

# The use of Jamaican cherry leaves solution to remove eggs adhesiveness in the production of Asian redbtail catfish fry (*Hemibagrus nemurus* Valenciennes, 1840)

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**Abstract.** Asian redbtail catfish (*Hemibagrus nemurus* Valenciennes, 1840) is one of the freshwater fish commodities that have high economic value and are popular with the community. The community's need for Asian redbtail catfish is still fulfilled from catches in nature. This fish farming business has been carried out but there are still obstacles, especially in the provision of fry through hatcheries. The obstacle is the high adhesion of the fish eggs which will affect the fertilization and hatching values obtained. The purpose of this study was to analyze the use of Jamaican cherry leaves solution with different doses on egg adhesion, fertilization, hatching, growth, and survival of Asian redbtail catfish larvae. This research was conducted from April to June 2022 at the Laboratory of Fish Hatchery and Breeding at the Faculty of Fisheries and Marine Science, Riau University. The design used was a completely randomized design with a dose of Jamaican cherry leaves solution treatment consisting of P0 (control treatment without soaking Jamaican cherry leaves solution), P1 (soaking dose of Jamaican cherry leaves solution of 3 g/L), P2 (soaking dose of Jamaican cherry leaves solution of 4 g/L), and P3 (soaking dose of Jamaican cherry leaves solution of 5 g/L). The results showed that the best treatment was obtained in treatment P2 (soaking dose of Jamaican cherry leaves solution of 4 g/L), which produced eggs that did not stick by 76.49%, fertilization value of 87.42%, hatching value of 87.94%, absolute weight growth of 1.3222 g, absolute length growth of 5.76 cm, specific growth rate of 14.5017 %/day, and the survival value for 42 days of rearing was 86.67%.

**Key Words:** absolute length growth, absolute weight growth, egg adhesion value, specific growth rate.

**Introduction.** The Asian redbtail catfish (*Hemibagrus nemurus* Valenciennes, 1840) is one of 31 species of economically important fish in the waters of the Kampar River, Riau (Sukendi 2014). Public consumption needs for this fish are still obtained from catches in nature. Although this fish farming business has started to be carried out, the provision of fry for the cultivation needs is still an obstacle that is always encountered. This is because the high adhesion of the fish eggs will affect the fertilization and hatching values obtained. To overcome the adhesiveness of the eggs, soaking the eggs using a solution of Jamaican cherry leaves can be used. This is because Jamaican cherry leaves (*Muntingia calabura*) contain 1.41% tannin compounds (Puspitaning 2012). Tannins can eliminate the adhesion of eggs because they can bind and precipitate protein compounds caused by the presence of some functional bond groups that will interact strongly with protein molecules which in turn will produce large and complex cross-links, namely tannin-protein (Zakes et al 2005). However, until now it is not known what dose of Jamaican cherry leaf solution is best for removing egg adhesion, increasing the value of fertilization, hatching, growth, and survival of Asian redbtail catfish larvae.

## Material and Method

**Description of the study.** This research was conducted from April to June 2022 at the Laboratory of Fish Hatchery and Breeding, Faculty of Fisheries and Marine Science, Riau University. The treatments in this study were the dosage of Jamaican cherry leaves solution consisting of P0 (control treatment without soaking the Jamaican cherry leaves solution), P1 (soaking dose of Jamaican cherry leaves solution of 3 g/L), P2 (soaking dose of Jamaican cherry leaves solution of 4 g/L), and P3 (soaking dose of Jamaican cherry leaves solution of 5 g/L).

The design used was a completely randomized design with 4 treatment levels and 3 replications to obtain 12 treatment units. In each treatment unit, around 1 g of eggs was used ( $\pm 753$  eggs). Determination of this dose is based on Baharudin et al (2016), which state that the effective tannin limit to reduce the adhesion of fish eggs is 6 gr/L with a soaking time of 4 minutes, and when it exceeds this limit, the activity of tannins in reducing protein can reach the chorion layer so that the chorion layer is easily broken, and premature larvae occur.

Soaking of the fertilized eggs is carried out in a basin container in which a coconut milk filter has been prepared for spreading eggs, after 4 minutes of soaking, the eggs are rinsed with clean water 2-3 times so that no Jamaican cherry leaves extract remains (Situmorang et al 2020). Then the coconut milk filter is lifted into an incubation container in the form of an aquarium for observing the measured parameters.

The parameters measured are the following: 1) egg adhesion was determined using the formula for the number of glued eggs/the total number of eggs  $\times 100\%$ ; 2) the fertilization value was determined using the formula: the number of fertilized eggs/the total number of eggs  $\times 100\%$ ; 3) the hatching value was determined using the formula: number of hatched eggs/number of fertilized eggs  $\times 100\%$ ; 4) absolute weight growth was determined using the formula: larval weight at the end of the study - larval weight at the beginning of the study; 5) absolute length growth was determined using the formula for larval length at the end of the study - larval length at the beginning of the study; 6) the specific growth rate (SGR) was determined using the formula:  $\ln$  weight of larvae at the end of the study -  $\ln$  weight of larvae at the beginning of the study / time  $\times 100\%$ ; 7) survival was determined using the formula: number of larvae at the end of the study / number of larvae at the beginning of the study  $\times 100\%$ . For the observation of absolute weight growth parameters, absolute length growth, daily weight growth rate, and survival rate, rearing was carried out for 42 days with observations every 7 days. During rearing the larvae were fed *Tubifex* sp. ad libitum with a frequency of 4 times a day.

**Statistical analysis.** The data obtained were tabulated and statistical tests were carried out with the SPSS 16 application. The statistical tests carried out were homogeneity of variances and one-way analysis of variance (ANOVA). If the results of the ANOVA test showed a significant difference ( $p < 0.05$ ), a further test was carried out using the Student-Newman-Keuls test to determine the differences between treatments.

## Results and Discussion

**Egg adhesion.** The results of observations on the adhesion of eggs from each dose of the given Jamaican cherry leaves solution soaking treatment can be seen in Figure 1.

Figure 1 shows that the smallest treatment resulted in egg adhesion successively in treatment P3 of 20.22%, P2 of 23.51%, P1 of 31.10%, and P0 of 49.47%. The results showed that the higher the dose of Jamaican cherry leaves solution given, the smaller the value of egg adhesion obtained. This fact is due to the higher concentration of tannin compounds, thus the more layers of glycoprotein are deposited by tannins. Tannins can remove adhesion by eroding the mucus layers in eggs, binding and depositing some protein molecules that bind to each other and form a complex compound, namely tannin-protein (Noga 1996; Zakes et al 2005). According to Badarullah et al (2020), the

percentage of very effective adhesion is 0-20%, the percentage of effective adhesion is 25-50% and the percentage of ineffective adhesion is almost 100%, namely > 75%.

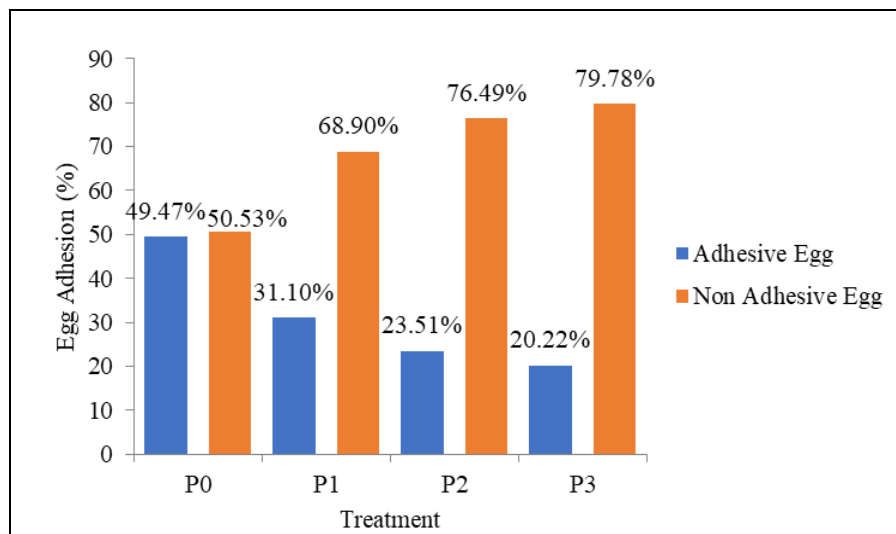


Figure 1. Histogram of the adhesiveness of Asian redbtail catfish eggs from each dose of the Jamaican cherry leaves solution soaking treatment (P0 = soaking solution of Jamaican cherry leaves of 0 g/L; P1 = soaking solution of Jamaican cherry leaves of 3 g/L; P2 = soaking solution of Jamaican cherry leaves of 4 g/L; P3 = soaking solution of Jamaican cherry leaves of 5 g/L).

The results of the analysis of variance (ANOVA) showed that the dose of Jamaican cherry leaves solution given had a significant effect ( $p < 0.05$ ) on the adhesiveness of Asian redbtail catfish eggs. The results of further tests using the Student-Newman-Keuls test showed that the treatments P0 and P1 were significantly different ( $p < 0.05$ ) from P2 and P3, but P2 and P3 were not significantly different ( $p > 0.05$ ).

**Fertilization rate (FR).** The results of observations on the value of egg fertilization from each dose of the given Jamaican cherry leaves solution soaking treatment can be seen in Figure 2.

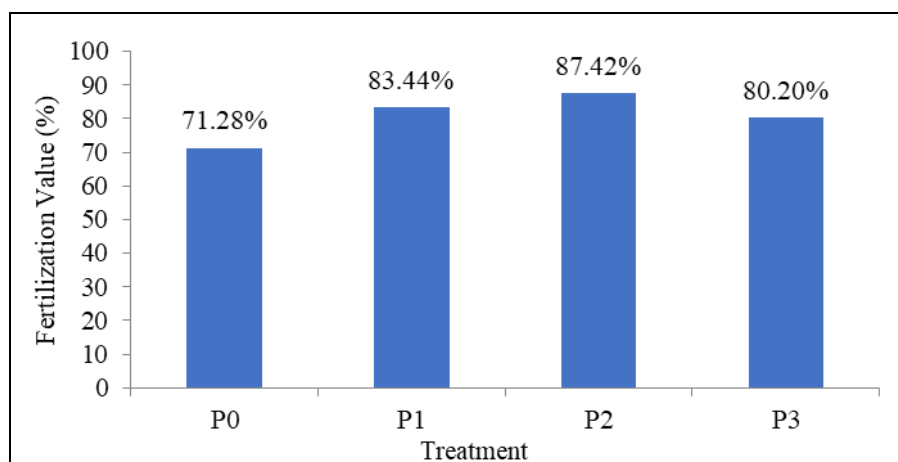


Figure 2. Histogram of the fertilization value of Asian redbtail catfish eggs from each dose of soaking with Jamaican cherry leaves solution.

Figure 2 shows that the highest treatment yields fertilization values sequentially in treatment P2 of 87.42%, P1 of 83.44%, P3 of 80.20%, and P0 of 71.28%. The greatest fertilization value obtained in this study was not related to the smallest adhesion value obtained previously, this indicates that the fertilization value is not only influenced by the egg adhesion value but other factors that influence it. The function of the Jamaican cherry leaves solution given is to utilize the tannin content contained in the Jamaican cherry leaves solution to decompose the glycoprotein layer into complex compounds for

the embryo development process (Mulyani & Johan 2020). Baharudin et al 2016 stated that the effective tannin limit to reduce egg adhesion is 6 g/L with a soaking time of 4 minutes for premature larvae. The highest fertilization value obtained was lower than the fertilization value of African catfish given a solution containing tannins at a dose of 10 g/L by 90% (Yustiati et al 2021), higher than the fertilization value of catfish given a solution containing tannins at a dose of 6 g/L of 15.86% (Pratiwi et al 2020).

The results of the analysis of variance (ANOVA) showed that the dose of Jamaican cherry leaves solution given had a significant effect ( $p < 0.05$ ) on the fertilization value of Asian redbtail catfish eggs. The results of further tests using the Student-Newman-Keuls test showed that the treatments P1 and P3 were significantly different ( $p < 0.05$ ) from P0 and P2, but P1 and P3 were not significantly different ( $p > 0.05$ ).

**Hatching value (HR).** The results of observations on the hatching value of each dose of the given Jamaican cherry leaves solution soaking treatment can be seen in Figure 3.

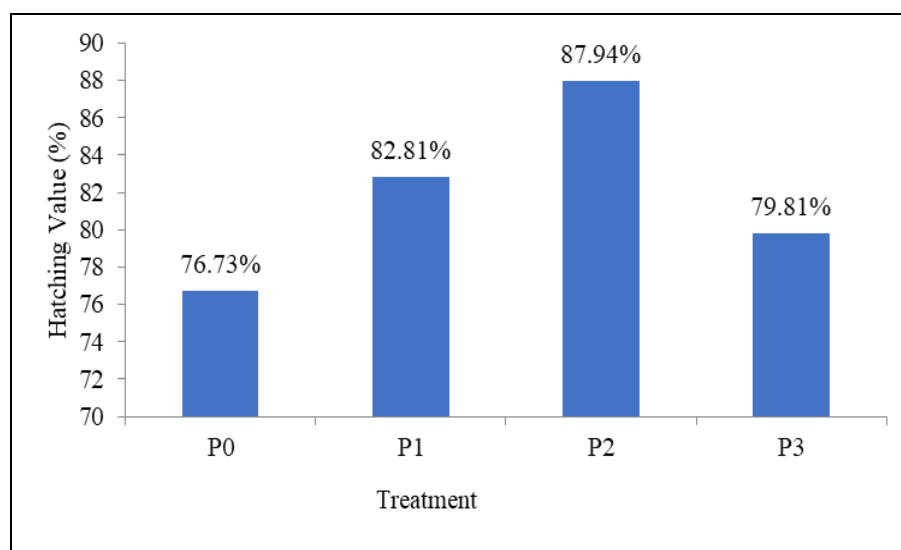


Figure 3. Histogram of the hatching value of Asian redbtail catfish eggs from each dose of soaking the Jamaican cherry leaves solution.

Figure 3 shows that the largest treatment that produces hatching values sequentially is the treatment P2 of 87.94%, P1 of 82.81%, P3 of 79.81%, and P0 of 76.73%. Giving excessive or low concentrations will affect hatchability resulting in the death of eggs and eggs do not hatch (Ihsan & Ibrahim 2004). The relatively high concentration of Jamaican cherry leaves solution results in too thin erosion of the mucus layer on the egg membrane so that eggs that are unable to tolerate the tannin compounds contained in Jamaican cherry leaves, can be toxic and result in the death of fish eggs (Mulyani & Johan 2020). The highest hatching value obtained was greater than the hatching value given the Jamaican cherry leaves solution with the same dose in African catfish, which was 64% (Mulyani & Johan 2020).

The results of the analysis of variance (ANOVA) showed that the dose of Jamaican cherry leaves solution given had a significant effect ( $p < 0.05$ ) on the hatching of Asian redbtail catfish eggs. Further test results using the Student-Newman-Keuls test showed that the treatments P0, P1, and P3 were significantly different ( $p < 0.05$ ) from P2, while P0, P1, and P3 were not significantly different ( $p > 0.05$ ), and P1 not significantly different ( $p > 0.05$ ) with P2.

**Absolute weight growth.** The results of observations on the weight growth of Asian redbtail catfish larvae for each sampling for 42 days of rearing from each dose of the given Jamaican cherry leaves solution soaking treatment can be seen in Figure 4.

From Figure 4, it can be seen that the weight growth of Asian Redtail Catfish larvae from the beginning to the 21st day was relatively the same but experienced a significant increase on the 28th to 42nd day of rearing. This is because the process of adaptation

and refinement of body organs has been completed so that the feed consumed can be used for growth.

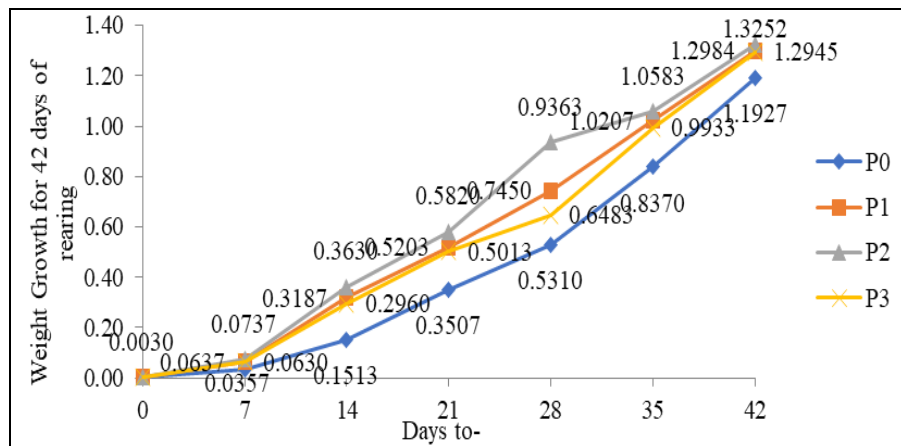


Figure 4. Weight growth of Asian redbtail catfish larvae for each sampling for 42 days of rearing from each treatment dose of soaking with Jamaican cherry leaves solution.

Aryzegovina et al (2015) states that growth will increase rapidly with the increasing frequency of feeding given, so that the more often the feed is given the better the results for fish growth, compared to infrequent feeding. In this study, feeding was done ad libitum with a frequency of 4 times a day. A histogram of the absolute weight growth of Asian Redtail Catfish larvae from each treatment for 42 days of rearing can be seen in Figure 5.

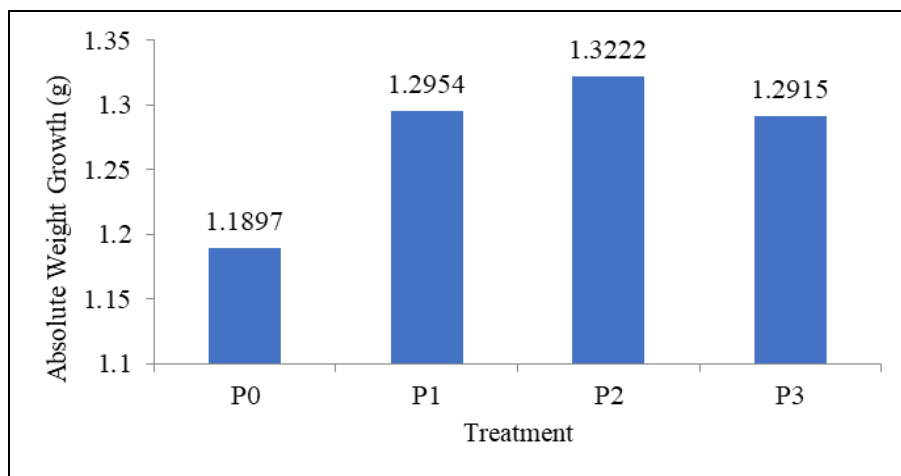


Figure 5. Histogram of absolute weight growth of Asian redbtail catfish larvae from each treatment dose of soaking with Jamaican cherry leaves solution.

Figure 5 shows that the largest treatment that produces absolute weight growth values sequentially is the treatment P2 of 1.3222 g, P1 of 1.2954 g, P3 of 1.2915 g, and P0 of 1.1897 g. Indirectly, the Jamaican cherry leaves solution did not affect the growth of larvae. The absolute weight growth of Asian redbtail catfish reared for 21 days reached 0.30 g (Syafitra et al 2018), ad libitum reached 0.98 g for 40 days (Firmansyah et al 2019) and reared for 30 days reached 0.44 g (Juliana et al 2016).

The results of the analysis of variance (ANOVA) showed that the dose of Jamaican cherry leaves solution given had a significant effect ( $p < 0.05$ ) on the growth of the absolute weight of Asian redbtail catfish. The results of further tests using the Student-Newman-Keuls test showed that the treatments P1 and P3 were significantly different ( $p < 0.05$ ) from P0 and P2, while P1 and P3 were not significantly different ( $p > 0.05$ ).

**Absolute length growth.** The results of observations on the length growth of Asian Redtail Catfish larvae for each sampling for 42 days of rearing from each dose of the Jamaican cherry leaves solution soaking treatment can be seen in Figure 6.

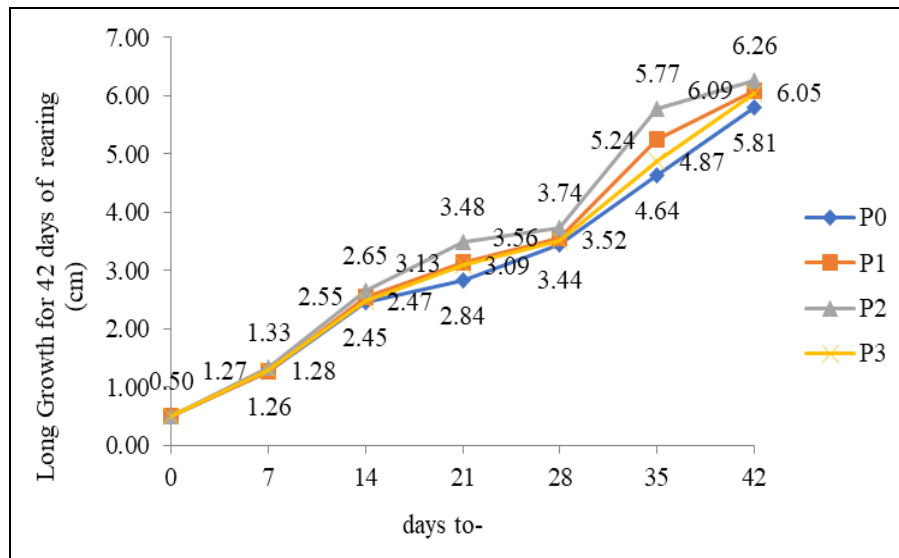


Figure 6. Length growth of Asian redbtail catfish larvae for each sampling for 42 days of rearing from each treatment dose of Jamaican cherry leaves solution.

From Figure 6 it can be seen that the length growth of Asian redbtail catfish larvae is the same as the previous observation of weight growth, where from the beginning of rearing until day 21 it was relatively the same and experienced a significant increase on day 28 to day 42 of rearing. Fish growth is influenced by internal factors (heredity, sex, and age) and external factors (water and feed quality). Feed is one component that has a major role in fish growth (Karimah et al 2018). A histogram of the absolute length growth of Asian Redtail Catfish larvae from each treatment for 42 days of rearing can be seen in Figure 7.

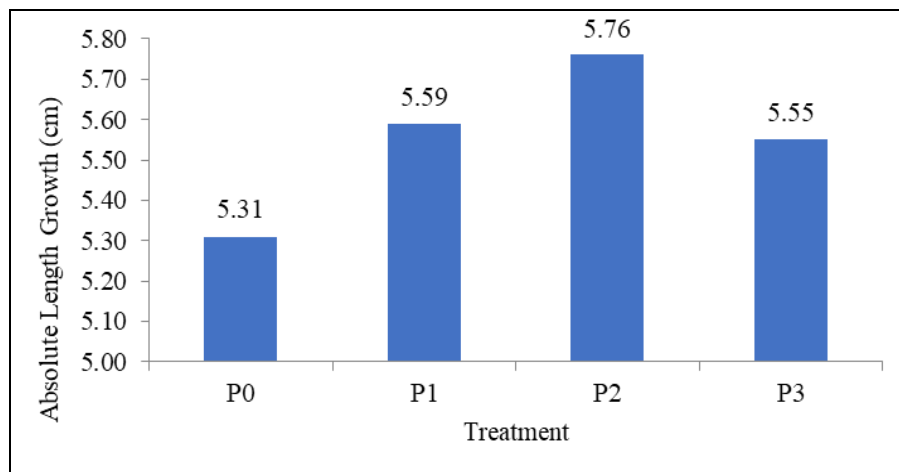


Figure 7. Histogram of absolute length growth of Asian redbtail catfish larvae from each treatment dose of Jamaican cherry leaves solution.

Figure 7 shows that the largest treatment yield in absolute length growth values is sequentially treatment P2 of 5.76 cm, P1 of 5.59 cm, P3 of 5.55 cm, and P0 of 5.31 cm. The protein used was visible in the growth of fish with the addition of length and body weight of fish. Indra et al (2014) stated that the content of proteolytic enzymes can stimulate faster embryonic growth. The absolute length growth of Asian redbtail catfish reached 2.55 cm for 21 days of rearing (Syafitra et al 2018), 4.01 cm for 21 days of

rearing (Yosmaniar et al 2007) and 3.45 cm for 40 days of rearing (Firmansyah et al 2019).

The results of the analysis of variance (ANOVA) showed that the dose of Jamaican cherry leaves solution given had a significant effect ( $p < 0.05$ ) on the absolute length growth of Asian redbtail catfish. The results of the further test using the Student-Newman-Keuls test showed that the treatment P0 was significantly different ( $p < 0.05$ ) with P2, while P0 was not significantly different ( $p > 0.05$ ) with P1 and P3, and P2 was not significantly different ( $p > 0.05$ ) with P1 and P3.

**Specific growth rate.** The results of observations on the specific growth rate of Asian redbtail catfish larvae from each dose of the given Jamaican cherry leaves solution soaking treatment can be seen in Figure 8.

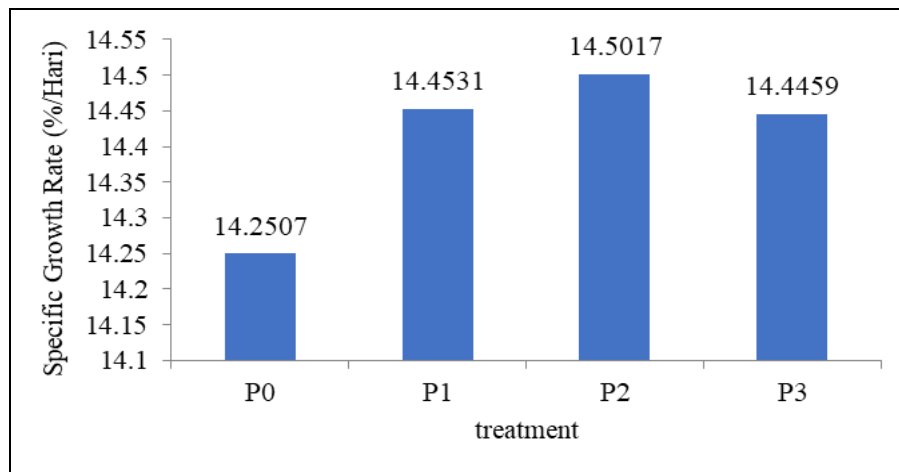


Figure 8. Histogram of the specific growth rate of Asian redbtail catfish larvae from each treatment of soaking with Jamaican cherry leaves solution.

Figure 8 shows that the largest treatment that produces the value of the specific growth rate sequentially is treatment P2 of 14.5017 %/day, P1 of 14.4531 %/day, P3 of 14.4459 %/day and P0 of 14.2507 %/day. The specific growth rate of larvae obtained is greater than the results of research by Syafitra et al (2018) by 11.00 %/day for 21 days of rearing, for 40 days of rearing it reaches 9.23% (Firmansyah et al 2019), smaller from Yosmaniar (2007) with 19.21% for 21 days of rearing.

The results of the analysis of variance (ANOVA) showed that the dose of Jamaican cherry leaves solution given had a significant effect ( $p < 0.05$ ) on the specific growth rate of Asian redbtail catfish larvae. The results of further tests using the Student-Newman-Keuls test showed that the treatments P1 and P3 were significantly different ( $p < 0.05$ ) from P0 and P2, while P1 and P3 were not significantly different ( $p > 0.05$ ).

**Survival.** The results of observations on the survival of Asian redbtail catfish larvae from each treatment can be seen in Figure 9.

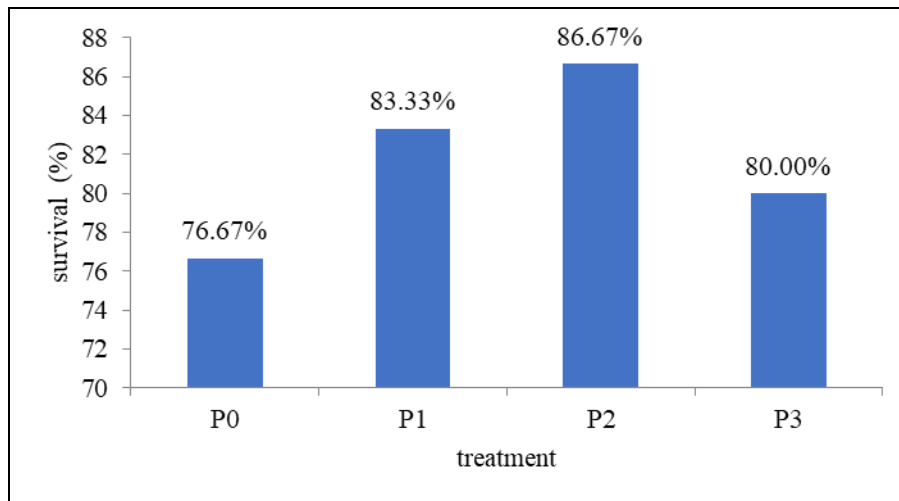


Figure 9. Histogram for the survival of Asian redbtail catfish larvae from each treatment with different doses of Jamaican cherry leaves solution.

Figure 9 shows that the highest treatment yields for survival value sequentially is treatment P2 of 86.67%, P1 of 83.33%, P3 of 80%, and P0 of 76.67%. The survival value of Asian redbtail catfish was not affected by the addition of Jamaican cherry leaves solution. Survival is influenced by biotic factors such as competitors, population density, age, and the ability of organisms to interact with the environment, while abiotic factors such as temperature, dissolved oxygen, pH, and ammonia content (Effendi 1997). Herath and Atapaththu (2013) said that the survival of fish larvae is determined by a combination of various factors including larval nutrition, environment, water quality immunity and stocking density, and the use of feed also influences growth and survival.

The results of the analysis of variance (ANOVA) showed that the dose of Jamaican cherry leaves solution given had a significant effect ( $p < 0.05$ ) on the survival of Asian redbtail catfish. The results of the Student-Newman-Keuls test showed that the treatment P0 was significantly different ( $p < 0.05$ ) with P2, while P0 was not significantly different ( $p > 0.05$ ) with P1 and P3, and P2 was not significantly different ( $p > 0.05$ ) with P1 and P3.

**Conclusions.** The use of Jamaican cherry leaves solution with different doses was able to reduce the adhesion of Asian redbtail catfish (*Hemibagrus nemurus* Valenciennes, 1840) eggs. From the results of the study, it was found that the best dose at P2 (4.0 g/L) produced 76.49% unglued eggs, 87.42% fertilization value, 87.94% hatching value, 1.3222 g absolute weight growth, absolute length growth of 5.76 cm, specific growth rate of 14.5017 %/day, and the survival value for 42 days of rearing was 86.67%.

**Conflict of interest.** The authors declare that there is no conflict of interest.

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