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Use of Durian leaf infused (*Durio zibethinus*) as natural anesthetic for Gouramy fish (*Osphronemus gouramy*) in dry transportation

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Abstrak

Penelitian ini bertujuan untuk mengetahui pengaruh pemberian infusum daun durian untuk proses anestesi ikan gurami yang mempunyai berat 100-150 gr dengan dosis yang telah ditentukan menggunakan uji ambang atas dan ambang bawah serta menggunakan rumus Effectivity Consentration (EC-100) terhadap waktu pingsan dan waktu sadar ikan. Pada uji ambang atas dan ambang bawah menggunakan konsentrasi 1000ppm, 2500ppm, 5000ppm serta 7500ppm. Diperoleh bahwa 7500ppm infusum daun durian sebagai ambang atas karena ikan gurami mati semua pada perendaman 24 jam dan 5000ppm sebagai ambang bawah karena ikan tetap hidup selama perendaman 48 jam. Selanjutnya dicari deret konsentrasi dengan acuan rumus EC-100 sehingga diperoleh dosis 5400ppm, 5900ppm, 6400ppm, 6900ppm dan 7400ppm. Dosis tersebut digunakan sebagai perlakuan yaitu P1: 5400ppm, P2: 5900ppm, P3: 6400ppm, P4: 6900ppm, P5: 7500ppm. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) yang terdiri dari 5 perlakuan dengan 3 ulangan. Parameter utama yang diamati waktu ikan gurami pingsan dan waktu ikan gurami sadar. Untuk waktu (menit) ikan gurami pingsan diperoleh P1: 200,67 P2: 198,33 P3: 196 P4: 193 P5: 186,67 sedangkan waktu (menit) ikan gurami sadar diperoleh pada P1: 22 P2: 22,67 P3: 23,33 P4: 28,33 P5: 30,33. Selanjutnya di uji statistik mengggunakan metode ANOVA dan uji BNT dengan taraf 5% menunjukkan beda nyata dan dengan taraf 1% menunjukkan sangat beda nyata. Dapat disimpulkan bahwa penelitian menggunakan infusum daun durian sebagai anestesi alami ikan gurami berbeda sangat nyata terhadap waktu pingsan dan waktu sadar. Perlakuan yang terbaik pada P5(7400ppm) karena ikan gurami cepat dalam proses pemingsanan dan lama dalam proses penyadarannya.

Kata kunci: *Anestesi; Daun Durian; Gurami; Infusum; Saponin*

Abstract

Purpose the research to know influence giving leaf durian infused to anesthesia process gurami fish with weight 100-150 gr use predetermined dosage by upper threshold test and lower threshold test. Then, use formula EC-100 (Effektivity Consentrastion) to know fainting time fish and conscious time fish. On threshold test use among others concentrate 1000ppm, 2500ppm, 5000ppm and 7500ppm. That at concentration 7500ppm is upper threshold because gurami fish die by soaking leaf Durian infused for 24 hours while at concentration 5000pm is lower threshold because gurami fish not die by soaking leaf Durian infused for 48 hours. Next to search concentration row with use formula EC-100 and than available concentrate 5400ppm, 5900ppm, 6400ppm, 6900ppm and 7400ppm. The concentration be used to research treatment, P1:5400ppm, P2:5900ppm, P3:6400ppm, P4:6900ppm and P5:7400ppm. The Method of this research is a complete random design (RAL) use five treatments and three replications. The main parameter observed lamely fainting time fish and conscious time fish. The result fainting time fish (minute) on P1: 200, 67 P2: 198,33 P3: 196 P4: 193 and P5: 186,67 while results conscious time fish (minute) on P1: 22 P2: 22,67 P3: 23,33 P4: 28,33 and P5: 30,33. Next statistic test use method ANOVA and BNT test with level 5% indicate significantly different, and 1 % indicate very differently real. The obtainable conclusion that research use leaf durian infusum to anesthesia process gurami fish very differently real to fainting time and conscious time. The best treatment on P5 (7400ppm) because gurami fish fast to fainting time and long a for conscious time.

Keywords: Anesthesia; Durian Leaf; Gouramy; Infused; Saponin

1. Introduction

Gouramy is one of the freshwater fish that has high economic value and has a high market demand among the community. The results of KKP data from 2015-2018, the amount of gouramy production increased from 169 tons to 356.53 tons. The increase in consumer demand is also offset by the increase in quality that must be fulfilled, such as providing fish to survive until the hands of consumers (de Oliveira et al. 2017). One of the methods used in the delivery of products is dry transport (Madan et al. 2018; Vanderzwalmen et al. 2021). Transportation can result in physical damage in the form of wounds that can

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potentially cause disease due to pathogenic infection (Priborsky & Velisek, 2018). Dry shipping makes use of an anesthetic agent to stun the fish during shipping to avoid stressing the fish (Rahmawan et al. 2020).

Many anesthetics have been used to stun fish (Purbosari, et al. 2019), ranging from chemicals and natural ingredients such as clove extract (Balamurugan et al. 2016), seaweed (Purbosari et al. 2021), Myrcene and linalool (Mirghaed, et al. 2016), but there are concerns that synthetic anesthetics will cause residues in fish meat and be harmful to consumers (Ventura et al. 2020). Gouramy, which is a consumption fish, is better off using natural ingredients so that the anesthetic agent used as an anesthetic agent does not dissolve in the fish's body and will endanger consumers. Anesthetic material that has been used as an anesthetic agent among cultivators is clove oil. However, clove oil now has a high selling price of IDR 175,000 / liter (Clove oil producer Malang, 2019). The high selling price requires cultivators to find other substitutes. In durian leaves, there are secondary metabolite compounds in the form of saponin tannins (Sonia, et al. 2020). Durian leaves contain high concentrations of tannin, saponin, and formic acid compounds, which can be potential as a natural anesthetic ingredient (Brown in Munandar, 2017).

The durian leaves in the plantation are only burned, and they are still considered garbage. Durian leaves can be used as an anesthetic agent by making them infused. This method is used to obtain secondary metabolites of durian leaves in the form saponins and antioxidant (Kam et al. 2018). Saponin compounds will be an anesthetic agent if the right concentration is used because if a little is not an effect and too much will be toxic (Brown in Munandar, 2017).

Previous research on the use of durian leaves as an anesthetic agent has been carried out on tilapia (Masi, 2014) and on pomfret (Munandar et al., 2017). Even though there are still many types of fish that have not been studied related to the use of durian leaf infusion as an anesthetic agent. Anesthetics in fish have many uses, among others; when injecting hormones into fish bodies, when taking fish blood, transporting fish, and reducing stress levels in fish. Therefore, this study aims to determine the effectiveness of durian leaf infusion (*Durio zibethinus*) is a natural anesthetic agent for gouramy (*Osphronemus gouramy*).

2. Materials and Methods

2.1. Preparation

Gouramy with a weight of 150 ± 1.03 g / fish as many as 120 fish taking from Tulungagung, then the fish are acclimatized in the fisheries laboratory to adjust the temperature. Taking dried durian leaves in the Ngantang area of Malang Regency, the Durian leaves taken dried durian leaves that have fallen on the ground.

This research was carried out in several stages, namely making infusion, determining the concentration limit using the upper and lower threshold test, determining the best concentration using the Effectivity Concentration (EC-100) test, determining the time of fainting and time of awakening, determining the survival rate and observing water quality.

2.2. Making durian leaf infused

They were making durian leaf infused using the infusion method. The infusion method is an extraction method that utilizes water as a solvent for metabolite compounds found in vegetable materials through boiling for 15-20 min at a temperature of 90℃ (Shiva et al. 2018). The results of making durian leaf infused are put on the aquarium.

2.3. Determination of upper and lower thresholds

Determination of the threshold concentration using the degree of concentration of durian leaf infusion ranging from 1000 ppm, 2500 ppm, 5000 ppm, and 7500 ppm with repetition of each concentration two times. Observations were carried out for 24 hours to determine the upper threshold test and 48 hours to determine the lower threshold test (APHA, 2005). Two fish were used per aquarium containing 20 liters of durian leaf infusion water. Obtained data that the concentration of 5000ppm as the lower threshold test and 7500 ppm as the upper threshold test.

2.4. Determination of the best concentration series

The results of the threshold test data are then carried out to determine the best concentration with a concentration series that can be obtained using the formula; (APHA, 2005).

$$Log\ \frac{N}{n} = \mathrm{K}\ (Log\ \frac{a}{n})$$

$$\frac{a}{n} = \frac{b}{c} = \frac{c}{d} = \frac{d}{c} = \frac{e}{d} = \frac{N}{e}$$

Notes:

N = upper threshold concentration
N = lower threshold concentration
K = The number of concentrations tested
A = the smallest concentration in the specified series

The concentration series is later used as a treatment and repeated three times. In each treatment, three gouramy fish were used in 20 liters of durian leaf infusion water.

2.5. Determination of conscious time and fish fainting time

Gouramy that was put on the durian leaf infusum concentration treatment was observed until fainting. After the fish reaches a fainting state, the time is recorded, and the fainting fish is marked as not responding to external stimulation (touch), and the fish is stationary on the edge of the aquarium. Furthermore, after the fish has passed out, it is transferred to an aquarium that has been given high aeration to bring the fish back to life. The fish regained consciousness marked by a response when given stimulation, and the fish were already swimming actively. When the fish is unconscious until the fish is conscious, record the time to enter the gouramy consciousness.

2.6. Water quality

Observation of water quality was carried out during the stunning fish process using durian leaf infusion. Parameters observed were temperature and pH. Temperature observations were to determine the metabolism of gouramy when put in the treatment of durian leaf infused concentration, while pH observations were to determine the effect of durian leaf infusion concentration in the water.

2.7. Survival rate

Observation of the survival rate parameters was carried out after the gouramy gouramy was awakened from a fainting state to a conscious state.

2.6. Data analysis

Data analysis used one-way ANOVA, and if there is an effect, it will be continued with the LSD test (Least Significant Difference) with a level of 5% and 1% (Kim, 2017).

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3. Results and Discussion

3.1. Results

The results of this study indicate that the infusum of Durian leaves with a concentration of 5000 ppm can be said to be the lower threshold because the fish stay alive soaked by infusion for 48 hours and the concentration of 7500 ppm as the upper threshold because the fish has died soaked by infusion for 24 hours can be seen in table 1.

If the upper and lower threshold concentrations are known, then the concentration series used as treatment can be found through the logarithmic equation Effectivity Concentration (EC-100). The effectiveness test resulted in 5 series of concentrations, including 5400 ppm, 5900 ppm, 6400 ppm, 6900 ppm, and 7400 ppm. The best concentration effectiveness test results for anesthetic power were at a concentration of 7400 ppm because the fish could faint after soaking for 186.67 minutes. It is suspected that secondary metabolites of durian leaves affect the anesthetic power of fish; it can be seen in Figure 1.

An anesthesia power test using secondary metabolite compound agents obtained observational data on the characteristics of the fish in a fainting state, including; the reduced response of the fish to external stimuli slows down movement and is more sedentary on the edge of the aquarium. After the fish is in a fainting state, then it is awakened, and data is obtained that the treatment with a concentration of 7400 ppm has the longest awareness process, which is 30.33 minutes, can be seen in Figure 2.

Tabel 1
Concentration threshold test for durian leaf infused.

Concentration of infused (ppm)	Sample	Mortality (%)	
		24 h	48 h
1000	6	0	0
2500	6	0	0
5000	6	0	0
7500	6	100	100

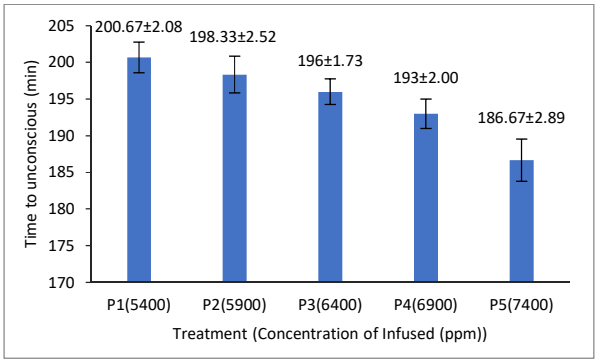

Figure 1. Gouramy unconscious time test.

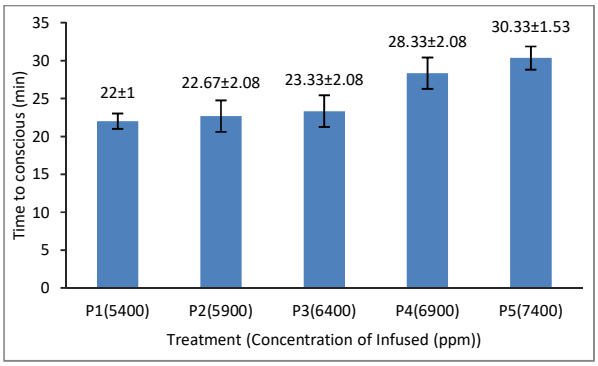

Figure 2. Gouramy conscious time test.

Observation of water quality was carried out during the stunning process of gouramy with temperature and pH parameters observed. From 5 treatments and three replications, the results obtained were that a temperature of 27-280C could be seen in Figure 3, and a pH of 7.3-7.4 can be seen in Figure 4. The calculation of the survival rate was carried out after the awakening process of the fish from the stunning process for all treatments was obtained 100% survival rate data.

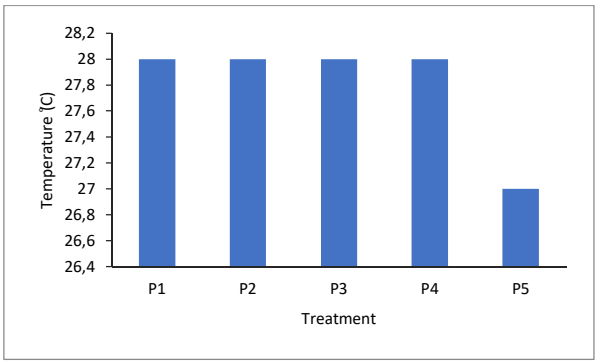

Figure 3. Observation of water temperature.

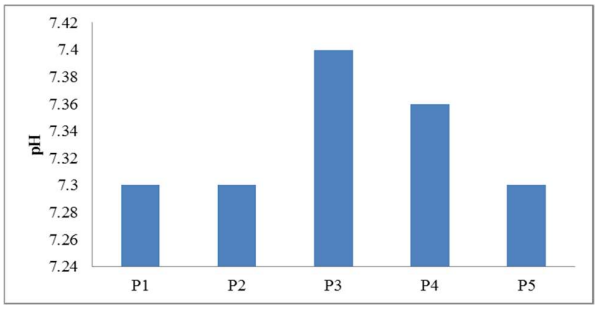

Figure 4. Observation of water pH.

3.2. Discussion

The concentration of durian leaf infused has certain limitations because if the concentration is too little, it does not affect the anesthetic power of gouramy, and if the concentration is too high, it will be toxic to gouramy. The anesthetic power of secondary metabolites, which has the potential as an anesthetic agent, is saponins. Saponin compounds can affect the balance in the brain because they interact with red blood cells and cause cell hemolysis, thereby reducing the amount of oxygen that acts as a source of energy for cell activity (Masithah et al. 2014). Decreased oxygen in the body will decrease the performance of the brain and paralyze the motoric nervous system of the fish,

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which can cause the fish to be unable to respond to external responses (Hu and Wu, 2001). Reduced oxygen in the brain will reduce metabolism in the fish's body and can even make the fish faint, which is indicated by slowing down the movement of the fish and not responding to the fish given to stimuli.

The high concentration will also increase secondary metabolite compounds from durian leaf infused in the water. The high concentration of secondary metabolite compounds will be a high anesthetic agent as well and will accelerate the onset (time of fainting) because it makes it easier for the molecules to work in their place in the body (Adriyanto et al. 2009). The anesthetic process is carried out by immersing the fish so that secondary metabolites such as saponins and tannins enter the fish's body through the osmoregulation system and circulate through the blood until it reaches the nerve center in the brain, which causes the fish to lose consciousness or faint.

In the process of raising fish from unconsciousness to consciousness, data was obtained that treatment with 7400 ppm required a long time. The use of an anesthetic in fish with different doses and lengths of contact will affect the level of awareness of the fish through the process of weakening the central nerve (Rahardja in Hasan, 2015). The process of moving these compounds through the osmoregulation system, osmoregulation is one of the activities to balance the concentration of the fish body with the aquatic environment through the gill membrane. Fish need time to remove compounds that become anesthetic agents that are absorbed in the body of the fish. Along with the transfer of anesthetic compounds in the body of the fish, the fish can gradually carry out the respiratory system and will obtain energy for metabolism. Fish reach a conscious state characterized by active movement and responding to stimuli (Sukmiwati, 2007). In the awareness process, fish blood circulation begins to stabilize along with the transfer of anesthetic substances from the bloodstream to the environment through the gills because the entry and exit pathways of anesthetic substances in fish are mostly through the gills, or it is called the osmoregulation system (Masithah et al, 2014).

Water quality parameters observed in the form of temperature obtained results from 27-28 ˚C. Gouramy cultivation is the optimal temperature between 28-32 ˚C because with low-temperature gouramy will experience a decrease in body metabolism resulting in delayed growth and development of fish bodies (SNI-KKP, 2006). Fish is a cold-blooded creature, so that fish metabolism is influenced by water temperature (Adriyanto, 2009). It can be concluded that gouramy has decreased metabolism due to the influence of the durian leaf-infused metabolite, which is absorbed in the body of the fish, not caused by a decrease in water quality characterized by the water temperature values that are not much different (Mashuda et al. 2020). The pH value of the waters during the stunning fish process was 7.3-7.4. The pH value does not show a significant change in untreated waters, namely 7.3. This shows that the concentration of durian leaf infused does not affect the pH in the waters, so that the pH value is relatively stable. The pH value of the waters that gouramy can tolerate is between 6.5 - 8.5 (SNI-KKP, 2006). The rise and fall of the pH value in the waters is influenced by the number of dissolved hydrogen ions. Acidic hydrogen ions whose number exceeds normal limits in waters will cause acidic waters (Handajani et al, 2018). It can be concluded that the administration of durian leaf infused treatment did not affect the pH value of the waters.

The process of immersing the fish with anesthetic ingredients is always controlled by providing tactile stimulation even to the point where it is held. If the fish does not respond, it can be concluded that the fish is already unconscious and then regained consciousness in an aquarium that has been given high aeration until the fish is conscious. The results of observations during the research on the effectiveness test of using different concentrations of durian leaf infusion, starting from the process of immersing the fish to fainting until the fish resuscitated smoothly, it was proven that gouramy had a 100 % survival rate for each treatment and its repetitions. The anesthetic process with an overdose or immersion time can affect the mortality rate.

4. Conclusion

The most effective leaf infused concentration for the anesthetic power of gouramy with reference to the EC-100 formula is at treatment 5 of 7400 ppm because, with a soaking time of 186.63 minutes, three fish with a weight of 100-150 have reached a fainting state and need 30.33 to bring the fish back to life. As well as the survival rate during the anesthesia process until they regain consciousness, the results are 100 % due to proper fish handling. That is if the fish has passed out, immediately transferred to the recovery aquarium.

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