Anesthetic effectiveness of tekelan leaves on tinfoil barb fish in closed transportation

Eva Ayuzar¹, Rachmawati Rusydi¹ *, Hayatun Muna², Muliani Muliani¹

¹Department of Aquaculture, Faculty of Agriculture, Universitas Malikussaleh, Aceh Utara, Indonesia.
²Sekolah Menengah Kejuruan Negeri 1, Juhok, Aceh Timur, Indonesia.

ARTICLE INFO

Keywords:
Anesthetic
Chromolaenaodorata
Transportation
Tinfoil barb

ABSTRACT

The mortality of tinfoil barb during transportation is relatively high due to the oxygen consumption rate and excretion of these fishes. Therefore, the use of an appropriate anesthetic drug becomes one of the solutions for solving this transportation problem of tinfoil barb. One of the natural anesthetic drugs with potential to be studied is Chromolaena odorata leaves. The purpose of this study is to evaluate the effectiveness of anesthetic compounds from Chromolaena odorata leaves in the closed transportation of tinfoil barb. The experimental design used was a non-factorial completely randomized design that consisted of four treatments and three replications, then followed by a Tukey test to see if there was a significant difference. The experimental treatments were A (0 ml/L of leaves filtrate), B (80 ml/L of leaves filtrate), C (90 ml/L of leaves filtrate), and D (100 ml/L of leaves filtrate). The observed parameters were fish behavior towards unconscious condition, onset time, immobilization time, survival rate, and water quality. The results showed that the shortest onset time was obtained from treatment D (100 ml/L leaves filtrate), namely 58 minutes towards unconscious condition, then the longest immobilization time was also shown by treatment D, which was 371 minutes. The highest survival rates were also shown by treatment D which were 94.33% during transportation and 76.33% during culture. The water quality parameters during the experiment were in an optimum condition of tinfoil barb.

Introduction

Tinfoil barb fish (Barbonymusschwanenfeldii) is an endemic ornamental fish from West Kalimantan with unique red eyes and a silver body (Kusmini et al. 2018). The uniqueness of tinfoil barb fish causes the fish to become a popular ornamental fish with relatively high market demand and price. According to Yosmani et al. (2015), the price of tinfoil barb fish in West Kalimantan as an ornamental fish at a size of 1 inch is IDR 1,000/ fish, while at consumption size, it is IDR 40,000-50,000/kg. Furthermore, marketing of tinfoil barb is carried out by transporting these fish to several areas carefully to prevent fish mortality during transportation.

The mortality rate of fish during transportation depends on the basal conditions of the fish during transportation, in addition to other external factors. Fish that cannot reach the basal conditions during transportation has a high risk of mortality. Fish activities requiring oxygen intake and the presence of their excretion during transportation process cause the fish to easily become stressed. According to Primadona et al. (2017), a decrease in metabolic rate is a determinant of success in the fish transportation process because the lower the metabolic rate in fish, the lower the fish respiration rate will also be. If the respiration rate is low, oxygen consumption will also be lower, which would then result in a reduced fish mortality rate during transportation.

The technique of stunning fish during transportation is a solution to reduce the rate of oxygen consumption and excretion rate of fish. Stunning is useful to maintain fishes at basal conditions with minimum oxygen intake and excretory activity. This fish stunning technique uses...
anesthetic substances that are expected to be safe for fish survival, consumer health, and the environment.

Hermawan et al. (2014) showed the results of their research that stunning using low water temperature of 7-8 ºC was able to sustain the life for 33% of their fish sample. Meanwhile, temperatures that are closer to room temperature, namely 13-14 ºC, can only sustain the life for as much as 16.67% of their fish sample. This shows that stunning can be used to maintain fish survival during transport.

Fish anesthesia is an action that causes the fish’s body to lose its ability to feel due to low respiratory and metabolic activities, allowing the fish to experience a physiological change from a conscious to an unconscious state (Abid et al., 2014). Anesthetics that can be used include natural anesthetics and synthetic anesthetics. Some natural anesthetics include clove oil, betel leaf, nutmeg, and so on, while synthetic anesthetics such as MS-222, quinaldine, benzocaine, and phenoxyethanol can affect consumer health and the environment.

Exploration of other natural anesthetic sources has become a point of interest to serve as an alternative to the use of synthetic anesthetics. One source of natural anesthetic that has the potential to be developed is tekelan leaves (Chromolaena odorata). Tekelan plants are weeds that are used as traditional herbs by people from developing countries. According to Vijayaraghavan et al. (2013), the ethanol leaf extract of C. odorata is rich in diglycosides, alkaloids, flavonoids, steroids, phenols, tannins, and saponins from methanol and water extracts. The leaves contain several main compounds such as alkaloid, tannins, phenols, flavonoids, saponins, terpenoids, and steroids. The essential oil from tekelan leaves contains α-pinene, cadinene, kampora, limonene, β-caryophyllene, and candinol isomers (Yenti et al., 2011). One of the phytochemical components in tekelan leaves is flavonoids which may possess bioactivity as anti-cancer, anti-viral, anti-bacterial, anti-inflammatory and anti-allergic. Flavonoids can also affect the speed of the inflammatory process in wound healing and can protect wounds from free radicals (Sundaryono, 2011).

Research conducted by Okoro et al. (2019) showed that variations in the concentration of tekelan leaf extract and variations in exposure time affected the behavior and hematology of juvenile Clariasgariepinus. However, the negative impact of tekelan leaf toxicity was stated by Ogbonne et al. (2018), that tekelan leaves are toxic to Clariasgariepinus, where the higher the concentration of the extract given, the higher the mortality of the fish.

Based on this background, research on the anesthetic effectiveness of tekelan leaves (C. odorata) in the closed transportation of tinfoil barb fish becomes interesting to study. This study aims to evaluate the anesthetic effectiveness of tekelan leaves in the closed transportation of tinfoil barb fish. The addition of tekelan leaf filtrate on wet transportation media is expected to minimize stress and mortality in fish.

Materials and Methods
Materials
The research objects used in this study were 72 tinfoil barb fishes (Barbonymusschwanenfeldii) with the length of 10-12 cm for all treatments. The condition of the fish was healthy and they were put in fasting conditions before transportation was conducted. Another research material was the leaves of tekelan (Chromolaena odorata) of which filtrate treatments were made using freshwater. Other supporting equipments included closed transportation equipment and measuring equipment.

Preparation of tekelan leaves filtrate
The preparation of the leaves filtrate was carried out based on the procedure of the bandotan leaf filtrate in transportation according to the modified method of Pramono (2002). Tekelan leaves were sorted to select dark green leaves and cleaned of dirt attached to the leaves. The sorted leaves were then dried. Furthermore, the dried leaves were weighed and crushed.

The smooth leaves were mixed with water in a ratio of 1:2 and homogenized to form a mixture. The mixture was stirred until evenly distributed for 24 hours. The mixture of ingredients was filtered using filter paper/cloth to obtain a filtrate that was free from dirt and dregs. The filtrate in the form of a crude extract was stored in a dark bottle before being used as an anti-metabolic substance (immotilization) (Pramono, 2002).

Implementation of tekelan leaves filtrate as anesthetic substance
Before the immotilization process was carried out, first the tinfoil barb fishes were kept for 24 hours in the prepared aquarium. This was done to reduce metabolic processes in the fish body during transportation.

Then, the tinfoil barb fish were immotilized by applying the tekelan leaves filtrate according to the treatment in each transport container (plastic bag). Observations of behavior before stunning and onset time were carried out after giving filtrate onto these...
fishes until they became stunned. The treatments of filtrate concentration of tekelan leaves were:
A: 0 ml / L of tekelan leaf filtrate
B: 80 ml / L of tekelan leaf filtrate
C: 90 ml / L of tekelan leaf filtrate
D: 100 ml / L of tekelan leaf filtrate
(Note: Determination of concentration was based on preliminary tests).

Packaging and transportation of fish

The transportation containers used were in the form of double plastic bags containing fish and anesthetic materials with a transportation media volume of 1.5 liters. The density of fish in one plastic bag was 9 fish/bag. The plastic bags were then given pure oxygen gas with water to oxygen ratio of 2:3. Then the plastic bags were tightly tied using rubber bands, and they were then put into a styrofoam box. The styrofoam box was then closed tightly.

The packed fishes were then transported for 6 hours. The length of immobilization was observed from the time the fish become stunned until they returned to a conscious state. The survival rate of the fish during transportation was observed until the end of the transportation. The observation procedure refers to Suwandi et al. (2007).

The process of observing consciousness recovery was carried out in the transportation process once every half an hour and observed until the fish were conscious, indicated by the fish's active movement and also the time to recover consciousness of the fishes.

Maintaining the fish after transportation

After the transportation process is finished, the fish were then moved to a container filled with 12 liters of water and aerated for 48 hours. The fish were kept for 7 (seven) days. The feed given was artificial feed with a frequency of twice a day, morning at 08.00 and afternoon at 16.00 WIB. Siphoning was done every day and 50% water was changed by additional water. After maintaining the fish, the degree of fish survival during maintenance was then observed by referring to the survival calculation formula in Heriyati and Kasman (2017).

Data analysis

The quantitative data obtained were analyzed using a completely randomized design (CRD) - non-factorial and continued with Tukey's follow-up test to see if there was a significant effect. Furthermore, data descriptions were presented in graphs and tables. Qualitative data on fish behavior before stunning were also described in the table.

Results

Fish behavior before stunning

The behavior of tinfoil barb fish that were given anesthetic substance from the tekelan leaves filtrate showed a change in behavior from normal (active) to slow and less responsive. The behavior of the fish before stunning can be seen in Table 1.

Duration of onset

The duration of onset is the time it takes for biota in normal circumstances to achieve the loss of consciousness. Anesthetics (immobilization) are a process carried out to reduce the activity, metabolism, and respiration of aquatic biota before being transported.

Based on the mean onset duration, each treatment showed a different time. The shorter the onset duration, the more effective the anesthetic is to sedate the fish. The different times for each treatment can be seen in Table 2.

The shortest time to stunning was obtained in treatment D at a dose of 100 ml / L with onset duration of 58 minutes. The longest onset duration was found in treatment B with a dose of 80 ml/L with the required mean onset duration of 103 minutes. Based on the results of the analysis of variance (ANOVA), it showed that the use of tekelan leaves filtrate affected the duration of onset of tinfoil barb fish in closed transportation where the value of F-count was 3.353> F-table (0.01) 7.59. The onset duration in each treatment very significantly differs from each other.

| Implementation of Filtrate | Treatment | Movement | Operculum | External Stimulus |
|---------------------------|-----------|----------|-----------|------------------|
| Before implementation     | A(control)| Normal   | Normal    | Responsive       |
|                           | B(80ml/l) | Normal   | Normal    | Responsive       |
|                           | C(90ml/l) | Normal   | Normal    | Responsive       |
|                           | D(100ml/l)| Normal   | Normal    | Responsive       |
| 58 minutes after implementation | A(control)| Normal   | Normal    | Responsive       |
|                           | B(80ml/l) | Lose balance | Slow    | Less Responsive |
|                           | C(90ml/l) | Lose balance | Slow    | Less Responsive |
|                           | D(100ml/l)| Settled  | Very slow | Non-Responsive   |
Table 1. Onset duration of tinfoil barb fish.

| No | Treatment   | Onset Duration (minutes) |
|----|-------------|--------------------------|
| 1  | A (Control) | 0a                       |
| 2  | B (80 ml/l) | 103 ± 2.80b              |
| 3  | C (90 ml/l) | 79 ± 1.53c               |
| 4  | D (100 ml/l)| 58 ± 0.58d              |

Note: Superscript alphabetic reveals a significant difference of the treatments at confidence level 99%.

Table 2. Duration of immotilization of tinfoil barb fish.

| No | Treatment   | Duration of Immotilization (minutes) |
|----|-------------|--------------------------------------|
| 1  | A (control) | 0a                                   |
| 2  | B (80 ml/l) | 290 ± 17.32b                         |
| 3  | C (90 ml/l) | 330 ± 0c                             |
| 4  | D (100 ml/l)| 371 ± 9.87d                          |

Note: Superscript alphabetic reveals significant difference of the treatments at confidence level 99%.

**Duration of fish immotilization**

The duration of fish immotilization is the length of time needed for the stunned fish until it returns to a conscious state. The results showed that the immotilization duration of tinfoil barb fish was different between treatments. The duration of immotilization of tinfoil barb fish can be seen in Table 3.

The shortest time for immotilization was obtained in treatment B with a dose of 80 ml/L with an average time of 290 minutes until they reach conscious state, while the longest immotilization time of tinfoil barb fish was obtained in treatment D with a dose of 100 ml/L and a mean time of 371 minutes.

Based on analysis of variance (ANOVA) at a confidence interval of 99%, it can be seen that the use of tekelen leaf filtrate as an anesthetic agent has a very significant effect on the length of time needed to keep fishes stunned during closed transportation, where the value of F-count was 857.748 > F-table (0.01) 7.59. Based on further tests using the LSD test, it showed that treatments A, B, C, and D showed very significant differences in the duration of immotilization.

**Survival rate of fish**

The survival rate during the transportation is the number of fish that live during transportation. The survival rate of tinfoil barb fish was influenced by the transportation media, in the form of water media containing tekelen leaves filtrate. The average survival rate during transportation can be seen in Figure 1.

The highest survival rate during transportation was found in treatment D at a dose of 100 ml/L with an average survival rate of 76.33%. The lowest survival rate during maintenance was found in treatment A (control) with an average survival rate of 53.33%. Based on the results of the analysis of variance (ANOVA), it was found that the anesthetic of tekelen leaf filtrate did not significantly affect the survival rate of tinfoil barb fish during maintenance, where the value of F-count was 3.077 < F-table (0.01) 7.59.

Figure 1. Survival rate of tinfoil barb fish during transportation.

Figure 2. Survival rate of tinfoil barb fish during maintenance.
Changes in fish behavior during stunning

The use of nutmeg oil to stun tilapia showed several changes in fish behavior during stunning, which include panic, stress, swimming sideway until finally sinking to the bottom of the container and not responding anymore. The results in this current study are in line with the results from the research conducted by Khalil et al. (2013) where the application of an anesthetic agent would cause changes in the behavior of the fish exposed to the substance.

Duration of onset

Onset duration is the time it takes for biota in a normal state to lose consciousness. Anesthetic (immobilization) is a process carried out to reduce the activity, metabolism, and respiration of aquatic biota before transportation. The administration of a high dose of the anesthetic tekelan leaves filtrate caused the onset duration of tinfoil barb fish to be shorter than the lower dose, according to the statement of Amirullah et al. (2014) stating that at higher concentrations of anesthetic agents, the fish fainted faster.

Treatment B with a lower dose of tekelan leaves filtrate showed a longer onset duration. The lower dose of tekelan leaves filtrate resulted in lower levels of anesthetic substance contained within, so that the decline in nerve function occurred more slowly and took longer. This follows the opinion of Sukmiwati and Sari (2007) which stated that in a study on the effect of the concentration of rubber seeds as an anesthetic for goldfish during transportation, the lower the concentration given indicated the longer the process took to reach stunning.

The characteristic of a good anesthetic substance is that it has an induction time (onset time) of less than 15 minutes (Yanto, 2012). The best results from this study showed that the shortest onset time was 58 minutes at a filtrate dose of 100 ml/L. The results from this study still required further research studies to obtain doses of anesthetic substances that met these characteristics.

### Water Quality

Observation of water quality during transportation and maintenance was carried out to determine the optimal range for fish survival. The water quality parameters that had been measured during transportation and maintenance can be seen in Tables 4 and 5.

### Discussion

#### Fish behavior before stunning

The administration of anesthetic from the tekelan leaves filtrate was found to cause changes in behavior of tinfoil barb fish. The anesthetic substance from the leaves filtrate entered the body through the osmoregulation system in the body of the fish, which involved the gill organs and skin. Furthermore, the anesthetic substance reached the target organ in the form of the central nerve through the blood circulation, causing the central nerve to weaken and the fish to lose consciousness. The body's response to weak central nerves was seen in the slowness of motion, body imbalance, and slow response to stimuli.

This follows the statement which stated that the behavioral responses that occurred in fish as a result of drugs dissolved in water is the reduction of fish respiration rate and their activity. This condition caused the fish to become nervous and often rising to the surface to find oxygen. This decrease in respiration rate causes the loss of all or part of the senses in the fish's body as a result of decreased nerve function, thus blocking the action and delivery of nerve impulses (Yanto, 2009).

Research conducted by Khalil et al. (2013) that used nutmeg oil to stun tilapia showed several changes in fish behavior during stunning, which include panic, stress, swimming sideway until finally sinking to the bottom of the container and not responding anymore. The results in this current study are in line with the results from the research conducted by Khalil et al. (2013) where the application of an anesthetic agent would cause changes in the behavior of the fish exposed to the substance.

#### Water quality

| Treatment | Temperature(°C) | pH | DO (mg/l) | Turbidity (NTU) |
|-----------|----------------|----|----------|----------------|
| A(control)| 27.0 – 27.3    | 7.2 – 7.3 | 4.5 – 4.7 | 1.10 – 1.45   |
| B(80 ml/l)| 27.2 – 27.3    | 7.3 – 7.4 | 4.6 – 4.7 | 42.3 – 43.33  |
| C(90 ml/l)| 27.1 – 27.5    | 7.1 – 7.2 | 4.7 – 4.7 | 99.97 – 106.67|
| D(100 ml/l)| 27.0 – 27.4   | 7.0 – 7.3 | 4.4 – 4.9 | 126 – 133.33  |

### Table 3. Water quality during transportation.

| Treatment | Temperature(°C) | pH | DO (mg/l) | Turbidity (NTU) |
|-----------|----------------|----|----------|----------------|
| A(control)| 26.4 – 28.1    | 7.6 – 8.0 | 3.7 – 5.6 | 1.46 – 1.93   |
| B(80 ml/l)| 26.2 – 28.2    | 7.7 – 7.9 | 3.9 – 5.1 | 1.07 – 1.53   |
| C(90 ml/l)| 26.4 – 28.1    | 7.7 – 8.0 | 3.5 – 5.2 | 1.72 – 2.49   |
| D(100 ml/l)| 26.5 – 28.2   | 7.6 – 8.0 | 3.9 – 5.3 | 1.65 – 2.36   |

### Table 4. Water quality during maintenance.
The administration of a high dose of anesthetic agents showing a faster onset time was also produced in the study of Syahvitri et al. (2018), whose results showed that the effectiveness of purple senduduk leaves at a dose of 25% was able to stun star pomfrets within 35-40 minutes. This onset time was not much different from the time of onset for tinfoil barb fish stunned with tekelan leaf anesthetic from this current study.

**Duration of fish immobilization**

The best immobilization time was indicated by the use of the highest dose of tekelan leaves filtrate, which was 100 ml/L. Tinfoil barb fish exposed to tekelan leaf filtrate 100 ml/L. experienced stunning for 371 ± 9.87 minutes or for > 6 hours. This was because the higher the dose given during the immobilization process, the longer the immobilization time achieved by the tinfoil barb fish. This follows the statement of Aini et al. (2014) which stated that there was a tendency that the higher the concentration of bandotan leaves as an anesthetic was given, the longer the time needed for fish to return to awareness.

The research results from Munandar et al. (2017) showed that a higher dose of durian leaves, which was 7500 ppm, was able to stun freshwater pomfret for longer than treatment with a lower dose, which was 102.5 minutes. Saponin compound from durian leaves was natural anesthetic ingredient that could stun freshwater pomfret fish.

**Survival rate of fish**

The high concentration of tekelan leaves filtrate resulted in a longer immobilization period of tinfoil barb fish so that the stress level and metabolic reactions were low. This supported the suppression of the mortality rate (mortality rate) in fish during transportation. Also, the 100 ml/L doses of tekelan leaf filtrate was shown to be a non-toxic dose that could be tolerated by tinfoil barb fish.

A different condition was observed in control treatment A, where without the provision of anesthetic agent from the leaf filtrate, the level of survival rate of tinfoil barb fish was found to be lower than that of the fish treated with tekelan leaves filtrate. These control fishes were not stunned, so they experienced oxygen competition and their excretory activity increased, resulting in stress and mortality.

Treatment B with a dose of 80 ml/L also obtained a level of survival that was not much different from control (A). The dose given was too small so that immobilization did not last until the end of transport, resulting in increased oxygen consumption and excretory activity of the tinfoil barb fish.

This was in line with the research of Aini et al. (2014) where the high concentration of bandotan leaf extract could maintain the viability of tilapia seeds, by reducing the metabolic rate and oxygen consumption. The administration of bandotan leaf extract was able to prevent an increase immortality rate of tilapia seeds during transportation.

The degree of survival during the maintenance period also showed results that were in line with the level of survival during transport. Providing the optimum dose of tekelan leaf filtrate (100 ml/L) during transportation reduced the potential for stress to the fish, until the fish were reared for 7 (seven) days from the time of transportation.

The changes in the environment from transportation media to maintenance media also increased the potential for stress on the transported tinfoil barb fish. This could be seen in the control treatment (without tekelan leaves filtrate) where the survival rate was lower than other treatments. In the absence of anesthetic substances, metabolic processes took place normally during transportation and increased stress on fish due to oxygen limitations and changes in the media environment.

Therefore, the administration of anesthetic agents helped in maintaining the survival rate of fish during transportation and rearing. This is supported by the statement that fish survival was found to be influenced by temperature, the health level of the fish being transported, and good handling during transportation (Mursalin, 2015)

**Water quality**

According to SNI (1999), water quality parameters in water ponds that support the enlargement of goldfish from the Cyprinidae family need the temperature of 25 °C - 30 °C, pH 6.5 - 8.5, dissolved oxygen> 5 mg/L, NH3 <0.02 mg/L, and brightness>30 cm.

Water quality during transportation for temperature and pH1 parameters was in the ideal range for the survival of tinfoil barb fish. However, dissolved oxygen and turbidity in the transport medium of tinfoil barb fish were not suitable to the water quality standard that supported the fish's life. Dissolved oxygen values were in the range of less than 5 mg/L. This could lead to fish mortality during transportation. Low dissolved oxygen in the transportation medium could be caused by a lack of oxygen delivery to the transport container before transportation.

The turbidity value of the transportation media was very high, reaching 133.33 NTU which
exceeded the ideal water quality limit. Based on Indonesian Government Regulation Number 82 (2001), the ideal water turbidity value for the environment is 2-30 NTU (February, 2020). According to Gusrina (2008), cloudy water media could lower the oxygen-holding capacity of fish, reduced fish visibility, reduced fish appetite, and difficulty of breathing in fish.

The value of water quality during maintenance was in the appropriate range for fish of the Cyprinidae family to live. Even though the dissolved oxygen was within the minimum limit of the quality standards of the rearing media, the fishes could survive the maintenance period. The fluctuation in the value of dissolved oxygen in the rearing media could be caused by the absence of additional aeration while the fish were kept in the aquarium. The ideal water quality value could support and maintain the degree of survival of the reared tinfoil barb fish. The high turbidity in the transportation media during the study did not cause a problem. Basal conditions of tinfoil barb due to immotilization lead to low oxygen consumption and metabolism.

Conclusion
The anesthetic agent of the tekelan leaves filtrate was effective in supporting closed transportation of tinfoil barb fish. The administration of 100 mL/L dose of tekelan leaves filtrate was able to provide the shortest onset duration and longest immotilization time. The survival rate of tinfoil barb fish was not significantly influenced by the administration of tekelan leaf filtrate, but a dose of 100 mL/L showed a higher level of survival.

References
Ahid, M.S., E.D. Masithah, Prayogo. 2014. The secondary metabolites potential of infusum durian’s (Duriozibethinus) leaves effect to survival rate of nila (Oreochromisniloticus) on the livefish dry transport system. Jurnal Ilmiah Perikanan dan Kelautan, 6(1): 93-99.

Aini, M., M. Ali, B. Putri. 2014. Application of the immobilization technique of tilapia (Oreochromisniloticus) seeds using got weed leaf extract (Ageratumsinuata) in wet transportation. e-Jurnal Rekayasa dan Teknologi Budidaya Perairan, 2(2): 218-226.

Amirullah, A., E. Efendi, M. Ali. 2014. Effective concentration (sc50-1 hour) of tuba root extract (Derris elliptica) as an anesthetic agent for carp (Cyprinuscarpio) seeds. Aquaculture-Jurnal Ilmu Perikanan dan Sumberdaya Perairan, 3(1): 227-232.

February, I. 2020. Supporting water quality support on catfish cultivation business in Banjar district, South Kalimantan. Jurnal Enviro Sciienceae, 16(1): 72-76.

Gusrina. 2008. Fish cultivation volume I. Direktorat Pembinaan Sekolah Menengah Kejuruan, Jakarta.

Heriyati, E., Kasman. 2017. Survival test of tiger grouper (Epinephelusfuscoguttatus) with low temperature immobilization technique in dry transport system. Zira’a’h, 42(1): 56-84.
Yosmaniar, E. Supriyono, S.K. Nurjanah. 2015. Growth and survival of tinfoil barb (*Barbonymusscwanenfeldii*) at different salinity. Prosiding Seminar Nasional Ikan ke 8. pp 419-428.

How to cite this paper:
Ayuzar, E., R. Rusydi, H. Muna, M. Muliani. 2021. Anesthetic effectiveness of tekelan leaves on tinfoil barb fish in closed transportation. *Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan*, 10(2): 142-150.