

**Original Research Article**  
**The Immunity Performance of Nile Tilapia  
(*Oreochromis niloticus*) After The Exposure of  
Botanical Insecticide with Azadirachtin Active  
Ingredient**

---

**ABSTRACT**

This study aims to determine the exposure effect of insecticide with the *azadirachtin* active ingredient on the immunity of Nile tilapia. This research was conducted in March and August for the preliminary test and September-October 2022 for the main test at Ciparanje Inland Aquaculture Hatchery, Padjadjaran University. This study used the experimental method of Complete Randomized Design with five treatments and three repeats. The Nile tilapia used is 6-7 cm. The treatment used is exposure to insecticide with different doses, namely A (0 ppm), B (26.25 ppm), C (52.5 ppm), D (78.75 ppm), E (105 ppm). Parameters observed included red blood cell count, white blood cell count, feed response, shock response, and macroscopic symptoms up to day 14. Red blood cell and white blood cell data were analyzed in a quantitative descriptive manner. Data on feed response, shock response, and macroscopic symptoms were carried out in a qualitative descriptive manner. Water quality data were analyzed in a quantitative descriptive manner. The results showed that exposure of insecticide with *azadirachtin* active ingredient interfered fish health by increasing white blood cell count, lowering red blood cell count, disrupting feed response and shock response. The higher the concentration of insecticide given, the more it affects the immunity of tilapia. Treatment without the addition of insecticides is the best treatment in this study.

*Keywords: Azadirachtin; Immunity; Insecticide; Nile tilapia; Oreochromis niloticus.*

## **1. INTRODUCTION**

Pesticides are widely used in the agricultural and plantation sectors to eradicate pests. Pest attacks can damage agricultural products and cause losses to farmers. Insecticide is one type of pesticide with chemical or botanical active ingredients used to control or eradicate insect type disturbing organisms [1]. The use of chemical insecticide in the long term can produce residues and have a negative impact on the environment so that farmers use botanical insecticide as an alternative to overcome plant pests [2].

Neem plants are one of the plants whose secondary metabolites can be used as botanical insecticide [3]. Leaf or seed extracts from neem plants contain *azadirachtin* compounds. *Azadirachtin* compounds have an influence on insect pests, such as inhibitors of feeding activity, inhibitors of skin development and turnover, rejection of ejection, and can cause death in insects [4].

Insecticides used in agriculture can pollute aquaculture waters. This is because the location of aquaculture is generally close to agricultural locations, there are even fishery activities located in rice fields (mina padi). The flow of water from agricultural areas will be distributed to water bodies with lower locations such as aquaculture ponds so that insecticides used in agricultural activities can be carried away by the flow of water. Insecticides that enter aquaculture water bodies can be a threat to aquatic biota, especially fish [5].

Fish is one of the animals that can be used as a bioindicator of pollution levels, water quality, and changes in the aquatic environment. One type of fish that can act as a bioindicator is Nile tilapia (*Oreochromis niloticus*). Tilapia is one of the aquatic biota recommended by USEPA (United States Environmental Protection Agency) as a toxicology test animal. This is because Nile tilapia has been widely cultivated, its distribution is quite wide, has a good ability to tolerate adverse environmental conditions, and is easy to maintain in the laboratory so it is good for use as a bioindicator [6].

The purpose of this study was to determine the exposure effect of insecticide with *azadirachtin* active ingredient on Nile tilapia immunity so that it can be used as a reference and information on tolerable concentrations and long-term effects after exposure of botanical insecticide with *azadirachtin* active ingredient on Nile tilapia immunity.

## 2. METHODOLOGY

### 2.1 Time and Place

This research was carried out at the Ciparanje Inland Aquaculture Hatchery, Faculty of Fisheries and Marine Science, Padjadjaran University in March and August 2022 for Preliminary Tests. Meanwhile, the main test was carried out from September to October 2022..

### 2.2 Tools and Materials

The tools used in this study were an aquarium measuring 50 x 29.5 x 35.5 cm<sup>3</sup>, a concrete tub 2 x 1 x 0.5 m<sup>3</sup>, blowers and aeration equipment, heaters, cup clips, millimeter blocks, digital scale, siphon device (water hoses), syringe, mercury thermometer, DO meter, pH meter, stopwatch, Thoma pipet, haemocytometer, cover glass, binocular microscope, scissor, labels, camera.

While the ingredients used, namely Nile tilapia nirwana measuring 6-7 cm, insecticide with *azadirachtin* active ingredient, feed, potassium permanganate (PK), Ethylene Diamine Tetra-Acetic Acid (EDTA) solution, Hayem solution, Turk's solution.

### 2.3 Research Methods

This study used a randomized design complete with five treatments and three repeats. The treatment given is:

- Treatment A = Control (Without addition of insecticide)
- Treatment B = Addition of insecticide 25% LC50 (26,25 ppm)
- Treatment C = Addition of insecticide 50% LC50 (52,5 ppm)
- Treatment D = Addition of insecticide 75% LC50 (78,75 ppm)
- Treatment E = Addition of insecticide LC50 (105 ppm)

### 2.4 Research Procedure

#### 2.4.1 Research preparation

The preparations carried out include the preparation of containers, the preparation of insecticides, and the acclimatization of fish. Preparation of containers is carried out by washing tools to be used such as aquariums, aeration hoses, aeration stones using Potassium Permanganate (PK), then the tools are dried. Next, the aquarium is laid out according to a predetermined layout and labeled. The aquarium was filled with 30 L of water in preliminary tests. While in the main test as much as 40 L. Aquarium is equipped with a heater and aeration device.

The insecticide used is prepared by being taken using a syringe according to a predetermined concentration. The syringe is inserted into the insecticide to be used and the plunger part is gently pulled so that the insecticide is sucked into the syringe. Then prepared cup clip as a container to store insecticide to be used. Then, the plunger on the syringe is gently pressed into the cup clip container, then the cup clip container is tightly closed.

The Nile tilapia that will be used are acclimatized for 3 days to ensure the condition of the fish is healthy. During acclimatization, fish are fed as much as 3% of the biomass. After acclimatization, the fish are introduced into the aquarium and observations are made. The preparatory stage of this research was carried out in the preliminary test and the main test.

#### 2.4.1 Preliminary Test

The treatment in the preliminary test refers to the USEPA recommendation, which is 0; 10; 100; and 1000 ppm. The test was carried out with 4 treatments and 2 repetitions. Tilapia used as many as 15 heads/aquarium. The aquarium used 8 pieces and the aquarium filled with water as much as 30 L. However, because the lower threshold and upper threshold ranges in the treatment were too large, additional treatment was carried out by reducing and enlarging the treatment concentration between 10 ppm and 1000 ppm. Additional concentrations are used, namely 50 ppm, 75 ppm, 100 ppm, 125 ppm, 150 ppm, 175 ppm, 250 ppm, and 500 ppm. Observations were made for 96 hours by observing the death of

fish every 24 hours. The results obtained were analyzed for regression using SPSS 2021 software. The results that have been obtained are concentrations that are thought to be deadly to test fish as much as 50%. After 96 hours of observation, an LC<sub>50</sub> value of 105 ppm was obtained.

### **2.4.1 Main Test**

The treatments to be used in the main test, namely 5 treatments and 3 repeats. Tilapia used as many as 20 heads/aquarium. The aquarium used as many as 15 pieces and filled with water as much as 40 L. The concentration to be used in each treatment refers to the LC<sub>50</sub> value that has been obtained in the preliminary test. The treatment to be used, namely control (without the addition of insecticide), 25% LC50 (26.25 ppm), 50% LC50 (52.5 ppm), 75% LC50 (78.75 ppm), and 100% LC50 (105 ppm). During the observations, the fish were fed as much as 3% of the biomass. Water quality maintenance is carried out by replacing water by water hoses as much as 10% every three days. The observations were conducted for 14 days by observing red blood cell count, white blood cell count, feed response, shock response, and macroscopic symptoms, and water quality.

### **2.4.1 Observation Parameters**

#### **Total Red Blood Cells (Eritrosit)**

Observation of the number of red blood cells was carried out on days 0, 3, 7, 10, and 14. According to [7], the calculation of the total of red blood cells is as follows :

$$RBC = \frac{A1 + A2 + A3 + A4 + A5}{5} \times 25 \times 10 \times 200$$

Description :

RBC = Red Blood Cells;  
 A1,A2,A3,A4,A5 = Observed box sample;  
 5 = Total of sample boxes;  
 25 = The entire total of red blood cells boxes;  
 10 = Box thickness (mm);  
 200 = Dilution (mm<sup>3</sup>)

#### **Total White Blood Cells (Leukosit)**

Observation of the total of white blood cells was carried out on days 0, 3, 7, 10, and 14. According to [7], the calculation of the total of white blood cells is as follows:

$$WBC = \frac{A1 + A2 + A3 + A4}{4} \times 16 \times 10 \times 20$$

Description :

WBC = White Blood Cells;  
 A1,A2, A3, A4 = Observed box sample;  
 4 = Total of sample boxes;  
 16 = The entire total of red blood cells boxes;  
 10 = Box thickness (mm);  
 20 = Dilution (mm<sup>3</sup>)

#### **Feed Response**

The observation of feed response refers to the research of [8] and carried out until the 14th day. Observation of feed response is calculated through the response time of fish when eating the given feed. The response rate of fish feed is as follows:

- (+++) = Fish respond to feed under 30 seconds
- (++) = Fish respond to feed 31 – 90 seconds
- (+) = Fish respond to feed 91 – 180 seconds
- (-) = Fish don't respond to feed

#### **Shock Response**

The observation of shock response refers to the research of [8] and carried out until the 14th day. The observation of the shock response is calculated through the number of active fish after the shock is given. The shock response rate of fish is as follows:

- (+++) = >80% of fish respond to shock
- (++) = 60 – 80% of fish respond to shock
- (+) = 30 – 60% of fish respond to shock
- (-) = Fish don't respond to shock

### Macroscopic symptoms

Observation of macroscopic symptoms was carried out after insecticide treatment by observing body damage to the outside, such as tearing fins, fading body color, bleeding, and distended abdomen until day 14.

### Water Quality

Water quality parameters observed include temperature, acidity (pH), and dissolved oxygen. Observations on water quality were made on days 0, 3, 7, 10, and 14.

## 2.5 Data Analysis

Red blood cell and white blood cell count data were analyzed in a quantitative descriptive manner. Data on feed response, shock response, and macroscopic symptoms were carried out in a qualitative descriptive manner. Water quality data is analyzed descriptively quantitatively and compared with Indonesian National standard.

## 3. RESULTS AND DISCUSSION

### 3.1 White Blood Cell Count

White blood cells are blood cells that function as non-specific defenses in the immune system of fish. Changes in white blood cell count are indicators of fish in poor condition [9]. The following is a graph of the results of observing the number of white blood cells.

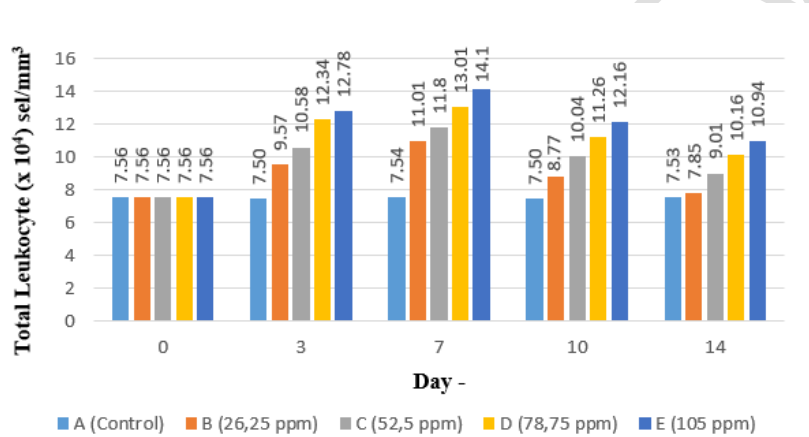


Figure 1. Graph of Total Leukocyte



	3	++	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
D	1	+	+	+	+	+	+	+	++	++	++	++	++	++	+++
	2	+	+	+	+	+	+	+	++	++	++	++	++	+++	+++
	3	+	+	+	+	+	+	+	++	++	++	++	+++	+++	+++
E	1	+	+	+	+	+	+	+	+	++	++	++	++	++	+++
	2	+	+	+	+	+	+	+	+	++	++	++	++	+++	+++
	3	+	+	+	+	+	+	+	+	++	++	++	++	+++	+++

**Description :** (+++) fish respond to feed under 30 seconds, (++) fish respond to feed 31-90 seconds, (+) fish respond to feed 91-180 seconds, (-) fish don't respond to feed

A decreased feed response is caused by exposure to insecticides and causes stress in fish. Stressful conditions experienced by fish cause behavioral changes, such as loss of appetite and rejection of feed [18]. This is in line with [19], who states that stress experienced by fish can cause disruption of physiological conditions characterized by loss of appetite of fish. This is supported by the condition of the amount of feed left when feeding. When associated with the results of observing the number of red blood cells, the low number of red blood cells causes a decrease in fish body activity. This causes the fish to become weak, fall silent at the bottom or hang below the surface of the water, and decreased appetite [20].

### 3.4 Shock response

Observation of the shock response is carried out by knocking on the walls of the treatment aquarium. This is done to determine the response or reflex of fish to stimuli given from outside [17]. If the fish is in a healthy condition, the fish will immediately move when given a surprise. The following is the result of observing the shock response.

**Table 2. Shock Response**

Treatment	Repetition	Day to-													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
B	1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
C	1	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++
	2	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++
	3	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++
D	1	+	+	+	+	+	++	++	++	++	++	++	++	+++	+++
	2	++	+	+	+	+	++	++	++	++	++	++	++	+++	+++
	3	+	+	+	+	+	++	++	++	++	++	++	+++	+++	+++
E	1	+	+	+	+	+	++	++	++	++	++	++	++	+++	+++
	2	+	+	++	+	+	++	++	++	++	++	++	++	+++	+++
	3	+	+	+	+	+	++	++	++	++	++	++	++	+++	+++

**Description:** (+++) >80% of fish respond to shock, (++) 60-80% of fish respond to shock, (+) 30-60% of fish respond to shock, (-) Fish don't respond to shock

Based on the observations, treatment A and B on days 1 to day 14 did not decrease shock response. Meanwhile, in treatment C, D, and E experienced a decrease in shock response. In treatment C, the response to shock decreased until day 6. Meanwhile, the shock response in treatment D was low until day 5 and treatment E was low until day 6. The decreased shock response is thought to be due to stress experienced by fish due to exposure to insecticides and decreased metabolism so that fish become weak and respond less to the shock given.

### 3.5 Macroscopic Symptoms

Macroscopic symptoms are observed from day 1 to day 14 by observing damage to the outside of the body of the fish. Observations were made after insecticide exposure was given at each treatment. The following are the results of observations of macroscopic symptoms

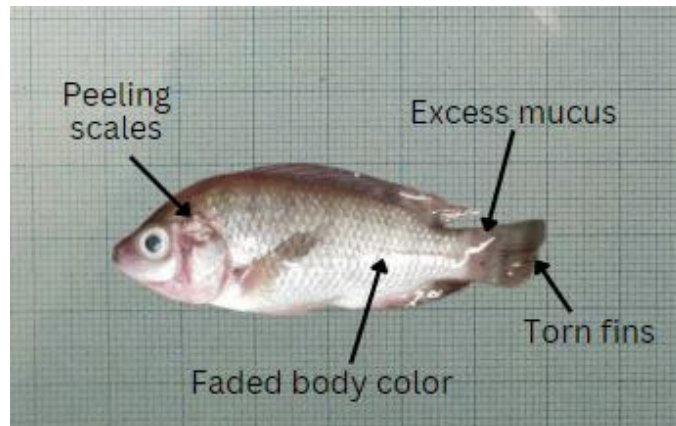


Figure 3. Macroscopic symptoms

Based on observations, the macroscopic symptoms that appear are excess mucus, faded body color, peeling scales, and torn fins. Exposure to insecticides causes fish to produce more mucus on their bodies to protect the exposed body from damage. The same was expressed by [21] which states that fish will produce mucus in their bodies as a form of defense due to exposure to pesticides which are pollutants in the fish's environment. However, this causes the supply of oxygen to the fish's body to decrease so that the fish's body weakens and causes the body color to fade. This is in line with [5], which states that exposure to insecticides can cause fish body color to fade and torn fin in fish due to weakening of the fish body due to insecticide exposure.

### 3.6 Water Quality

Water quality observed during the study included temperature, pH (acidity degree), and dissolved oxygen. The following are observations of water quality.

Table 3. Water Quality

Treatment	Day 3, 7, 10, dan 14			Day 21 dan ke-28		
	Temperature (°C)	pH	DO (mg/L)	Temperature (°C)	pH	DO (mg/L)
A	29,8 - 30,7	6,7 - 6,9	5,6 - 6,1	29,8 - 30,9	6,8 - 6,9	5,7 - 6,1
B	29,9 - 30,7	6,6 - 6,8	5,4 - 5,8	30,1 - 30,9	6,6 - 6,8	5,5 - 5,9
C	29,9 - 31	6,7 - 6,9	5,7 - 6,1	29,9 - 30,5	6,6 - 6,7	5,6 - 6,1
D	29,7 - 30,9	6,5 - 6,9	5,6 - 5,9	30,1 - 31	6,5 - 6,8	5,7 - 5,9
E	30,1 - 31	6,6 - 6,8	5,5 - 5,8	30,4 - 31	6,5 - 6,8	5,6 - 5,9
Optimal [22]	25 - 32	6,5 - 8,5	≥ 5	25 - 32	6,5 - 8,5	≥ 5

Based on the results of water quality observations (temperature, pH, dissolved oxygen) that have been carried out show that the value of water quality during the study was within the optimal range for tilapia according to [22]. In addition, exposure to insecticide provided does not affect the water quality of maintenance media because water quality is controlled according to the demands of a complete randomized design method that is not allowed for other factors other than the treatment given (exposure of botanical insecticide with *azadirachtin* active ingredient).

## 4. CONCLUSION

Based on the results of research that has been carried out, exposure to insecticides affects the health of tilapia, the higher the exposure to insecticide given, the more decreased the health of tilapia. The peak of disruption of fish health on the 7th day and after that the health condition of the fish gradually improves. Treatment without insecticide is the best treatment in this study

## REFERENCES

- [1] Isnaini, M., Pane, E. R., and Wiridianti, S. Testing Several Types of Vegetable Insecticides Against Rice Lice (*Sitophilus oryzae* L). *J. Biota*. 2015;1(1):1–8.
- [2] Suhartini, P. Suryadarma, and Budiwari, Utilization of Pesticides Vegetable On Pest Control *Plutella Xylostella* On Plants Mustard Greens (*Brassica juncea* L.) Towards Sustainable Agriculture. *J. Basic Science*. 2017;6(1): 36–43.
- [3] Sumaryono, Latifah, and Sedyawati, S. M. R. Identification and Toxicity Test of Azadirachtin from Neem Leaves as Walang Sangit Bioinsecticide. *J. Chemical Science*. 2013;2(1).
- [4] Hartanto, E. S., and Hutajulu, T. F. The Use Of Azadirachtin Of Neem (*Azadirachta indica* A. Juss) For Preparation Anti Ants. *J. Industrial Technology Research*. 2014;8(16): 84–94.
- [5] Adharini, R. I., Suharno, S., and Hartiko, H. Contamination Effect of Profenofos Insecticide on Physiology of Red Tilapia (*Oreochromis sp.*),” *J Humans and the Environment*. 2016;22(2):365–373.
- [6] Nirmala, K., Hastuti, Y. P., and Yuniar, V. Toxicity of Mercury (Hg) on Survival and growth Rate, Hemato and Histopathological Parameters of *Oreochromis niloticus*. *J. Indonesian Aquaculture*. 2012;11(1):38–48.
- [7] Sukenda, Rafsyanzani, M.M., and Hidayatullah, D. Performance of *Bacillus* sp. Probiotic in Catfish Juvenile *Clarias* sp. Infected by *Aeromonas hydrophila*. *J. Indonesian Aquaculture*. 2016;15(2):162–170.
- [8] Suryadi, I. B. B., Bari, I. N., & Lal, M. T. M. Sublethal Effects Of Fungicide *Bacillus amyloliquefaciens* On Tilapia (*Oreochromis niloticus*) And Common Carp (*Cyprinus carpio*) Fingerlings. *J. Indonesian Swamp Aquaculture*. 2021;9(1):185–199.
- [9] Riau waty, M and Syawal, H. Blood Description of *Oreochromis niloticus* in Pond Cultures in Marpoyan Damai Subdistrict of Pekanbaru City. *J. Fisheries and Maritime Affairs*. 2016;21(1):1–6.
- [10] Rahmi, A. Hematological Response of Milkfish, *Chanos chanos* (Forskall, 1755) Exposed to Lead (Pb) at Subchronic Concentrations. 2020.
- [11] Singh, N. N., and Srivastava, A. K. Haematological Parameters as Bioindicators of Insecticide Exposure in Teleosts. *Ecotoxicology*. 2010;19:838–854.
- [12] Lukmini, A., Supriyono, E., and Budiardi, T. Toxicity of Fentin Acetate Molluscicide on Haematological and Growth of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), "J. Ikhtologi Indonesia. 2016;17(1):1–9.
- [13] Hartika, R., Mustahal, and Putra, A. N. Tilapia Blood Parameters with The Addition of Different Dose of Prebiotics in Feed. *J. Fisheries and Maritime Affairs*. 2014;4(4):259–267.
- [14] Mengi, L., Jasmanindar, Y., and Liufeto, F. C. "Prevention of *Vibrio* sp. on Milkfish (*Chanos chanos*) with *Cinnamomum burmannii* (*Cinnamomum burmannii*) decoction," *J. Vocational Fisheries Sciences*. 2022;3(1): 9–13.
- [15] Syahbana, R. A., Purwanti, E., Nurwidodo, Prihanta, W., and Husamah, H. The Effect of Diazinon 600 EC Contamination on Mortality Levels and Erythrocytes of Tilapia (*Oreochromis niloticus*). *Monday. Nas. V*. 2020: 228–233.
- [16] Tanbiyaskur, Yulisman, and Yonarta, D. LC50 Tuba Root Extract Test and Its Effect on Nile Tilapia (*Oreochromis niloticus*) Health Status. *J of Aquaculture and Fish Health*. 2019;8(3).

- [17] Rosidah, Buwono, I. D., Lili, W., Suryadi, I. B., and Triandika, A. R. The resistance of Sangkuriang Catfish (*Clarias gariepinus* Burchell 1822) against *Aeromonas hydrophila* Bacteria Given Moringa Leaf Extracts (*Moringa oleifera* L.) Through the Feed. *J. Indonesian Ichthyology*. 2019;19(1):97–113.
- [18] Fauzi, A. R., Hasan, H., and Prasetio, E. The Effectiveness of Ethanol Extract of Hibiscus Leaves (*Hibiscus rosa sinensis* L.) as Immunostimulants of Jelawat Fish (*Leptobarbus hoevenii* Blkr.) Infected with Bacteria *Aeromonas hydrophila*. *J. Ruaya*. 2019;7(1):28–37.
- [19] Irianto, A. *Pathology of Teleost Fish*. Yogyakarta: Gadjah Mada University Press, 2005.
- [20] Prasetio, E., Fakhrudin, M., and Hasan, H. The Effect of Powder (Aloe vera) Fish on Jelawat Hematology (*Leptobarbus hoevenii*) Tested the Challenge Bacteria *Aeromonas hydrophila*. *J. Ruaya*. 2017;5(2):44–54.
- [21] Hafiz, F., Prasetyono, E., and Syaputra, D. Toxicity of Glyphosate Isopropylamine-based Herbicide on The Growth of Catfish (*Clarias gariepinus* Burchell, 1822) Juveniles. *Oceanology and Limnology in Indonesia*. 2018;3(3):235–244.
- [22] SNI. *Production of Tilapia (*Oreochromis niloticus*) Growing Class in Calm Water Ponds*. 2009.