



Effect of Soaking Java barb (*Baryoniums gonionotus*) Eggs in *Jatropha* Leaf Solution (*Jatropha curcas* L.) on Their Hatching and Survival Rate

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ABSTRACT

The biggest problem that often found in barb hatcheries are the poor hatching rate of eggs. One of the causes of low hatchability is infestation of fungi such as *Saprolegnia* sp. Prevention of the infestation of fungi could be done by adding natural compounds such as antifungal substances from plants leaves, for example *Jatropha* leaf. Soaking *Jatropha* leaf solution aims to protect the chorion from being infested by fungi so that it can minimize hatching failure of eggs. The purpose of this study was to determine the effect of soaking *Jatropha* (*Jatropha curcas* L.) leaf solution on the hatching rate and survival rate of Java barb (*Barbonymus gonionotus*) eggs. The testing eggs which were used on this study were originated from male and female broodstock aged 1-1.5 years. Experimental method was carried out using a completely randomized design (CRD) consisting of 4 treatments and 5 replications. The treatments were *Jatropha* leaf solution at dosages of A (0 g/L), B (2 g/L), C (4 g/L), and D (6 g/L). The data observed were embryonic development, hatching rate (HR), survival rate (SR), and water quality. The results showed that treatments C and D demonstrated fastest embryonic development and hatching phase achieved at 475 minutes. Furthermore, treatment C showed the best value with HR $84.00 \pm 3.16\%$ and SR $85.24 \pm 1.00\%$. It can be concluded that soaking eggs using *Jatropha* leaf solution at concentration of 4 g/L significantly improved hatching rate and survival rate of Java barb (*Barbonymus gonionotus*).

Keywords: *Jatropha*, Java barb eggs, hatchability, fungi

ABSTRAK

Masalah terbesar yang sering ditemui dalam kegiatan pembenihan adalah tingginya angka kematian pada penetasan telur. Salah satu penyebab rendahnya daya tetas telur adanya jamur *Saprolegnia* sp. yang menempel pada telur. Pencegahan terhadap serangan jamur *Saprolegnia* sp. yaitu dengan menambahkan bahan alami seperti tanaman yang mengandung anti jamur yaitu daun jarak pagar. Perendaman menggunakan daun jarak pagar bertujuan untuk melindungi korion agar tidak mudah terserang jamur sehingga dapat meminimalisir rendahnya daya tetas telur. Tujuan penelitian ini untuk mengetahui pengaruh perendaman dengan larutan daun jarak pagar (*Jatropha curcas* L.) terhadap daya tetas telur ikan java barb (*Barbonymus gonionotus*). Ikan uji yang digunakan adalah induk jantan dan betina yang berumur 1-1,5 tahun. Penelitian ini menggunakan metode eksperimental dengan Rancangan Acak Lengkap (RAL) terdiri dari 4 perlakuan dan 5 pengulangan. Larutan daun jarak pagar terdiri dari dosis A (0 g/L), B (2 g/L), C (4 g/L), dan D (6 g/L). Data yang diamati adalah perkembangan embrio, daya tetas telur (HR), kelulushidupan (SR), dan kualitas air. Hasil penelitian menunjukkan perlakuan C memberikan nilai terbaik dengan HR $84.00 \pm 3.16\%$ dan SR $85.24 \pm 1.00\%$. Kesimpulan perendaman telur menggunakan larutan daun jarak pagar dengan dosis berbeda pada berpengaruh nyata ($P 0.05$) pada daya tetas dan kelulushidupan ikan java barb (*Barbonymus gonionotus*).

Kata kunci: Jarak pagar, telur tawes, daya tetas, fungi

1. Introduction

Hatchery is an important part of aquaculture activities. Success in producing larvae will have a good impact on the next stage of cultivation. Java barb (*Barbonymus gonionotus*) is a freshwater fish that's quite popular in a community and is classified as an endemic native species in Indonesia. The Fulfilment for seeds is a very important factor in the success of aquaculture business (Rahmadini, 2017). One of the biggest problems encountered in some hatchery is the high mortality and poor hatching rates (Ikramuddin et al., 2023). So, it is necessary find a way to increase fertilization and hatchability of eggs. One of challenge is how to reduce and inhibit infestation of parasites and fungi that become first obstacle of eggs development and hatchability prevention and treatment against fungal infestation such as *Saprolegnia* sp. often used synthetic and chemical compounds such as bethadine or methylene blue (Mahyuddin et al., 2020). On the other hand, continuous and improper use of chemicals and antibiotics will cause new problems, such as polluted the environment and improve microbial resistance, therefore optimizing and/or controlling the use of antibiotics are key elements in reducing environmental contamination (Polianciuc et al., 2020). To overcome this problem, there is a need for alternative prevention against fungal infestation by adding natural ingredients such as plant leaves that contains antifungal. One of plant leaf that contains antifungal is *Jatropha* leaf (*Jatropha curcas* L.). *Jatropha* leaf contain saponins, tannins, terpenoids, alkaloid, steroids, glycosides, phenolic compounds and flavonoids which can inhibit the growth of fungi (Sharma et al., 2012).

The current finding on activity of *Jatropha curcas* plant exhibited not only excellent antibacterial agent as well as their different parts also strongly inhibited the growth of two tested fungal species *Aspergillus niger* and *Penicillin notatum* and found best inhibitor of the growth of fungus or best antifungal agent (Rahu et al., 2021). The maximum and minimum antifungal activity of various parts of *J. curcas* was measured as in water stem extract maximum (30.5 ± 0.70), while minimum activity was showed in leaf (15 ± 0.0 mm). Antioxidant properties of stem, root and leaves of *J. curcas* had exhibited antioxidant activity, antioxidant models as DPPH radical, scavenging activity, nitric oxide radical scavenging (Sarker et al., 2020)

The treatment that can be done to increase hatchability of the eggs and the survival of the Java barb larvae is by immersing the fish eggs in a solution of *Jatropha* leaf using a variety of different dosages. Previous research has been conducted by Mahyuddin et al., (2020) with the title the effect of soaking carp (*Cyprinus carpio* L.) eggs in *jatropha* leaf solution (*Jatropha curcas* L.) on egg hatchability, and provides results that *jatropha* leaf solution has an influence in inhibiting the growth of *Saprolegnia* sp. in carp eggs. The gap from previous studies with this study is in the species used. This research was conducted to determine the effect of different doses of soaking *Jatropha* leaf on hatchability of eggs and survival of Java barb larvae. Furthermore, to find out the best concentration of *Jatropha* leaf solution on hatchability and survival of Java barb (*Barbonymus gonionotus*) larvae.

2. Materials and methods

2.1. The broodfish

The broodfish used in the study were java barb fish that had already been spawned and had their fertilized eggs taken. The java barb was obtained from the Balai Budidaya Ikan Air Tawar Muntilan, Magelang, Central Java.

2.2. Egg samples and the maintenance

The eggs samples were obtained by spawning a pair of broodstock stimulated by LHRH (sGnRH) hormone 'ovaprim'. A total of 1,000 java barb eggs were used as test subjects. Twenty plastic jars were used as incubation containers, each equipped with an aerator to maintain oxygen circulation. Egg development was observed under a microscope to determine the embryogenesis process.

2.3. Water quality measurements

Water quality was observed using a Water Quality Checker (WQC), which included monitoring of the temperature, dissolved oxygen, and pH. This was carried out every morning and evening during the work to ensure and control suitable water quality during the larval rearing process.

2.4. Preparation of *Jatropha* leaf solution

Jatropha leaf solution (*Jatropha curcas* L.) follows the methods outlined in the research conducted by Maryani et al. (2020) and Mahyuddin et al. (2020). One kilogram of *Jatropha* leaves collected from Semarang was dried in shade, grinded, and sieved to obtain a fine powder. The leaf powder was suspended in distilled water according to the treatment

concentration. A mixture was brewed at 50°C for 15 minutes, allowed to cool, and then used for soaking.

2.5 Phytochemical test

Phytochemical test was conducted as a qualitative preliminary test to determine the presence of chemical compounds (secondary metabolites) in *Jatropha curcas* leaves. The phytochemical content evaluation of *Jatropha* leaves (*Jatropha curcas* L.) included flavonoids, phenols and tannins, following the methods performed previously by Andriyanto *et al.* (2016).

2.7. Experimental design

The experimental design of utilized a completely randomized design (CRD) with four treatments and five replications. The *jatropha* leaf solution used followed the research conducted by Mahyuddin *et al.* (2020). The treatment in this study involved soaking eggs in *jatropha* leaf solution at different doses: A (0 g/L, as a control), B (2 g/L), C (4 g/L), and D (6 g/L).

2.8. Egg soaking process

The egg soaking process took place in buckets with a volume of 5 liters of water, with 50 Java barb eggs per container. The soaking java barb eggs in *jatropha* leaf solution followed to the research of Mahyuddin *et al.*, (2020). After the spawning process, fertilized eggs were manually counted, with each treatment using 50 egg samples. Soaking was carried out for 4 minutes. After soaking, the eggs were transferred to a hatchery container filled with clean water with a volume of 3 liters, equipped with aeration and allowed to stand for 13 to 20 hours or until the eggs hatched. Subsequently, the hatched larvae were counted and recorded for all treatments, and the data obtained were subjected to analysis.

2.9. Data Analysis

Data analysis in this study included observational data on Java barb's embryonic development (embryogenesis), hatching rate, survival rate, and water quality. The collected data were analyzed statistically using variety analysis processed with the IBM SPSS 21 program. Normality, homogeneity, and additivity tests were conducted, and if the

analysis revealed significant effect, the Duncan test was carried out with a confidence level of 95%. Data on egg development and water quality were analyzed descriptively.

3. Results and Discussion

3.1. Phytochemical Test

Phytochemical test was carried out as a qualitative preliminary test to determine the content of chemical compounds (secondary metabolites) of *Jatropha* leaf. The results showed that *Jatropha* leaf solution contained flavonoids at 0.005% - 0.014%; phenol at 0.03% - 0.09%; and tannin 0.033% - 0.098% according to concentration of *Jatropha* leaf (Table 1).

In research conducted by Mahyuddin *et al.*, (2020) soaking *jatropha* leaf solution on the hatchability of goldfish (*Cyprinus carpio* L.) has a significant effect. The high hatchability of eggs is due to the content of secondary metabolite compounds contained in the solution of *jatropha* leaves which provide protection for eggs during soaking from fungal attacks that can make eggs fail to hatch. Among them are Alkaloid compounds, flavonoids and tannins have activity as an antifungal. Mustikasari and Ariyani (2010) stated that the compound alkaloids have antimicrobial activity by damaging the cell walls of microbes. Furthermore, Sabir (2005), explained that flavonoid compounds could damage permeability of microbial cell walls, binding with cell functional proteins and DNA so that able to inhibit the growth of microbes. Flavonoid compounds contained in *jatropha* leaf extract belongs to the phenol group which can serve as anti-fungi. Compound phenols work in cells mainly denatured cell proteins and damage to the cell walls of fungi (Volk and Whileer, 1993). Damaged cell walls cause the absence of energy reserves thereby inhibiting the growth of fungal hyphae.

Jatropha leaf (*Jatropha curcas* L.) contains compounds that influence the development process of Java barb eggs. Flavonoids can degrade the egg cell membrane or cell wall so that the egg wall become thinner and fish larvae could come out more quickly. This is in accordance with Inaya *et al.* (2015), that flavonoid compounds can damage cell membranes which play a role in cell integrity by

Table 1. Phytochemical Test Table of *Jatropha* Leaves Solution (*Jatropha curcas* L.)

| Treatment | Dosage (g/L) | Flavonoid (%) | Phenol (%) | Tannin (%) |
|-----------|--------------|---------------|------------|------------|
| B | 2 | 0.005 | 0.030 | 0.033 |
| C | 4 | 0.009 | 0.060 | 0.065 |
| D | 6 | 0.014 | 0.090 | 0.098 |

Source: Personal Research Results Lab Test. Chem-Mix

denaturing proteins in the cell membrane. This membrane will disrupt its permeability and cause leakage of cell contents so that the fluid inside the egg cell comes out. However, this can also potentially cause the eggs to die when the embryo in the eggs were not yet fully formed and not ready to hatch, or if the larvae have hatched, the larvae were premature which could die within few hours.

Phenolic compounds in the *Jatropha* leaf solution also plays an important role in denaturing proteins and damaging cell membranes. According to Baharudin *et al.* (2016), phenolic compounds can damage cell membranes and denature proteins by dissolving fats in cell walls. If the solution of *Jatropha* leaf is too high, the phenolic activity in reducing protein could reach the chorion layer, which makes it break easily and causes the larvae to hatch prematurely. Tannin compounds can also trigger the processes of chorionase enzyme to soften the chorion layer (Baharudin *et al.*, 2016). This is possible because the chorionase enzyme is more active at low pH and tannins are acidic so that tannins can help the chorionase enzyme to accelerate the process of softening the chorion.

The mechanism of the phytochemical content in the *Jatropha* leaf solution can affect Java barb eggs, where there is a vitelline membrane in the middle of the Java barb eggs covering the fluid surrounding the embryo. Oxygen in water will diffuse, which is move down its concentration gradient through the jelly layer, across the membrane, through the perivitelline fluid and into the embryo (via the skin and/or external gills). Carbon dioxide also moves in the opposite direction by diffusion. No energy is required because diffusion is a passive process. Cilia on the surface of the Java barb embryo moves to create a current of water in the perivitelline fluid and helps accelerate diffusion. This is how Java barb eggs interact with the external environment, which the substance content in the *Jatropha* leaf solution at the moment is able to protect the eggs from fungal infestation.

3.2. Embryo Development

Based on the observation the process of embryogenesis using a microscope, it was demonstrated that the results between treatments A, B, C, and D had differences. Embryo development in treatment C and D at dosages of 4 g/L and 6 g/L was faster than at 2 g/L and 0 g/L. Morula phase occurred at 55 minutes where there was no difference in time with other treatments. The fastest blastula

phase occurred in treatments B, C, and D at 175 minutes while in treatment A at 235 minutes. Gastrula phase in treatments B, C and D achieved at 235 minutes while treatment A at 295 minutes. Neurula phase achieved at 355 minutes for treatment C and D, whilst treatments A and B at 415 minutes. The organogenesis phase in treatment C and D occurred at 415 minutes, which was faster than in treatments A and B, namely at 475 minutes. The hatching phase in treatments C and D occurred in 475 minutes, faster than treatment B which is 535 minutes, while treatment A occurs at 595 minutes (**Table 2**) and the phases of embryogenesis are shown below (**Table 3**).

Embryo development is the most sensitive stage in the entire life cycle of fish (Triwardani *et al.*, 2022). Soaking Java barb eggs in *Jatropha* leaf solution using a predetermined dose showed differences in each treatment. High dosages could accelerate the process of Java barb embryo development. The higher the immersion solution, improve the hatching rate and faster the hatching occur. This was because the higher the dose of the solution used, the higher the flavonoid content. The high content of flavonoids can degrade the egg cell membrane or wall, which makes the larvae in the egg come out quickly.

Flavonoids and high phenolic compounds can degrade the membrane or egg wall so that the egg could hatch quickly. On the other hand, Inaya *et al.*, (2015) described that flavonoid compounds could also able to damage cell membranes that play a role in cell integrity by denatured proteins in cell membranes. As a result, cell membrane is disturbed its permeability which causes leakage and egg fluid dispersed. In addition, the role of tannin compounds can also trigger the chorionase enzyme process to soften the chorion layer (Baharudin *et al.*, 2016). This is possible because the chorionase enzyme is more active at low pH and tannins are acidic, so it can accelerate the chorion softening process. The soft chorion results in breaking easily and causes the larvae to hatch prematurely. On the other hand, tannins have the function of eliminating stickiness in eggs so that eggs do not stick together and can get the oxygen needed in the embryogenesis process properly. This is in accordance with Woyanovich and Horvarth (1980), tannin levels that are effective to reduce the stickiness of fish eggs ranging from 3.0 g/L - 4.8 g/L.

Table 2. Java barb Embryo Development (*B. gonionotus*)




















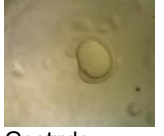















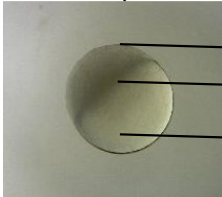
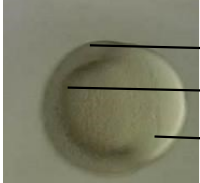
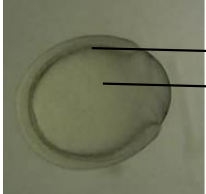
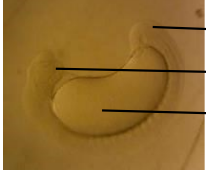
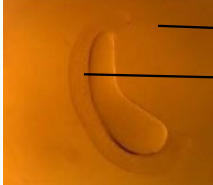


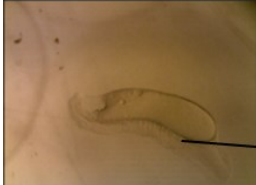
| Hour | Treatment | | | | Length of time (minutes) |
|-------|--|--|--|--|--------------------------|
| | A (0 g/L) | B (2 g/L) | C (4 g/L) | D (6 g/L) | |
| 20.00 |  Morula |  Morula |  Morula |  Morula | 55 |
| 21.00 |  Blastula |  Blastula |  Blastula |  Blastula | 115 |
| 22.00 |  Blastula |  Blastula |  Blastula |  Blastula | 175 |
| 23.00 |  Blastula |  Gastrula |  Gastrula |  Gastrula | 235 |
| 00.00 |  Gastrula |  Gastrula |  Gastrula |  Gastrula | 295 |
| 01.00 |  Gastrula |  Neurula |  Neurula |  Neurula | 355 |
| 02.00 |  Neurula |  Neurula |  Organogenesis (eyespot) |  Organogenesis (eyespot) | 415 |
| 03.00 |  Organogenesis |  Organogenesis (eyespot) |  Hatch |  Hatch | 475 |
| 04.00 |  Organogenesis (eyespot) |  Hatch | | | 535 |
| 05.00 |  Hatch | | | | 595 |

Table 3. The Phases of Embryogenesis *Barbonymus gonionotus* Eggs

| Phase | Information |
|--|--|
| <p>a. Morula phase</p>  | <p>The morula phase is marked by the presence of a second layer, which is still faint at the poles of the anima called the blastomeres.</p> |
| <p>b. Blastula phase</p>  | <p>The blastula phase is marked by the presence of a condensed second layer at the poles of the anima called the blastomere and is seen more clearly.</p> |
| <p>c. Gastrula phase</p>  | <p>The gastrula phase is characterized by a layer that covers the yolk called the blastomeres and the layer that is not covered by the yolk called the blastodisc.</p> |
| <p>d. Neurula phase</p>  | <p>The neurula phase is marked by an embryo that is already visible and a head candidate is starting to form and the shape of the yolk is getting clearer.</p> |
| <p>e. Organogenesis phase</p>  | <p>The organogenesis phase is marked by the formation of organs that are increasingly visible, such as heartbeats, eyespots, and spine.</p> |
| <p>f. Hatch Phase</p>  | <p>The hatching phase is marked by the release of the tail, followed by the head and increasingly active movement of the larvae.</p> |
| <p>g. <i>Saprolegnia</i> sp. -infected egg</p>  | <p><i>Saprolegnia</i> sp. -infected egg is characterized by the appearance of fine threads (hyphae) like cotton on the surface of the egg.</p> |
| <p>h. Abnormally hatched eggs</p>  | <p>Eggs that hatch into larvae show abnormal development characterized by a crooked spine and the shape of physiological organs that look deformed</p> |

3.3. Hatching Rate (HR)

Based on the results of Java barb's hatching rate after the ANOVA test, showed that there were significant differences among the treatments to the degree of hatching rate of Java barb eggs. The hatching rate from the highest to the lowest were treatment C, B, A and D were $84.00 \pm 3.16\%$, $70.40 \pm 2.61\%$, $64.40 \pm 2.97\%$, and $61.20 \pm 2.28\%$ respectively (Figure 1).

Soaking the eggs using *Jatropha* leaf solution has an important effect on Java barb's hatching rate. The rate was influenced by the dose given in each treatment so that the optimal dose could be assessed. Higher *Jatropha* leaf concentration could increase the hatching rate. However, if the condition of the larvae was still weak and difficult to further develop, it was possible that the larvae would perish or become abnormal. According to Azizi *et al.* (2021), weak embryos will not hatch into larvae and eventually die due to not strong enough to break the shell of the egg wall to hatch. Embryos that are weak but successfully hatch have the risk to become an abnormal-larvae. In addition, the content of tannins and flavonoids in the solution of *Jatropha* leaves has an influence on the hatchability of Java barb eggs, because these compounds are useful for preventing fungal infestation on fish eggs. According to Malik and Inriyani (2015), solutions containing antibacterial compounds such as tannins and saponins can prevent fungal infections in fish eggs. Furthermore, according to Mahyuddin *et al.* (2020), *Jatropha* leaf (*Jatropha curcas* L.) solution has an effect on the hatching rate of

fish eggs because it contains secondary metabolites such as tannins which protects the eggs from fungal infestation that could cancel its hatching. The eggs will be protected by an antifungal substance contained in the solution of *Jatropha* leaves, so that the fungus does not easily infect them. According to Fitri (2007), without the presence of antifungal compounds, egg resistance against fungal infestation merely relies on the strength of the chorion alone.

Based on the research results above, it was found that the highest value of hatching rate (84.00%) was in treatment C by immersing *Jatropha* leaf solution as much as 4 g/L. Whilst the lowest value was in treatment D (6 g/L) which was 61.20%. This study confirmed that proper dosage of *jatropha* leaves solution positively affected the hatching rate of Java barb eggs. Mahyuddin *et al.* (2020) trials in goldfish eggs (*Cyprinus carpio* L.) obtained that the highest hatching rate (86.60%) was achieved at dosage of 4.0 g/L, while the lowest was obtained in the control treatment (0 g/L *Jatropha* solution) with hatching rate 23.30%. Those results above were different compared to the research conducted by Mahyuddin *et al.* (2020). The difference of hatching rate might be due to eggs quality, brood stock fertility and age because Radon *et al.* (2013) stated that hatching rate of eggs was a hereditary trait from the parent. Lismawati *et al.* (2016), found that hatching rate of Java barb was 64.30%, whereas Alfath *et al.* (2020) found as high as 89.80%.

Eggs that are not soaked with *Jatropha* leaf solution have lower value of hatching rate

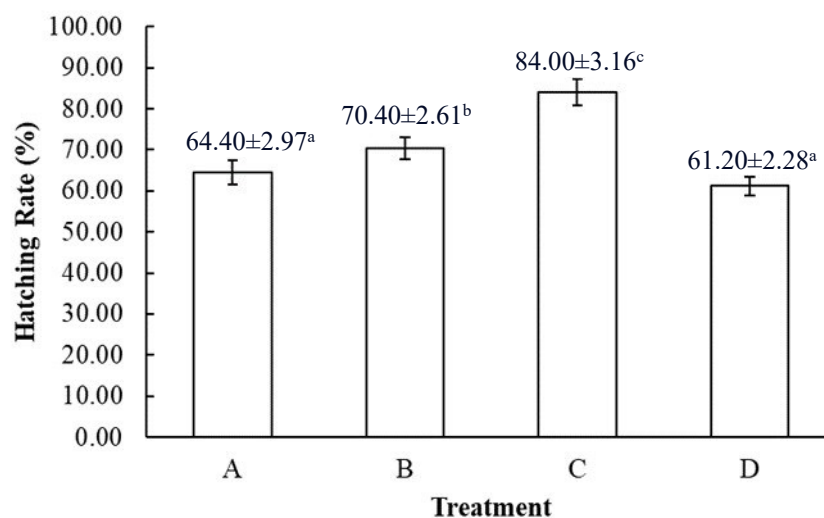


Figure 1. Hatchability histogram (hatching rate) of Java barb Eggs (*B. gonionotus*). Description: A (Control), B (Dosage 2 g/L), C (Dosage 4 g/L), D (Dosage 6 g/L). Different superscripts in the same graphic shows that there are significant differences ($p < 0.05$).

(Mahyuddin *et al.*, 2020), because these eggs are not protected by the antifungal substance contained in *Jatropha* leaf solution, so the fungus will easily infect Java barb eggs. According to Rosida *et al.* (2017), in the absence of antifungal compounds, egg resistance to fungal infestation such as *Saprolegnia* sp. will only rely on the strength of the chorion alone. The fungi infestation will cause weakening of the egg chorion, resulting in the high chance being infected. The infested eggs will allow fungal hyphae to grow and penetrate the chorion of the egg. The nutrients in the eggs will be absorbed by the fungus and cause the eggs stop to develop and eventually die. However, in this study, soaking eggs using an excessive dose of *Jatropha* leaf solution caused premature to hatch, and eventually died because they have not been able to adapt to the environment properly.

The hatching rate of Java barb is influenced by several factors. Zairin *et al.* (2005), stated that low hatching rates was a phenomenon that eggs did not develop after being fertilized and changes in their physiological abilities during embryogenesis. While Setyono (2009), stated that not all fertilized eggs will hatch into larvae. Eggs that don't hatch can be caused by unfavorable egg conditions due to the presence of a mixture of water when collecting eggs. Other factors are the quality of the broodstock, the quality of the feed given to the broodstock, the metabolism in the development of fish eggs, and the condition of media used for spawning and hatching the eggs. Maleko *et al.* (2008) added that broodstock with good growth will potentially

have a good reproductive rate, so the quality of the fish eggs and larvae produced will also be good. According to Hardaningsih *et al.* (2008), embryos that are unable to carry out metabolic processes and development will die due to the inability to form tissues in prospective organs.

3.4. Survival Rate (SR)

Based on the ANOVA test, it was demonstrated that immersion of *Jatropha* leaf solution significantly affected the survival of Java barb larvae. The highest to lowest survival rate of Java barb larvae were on treatments C ($85.24 \pm 1.00\%$), B ($75.65 \pm 1.40\%$), A ($65.82 \pm 1.50\%$), and D ($60.84 \pm 2.09\%$) respectively (Figure 2).

The best results were treatment C. It was assumed that the flavonoids, phenols, and tannins contained in the *Jatropha* leaf solution could provide good protection to eggs shell so that the eggs were protected from fungal infestation. A high hatchability of eggs indirectly supports the survivability of Java barb larvae. Gusrina (2008), stated that the number of larvae that live at the end of rearing is inseparable from hatching rate. Egg quality is important factor to produce viable larvae. Immunity of fish, both in the larval and broodstock stages are inseparable from the role of antimicrobial compounds. Thus, can increase hatching and suppress abnormalities in the larvae (Haser *et al.*, 2018). In treatment D actually gives decreased results because giving doses that are too high can accelerate egg hatching. However, it is very possible if the condition of the larvae is still weak and difficult to develop longer so that the larvae die or will become abnormal larvae. According to Azizi *et al.*,

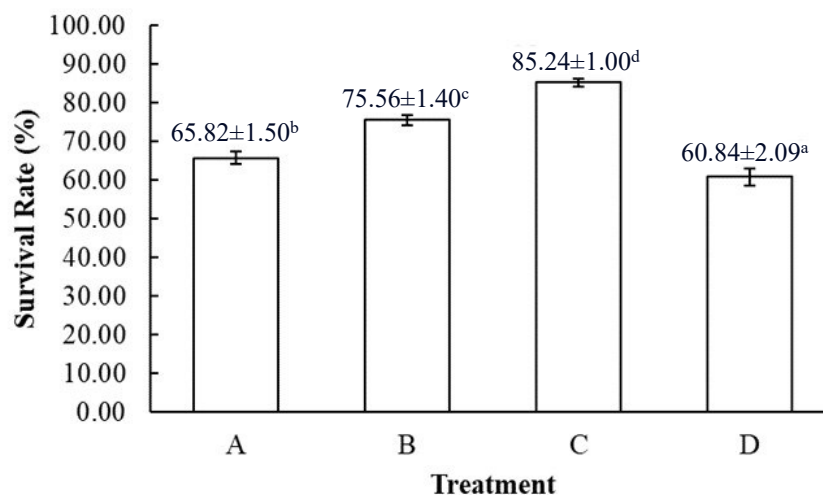


Figure 2. Lifetime histogram (survival rate) Java barb Eggs (*B. gonionotus*).

Description: A (Control), B (Dosage 2 g/L), C (Dosage 4 g/L), D (Dosage 6 g/L). Different superscripts in the same graphic shows than there are significant differences ($p < 0.05$).

(2021) that weak embryos will not hatch into larvae and eventually die, this is because the embryo is not strong enough to break the shell of the egg wall to hatch, weak and successfully hatched embryos have the opportunity to become abnormal larvae. This is what causes a decrease in SR in treatment D.

The survival rate of Java barb larvae is quite high. It is influenced by 2 factors, namely internal factors and external factors. Internal factors include the age and size of the fish, as well as adaptability to its environment. While the external factors namely the quality of rearing medium and the availability of food. Diana and Safutra (2018) reported that Java barb larvae fed with natural food obtained a survival rate of 64.40%, while in Winata *et al.* (2018), the survival rate of Java barb was 85.00%. One of the factors for the high survivability of Java barb larvae is thought to be due to proper and intensive natural feeding during maintenance, so that the diet needs of the larvae can be met and the larvae can produce energy for growth. According to Maleko *et al.* (2014), after hatching, fish larvae depend on food sources as energy for growth. Poor feed quality will interfere larval development, which can lead to death.

3.5. Water Quality

The water quality in this research shows within optimal condition and in accordance with fish maintenance standards (Table 4). Water quality has a very important role for aquatic organisms to support their life. Water quality management during research aims to reduce the risk of death that causes failure (Triwardani *et al.*, 2022). Water quality that is outside the optimum range will cause fish stress, resulting in susceptible to disease. According to Ardit *et al.* (2015), the survival rate of fish is influenced by good water quality management. Water quality parameters measured in this research included pH, dissolved oxygen (DO), and temperature.

During the research, average water pH for

outside the optimum range could cause poor growth and even death. According to Yumame *et al.* (2013), the water pH between 6.5 and 9 is considered suitable for fish farming. Manunggal *et al.* (2018) also stated that a pH value between 7 and 9 is very adequate for pond water. Ivan *et al.* (2019), added that pH changes to acid or base will disrupt the survival of fish because it disrupts the respiration process. Java barb has an optimal pH range between 6.7 and 8.6 (Boyd, 1979). Thus, the water pH during the test was within normal range.

The water temperature during the study ranged from 21.0 to 27.0 °C, where these range were still within the normal range. The daily temperature changes were not exceeded 6°C, so it's still safe for fish life. This result was in line with Oktafiansyah (2015) and Zulkarnain *et al.* (2017), that daily temperature changes up to 10°C did not cause fish stress. Furthermore, Kottelat *et al.* (1993), stated that Java barb live in freshwaters at temperatures of 22 - 28°C. According to Muarif (2016), water temperatures between 22 - 30°C are still considered suitable for aquaculture. Nugraha *et al.* (2012) stated that temperatures below the minimum limit increase the duration of eggs hatching and reduce the performance of enzymes in the eggshell (chorion). Conversely, temperatures above the maximum limit can result in premature hatching and difficult to survive.

Based on the research results, it was found that the dissolved oxygen level was between 5.6 and 8.5 mg/L, which were within the optimum range. The dissolved oxygen could help metabolic processes to produce energy for survival and growth. The higher dissolved oxygen level, the better the water quality. According to Government Degree No. 82 (2001), the safe dissolved oxygen in aquaculture is ≥ 4 mg/L. Furthermore, Mahendra (2018), described that fish can live normally when dissolved oxygen content ≥ 3

Table 4. Water Quality Data on Java Barb During Research

| Treatment | Range of water quality parameter values | | |
|---------------|---|------------------|---------------------------|
| | Temperature (°C) | pH | DO (mg/L) |
| A | 21,0 - 25,9 | 7,0 - 7,9 | 5,6 - 8,5 |
| B | 21,1 - 27,0 | 7,0 - 7,9 | 5,8 - 8,4 |
| C | 21,2 - 27,0 | 7,0 - 7,8 | 6,1 - 8,5 |
| D | 21,0 - 27,0 | 7,0 - 7,9 | 6,2 - 8,1 |
| Qualification | 22 - 28 | 6,5 - 8,5 | ≥ 3 (SNI 7550, 2009) |
| Value | (Kotelatet <i>et al.</i> , 1993) | (SNI 6133, 1999) | |

Description: A (Control), B (Dosage 2 g/L), C (Dosage 4 g/L), D (Dosage 6 g/L)

larval rearing was between 7.0 and 7.9. pH

mg/L, and increase fish productivity when dissolved oxygen above 5 mg/L.

4. Conclusion

It was concluded that soaking of Java barb eggs in *Jatropha* leaf solution significantly affected egg development, hatchability, and survival rate Java barb fish. Soaking using *Jatropha* leaf solution accelerated the hatching time from 595 minutes (control) to 475 minutes (treatment D). The best hatchability and survival rate were demonstrated in treatment C (soaking in 4 g/L *Jatropha* leaf solution) which were $84.00 \pm 3.16\%$, and $85.24 \pm 1.00\%$ respectively.

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