INFLUENCE DURIAN LEAVES (*Durio zibethinus*) INFUSION AS NATURAL ANESTHESIA OF STRIPED CATFISH JUVENILE (*Pangasius* sp.)

Dedi Rohendi, Taufik Budhi Pramono, Purnama Sukardi

Program Studi Akuakultur, Fakultas Perikanan dan Ilmu Kelautan, Universitas Jenderal Soedirman, Purwokerto, 53123, Indonesia
Email: dedirohendi0@gmail.com

Abstract

The use of anesthesia in the process of living fish transportation was an effort in keeping the physiology of fish alive and healthy to the destination. Durian leaves were one of the natural ingredients that can be used as fish anesthesia. The purpose of this research known the effective concentration of infusion durian leaves as anesthetic seeds of patin fish based on inductive time, sedative time and survival rate. Experimental studies used complete randomized designs, four treatments and threefold. The treatment of infusion concentrations of durian leaves 10%, 20%, 30%, and 40%. The time when fish begins to faint when inserted into the anesthesia solution was recorded as inductive time, whereas the time the fish began to recover back or knowingly recorded as a sedative time. The survival calculation was done on the number of fish surviving post-anesthesia with maintenance for 3 days. The results of this study showed that the effective concentration gained was 10%, with the inductive time (71.67 minutes) distinct from the apparent 20% concentration, but no apparent difference with the concentration of 30% and 40%. Meanwhile, the sedative time (0.25 minutes) differs from the concentration of 40%, but did not differ with concentrations of 20% and 30%. The survival rate of the fish seed Patin at a concentration of 10% showed real distinct results and reached 100%. Infusion concentration of durian leaves by 10% was an effective concentration in the anesthetic process of striped catfish juvenile.

Keywords: *durian leaves, natural anesthesia, infusion, Pangasius sp.*

Introduction

Catfish is a leading freshwater fishery commodity in Indonesia which has high economic value and market demand in the community (Sunarma, 2007; Meilisza, 2009). The selection of catfish as a superior commodity in aquaculture is due to its relatively easy maintenance, affordable prices, high stocking density (Septimesy et al., 2016) and tolerant of high environmental temperatures (Trong et al., 2002). These advantages make catfish farming more and more attractive and continue to grow. The development of catfish culture has continued to increase, both in hatchery and nursery efforts (Karim et al., 2016), as well as enlargement in ponds and floating net cages (Marthin et al., 2018). Increased production of catfish needs to be supported by the availability of seeds in the right amount, time and quality (Meilisza, 2009; Syahputra, 2010). Therefore, it was necessary to make efforts to manage the supply of seeds through good fish transportation.

Fish transportation is the transport of fish from one place to another with a certain number with low mortality and loss to the destination (Junianto, 2003). Fish transportation with long-distance destinations and certain types of fish, such as wild, rare and sharp fish, requires special handling, namely anesthesia or anesthesia (Saputra et al., 2017; Soedibya and Pramono, 2018). Anesthesia in fish has the aim that the condition of the fish is unconscious and its response to movement is reduced through the respiratory system, and then circulates to all body tissues (Soedibya and Pramono, 2018).

Anesthetics can use commercial chemical anesthetics such as Tricaine Methane Sulphonate / MS-222 (Pramono, 2002), 2-phenoxyethanol, quinaldine, and metomidate (Lepic et al., 2014). However, they are relatively expensive, difficult to obtain and

still leave residues in the body (Sukarsa, 2005). Efforts to use anesthetics from natural ingredients are currently being developed by several researchers including marine algae (Sukarsa, 2005), clove oil (Riesma et al., 2014), keben fruit seed extract (Septiarusli et al., 2012), betel leaves extract green (Nur'aini, 2016), and guava leaves (Karim et al., 2016). Natural anesthetic ingredients have advantages, including easy access, relatively affordable prices, easy to apply and safe for fish health (Saskia et al., 2012; Temitope, 2014; Yudhistira et al., 2020).

Durian leaves were widely available in nature, but their potential was not widely used. Information on the use of durian leaves as a natural anesthetic agent in the transportation process is still relatively small, namely the infusion of durian leaves as an anesthetic agent for catfish *(Clarias gariepinus)* (Yudhistira et al., 2020) and pomfret (Munandar et al., 2017). However, the use of durian leaves infusion to anesthetize catfish seeds has never been carried out. This study aimed to determine the effective concentration of durian leaves infusion on fainting time (inductive), conscious time (sedative) and survival rate of catfish fry.

Materials and methods

Research implementation

This research was conducted in March 2020. The research site was in the Fish Cultivator Group (POKDAKAN) Mina Dadi Rejeki, Gumiwang Village, Purwanegara District, Banjarnegara Regency. The average weight and length of catfish seeds used was 9.11 ± 0.78 g and 10.53 ± 1.13 cm obtained from fish farmers in Gumiwang Village, Purwanegara District, Banjarnegara Regency. This study used an experimental method with a Completely Randomized Design (CRD). The treatments were given using durian leaves infusion solution with different concentrations, namely 10% (P1), 20% (P2), 30% (P3) and 40% (P4) with repetition of each treatment 3 (three) times.

Phytochemical Analysis

Analysis of phytochemical compounds refered to Harborne (1987) included testing of flavonoids, saponins and tannins. The flavonoids were tested by means of 2 mL extract solution added with a little mg powder and 2 mL concentrated of HCl. Flavonoid compounds characterized by the formation of a reddish or orange color. Saponin testing was done by adding distilled water and shaked it vigorously in the extract solution. The presence of saponin compounds is indicated by the formation of foam 1-10 cm which was stable and did not less than 10 minutes. While the tannins by means of a 2 mL extract solution were added 10 mL of distilled water then bring to a boil. After cooling the filtrate was added with 5 mL of 1% FeCl. If the color changed to dark blue, it indicated that the sample contained tannin.

Making Durian Leaves Infusion Solution

The solution used was a mixture of water (diluent) with boiled durian leaves, after referred to as infusion. The making of durian leaves infusion solution refered to Nur'aini (2016) and Yudhistira et al., (2020) that fresh durian leaves were cleaned of stuck dirt, then weighed as much as 100 g, 200 g, 300 g, and 400 g. The weighed durian leaves were then cut into small pieces 3-4 cm and then boiled in 1 L of water at a temperature of ± 90^{o}C. After the solution returned to normal temperature, the solution was left closed for three days. The concentration of the anesthetic solution was obtained from the calculation of the quotient between the weight of durian leaves (g) with the amount of water used as an anesthetic medium (mL) then multiplied by 100% as shown in the formula below:

$$\text{Infusion Concentration (\%)} = \frac{\text{weight of durian leaves}}{\text{amount of water for anesthesia}} \times 100\%$$

The concentrations obtained were 10%, 20%, 30%, and 40%, respectively. Furthermore, each concentration of anesthetic solution was tested on catfish seeds with an average length and weight of 10.53 ± 1.13 cm and 9.11 ± 0.78g to determine the most effective anesthetic concentration.

Maintenance and Preparation of Seeds

The maintenance of catfish seeds was carried out for 3 days after purchase and given pellet feed. The selection of catfish seeds for research was carried out by selecting active and healthy seeds. Catfish seeds before being anesthetized were fasted or not fed for 24 h. This fasting aimed to reduce feed input, so that

when anesthetized it did not released much metabolic waste.

Observation of Inductive and Sedative Anesthesia of Patin Fish Seeds

The catfish seeds that have been kept and fasted were then tested on the durian leaves infusion solution. The test fish were put into an aquarium with a size of 20x12x16 cm which contained an anesthetic solution for durian leaves infusion with predetermined concentrations of 10%, 20%, 30%, and 40% until fainting. The time when the test fish began to faint when put into the anesthetic solution was recorded as inductive time (minutes), while the time the fish began to recover or regain consciousness and experience movement after restoration by placing it in aerated water media was recorded as sedative time (minutes).

Survival Rate

The calculation of the survival rate was carried out during the anesthesia process and after anesthesia with maintenance for 3 days. The maintenance of catfish seeds aimed to determine the effect and concentration of an anesthetic solution of durian leaves infusion on the survival of catfish seeds. According to Effendie (1978) fish survival can be calculated by the formula:

$$SR = \frac{\text{amount of fish alive}}{\text{initial stocking amount}} \times 100\%$$

Data analysis

The data in the form of a percentage was transformed in the form of arcsin transformation. The data obtained inductive time, sedative time and survival rate were analyzed statistically using analysis of variance (ANOVA) in Statistical Product and Service Solutions (SPSS) 17.0 software. Data shown a significant effect was continued with the Honestly Significant Difference test (BNJ) or the Tukey test. The data from the phytochemical test results of durian leaves infusion solution were analyzed descriptively.

Result and discussion

Phytochemical Test of Durian Leaves Infusion

Based on the phytochemical test in this study, the durian leaves infusion solution showed positive results for the presence of secondary metabolites such as saponins, tannins and flavonoids (**Figure 1**). These results were in line with Brown's (1997) research which states that saponins, tannins and flavonoids were secondary metabolites which were relatively abundant in durian leaves. These secondary metabolite compounds in durian leaves have potential as natural anesthetic agents (Maradona, 2013; Abid et al., 2014; and Munandar et al., 2017).

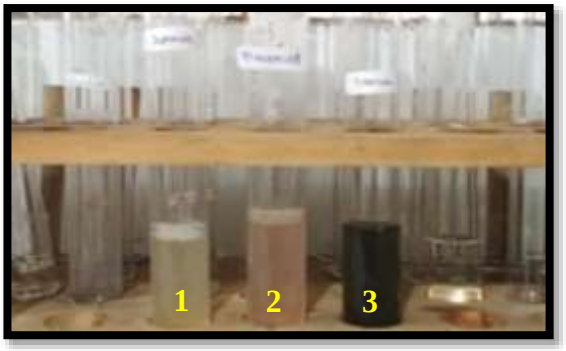

Figure 1. Phytochemical Test Results of Durian Leaves Infusion (1) Saponin Compounds (2) Flavonoid (3) Tannin

Based on the research results in Figure 1, it shown that the presence of secondary metabolite compounds in the durian leaves infusion solution was characterized by the formation of foam for saponins (Muyasaroh, 2018), the formation of a turquoise color for tannins (Arlofa, 2011), and a yellowish red color in the tube. reactions to flavonoids (Insanu, 2011). This was in accordance with Harborne's (1987) statement which states that the presence of flavonoid compounds was indicated by the formation of reddish or orange colors, tannins by the formation of dark blue, and saponins were characterized by the formation of foam.

Tannins were secondary metabolites of polyphenols that dissolved in water and were able to accelerate the deposition of proteins with molecular weights ranging from 1000 to 3000 and can bind to other macromolecules. Saponins were compounds which were commonly found in plants as anti-viral / bacterial types of glycosides with a foamy character when reacted with water, but with excessive used, these compounds can be toxic (Suteja, 2018). Other secondary metabolite compounds of polyphenols, namely flavonoids, were useful for body health, as anti-viruses and were able to inhibit enzyme activity in fish meat resulting in decreased

metabolism (Apriyanti, 2007; Arifin and Sanusi, 2018).

Inductive Time

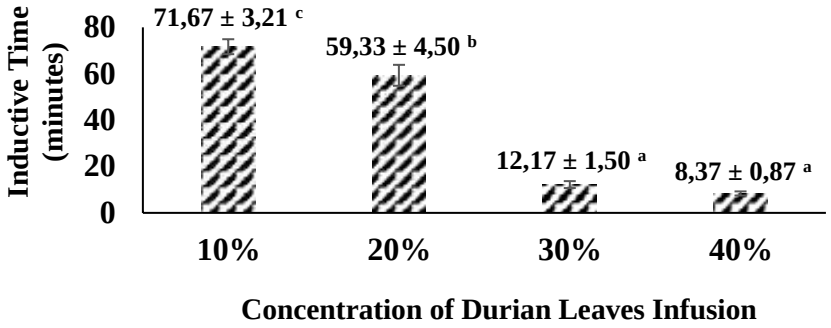

Figure 2. The average inductive time of catfish seeds in the administration of durian leaves infusion solution. Numbers followed by different superscript lowercase letters indicated significantly differences (P <0.05)

ANOVA statistical test results showed that differences in the concentration of durian leaves infusion treatment on inductive time had a significant effect (P <0.05). Based on the results of Tukey's test, the treatment of durian leaves infusion with a concentration of 30% and 40% showed no significant difference, but significantly different from the concentrations of 10% and 20% (**Figure 2**). The difference in the speed of inductive time in this study was thought to be influenced by the higher concentration of infusion.

Similar conditions were also shown in the study of Riesma et al., (2014), which stated that the inductive time of catfish seeds anesthetized using clove oil with a concentration of 0.010 mL / L with control showed no significant difference, but significantly different with a concentration of 0.015 mL / L and 0.020 mL / L. Research by Abid et al., (2014) also reported that the higher the infusion concentration of durian leaves, the faster the inductive time in the anesthetic process of tilapia. However, it was different from the results shown by Hidayat's (2010), which stated that catfish anesthetized with a chemical anesthetic agent, namely ketamine with doses (10, 20, 30, 40 and 50 ppm) did not showed a significant difference to the inductive time.

The fastest inductive time of catfish seeds during the anesthetic process in this study was 8.37 minutes with a concentration of 40% durian leaves infusion. While the longest inductive time at a concentration of 10% durian leaves infusion with a time of 71.67 minutes. The decrease in consciousness and speed of fainting in this study was thought to be the effect of metabolite compounds that were getting more concentrated along with the high concentration of durian leaves infusion. Research by Abdullah (2012) and Abid et al., (2014) also stated the same thing that the higher the concentration of the test material given, the higher the concentration of compounds in the extract in it, caused the fish to faint more quickly.

Sedative Time

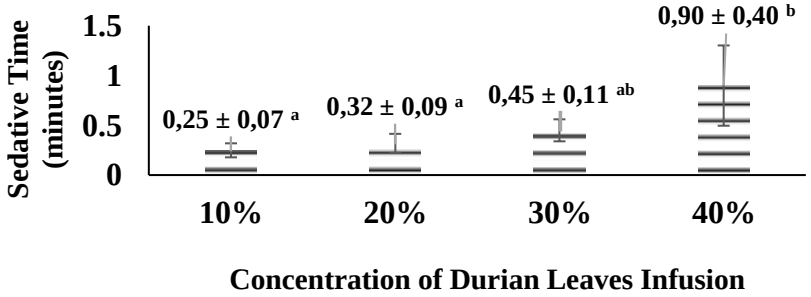

Figure 3. The average sedative time of catfish seeds was given the durian leaves infusion solution. Numbers followed by different superscript lowercase letters indicated significantly differences (P <0.05)

The results of the ANOVA statistical test showed that differences in the concentration of durian leaves infusion treatment on sedative time had a significant effect (P <0.05). Based on the results of Tukey's test, the treatment of durian leaves infusion with a concentration of 10%, 20% and 30% showed no significant difference, but significantly different from a concentration of 40% (**Figure 3**). The different length of sedative time in this study was thought to be influenced by the high metabolite compounds contained in the durian leaves infusion.

The same condition was also reported in the study of Riesma et al., (2014), which stated that the sedative time of catfish seeds anesthetized using clove oil with a concentration of 0.010 mL / L was not significantly different, but significantly different with a concentration of 0.015 mL / L and 0.020 mL / L. Hasan et al., (2015) also reported significantly different results on sedative time in prospective broodfish using keben seed extract. However, the research of Yudhistira et al., (2020) and Hidayat (2010) showed results that were not significantly different from sedative time in catfish anesthesia used durian leaves infusion and catfish seeds used ketamine chemicals.

The fastest sedative time or recovery of catfish seeds in this study was at a concentration of 10% durian leaves infusion with a time of 0.25 minutes. While the longest sedative time was 0.90 minutes with a concentration of 40% durian leaves infusion. Based on the sedative time, all treatments including the concentration of 40% with a mean time of awakening of 0.90 minutes were included in the effective criteria. Effective anesthesia was an anesthetic that can revive fish in less than 5-10 minutes (Aini et al., 2014; Soedibya and Pramono, 2018).

Recovery time was influenced by the absorption rate of metabolite compounds that were absorbed in the body (Abid et al., 2014; Munandar et al., 2017). The absorption rate of the anesthetic substance metabolite compounds was highly dependent on the condition of the blood in the tissue and the fat content of the tested fish (Soedibya and Pramono, 2018). In addition, the availability of oxygen and the ability of fish play an important role in cleaning anesthetics from the body through the respiratory tract such as gills (Riesma et al., 2014).

Post Anesthesia Survival

ANOVA statistical test results showed that differences in the concentration of durian leaves infusion treatment had a significant effect on the survival of catfish seeds (P <0.05). Based on the results of Tukey's test, each treatment of durian leaves infusion with different concentrations (10%, 20%, 30% and 40%) showed significantly different results. The survival of catfish seeds that were maintained for 3 days after the anesthetic treatment in this study decreased along with the increase in the concentration of the durian leaves infusion given.

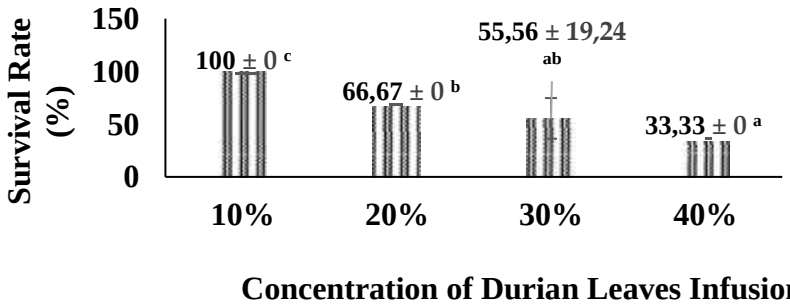

Figure 4. The average survival of catfish seeds after giving the durian leaves infusion solution for 3 days. Numbers followed by different superscript lowercase letters indicated significantly differences (P <0.05)

Similar research was also reported by Hasan et al., (2015) which stated that the survival of prospective goldfish broodfish which was anesthetized using the keben seed

extract with higher concentrations showed significantly different results. A different thing was found in the study of Yudhistira et al., (2020) that durian leaves infusion with concentrations (5%, 15%, 25% and 35%) in catfish showed no significant difference in survival rates. Another study by Hidayat (2010) on catfish seeds with the use of ketamine chemical anesthetics showed significantly different results.

The survival of catfish seeds in this study was thought to be influenced by the content of saponin secondary metabolites in the durian leaves infusion. Saponins were toxic to cold blooded animals because they interfere with the nervous system and affect the balance in the brain so that it can cause cell hemolysis due to their interaction with red blood cells (Septiarusli et al., 2012). This can cause obstruction of the binding process of oxygen in the blood vessels excessively and potentially lead to death.

Conclusion

The conclusion that can be obtained based on the research results was that the effective concentration of durian leaves infusion as a natural anesthetic agent in the stunning process of catfish seeds was a concentration of 10% (100 g of durian leaves in 1 L of boiled water) with an inductive time of 71.67 minutes, sedative time 0, 25 minutes and survival at 100%.

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