The Use of Different Natural Plant Extract to Remove Egg Adhesiveness in The Procution of Asian Redtail Catfish Fry (*Hemibagrus nemurus* CV)

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Abstract. The provision for the cultivation Asian redtail catfish fry needs is still limited, because adhesion of the eggs is strong. The solutions for this problem is to immersion of the eggs using natural plant extracts which are easy to obtain and cheap. The aim of of this research was to assess the impact of different natural plant extract on the adhesion on eggs of Asian redtail catfish fry. The study was carried out at the Fish Hatchery and Breeding Laboratory, Riau University from June to July 2023. The design used was a completely randomized design, with various natural plant extracts: P0 (control), P1 (1.00 ml pineapple solution per liter of water), P2 (4.00 bilimbi solution per liter of water), and P3 (4.00 ml Jamaican cherry leaves solution per liter of water). The results showed that the best treatment was observed in P3, resulting in a fertilization rate of 90.40%, hatching rate 87.13%, egg adhesion rate of 14.28, absolute weight growth of 1.2611 g, absolute length growth of 5.54 cm, daily weight growth of 13.17% per day, and a survival rate of 83.52%.

1 Introduction

One of the commodities useful for the freshwater fisheries in the province of Riau is Asian redtail catfish (*Hemibagrus nemurus*), which is one of the important cost fish in the waters of the Kampar Riau river [1]. The development of the *H.nemurus* fish farming business is very rapid, but the availability of fry to consumer needs is still not sufficient. This is due to the hatchery which is carried out through artificial spawning because of the adhesive nature of fish eggs or having adhesion between one egg and another. The adhesive of eggs fish cause the fertilization value of the fish to be low as well as the hatchability values of the eggs to be low, so the resulting fry production is low. To overcome this requires a treatment that eliminates the adhesive of the high egg. One way is immersion the egg in a solution containing compound that function to remove the stickness of the eggs.

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The compound used in eliminating egg areas is tannin or bromelin. Tannin has ability to bind the protein and precipitate the protein of eggs that covered by the gluprotein layers, so that the adhesiveness the egg will be lost [2]. On the other hand, the bromelin enzyme has the ability to break protein molecules into simpler forms [3]. These compounds are contained in several types of natural plants that are easily found around the environment and the prices are cheap. These plants include pineapples (*Ananas comosus*), bilimbi (*Averrhoa bilimbi* L), and jamaican cherry leaves (*Muntingia calabura*).

The pineapple plant contains a bromelin enzyme found in stems, bark, leaves, fruit, and stems of a pineapple plant (*A. comosus*) [4]. Bromelin enzyme content is the most common in the pineapple hump. According to [5], the concentration of the pineapple enzymes found in the pineapple hump amounts to 0,10-0,60%. This is the highest number of bromelin enzyme content obtained from other parts of the fruits [6]. Bilimbi contains active compound of saponin, flavonoid, triterpenoid, and tannin. Tannin compounds have the benefit of reducing the eggs adhesive. Cherry leaves (*Muntingia calabura*) contain compounds including flavonoid, saponin, and tannin. Similar to the previously described pineapple plant, the functions of flavonoid and saponin can serve as both an antibacterial and an antiinflammatory. Whereas tannins have the benefits of reducing the adhesive of the egg. From this description, it is necessary to find the best types of plants that can eliminate the adhesive forces of the *H.nemurus* fish at the time of fertilization, which will enable the value of the fertility as well the power of hatching, and the survival of the larvae so that production of fry in artificial spawning process is both possible.

2 Materials and Method

The study was conducted from June to July 2023 at the Faculty of Fisheries and Marine's Fish Hatchery and Breeding Laboratory at the University of Riau. The treatment in this study consisted of four treatment groups: P0 (control, no immersion in natural ingredient solution), P1 (immersion in 1 ml pineapple solution per liter of water), P2 (immersion in 4.0 ml bilimbi solution per liter of water), and P3 (immersion in 4.0 ml Jamaican cherry leaves solution of water). The design used was a randomized design with 4 treatment levels with three repetitions and 12 treatment units. This dosage is based on the results of research [7] that identified effective doses of natural plant substances for remove the adhesive of Asian redtail catfish egg. For instance, the optimal dosage for pineapple solution was determined to be 1.0 ml/liter of water, resulting in a 73.84% non-adhesive egg rate. Likewise, bilimbi solution showed the best effect at 4.0 ml/lite of water, achieving a 75.90% non-adhesive egg rate, while Jamaican cherry leaves solution yielded a 76.49% non-adhesive egg rate at the same 4.0 ml/liter of water dosage.

The fertilized eggs were subjected to a 4-minute immersion within a 1-liter basin containing 1 liter of water specific to each treatment, each with a predetermined dose. Following immersion, the eggs were transferred to an incubation container to monitor the measured parameters. These parameters encompassed:

1) Egg Adhesion = (total adhered eggs / total eggs) x 100%.
2) Fertilization Value = (total fertilized eggs / total eggs) x 100%.
3) Hatching Value = (total hatched eggs / total fertilized eggs) x 100%.
4) Absolute Weight Growth = larvae weight at end of research – larvae weight at start of research
5) Absolute Length Growth = larval length at end of research – larval length at start of research.
6) Specific Growth Rate (SGR = (Ln larvae weight at end of research – Ln larvae weight at start of research) / time x 100%

7) Survival Rate = (total larvae at end of research / total larvae at start of research) x 100%.

Observations for absolute weight growth, absolute length growth, daily growth rate, and survival rate were conducted over 42 days, with assessments made every 7 days. Collected data was organized into tables, and statistical analyses were conducted utilizing the SPSS 22 application. These analyses encompassed tests for homogeneity of variances and one-way analysis of variance (ANOVA). If the ANOVA results indicated significance ($P < 0.05$), subsequent analysis was performed using the SNK test to determine variations between treatments.

3 Result and Discussion

3.1 Egg Adhesion

The results of observations on the adhesion of eggs from each given treatments can be seen in Figure 1.

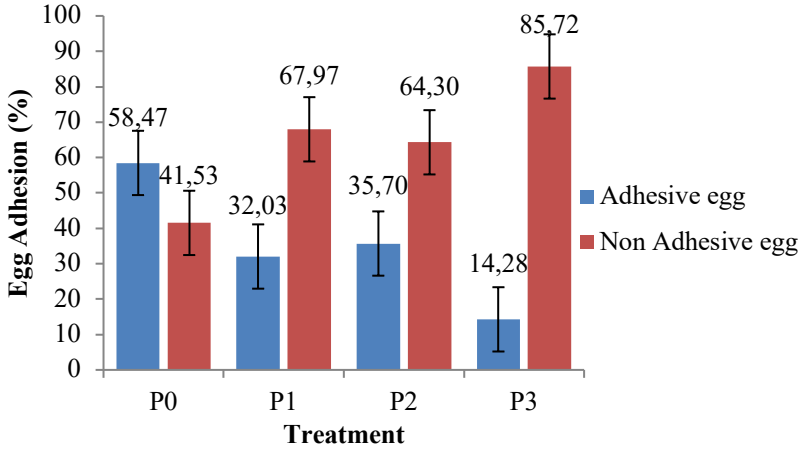

Fig. 1. Histogram of Adhesiveness *H.nemurus* Eggs from Different Types of Natural Plants.

Description :
P0 = Control treatment without immersion in natural ingredients solution
P1 = Immersion treatment with pineapple solution dose of 1 ml/ liter of water
P2 = Immersion treatment with wuluh starfruit solution dose of 4,0 ml/ liter of water
P3 = Immersion treatment with a dose of cherry leaf solution of 4,0 ml/liter of water

From Figure 1. it shows the smallest treatment that produces egg adhesion sequentially is in the P3 treatment is 14,28%, P1 is 32,03%, P2 is 35,79%, and P0 is 58.47%. The results showed that immersion *H.nemurus* eggs with cherry leaves solution using a dose of 4,0 ml/liter has the smallest adhesion of eggs sticking to each other compared to other treatments of 14,28%. The solution from the cherry leaves plant is more effective to eliminating the stickiness of the *H.nemurus* fish eggs, because it has a higher tannin content compared to the wuluh starfruit plant, while the pineapple plant contains the enzyme bromelin. According to [8] the tannin content contained in cherry leaves is 13,715 mgGAE/g extract or for every one gram of cherry leaves extract there is tannin which is equivalent to 13,715 mg of gallic acid, [9] declare the tannin content in wuluh starfruit was 0,914 mgQE/g, whereas according to [10] the tannin content of the pineapple hump was

0,63028 mgGAE/g extract, the bromelin enzyme content was most commonly found in the pineapple hump. According to [5], the bromelin enzyme content in pineapple plants obtained from pineapple humps is 0,10-0,60%.

All of these ingredients have a function to remove the adhesion to fish eggs. Tannin has the ability to remove adhesion with eroding the mucus layers on eggs interaction of tannins with protein occur due to the presence of phenolic hydroxy groups which easily from effective cross-links with proteins and other molecules through hydrogen bonds or covalent bonds tannin-protein complex compounds [11 *in* 12]. The bromelin enzyme has the ability to breakdown protein molecules into more basic structures [3] in proportion to its capacity to corrode the protein coating contained in the *H.nemurus* fish eggs [13 and 6].

The ANOVA analysis results indicated that immersing eggs in various natural plant species had a significant effect (P<0.05) on the adhesive of *H. nemurus* fish eggs. The Student-Newman-Keuls test showed that the P0 and P3 was significantly different from P2 and P1 (P<0,05) while P2 was not significantly different (P>0,05) from P1.

3.2 Fertilization Value

The results of observations on the value of egg fertilization from each given treatment can be seen in Figure 2.

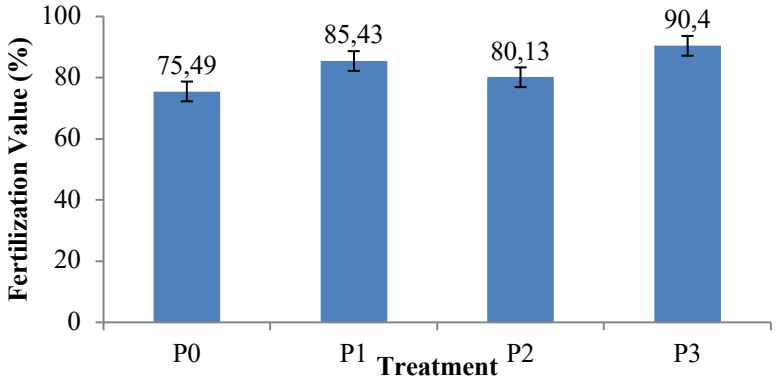

Fig. 2. Histogram of the Fertilization Value of H.nemurus Fish Eggs from Each Different Types of Natural Plants.

From Figure 2. Can shows that the largest treatment fertilization values sequentially was in the P3 treatment is 90,40%, P2 is 85,43%, P2 is 80,13%, and P0 is 75,49%. The fertilization value is in line with the previous adhesion egg value. The purpose of the cherry leaves solution is to employ the tannin content within it to disintegrate the glycoprotein layer into intricate compounds in order to achieve the process of embryo development [14]. The loss of the adhesive layer which causes the opportunity for cells to divide and develop is not disturbed by a lack of oxygen intake so that the fertilization value of the egg also increases.

The ANOVA analysis results indicated that immersing eggs in various natural plant species had a significant effect (P<0.05) on the fertilization value of *H.nemurus* fish eggs. The Student-Newman-Keuls test showed that all treatments significantly different (P<0,05).

3.3 Hatching Value

The results of observations on the value of egg hatching from each given treatment can be seen in Figure 3.

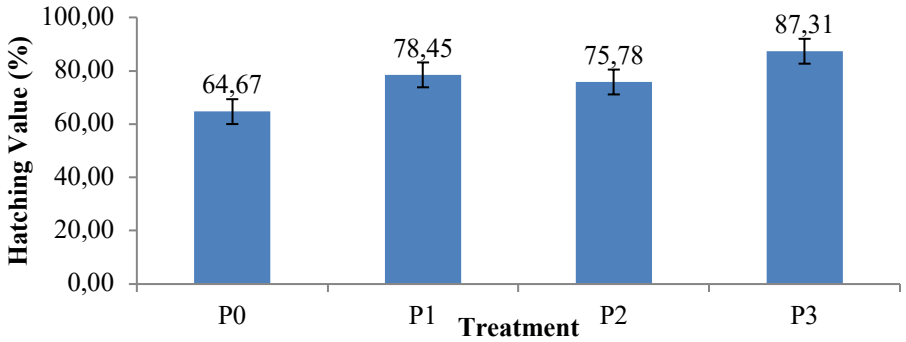

Fig. 3. Histogram of the Hatching Value *H.nemurus* Fish Eggs from Each Different Types of Natural Plants.

Figure 3. it shows the highest treatment hatching value sequentially was in treatment P3 is 87,31%, P2 is 78,45%, P2 is 75,78%, and P0 is 64,67%. This is because the egg adhesion decreases due to the greater tannin content in cherry leaves, causing many eggs to be fertilized resulting in the highest egg hatching rate. This is in accordance with [15] declare a high fertilization rate will result in substantial increase in egg hatching rate. [16] declare the eggs are attached to each other this will lead to an imbalanced dispersion of oxygen, culminating in fatality. Furthermore, if the eggshell layer undergoes erosion, it will prompt untimely larval hatching, leading to mortality.

The ANOVA analysis results indicated that immersing eggs in various natural plant species had a significant effect ($P<0.05$) on the hatching of *H.nemurus* fish eggs. The Student-Newman-Keuls test showed the treatment P0 and P3 were significantly different from P2 and P1 ($P<0,05$) while P2 was not significantly different ($P>0,05$) from P1.

3.4 Absolute Weight Growth

The results of observations on the growth of the weight *H.nemurus* fish larvae for each sampling for 42 days of maintenance of each given treatment can be seen in Figure 4.

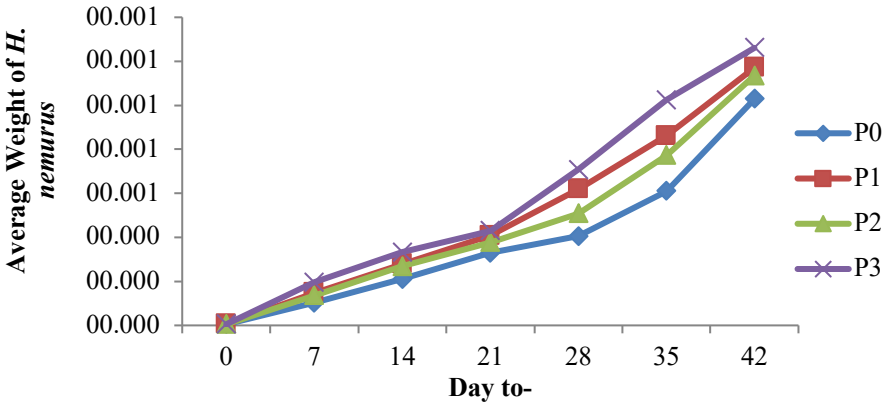

Fig. 4. Average Weight Growth of *H.nemurus* Fish Larvae.

From Figure 4. it shows the 21st day was relatively the same in each treatment given. This outcome emerged due to the larvae utilizing the consumed feed until the 21st day to accommodate and enhance their bodily functions. Furthermore, day-28 until day-42 of maintenance, The growth of the larvae has elevated as the process of acclimatization and enhancement of the body's organs has concluded, enabling the consumed feed to be utilized for development. [17] declare the growth will rapidly increase with more and more frequency of feeding given, so that the more often the feed is given the results will be better for fish growth, compared to rare feeding. The histogram of absolute weight growth of *H.nemurus* fish larvae from each treatment for 42 days of rearing can be seen in Figure 5.

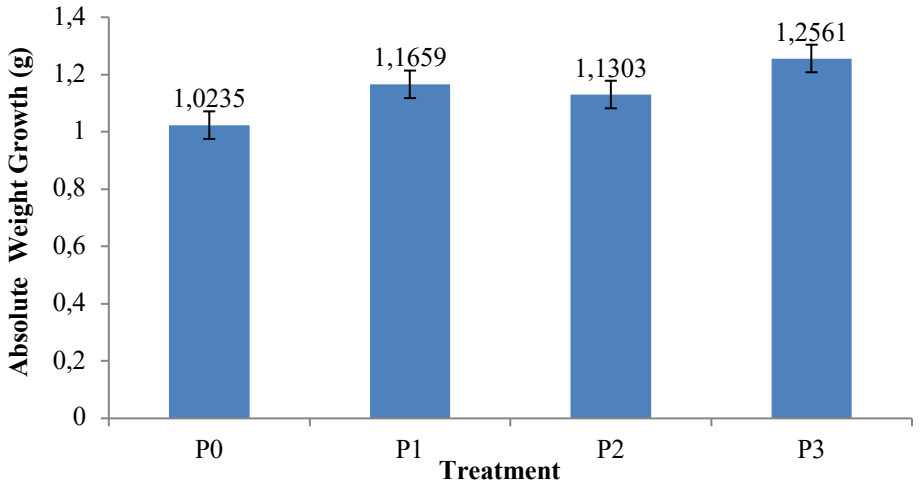

Fig. 5. Histogram of Absolute Weight Growth *H.nemurus* Fish Larvae from Each Egg Immersion Treatment Using Different Types of Natural Plants.

From Figure 5. it shows the largest treatment absolute weight growth values sequentially was in treatment P3 of 1,2561 g, P1 is 1,1659 g, P2 is 1,1303 g, and P0 is 1,0235 g. Feeding affect larvae growth. [18] declare feed given by looking at the protein content produces a positive linear to absolute weight. In addition, *Tubifex sp*. has autolysis properties and contains various enzymes, exogenous which can stimulate the formation of digestive enzymes which can result in better growth of *H.nemurus* fish larvae [19]. The absolute weight growth of the *H.nemurus* fish maintenance for 21 days reached 0,30 g [20], adlibitum reached 0,98 g for 40 days [21], rearing for 30 days reached 0,4369 g [22].

The ANOVA analysis results indicated that immersing eggs in various natural plant species had a significant effect ($P<0.05$) on the absolute weight growth of *H.nemurus* fish. The Student-Newman-Keuls test showed that all treatments had an effect significantly different ($P<0,05$).

3.5 Absolute Length Growth

The results of observations on the growth of the length *H.nemurus* fish larvae for each sampling for 42 days of maintenance from each given treatment can be seen in Figure 6.

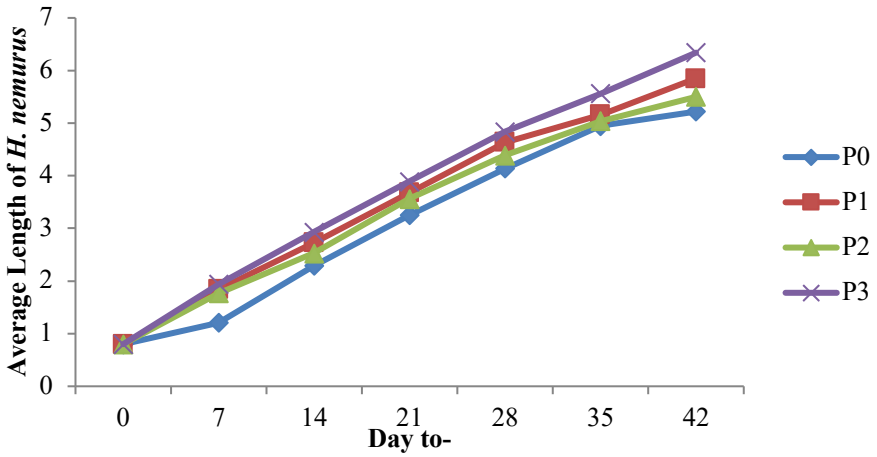

Fig. 6. Average Growth Length of *H.nemurus* Fish.

From Figure 6. it can be seen that from the beginning of maintenance to day 21st it was relatively same, and experienced a significant increase on day-28 until day-42 of maintenance. The growth fish is influenced by internal factors (heredity, sex, and age) and external factors (water and feed quality). Feed is a supporting factor in cultivation, which one of the elements important for supporting fish growth [23]. The histogram of absolute length growth of *H.nemurus* fish larvae from each treatment for 42 days of rearing can be seen in Figure 7.

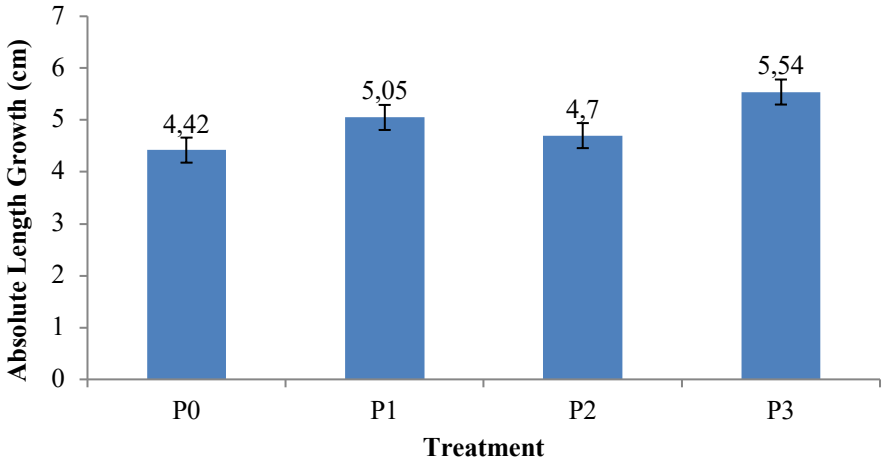

Fig. 7. Histogram of Absolute Length Growth *H.nemurus* Fish Larvae from Each Egg Immersion Treatment Using Different Types of Natural Plants.

From Figure 7. it can shows that largest action resulting in an absolute length growth value sequentially was in the P3 treatment is 5,54 cm, P1 is 5,05 cm, P2 is 4,70 cm, and P0 is 4,42 cm. The protein used shows growth in fish with the addition of length and body weight of fish. According to [24] declare the growth is influenced by nutritional factors

obtained from feed which can support the growth of fish larvae. The protein used shows growth with the addition of length and body weight of fish. *Tubifex sp*. worms is a natural food that contains high nutritional value. The content of *Tubifex sp*. is 57% protein, 13,30% fat, 2,04% carbohydrates [25]. The absolute length growth of *H.nemurus* fish reached 2,30 cm for 16 days of rearing [26], 1,85 cm for 14 days [27].

The ANOVA analysis results indicated that immersing eggs in various natural plant species had a significant effect (P<0.05) on the absolute length growth of the *H.nemurus* fish. The Student-Newman-Keuls test showed that all treatments had an effect significantly different (P<0,05).

3.6 Specific Growth Rate

The results of observations on the specific growth rate *H.nemurus* fish larvae from each given treatment can be seen in Figure 8.

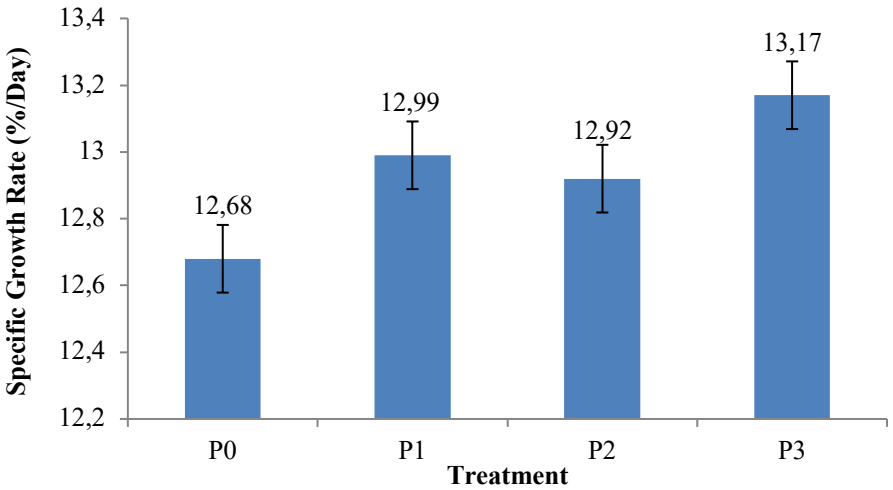

Fig. 8. Histogram of Specific Growth Rate *H.nemurus* Fish Larvae from Each Egg Immersion Treatment Using Different Types of Natural Plants.

Figure 8. can shows the largest treatment specific growth rate values sequentially was in treatment P3 is 13,17%/day, P1 is 12,99%/day, P2 is 12,92%/day, and P0 is 12,68 %/day. The growth rate is also influenced by competition for food such as the quantity of different fish appetites. In accordance with the statement of [28] The growth rate can be impacted by both living and non-living factors. Living factors encompass the fish's age and feeding capabilities, while non-living factors include food availability and the quality of the environment. However, the specific growth rate achieved in this study was lower than the findings of prior research, in which the H. nemurus fish attained a specific growth rate of 19.12% over a 21-day period [29] for 40 days reaching 9,23% [21].

The ANOVA analysis results indicated that immersing eggs in various natural plant species had a significant effect (P<0.05) on the specific growth rate of *H.nemurus* fish larvae. The Student-Newman-Keuls test showed that all treatments provided significantly different effect (P<0,05).

3.7 Survival Rate

The results of observations the survival rate *H.nemurus* fish larvae from each given treatment can be seen in Figure 9.

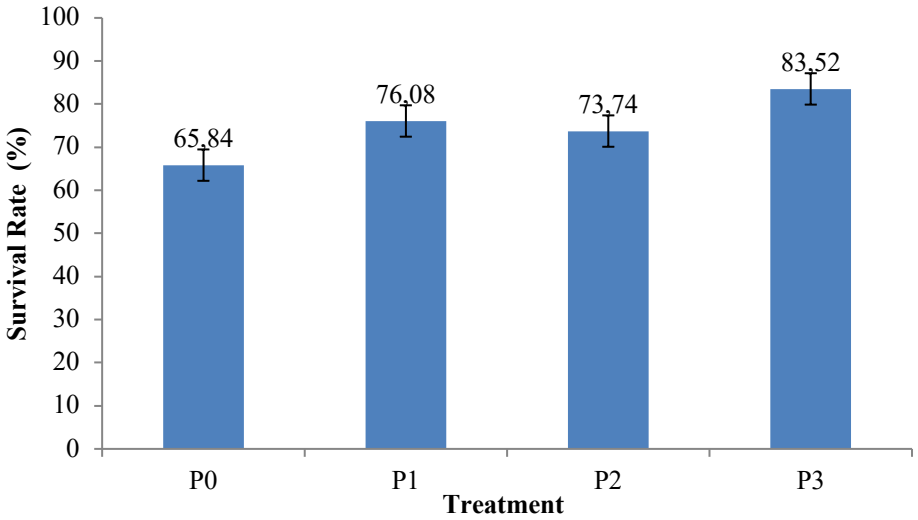

Fig. 9. Histogram of Survival Rate *H.nemurus* Fish Larvae for 42 Days of Rearing from Each Egg Immersion Treatment Using Different Types of Natural Plants

From Figure 9. it shows the largest treatment survival rate values sequentially was in the P3 treatment is 83,25%, P1 is 76,08%, P2 is 73,74%, and P0 is 65,84%. [30] declare the Survival rates are shaped by a combination of intrinsic and extrinsic elements, including inter-species competition and insufficiencies in both the quality and quantity of food resources. [31] declare the survival rate of larvae after hatching is also influenced by the quality of the eggs produced by the parents. The better egg quality, so the hatching rate and larval survival will also increase.

The ANOVA analysis results indicated that immersing eggs in various natural plant species had a significant effect ($P<0.05$) on the survival of *H.nemurus* fish larvae. The Student-Newman-Keuls test showed that the P0 and P3 treatments were different. Significantly with P2 and P1 ($P<0,05$) while P2 was not significantly different ($P>0,05$) with P1.

4 Conclusion

The use of different natural plant species can reduce the stickiness of Asian redtail catfish eggs (*Hemibagrus nemurus*). The results, it was found that the best plants were at P3 (immersion treatment with a dose of cherry leaves solution of 4,0 ml/liter of water) with a fertilization value is 90,40%, hatching value is 87,13%, egg adhesion value is 14,28%, absolute weight growth is 1,2611 g, absolute length growth is 5,54 cm daily weight growth rate is 13,17%/day, and survival rate is 83,52%.

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