

## **Anesthesia application of holy basil (*Ocimum tenuiflorum*) leaf essential oil on catfish (*Pangasius* sp.) seed transportation**

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**Abstract.** This study aims to know the effect of the different anesthesia doses of holy basil leaf (*Ocimum tenuiflorum*) oil on the survival rate of the catfish (*Pangasius* sp.) seed in transportation activity. The experiment used a complete randomized design with 4 treatments, namely 0 mg L<sup>-1</sup>, 25 mg L<sup>-1</sup>, 50 mg L<sup>-1</sup>, and 75 mg L<sup>-1</sup> and 3 replications. It was conducted in the Laboratory of Aquaculture, Fisheries Department, Faculty of Agriculture, Palangka Raya University, Central Kalimantan. Results showed a non-significant effect of the different doses of the holy basil leaf oil on the survival rate of the catfish during transportation and acclimatization, 100% and 98-100%, respectively.

**Key Words:** active compound, induction time, sedative duration, survival rate.

**Introduction.** Fish transportation is one of the important activities in fish seed supply from the original habitat to different localities so that certain handling treatments are required to make the fish survive to reach the target localities (Pratisari 2010; Bakrie & Olgani 2020; Nakalelo 2020). Constraints in fish transportation are high mortality and a limited number of fish that can be transported because of high fish activity and metabolic rate that reduces the dissolved oxygen and makes ammonia accumulate in the water during the transportation (Arfah & Supriyono 2002). Thus, other handling treatments are needed to overcome the fish from stress and mortality, i.e. anesthesia.

Anesthesia is a condition of temporary loss of consciousness from pressure on the function of local or entire nerve system. Anesthesia occurs in the central nervous system which makes an organism unconscious (Achmadi 2005; Suwandi et al 2013; Yudhistira et al 2020). Anesthesia can reduce fish movements and suppress metabolic activity during transportation (Saputra et al 2017). Therefore, the selection of the anesthetic substances needs to consider various characteristics, such as high solubility, fast induction and recovery time, no residuals left in the fish body, inexpensive price, and easiness to find (Saskia et al 2013; Jegede 2014). A natural anesthetic substance, such as the essential oil of clove (*Syzygium aromaticum*), the lemon grass (*Cymbopogon citratus*) oil, and the nutmeg (*Myristica fragrans*) mace oil, that does not have a negative side effect, but is effective in anesthesia, has become an alternative and appropriate option compared with chemicals (Sukarsa 2005).

Holy basil (*Ocimum tenuiflorum*) is also known as ruku-ruku and contains flavonoid, triterpenoid, essential oil, alkaloid, tannin, and saponin. The holy basil plant has an efficacy as anti-oxidant, anti-microbial, anti-mutagen, and anti-allergy (Kardinan 2001; Gajula et al 2009; Shafqatullah et al 2013; Efendi et al 2015; Siva et al 2016).

The application of holy basil essential oil for catfish (*Pangasius* sp.) seed anesthesia is rarely done and there is no information on the optimum dose and the effective time in fish transportation. This study focuses on the effect of holy basil oil on

fish behavior, induction time, sedative duration, and survival rate. This study is also intended to find the optimum dose of the holy basil leaf oil to enhance the survival rate of the catfish seeds during transportation.

**Material and Method.** This study was carried out in October 2021 including the preparation of the experimental animals, acclimatization, preliminary study, and transportation. During acclimation, the fish were fed twice a day, in the morning and afternoon, then feeding was terminated a day before the anesthetization. The parameters observed were fish behavior, survival rate, induction time (time estimated from the anesthesia to the unconsciousness), and sedative duration (recovery from the unconsciousness).

**Preliminary experiment.** Materials used in this phase were 40 ind. catfish collected from fish farmers around Palangka Raya city, and the anesthetic oil of the holy basil leaf was prepared at the doses of 100 mg L<sup>-1</sup>, 150 mg L<sup>-1</sup>, and 200 mg L<sup>-1</sup>. The preliminary experiment used 4 treatments each of which had 2 replications at the stocking density of 5 individuals per jar.

**Transportation preparation.** This study utilized catfish seeds. In this phase, the container was labeled plastic bag filled with 1 L of water. Each treatment contained 50 catfish. The fish seeds were anesthetized with the holy basil oil at doses of 0 mg L<sup>-1</sup>, 25 mg L<sup>-1</sup>, 50 mg L<sup>-1</sup>, and 75 mg L<sup>-1</sup>. The fish seeds were transported by car for 8 hours at a route of Palangka Raya-Banjarmasin-Palangka Raya from 15.00 pm to 23.00 pm. The fish condition was monitored once in 2 hours. After the transportation, the fish were placed in aerated clean water. The catfish seed's behavior was observed and the sedative time was counted as the time the fish were put in the water until the fish start the movement.

**Water quality.** Water quality parameters (dissolved oxygen - DO, ammonia-N, pH, and temperature) in the plastic bag were measured before and after the transportation. These measurements used a thermometer TP3001, pH-meter AMTAST AMT28F, DO-meter Hanna HI98193.

**Data analysis.** The survivorship of the catfish seeds and the water quality data (temperature, DO, ammonia, and pH) were qualitatively described, while the induction time and the sedative time were quantitatively analyzed. The results were presented in tables and graphs.

## Results and Discussion

**Preliminary experiment.** Before running the experiment, a preliminary test was done to observe the activity of the catfish seeds.

The catfish seed anesthetized with the holy basil essential oil at the doses of 100 mg L<sup>-1</sup>, 150 mg L<sup>-1</sup>, and 200 mg L<sup>-1</sup> could totally be unconscious for about 15 min indicated with falling down to the bottom in upside down position and slow operculum movements. In the transportation phase, the highest mortality (> 99%) occurred at the fourth hour, while the transportation time used in this study was 8 hours, then the second experiment was done by reducing the dose levels to 25 mg L<sup>-1</sup>, 50 mg L<sup>-1</sup>, and 75 mg L<sup>-1</sup>, respectively.

The use of these dose levels did not cause the catfish seeds to be totally unconscious, but the fish were in a light unconsciousness condition indicated by slow movements and being reactive against the external stimulus with a 100% survival rate, and thus, the major experiment applied 0 ppm (control), 25 mg L<sup>-1</sup>, 50 mg L<sup>-1</sup>, and 75 mg L<sup>-1</sup> as treatment concentrations.

### Transportation experiment

*Behavior, induction time, and sedative duration of the catfish seeds.* In general, the catfish seeds placed in the jar added with the anesthetic material of the holy basil leaf showed the same behavior in all treatments, i.e. the fish became weak, activity was slow, operculum movements slowed down, and loss of swimming balance appeared, whereas in the control (K) treatment, the catfish seeds had normal movements, behavior, reaction, and good response. The fish behavior with treatments is presented in Table 1.

Table 1

Fish behavior in induction time

Time (minute)	Treatments			
	K	A	B	C
0-10	Active movement	Active movement	Active movement	The movement of the body and operculum starts slowing down
11-20	Active movement	Active movement	The movement of the body and operculum starts slowing down	Loss of balance and the movement of the operculum slows down
21-30	Active movement	Slow movement	Loss of balance and the movement of the operculum slows down	Unconscious
30-40	Active movement	Loss of balance and the movement of the operculum slows down	Unconscious	Unconscious
40-50	Active movement	Unconscious	Unconscious	Unconscious

In all media added with the holy basil leaf oil, the catfish seeds began to show stress symptoms several minutes after the anesthesia by showing disoriented swimming, swimming up to the surface to get oxygen, and slow movement of the operculum. Then, the fish were in unbalanced condition with slant and even upside down swimming position. The operculum was still moving but had no response to the environmental stimuli reflecting the fish's unconsciousness (Daud et al 1997; Hu & Wu 2001).

The difference in induction time occurs among the treatments. The fastest induction time was recorded in treatment C (75 mg L<sup>-1</sup>), 21-30 min., while treatment A (25 mg L<sup>-1</sup>) needed a longer induction time, 40-50 min. It means that the use of a higher dose yields a faster induction time. It is in line with Hariyanto et al (2008) and Rendra (2005) that the higher the dose of an anesthetic substance is, the faster the induction time will be. Daud et al (1997) stated that a relatively fast induction time in fish anesthesia can reduce the length of stress.

The behavioral response of the catfish seeds from the holy basil leaf anesthesia is caused by the respiration rate decline of the fish. This condition causes the fish to be nervous and go to the surface for oxygen intake. The decrease in the respiration rate results in the loss of body sense, partly or entirely, as a result of nerve function decline that blocks the action and the transmission of the nerve impulse (Bose 1991; Schreck & Moyle 1990). The anesthetic ingredients can also directly or indirectly disrupt the ionic equilibrium in the fish brain due to a decrease in cation K<sup>+</sup> and an increase in cations Na<sup>+</sup>, Fe<sub>3</sub><sup>+</sup>, and Ca<sub>2</sub><sup>+</sup>. This disturbance will then affect the work of the motor nerves and respiration resulting in numbness or fainting (Yanto 2009).

In an unconscious state, the fish metabolism, oxygen consumption, and carbon-dioxide release also decrease so that the water quality remains in optimum condition. It is in agreement with Tahe (2008) that anesthesia during transportation aims to calm

down the fish to reduce fish activity, oxygen consumption, and carbon-dioxide production which can negatively impact the fish.

The active compounds of the holy basil leaf that work at the induction time of the catfish seeds belong to the alkaloid group. It is one of the secondary metabolites that have sedative, antimicrobial, and nerve system triggering effects (Aksara et al 2013). Holy basil leaf also contains flavonoid that works in anesthesia. Flavonoid is an analgesic agent that causes calmness and decreases the movement up to fainting (Ravi et al 2012; Septiarusli et al 2012; Mamun-Or-Rasid et al 2013; Sunarno et al 2019).

The sedative duration is affected by the anesthetic dose of the holy basil leaf in the catfish seed transportation (Table 2). The higher the anesthetic dose of the holy basil leaf is, the longer the sedative duration. Davis & Griffin (2004) stated that anesthesia works as an ingredient that can suppress respiration and the autonomous function between the sympathetic and parasympathetic nervous systems. It could result from the increased concentration of the anesthetic material that accelerates the fish's faint time. The higher the concentration is, the faster the absorption of the anesthetic substance in the blood which is then distributed to the entire fish body. The anesthetic material absorbed in the blood vessels will be transferred to the central nervous system, i.e. brain and medulla spinalis. It will then block the dopamine receptors postsynaptic, inhibit the release of dopamine, and suppress the central nervous system which results in the sedative effect, muscular relaxation, and low spontaneous reaction of the catfish seeds to the external stimuli, then makes the fish faint. Such a condition is highly expected in fish transportation (Arfah & Supriyono 2002; Andriyanto et al 2010).

Table 2

Fish behavior in the sedative time

Time (minute)	Treatments			
	K	A	B	C
1-5	Active movement	Active movement	Movement and operculum start being active	Movement and operculum start being active
6-10	Active movement	Active movement	Active movement	Active movement

The sedative duration of the catfish seeds was 1-5 minutes in treatment A (25 ppm) and 6-10 minutes in treatments B (50 ppm) and C (75 ppm). Treatment A is the ideal dose with a sedative duration of 1-5 min, whereas other treatments take more than 5 min. This finding is in agreement with Firdaus et al (2022) that a good sedative duration is approximately 5 minutes. Arlanda et al (2018) reported that the use of a higher dose could yield a longer recovery time and vice versa.

After the transportation, the fish were recovered by replacing them in the aerated water media. The availability of sufficient dissolved oxygen could help the fish recover. Yanto & Raharjo (2009) stated that fish recovery after the anesthesia results from sufficient oxygen-containing water entering the body through the gills into the bloodstream to clean up the anesthetic substances and removes it through the fish drain. The fish recovery is followed by an increase in metabolism and oxygen demand.

*Catfish survivorship.* The survivorship of the catfish anesthetized with the holy basil leaf oil during 8-hour-transportation reached 100% (Figure 1) indicating that the catfish seeds can tolerate each treatment concentration. The survival rate is influenced by species, the dose of the anesthetic material, fish density, and transportation duration. Catfish do not have scales and the anesthetic material could easily penetrate into the body through their skin so that the anesthesia process could occur faster and reduce the body's metabolism. However, the use of a high dose could make the detoxification of the anesthetic material could last longer in the body and could make the kidney and liver work harder to eliminate the anesthetic compound. As a result, both organs could fail due to the excess of the anesthetic dose and long transportation time that could cause mortality (Andriyanto et al 2010; Verma 2016).

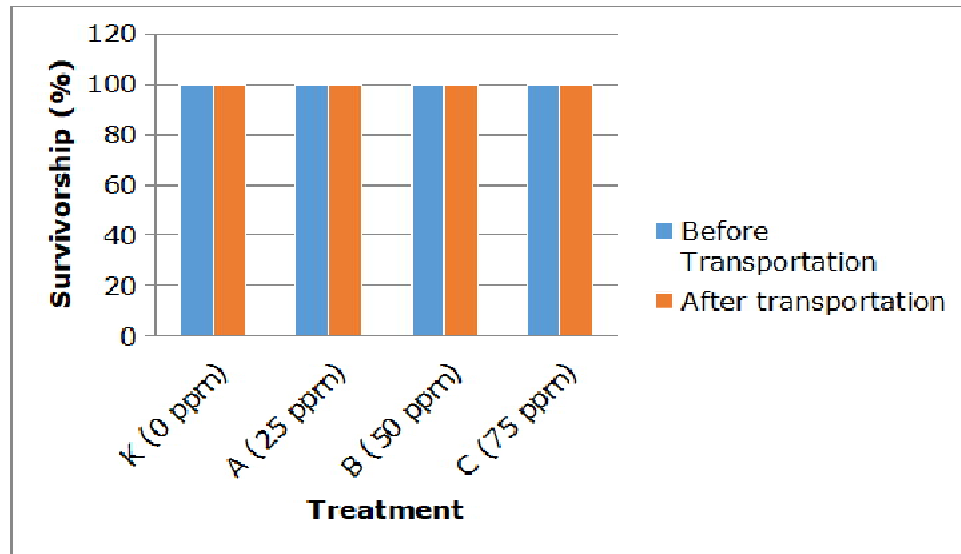


Figure 1. Fish's survival during transportation.

The present study utilized the essential oil of the holy basil leaf at a low concentration so that it did not make the body organ work hard, prevented a disruption or infection of the body organ, and yielded high survivorship. According to Sumahiradewi (2014), live fish transportation in fainting conditions can reduce the mortality rate so that longer transportation could be done. In fainting conditions, the fish metabolism declines, oxygen consumption decreases, and reduces carbon-dioxide released so that good water quality could be maintained in optimal conditions. It is in agreement with Tahe (2008) that anesthetics used during the transportation are to reduce fish activity, oxygen consumption, and carbon-dioxide production.

The length of trip time also influences the fish survivorship in transportation. The catfish seed transportation with or without anesthesia at low density can still support the fish's survival. The present study found that for approximately 8-hour-transportation, all treatments could yield 100% fish survival. Kurniansyah (2020) reported that the 7-9 cm-catfish seed transportation at the density of 50 ind L<sup>-1</sup> had 100% survivorship for about a 12-hour-trip.

**Water quality.** Water quality conditions before and after the transportation were very good for the survivorship of the catfish (Table 3). This result is in line with Jainudin (2018) that water temperature of 26-30°C, pH of 6.0-8.0, and DO > 4 mg L<sup>-1</sup> are recommended in the transportation of fish seeds. Water DO, pH, and temperature conditions are in a good range for the growth and survival of the catfish seeds (Table 3). DO from 4.38 to 4.64 mg L<sup>-1</sup> before transportation and 3.38 to 3.63 mg L<sup>-1</sup> after transportation. These ranges are suitable for fish seed packaging and supported by Ghufran et al (2007) that the best DO concentration for fish seed growth and survival ranges from 3 to 6 mg L<sup>-1</sup>. Oxygen is highly needed by fish for respiration, and thus, the availability of oxygen in the water will highly influence the fish's survival, growth, and metabolism.

Table 3

Water quality during the transportation

Observation	Treatments							
	Before transportation				After transportation			
	K	A	B	C	K	A	B	C
Temperature (°C)	28.83	28.87	29.80	29.63	26.40	26.70	26.60	26.63
pH	6.83	6.70	6.37	6.13	6.43	6.40	6.27	6.10
DO (mgL <sup>-1</sup> )	4.64	4.49	4.38	4.53	3.52	3.41	3.38	3.63

The pH values before and after transportation were also appropriate for the survival of the catfish seeds (Table 3). According to Ghufron et al (2007), good pH for the catfish seed survival ranges from 6 to 8.

The water temperatures before the transportation of the catfish seeds (28.83-29.80°C) and after transportation (26.40-26.70°C) were in the optimal range for the survival of the catfish seeds (25-30°C) (Rosmawati & Muarif 2010) so that the water temperature in the packaging media is highly recommended for the catfish transportation.

**Conclusions.** The present study found that the holy basil leaf is very potential as an anesthetic substance. The fastest induction time was recorded in treatment C at the anesthetic dose of 75 mg L<sup>-1</sup>, 21-30 minutes, while the fastest sedative duration was found in treatment A at the dose of 25 mg L<sup>-1</sup>, 1-5 minutes, with 100% survival rate. Water quality conditions before and after the transportation activity were optimum for the survivorship of the catfish seeds.

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

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