Sublethal toxicity of organophosphate pesticides on development organ and survival rate of silver rasbora (Rasbora argyrotaenia)

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Abstract. The use of pesticides in agriculture only functions as much as 90%. The rest has polluted the surrounding environment, especially fish. Organophosphates are active neurotoxins. The organophosphate does not require any other conversion to inhibit the acetylcholinesterase enzyme. Organophosphates have the ability inhibit the action of the acetylcholinesterase (AChE) enzyme which results in disruption of acetylcholine when it provides stimulation impulses from pre-synapse to post-synapse (neurotransmitter), this results in impaired muscle work. So that the work of the undirected muscles that can cause symptoms of poisoning that affect the work of the whole body. This study aims to know the effect of sublethal toxicity of organophosphate pesticides on development organ and the survival rate of silver rasbora larvae, and to determine the exposure of organophosphate pesticides that can affect development organ and the survival rate of larvae. The research method was an experiment with a Completely Randomized Design as an experimental design. The treatments used were different organophosphate concentrations, that is 0, 0.5, 1, 1.5, and 2 ppm. Each treatment was repeated four times. The main parameters observed were the development organ of larvae, egg yolk absorption rate, abnormality larvae. The results showed that organophosphate pesticide exposure of 0.5 - 2 ppm could affect the development of the organ, survival rate, and abnormality silver rasbora larvae. Exposure to organophosphate pesticides can also increase the absorption rate of egg yolks in larvae. Exposure dosage to organophosphate pesticides is directly proportional to the percentage of larva abnormalities and inversely proportional to the value of survival rate larva.

1. Introduction

Pesticides in agriculture only functions 90%, the rest has polluted the surrounding environment, especially fish [1]. The organophosphate does not require any other conversion to inhibit the acetylcholinesterase enzyme [2]. Organophosphates have the ability inhibit the action of the acetylcholinesterase (AChE) enzyme which results in disruption of acetylcholine when it provides stimulation impulses from pre-synapse to post-synapse (neurotransmitter). These results in impaired muscle work, so that the work of undirected muscles can cause symptoms of poisoning that affect the work of the whole body [3].
Pesticide sublethal dose of 0.05-0.001 ppm can affect the hematology of silver rasbora fish [4]. Pesticide can interfere with the brain function of fish larvae during a critical life-history transition, potentially influencing recruitment success [5]. Based on the above explanation, research is needed to determine the effect of sublethal toxicity of organophosphate pesticides with variations in concentration on the developing organs and the survival rate of silver rasbora larvae.

2. Material and methods

2.1. Research methods and Design

The research method is an experimental research method. The study used a completely randomized design (CRD) with 5 treatments and 4 replications. All treatments were exposed to organophosphate pesticides except controls.

2.2. Preliminary Test

A preliminary test is carried out to get the dose for the actual one. The test container is a 5 liter jar and filled with 1.5 liters of water equipped with aeration. The dosage used in the preliminary test includes; 0 mg / l; 0.0001 mg / l; 0.001 mg / l; 0.01 mg / l; 0.1 mg / l; 0.25 mg / l; 0.5 mg / l; 0.75 mg / l and 1 mg / l (Kienle et al., 2009). Larval deaths are recorded every 24 hours until the yolk in the larvae runs out.

2.3. Sublethal Test

This test is carried out by using doses including; 0, 0.5, 1, 1.5 and 2 ppm with 4 replications. The research place was a 5-liter tube with aeration and filled with 1.5 liters of water. Each jar is filled with organophosphate pesticides according to the prescribed dose.

2.4. Main Parameters and Supporting Parameters

Observations in this study were carried out, starting from hatching larvae until the yolk in the larvae was used up. The main parameters in the study were the development of organs and the survival of silver rasbora larvae. The development organ was observed using a binocular microscope with 100x magnification. While the survival rate is calculated at the end of the study using the formula:

\[
\text{Survival Rate} = \frac{\text{the number of late larvae of the study}}{\text{hatching rate larva}} \times 100
\]

Supporting parameters include the absorption rate of egg yolk, abnormalities of larvae, and water quality. The water quality calculated includes DO, temperature, and pH. Measurements were carried out in the morning and evening during the study. The absorption rate of egg yolk was observed using a Trinocular microscope using the formula[6], namely:

\[
\text{egg yolk absorption rate} = \frac{V_0 - V_t}{T}
\]

Description:
- $V_0$ = initial egg yolk volume (mm$^3$)
- $V_t$ = final yolk volume (mm$^3$)
- $T$ = time (hours)

The volume of egg yolks can be calculated using the formula [7] in [8]:

\[
V = 0,1667 \pi LH^2
\]

Description:
- $V$ = volume of egg yolk (mm$^3$)
- $L$ = diameter of elongated egg yolk (mm)
- $H$ = diameter of egg yolk shortens (mm)
Observation of abnormalities in this study included the shape of the head, body shape, and shape of the tail. The percentage of abnormalities can be calculated using the formula:

$$\text{Abnormality} = \frac{\sum A}{N_t} \times 100$$

Description:
\(\sum A\) = number of abnormal larvae
\(N_t\) = number of larvae at the end of treatment

3. Result and discussion

Based on observations of the first organ that is formed, namely the presence of body pigmentation. In treatment 1.5 and 2 ppm, egg yolks run out at 84 HPF while the mouth opening is not open. This shows the development of organs in imperfect larvae.

| Development Organ          | (0 ppm) | (0.5 ppm) | (1 ppm) | (1.5 ppm) | (2 ppm) |
|----------------------------|---------|-----------|---------|-----------|---------|
| Gass Blader                | 48 HPF  | 48 HPF    | 48 HPF  | 48 HPF    | 48 HPF  |
| Pigmentation               | 42 HPF  | 42 HPF    | 42 HPF  | 42 HPF    | 42 HPF  |
| Mount                      | 84 HPF  | 84 HPF    | 84 HPF  | 84 HPF    | 84 HPF  |
| The end of egg yolk        | 84 HPF  | 84 HPF    | 84 HPF  | 78 HPF    | 78 HPF  |

Information: HPF (Hour Post Fertilisation)

The higher the exposure to the pesticide dose given, the faster the absorption of the yolk. This is related to the energy used by larvae to adapt. Besides, the higher the exposure to organophosphate pesticides, the higher the percentage of abnormal larvae. But it is inversely proportional to the survival rate of silver rasbora fish. The higher the percentage of eating larvae abnormality, the lower the value of the resulting survival rate. Data on the percentage value of abnormality and survival of silver rasbora fish larvae can be seen in tables 2 and 3.

| Concentration (ppm) | Survival rate (%) ± SD |
|---------------------|------------------------|
| 0                   | 86 ± 1.41              |
| 0.5                 | 80 ± 0.96              |
| 1                   | 65 ± 1.7               |
| 1.5                 | 32 ± 1.26              |
| 2                   | 18 ± 1.4               |

| Concentration (ppm) | Abnormalities (%) ± SD |
|---------------------|-------------------------|
| 0                   | 1.5 ± 1.91              |
| 0.5                 | 25 ± 0.81               |
| 1                   | 37.25 ± 0.95            |
| 1.5                 | 43.75 ± 0.95            |
| 2                   | 62.25 ± 1.7             |

Organophosphate pesticides work by inhibiting the acetylcholinesterase enzyme. Normally acetylcholinesterase functions to hydrolyze acetylcholine to acetate and choline. When the enzyme is inhibited, acetylcholine accumulates in synapses and binds to muscarinic and nicotinic receptors in the central and peripheral nervous systems [9].
The results of the study showed that larvae in treatment 1, 5 and 2 ppm had the fastest record time of larval development, which was 78 HPF. Although the treatment shows the fastest organ development, the mouth opening of the larvae has not yet formed when the egg yolk runs out. This indicates an imperfect organ development. Whereas in treatments 0, 0.5, and treatment 1 ppm, the development of organs in larvae up to 84 HPF with a marked mouth that starts to open and there is still a little remaining egg yolk. According to [10], the inhibition of acetylcholinesterase due to organophosphate exposure causes a disruption in the development of nerve and muscle fibers resulting in hyperstimulation, which ultimately affects the development of organs in fish larvae.

Fish larvae use egg yolks as an energy source before entering exogenous feeding. The high rate of absorption of egg yolk is due to the use of egg yolk as a nutrient and energy for larvae so that egg yolk runs out faster [11]. The results of the study show that the higher the dose of organophosphate pesticide exposure followed by the acceleration of the rate of absorption of egg yolk in silver rasbora larvae. The difference in the absorption speed of egg yolks occurs due to inappropriate environmental influences and stressful fish. So that fish larvae need more energy to adapt. This adaptation process causes the metabolic activities in the body of the fish to run higher and requires greater energy so that the rate of absorption of the yolk becomes greater [12].

Increasing the dose of organophosphate pesticide exposure is also directly proportional to the increase in abnormal larvae. The higher the exposure to pesticides, the higher the abnormal larvae produced. The highest value of abnormalities is in treatment 2 ppm of 62.25% ± 0.52, and the lowest value in treatment 0 ppm is 0%. Abnormalities found include spinal malformations, caudal fins, and edema in the yolk sac. Organophosphate exposure causes cell proliferation failure, which then influences morphogenesis [13].

Larvae abnormalities can be caused by gene mutations and from teratogenic effects, environmental damage or toxicity, parasites, and nutritional deficiencies [14]. Abnormalities in bone malformation occur due to a disturbance during the axial formation and hardening of the vertebrae. In contrast, edema in the egg sacs occurs due to the failure of the osmoregulation system associated with the accumulation of pesticides [15]. A decrease in the survival value of silver rasbora fish larvae followed the increase in the number of abnormal larvae. The lowest survival rate in treatment 2 ppm of 18% ± 1.4, and the highest survival rate in treatment 0 ppm is 86% ± 1.41. The value of larval abnormalities is inversely proportional to the value of survival of silver rasbora larvae. The higher the number of abnormal larvae, the lower the value of survival. These results are relevant because the abnormalities that occur in the silver rasbora fish larvae caused the larval organs to not develop properly, this has an impact on the low value of larval survival. In addition, the low value of livelihood can also be caused by the use of more egg yolk for larval adaptation activities than the process of formation and development of organs in larvae. So that when the egg yolk has run out, organ formation in fish larvae is not perfect. It causes the larvae to not get energy for their survival. [16] added, organophosphate pesticides cause neurotoxicity in the early stages of fish development and death in brain cells that can cause death.

4. Conclusion

4.1. Conclusion

The conclusion of this study is sublethal exposure to organophosphate pesticides influencing the development of organs and the survival rate of silver rasbora fish larvae.

4.2. Suggestion

Suggestions can be given from this study is that the pesticide pollution threshold in the water is not more than 0.5 ppm.

5. References

[1] Sun, Z., Zhang, H., Wei, Z., Wang, Y., Wu, B., Zhuo, S., & Yang, H. 2018. Journal of Natural Gas Science and Engineering, 51, 27-36
[2] Shoaib, N., Siddiqui, P. J. A., & Ali, A. (2012). *PAK J ZOOL*, 44(2), 569–572.
[3] Setyawati, I., Wiratmini, N. L., & Wiryatno, J. (2011). *J BIOL*, 15(2), 44–48.
[4] Richendrfer, H., & Creton, R. (2015). *NEUROTOXICOLOGY*, 49, 50–58.
[5] Lutfiyah, L., Sulmartiwi, L., Rahardja, S. B., Budi, D. S., Wirawan, M. F. 2020. JOAS.5(2):1-8.
[6] Besson, M., Gache, C., Bertucci, F., Brooker, R. M., Roux, N., Jacob, H., Berthe, C., Sovrano, V., A., Dixson, D. L & Lecchini, D. 2017. Scientific Reports | 7: 9165 |
[7] Kendall, A. W. Jr. Ahlstrom, E. M., Moser, H. G. 1984. *Early Life History Stages of Fishes and Their Characters. Ontogeny and Systematics of Fishes*. Am Soc Ichthyol Herpetol Spec Publ No. 1. Allen Press. Lawrence. Pp 11-22.
[8] Blaxter, J and Hamming, S. 1988. Pattern and Variety in Development, 1-58 in Hoar, W. S dan D. J, Randall. 1969. *Fish Physiology*. Academic Press INC. London. 293-309. pp.
[9] Nacario, J. 1983. *AQUACULTURE*. 34:73-83.
[10] Arufe, M.I., J.M, Arellano., Letia, G., Gemma, A., C, Sarasquete. 2007. *AQUAT TOXICOL.*(84) : 328-336.
[11] Behra, M., Cousin, X., Bertrandc., Vonesch., Biellman, D., Chatonnet, A. 2009. *NAT NEUROCI* (5): 11-18
[12] Morison, C. M., T, Miyake dan J, R, Wright. 2001. *J MORPHOL*, 247: 172-195.
[13] May, C. R. 1974. *J EXP MAR BIOL ECOL*, 16: 213-225.
[14] Curtin, E., Hickey, G., Kamel, G., Davidson, A.J., Liao, E.C. 2011. MECH DEV (128) : 104-115.
[15] Jawad, L. A. 2004. *TUHINGA*, 15: 121-124
[16] Cook, L.W., Paradise, C.J., Lorn, B. 2005. *ENVIRON TOXICOL.*24(7):1745-1750.
[17] Parlak, V. 2018. *CHEMOSPERHE* (207): 397-403

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