Sublethal Toxicity Insecticide Organochlorine Endosulfan on The Value of Blood Sugar Levels and The Level of Life in Tilapia (Oreochromis niloticus) Seeds

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\textbf{Abstrak.} The use of pesticides in agriculture continues to increase. One of the classifications of pesticides is organochlorine endosulfan insecticide that can cause river pollution. One type of fish that is often cultivated in ponds is tilapia. River pollution can trigger stress and even fish death. The stress level of the fish can be seen from the value of its blood sugar levels. This research was conducted on March 20 - April 28, 2021, in Microbiology Laboratory and Anatomy-Aquaculture Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga, to determine the toxicity effect of sublethal endosulfan organochlorine insecticide on blood sugar level value and tilapia seed life rate. This study used experimental methods with CRD through exposure to different organochlorine concentrations, namely P0 (0%), P1 (10%), P2 (30%), P3 (50%), P4 (70%) with four replays. Analysis of the data was processed using ANOVA and DMRT, which showed that sublethal toxicity of endosulfan organochlorine insecticides had an effect (p<0.05) on blood sugar levels and the level of tilapia seed resistance. Using only a concentration of 10% has increased blood sugar levels significantly on the 7th day and continues to increase until the 28th day (114.50 mg/dL). Also, the level of fish life insecurity indicates a value below the Indonesian National Standard (72.50%).

1. Introduction
The use of pesticides in agriculture is increasing. Pesticides are chemicals useful to kill or restrain the growth of pests and pathogens [1]. One of the classifications of pesticides is the insecticide organochlorine endosulfan which is strictly prohibited in some countries [2][3], but in Indonesia is still used. Excessive use of pesticides can cause water pollution in rivers [5], where the river serves as a water source in the aquaculture pond [6]. Fish farming in Indonesia generally uses an extensive system [7]. One species of fish cultivated in aquaculture ponds is tilapia (Oreochromis niloticus) which is of high economic value [8] and is one of the freshwater fishery products that people are interested in [9]. The presence of water pollution can decrease the quality of water that triggers the occurrence of stress until fish death [10]. The stress level of the fish can be seen from the value of its blood sugar levels [11]. Not only that, but pollution caused by organochlorine endosulfan insecticides can also result in acute toxicity and sublethal toxicity in aquatic biota. The research results on sublethal toxicity test in tilapia exposed to organochlorine endosulfan conducted by Putri et al. [1] showed that organochlorine...
endosulfan has a noticeable effect on egg hatching power but has no noticeable effect on the abnormality of larvae in tilapia. Based on the exposure, it is necessary to test the toxicity of sublethal insecticide organochlorine endosulfan on tilapia seeds. It is exposed using endosulfan organochlorine insecticide with trademark Akodan 35EC, active endosulfan 350 grams / L, which aims to determine the influence of toxicity sublethal endosulfan organochlorine insecticide on the value of blood sugar levels and the level of tilapia seed.

2. Methods
2.1. Place and Time
This research was conducted on March 20 – April 28, 2021, at the Anatomy-Aquaculture Laboratory and Microbiology Laboratory of the Faculty of Fisheries and Marine Universitas Airlangga, Surabaya.

2.2. Materials
2.2.1 Research Equipment
Research equipment used in the study is an aquarium measuring 20 x 20 x 30 cm³ as much as 20 units, measuring cups, measuring pipettes, stopwatches/wall clocks, hoses, water filters, beaker glass, basins, seser, glass mixers, stars, pH meters, DO meters, blood glucose test kits, syringes, and stationery.

2.2.2 Research Materials
The research material used in this study is tilapia as many as 400 heads with a length of 10 to 12 cm derived from UPT Balai Benih Ikan Penataan, Winongan, Pasuruan, East Java.

2.3. Research Methods
The research method in this study is experimental.

2.3.1 Research Design
The research design used is The Complete Randomized Design (CRD). This study used five treatments, namely P0, P1, P2, P3 and P4 with 4 repeats each: P0 = Concentration 0%, P1 = Concentration 10% x LC50-96 hours, P2 = Concentration 30% x LC50-96 hours, P3 = Concentration 50% x LC50-96 hours and P4 = Concentration 70% x LC50-96 hours. In their research, Kumar et al. [12] stated that the LC50-96 hour value of tilapia exposed to endosulfan organochlorine insecticide was 12,795 μg/L. Observed variables include free variables: dose/concentration of endosulfan organochlorine insecticide, bound variables: blood sugar level value and tilapia seed excision rate, and control variable: water quality.

2.3.2 Working Procedures
a) Preparation of containers: starting with cleaning the aquarium using soap, then rinsed and soaked with chlorine as much as ten ppm for one day, followed by lightening in the sun and labeling on the aquarium, then done water replenishment as much as 20 L per aquarium and the addition of sufficient aeration to ensure the dissolved oxygen content and temperature can be optimal [13]. b) Seed preparation: fish seed used in denial size (10-12 cm) which is then done uniformizing size by sorting, after which the seed is criticized for seven days, feeding is done at satiation with pellets for two times a day. c) Sublethal toxicity test, conducted for 28 days of maintenance with a predetermined concentration, the change of test media is conducted every two days as much as 100% by feeding as much as two times a day and still using aeration, not forgetting to observe changes in fish behavior for 96 hours and measurement of water quality every morning and afternoon during the study. d) Fish blood sampling is done every seven days by using the "Cardiac Puncture Technique," with the first step of the fish is annexed using clove oil at a dose of 5 ppm, then the fish is wiped with tissue and performed blood sampling right on the heart hump with a syringe that already contains EDTA 10%. e) Measurement of fish blood sugar levels using digital blood glucose kit, so the blood that has been taken is dripping as much as ≥ four μL on the strip paper connected to the tool, and waited ±10 seconds until the results appeared on the screen. f) Observation of the level of fish survival, carried out by observing the fish that experienced death every day in the morning and afternoon before feeding. The results obtained are calculated using the formula
"Survival Rate," which compares the number of fish that can survive at the end of the period with the number of fish that live at the beginning of the maintenance period.

2.3.3 Research Parameters
a) The main parameters observed are the value of blood sugar levels and the level of life of tilapia seeds, and b) The observed supporting parameters are changes in the behavior of tilapia seeds and water quality, which include DO, pH, and temperature.

2.3.4 Data Analysis
Data Analysis The data were analyzed using analysis of variance (ANOVA) tests to determine the effect of the treatment. If it shows a significant difference result, it will be continued with Duncan's Multiple Range Test with a significant level of 5%.

3. Results and Discussion
3.1. Blood Sugar Level Value of Tilapia Seed

| Treatment (Concentration) | Tilapia Seed Blood Sugar Levels (mg/dL ± SD) |
|--------------------------|-----------------------------------------------|
|                          | Day 7      | Day 14        | Day 21        |
| P0 (0%)                  | 50.75 ± 1.708 | 51.50 ± 1.291 | 53.00 ± 1.414 | 54.75 ± 0.957 |
| P1 (10%)                 | 89.75b ± 9.912 | 92.00b ± 9.967 | 106.75b ± 10.468 | 114.50b ± 17.445 |
| P2 (30%)                 | 110.75c ± 13.97  | 113.25c ± 13.769 | 118.25b ± 0.957 | 118.75b ± 11.087 |
| P3 (50%)                 | 132.00d ± 12.675 | 133.00d ± 12.675 | 133.50c ± 1.915 | 195.50c ± 17.991 |
| P4 (70%)                 | 151.00e ± 16.432 | 152.25e ± 16.070 | 153.25d ± 18.518 | 234.25e ± 56.612 |

Description: Different superscript values show significantly different results (P<0.05).

Based on the results of the Analysis of Variants (ANOVA) on the 7th to 28th day of the study, showed that sublethal toxins test of endosulfan organochlorine insecticide had a significant effect (p<0.05) on fish blood sugar levels. DMRT advanced test results in Table 1. On the 7th and 14th days of the study, there was a noticeable difference (p<0.05) between each treatment: P0, P1, P2, P3, and P4. On the 21st day of the study, it showed that P0 differed markedly (p<0.05) with all treatments (P1, P2, P3, and P4). Meanwhile, P4 has a noticeable difference (p<0.05) with each treatment (P0, P1, P2, and P3). Day 28 research shows that P0 differs markedly (p<0.05) with all treatments (P1, P2, P3, and P4). for P1 and P2, there is no real difference (p>0.05), but they are different (p<0.05) with P0, P3, and P4. While for P3 and P4, there is no real difference (p>0.05), but they are significantly different (p<0.05) with P0, P1, and P2. Thus, it can be known that with a concentration of 10 % (P1) endosulfan organochlorine insecticide alone, it has been able to increase the value of blood sugar levels significantly on the 7th day and continue to increase until the end of the study reached 114.50 mg/dL. The graph of the average blood sugar level value of tilapia seeds can be seen in Figure 1.
Based on the results of the Analysis of Variants (ANOVA) on the 7th to 28th day of the study, showed that the toxicity test of sublethal insecticide organochlorine endosulfan had a significant effect (p<0.05) on the blood sugar level value of tilapia seeds. Figure 1 indicates an increase in tilapia seeds' blood sugar levels and increased concentration and time of exposure. The observation of fish blood sugar levels on the 0th day of the study showed normal values ranging from 50.25 mg/dL - 52.00 mg/dL. However, Shabrina et al. [14] revealed that normal fish blood sugar levels are in the range of 40-90 mg/dL. This is because there is still no exposure to endosulfan organochlorine insecticides so that the water quality is still in good condition by the Indonesian National Standard [15].

A significant increase in blood sugar levels began to occur on the 7th day, especially in P1, followed by P2, P3, and P4. However, blood sugar levels showed abnormal treatment for P2, P3, and P4. This significant increase in blood sugar levels is thought to be caused by environmental shock caused by stress material in the form of exposure to endosulfan organochlorine insecticides resulting in gluconeogenesis. This is supported by Lukmini [16] in his research that exposed the pesticide fetin acetate in tilapia stated that the rise in fish blood sugar levels is caused by gluconeogenesis. This results from the presence of stress material in the form of pesticides that further stimulate the hypothalamus to release cortisol hormones that trigger lipolysis, glycogenolysis, and gluconeogenesis. Gluconeogenesis is a term to include all mechanisms and pathways responsible for converting noncarbohydrate compounds into glucose.

P1 to P4 treatment begins to show abnormality (stress) from the 14th to the 28th day. However, on the 14th to 21st day, each treatment experienced a not very high increase. Therefore, it is suspected that tilapia have started to make adjustments to negative environmental changes, commonly referred to as adaptation processes. This is in accordance with what is expressed by Firdaus et al. [17], which states that there is a negative/bad environmental change, will result in the onset of fish response with gradual poses. This process begins with the fish by avoiding stressors. If the fish can not avoid, then the fish will try to continue the adaptation process with the available environment.

Day 28 of this study showed a significant increase in blood sugar levels in the treatment of P4 (234.25 mg/dL) and P3 (195.50 mg/dL), both of which showed no noticeable difference. The increase in blood sugar levels is thought to be due to changes in water quality that decreased under the Indonesian National Standard [15], so the impact is that fish become stressed. This is by Hardi [18] that sudden changes in water quality to extreme waters can cause stress that decreases fish resistance. Including water, pollutants will negatively affect the immune system. Meanwhile, according to Diansari et al. [19], the decrease in water quality will impact the occurrence of stress in fish that can inhibit fish's growth and even death. This will certainly affect the value of the level of fish life.

**Figure 1.** Graph of average blood sugar levels of tilapia seeds
3.2. Tilapia Seed Life Rate

Table 2. Average data on the survival rate of tilapia seeds in each treatment

| Treatment (Concentration) | Fish Life Rate (SR ± SD) |
|--------------------------|--------------------------|
| P0 (0 %)                 | 92.50± 6.455             |
| P1 (10%)                 | 72.50± 6.455             |
| P2 (30%)                 | 65.00± 4.082             |
| P3 (50 %)                | 53.75± 8.539             |
| P4 (70%)                 | 43.75± 8.539             |

Description: Different superscript values show significantly different results (P<0.05).

Based on the results of the ANOVA calculation, the toxicity test of sublethal insecticide organochlorine endosulfan has a noticeable effect (p<0.05) on the level of tilapia seed smoothness. The further dirt test in Table 2. showed that the P0 treatment differed markedly (p<0.05) with P1, P2, P3, and P>4 treatment. Meanwhile, P3 treatment is no real difference (p>0.05) from P4 treatment but significantly different from P0, P1, and P2 treatment. Thus, it can be known that with a concentration of 10% (P1) endosulfan organochlorine insecticide only, it has shown a value below the Indonesian National Standard [15] which is 72.50%. The graph of the average level of tilapia seed life can be seen in Figure 2.

![Percentage of Fish Life Rate](image)

Figure 2. Graph of average levels of tilapia seed flexibility

The level of fish survival is a comparison of the number of fish that lived [20]. Fish's survival can be used to determine the fish's ability and tolerance in terms of maintaining its life [21]. Based on the results of the Analysis of Variants (ANOVA) showed that the toxicity test of sublethal insecticide organochlorine endosulfans had a real influence (p<0.05) against the level of tilapia seed resistance. Based on Figure 2, it can be known that against the decrease in the level of fish survival tilapia seeds and the increasing concentration of exposure given. This statement was reinforced by Connell and Miller [22] that high concentrations of pesticides in the waters would affect the physiological processes of fish with peak conditions resulting in death. Thus, the number of dead fish will affect the level of life of the fish.

The fish's flexibility rate results showed that the P0 treatment had a high fish life rate of 92.50%. SR value is relatively excellent and normal because it exceeds the minimum value of the Indonesian National Standard [15], where the survival value (sintasan) for the production of tilapia seed is at least ≥ 75%. The high value of SR in this control treatment, allegedly caused by good water quality due to the absence of exposure to endosulfan organochlorine insecticides given and by Indonesian National Standards [15]. By the statement of Panggabean et al. [23], good water quality will support the survival and growth of fish.

Average SR values showed a decrease and abnormality (≤ 75%) P1 treatment followed by P2, P3, and P4. The decrease and abnormality of SR value are thought to be caused by the test fish experiencing stress; this is evidenced by the increasing value of fish's blood sugar levels, changes in behavior, and
decreased water quality. Nasir and Khalil [24] stated that poor water quality could cause disease in fish as well as death. Nwani et al. [25] added that fish exposed to pesticides in sublethal concentrations could exhibit changes in behavior and hematological characteristics that would eventually affect survival rates.

3.3. Tilapia Seed Behavior Changes

Table 3. Score tilapia seed behavior for 96 hours

| Treatment (Concentration) | Fish Reflex Response | Behavioral Responses | Swimming Response | Swarming Response |
|---------------------------|----------------------|----------------------|-------------------|-------------------|
| P0 (0 %)                  | +++                  | ++                   | +++               |                   |
| P1 (10%)                  | ++                   | ++                   | +++               |                   |
| P2 (30%)                  | ++                   | ++                   | +++               |                   |
| P3 (50 %)                 | +                    | +                    | +++               |                   |
| P4 (70%)                  | +                    | +                    | +++               |                   |

Description: (-) indicates no response, (+) indicates there is a low response, (++) indicates there is a medium response, (+++) indicates there is a high response.

Table 3 showed that the behavior response of tilapia seeds decreased gradually, and there were differences in behavior between the treatment of P0 and P1, P2, P3, and P4. Where is the P0 treatment shows normal behavior characterized by a high response in fish. Meanwhile, for the treatment of P1 to P4, there is a difference in behavior, namely a decrease in response. Fish behavior is an important part of the fish to maintain its life and return to normal in a short period [26]. Clinically tilapia exposed to endosulfan organochlorine insecticides show symptoms of stress in the presence of unstable movements and are on the ground. The movement of fish appears different in each treatment; this is thought to be caused by the administration of endosulfan organochlorine insecticides that can affect fish movement. This is supported by Taufik and Setiadi's statement [27] that endosulfan can influence the movement and behavior of fish. Shah [28] added that fish exposed to toxic substances could be seen from the behavior of fish. As with any hyperactive movement, paralyzed, it was floundering and even dead. This is a way to minimize biochemical processes in the body that have been poisoned so that lethal effects can be slower. Niti [29] added that the behavior changes in each treatment vary due to differences in concentration between treatments that cause environmental changes in the live media of fish.

3.4. Water Quality

Table 4. Average water quality data during the study

| Treatment (Concentration) | DO (mg/L) | Parameter pH | Temperature (°C) |
|---------------------------|-----------|--------------|------------------|
| P0 (0 %)                  | 3.00 – 3.28 | 7.05 – 8.50  | 26.20 – 30.95    |
| P1 (10%)                  | 1.24 – 3.39 | 6.21 – 8.55  | 26.32 – 31.72    |
| P2 (30%)                  | 1.29 – 3.52 | 6.28 – 8.35  | 26.07 – 29.70    |
| P3 (50 %)                 | 1.14 – 3.27 | 6.18 – 8.50  | 26.40 – 31.52    |
| P4 (70%)                  | 1.12 – 3.42 | 6.15 – 8.50  | 26.15 – 31.90    |
| Indonesian National Standard [15] | ≥ 3 | 6.5 – 8.5 | 25 – 32 |

Table 4. The results of water quality measurement in each treatment show a difference between the control treatment (P0) and the other treatment; it could happen due to the administration of different doses of endosulfan organochlorine insecticide. Water quality measurement is done to determine the environmental condition and sustainability of cultivation [30]. Water quality can also affect the value of blood sugar levels in fish [31]. Do measurement results get a value between 1.12 mg/L – 3.52 mg/L. Do value can be said to be less good but can be tolerated by fish. The lowest DO in all treatments except controls of ≤ 2 mg/L, meaning the waters are already severely contaminated due to exposure to endosulfan organochlorine insecticides. Patty [32] classifies the waters according to dissolved oxygen as follows: unpolluted (≥ 6.5 mg/L), moderately polluted (4.5 – 6.5 mg/L) and heavily polluted (< 2.0 mg/L). Meanwhile, all treatments’ highest DO range value exceeded the normal range limit (≥ 3 mg/L).
The results of the pH measurement in this study showed that the range of degrees of water acidity (pH) ranged from 6.15 – 8.55. This pH shows a value that is not by the Indonesian National Standard [15], meaning that this pH is not suitable for the survival of tilapia seeds, so it can be said that the water quality is poor. The pH value is influenced by the toxicity of a chemical compound [33]. The temperature obtained in this study shows a range of 26.07°C – 31.90°C. The temperature value is included in the normal limit of The Indonesian National Standard [15]. Temperature is volatile; if the water temperature is low, then fish's growth rate, appetite, and metabolic rate will drop [34]. Sahetapy [35] added that fish appetite could be reduced because the energy needed by the fish has been fulfilled so that there is an increase in blood glucose levels.

4. Conclusion
Based on the results of studies conducted, conclusions can be drawn that the toxicity of sublethal insecticide organochlorine endosulfan effect (p<0.05) on blood sugar levels and the level of excess life tilapia seeds. Using only a concentration of 10% has increased blood sugar levels significantly on the 7th day and continues to increase until the end of the study reached 114.50 mg/dL. Meanwhile, a concentration of 10% has shown a value below the Indonesian National Standard of 72.50%. From this study, it can also be known that fish blood sugar levels increase as the concentration and time of exposure increases. While the level of life of fish decreases along with the increasing concentration of exposure given.

5. References
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