.

5. Recommendation

The findings of this study support the use of Jatropha curcas leaf extract at a concentration of 4 g/L as a natural and effective agent to enhance hatching and survival rates in Java barb (Barbonymus gonionotus) culture. For broader application in aquaculture, further studies are recommended to evaluate the efficacy of this treatment across different fish species and developmental stages, as well as to determine any potential long-term effects on larval health and performance. Optimization of dosage and exposure duration is also suggested to maximize benefits while ensuring environmental safety.

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