Effect of sublethal dose organophosphate pesticides on embryogenesis and hatching rate of silver rasbora eggs (*Rasbora argyrotaenia*)

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Abstract. Silver rasbora is a fish that lives in freshwater, especially in river waters. However, the presence of silver rasbora fish is currently difficult to find due to environmental pollution. One of them is organophosphate pollution. This research aims to know how the effect of organophosphate pesticides with sublethal doses on embryo development and the hatchability of silver rasbora fish eggs. The research method was an experiment with a Completely Randomized Design as an experimental design. The treatments used were different organophosphate concentrations: 0, 0.5, 1, 1.5, and 2 ppm, each treatment repeated four times. The main parameters were embryo development and hatchability of silver rasbora fish eggs. Statistical analysis using Analysis of Variance and to find out the difference between one treatment and the other treatment is Duncan’s Multiple Range Test. The results showed that the treatment of different organophosphate concentrations had a significant effect (P <0.05) on the speed of embryo development and the hatchability of silverfish eggs. The fastest embryo development occurred in treatment two ppm and the slowest at treatment 0 ppm. The highest hatchability of silverfish eggs was found in treatment K (93%) and the lowest in treatment two ppm (19.5%), which was not significantly different from treatment 1.5 ppm (26%).

1. Introduction

Silver rasbora (*Rasbora argyrotaenia*) is a fish that lives in freshwater, especially in river waters [1]. Silver rasbora fish is also a fish resource that is in great demand in the market. The market demand for this fish is very high, so it is economically potential to be cultivated. So far, the supply of silver rasbora fish has only relied on catches from the wild so that the supply is often unstable. In addition, continuous
fishing of Silver rasbora fish in their natural habitat is able to threaten the sustainability and disrupt aquatic ecosystems. From the information available, currently, the existence of silver rasbora fish is increasingly difficult to find in nature, and its size is relatively small [2].

The use of pesticides in agriculture only functions 90%. The rest has polluted the surrounding environment, especially fish [3]. Organophosphate is an active neurotoxin. It doesn't even require any conversion to inhibit the enzyme acetylcholinesterase [4].

The determination of the amount of fish production comes from the success of the hatchery process, especially the process of hatching eggs [5]. However, due to environmental pollution can affect the production of fish eggs. The effect of pesticide on critical parameters, including deformation, mortality, and locomotor activity, were dependent on exposure time in subchronic exposures [6].

In our study, we conducted observations on embryogenesis and hatchability of silver rasbora fish eggs that were exposed to sublethal doses of organophosphate. The purpose of this study was to know the effect of sublethal doses of organophosphate on embryogenesis and the hatchability of silver rasbora fish eggs.

2. Material and methods

The tools used in this study are include aquariums, hatching racks, 5-liter jars, aeration hoses, aerator stones, alcohol thermometers, pH meters, DO meters. Dropper pipette, micropipette, tweezers, binocular microscope, concave glass object, and digital camera. The materials used are sterile silver rasbora fish eggs, water, kakaban made from palm fiber, label paper, microtip, and diluted organophosphate profenofos solution.

It was spawning female and male fish with a ratio of 2:1 in an aquarium that has been filled with water, aeration, and kakaban. After laying eggs, the eggs used are fertilized eggs. Water used in each treatment has the addition of organophosphate solutions with 5 treatment; 0, 0.5, 1, 1.5, and 2 ppm. The experimental container used was a 5-liter volume plastic jar of 20 pieces and was equipped with aeration.

3. Result and discussion

The process of embryo development and the length of time each phases of silver rasbora embryos exposed to different concentrations of organophosphates can be seen in Table 1. Embryonic development that can be seen from the results of observations starts from the cleavage and morula phases, blastula phase, gastrula phase, neurula, organogenesis phase. Finally, the eggs hatch into larvae.

The cleavage phase is characterized by the presence of blastomere cells formed, as in Table 1. The observations made can be seen that in the cleavage phase, treatment 2 ppm is 5 minutes faster than treatment 0.5 ppm. Treatment 0.5 ppm is 2 minutes faster than treatment 1 ppm, treatment 1 ppm 3 minutes faster than treatment 0 ppm, and treatment 1.5 ppm 5 minutes later than treatment 0 ppm.

In the next phase, the blastula phase can be seen in Table 1. The blastula phase is characterized by the formation of empty cavities [7]. The observations showed that the blastula phase of treatment 2 ppm was 3 minutes faster than treatment 0.5 ppm, while treatment 0.5 ppm was 3 minutes faster than treatment 1.5 ppm. Treatment 1.5 ppm was 12 minutes faster than treatment 1 ppm, and treatment 0 ppm was the slowest treatment with a difference of 3 minutes from treatment 1 ppm.

After the blastula phase, then the gastrula phase is continued, as in Table 1. According to [8] gastrulation is the process of embryonic development, where the organ cells that have been formed at the stage of blastula develop further. The results of the observation showed that treatment 2 ppm was the fastest treatment. Treatment 2 ppm was 21 minutes faster than treatment 1.5 ppm, while treatment 1.5 ppm was 5 minutes faster than treatment 1 ppm. Treatment 1 ppm was 3 minutes faster than treatment 0 ppm, and treatment 0.5 ppm was 1 minute later than treatment 0 ppm.

The neurula phase can be seen in Table 1. The neurula phase is characterized by nerves that begin to form in the midline of the embryo’s back, and the blastoderm covers around 4/5 yolk sacs [8]. Based on
observations on the neurula phase of treatment 2 ppm experienced a faster development of 6 minutes from treatment 1.5 ppm, while treatment 1.5 ppm was 6 minutes faster than treatment 1 ppm. Development of treatment 1 ppm was 4 minutes faster than treatment 0 ppm/liter, and treatment 0.5 ppm was 3 minutes later than 0 ppm treatment.

The organogenesis phase is an embryonic development phase that has a long enough time for the egg to hatch. In the opinion of [9], the organogenesis phase is the process of forming organs of living things that are developing. Based on observations on the organogenesis phase, treatment 2 ppm was 14 minutes faster than treatment 1.5 ppm, while treatment 1.5 ppm was 4 minutes faster than treatment 1 ppm. Development of treatment 1 ppm was 1 hour faster than treatment 0.5 ppm and treatment 0 ppm 20 minutes slower than treatment 0.5 ppm.

After passing through these phases, the silver rasbora eggs hatch into larvae. Eggs in treatment 2 ppm with organophosphate concentration of 2 ppm hatched 5 minutes faster than treatment 1.5 ppm, while treatment 1.5 ppm was 2 minutes faster than treatment 1 ppm. In treatment 1 ppm, only a difference of 1 minute was faster than treatment 0.5 ppm, and treatment 0 ppm was 2 minutes slower. From treatment 0.5 ppm.
| Embryogenesis          | Treatment and duration of embryo development phase |
|------------------------|---------------------------------------------------|
|                        | 0 ppm   | 0.5 ppm | 1 ppm | 1.5 ppm | 2 ppm |
| Cleavage Phase         |         |         |       |         |       |
|                        | 1 h 47 m| 1 h 42 m| 1 h 44 m| 1 h 52 m| 1 h 37 m|
| Blastula Phase         |         |         |       |         |       |
|                        | 3 h 39 m| 3 h 12 m| 3 h 27 m| 3 h 15 m| 3 h 9 m |
| Gastrula Phase         |         |         |       |         |       |
|                        | 6 h 47 m| 6 h 48 m| 6 h 44 m| 6 h 39 m| 6 h 18 m|
| Neurula Phase          |         |         |       |         |       |
|                        | 8 h 15 m| 8 h 18 m| 8 h 11 m| 8 h 5 m  | 7 h 59 m|
| Organogenesis Phase    |         |         |       |         |       |
|                        | 10 h 41 m| 10 h 21 m| 9 h 21 m| 9 h 17 m| 9 h 3 m |
| Larvae                 |         |         |       |         |       |
|                        | 25 h 20 m| 25 h 18 m| 25 h 17 m| 25 h 15 m| 25 h 10 m|

Table 1. Development of silver rasbora fish embryos and length of hatching time
Egg hatchability is calculated after all the eggs hatch. Hatching is an embryo that passes through several phases of development until the embryo exits its shell and becomes a larva. The results of this study can be seen in Table 2.

| Treatment | Hatching Rate (%) ± SD |
|-----------|------------------------|
| 0 ppm     | 93 ± 3.10              |
| 0.5 ppm   | 51 ± 4.35              |
| 1 ppm     | 29.5 ± 2.50            |
| 1.5 ppm   | 26 ± 2.16              |
| 2 ppm     | 19.5 ± 2.50            |

ANOVA test results showed that the treatment of different organophosphate concentrations in the hatching of silver rasbora fish eggs had a very significant effect (P < 0.01) on the hatchability of silver rasbora fish eggs. The test results showed that treatment 0 ppm produced the highest hatchability, which was significantly different from treatment 0.5 and 1 ppm. Treatment 1.5 ppm was not significantly different from treatment 1 ppm, and treatment 2 ppm had the lowest hatchability of eggs.

Organophosphates are a widely used class of neurotoxic pesticides. These pesticides work by inhibiting acetylcholinesterase (AChE) activity [10]. Organophosphates cause neurotoxicity at the zebrafish embryogenesis stage with effects on cell apoptosis activation in the brain [11]. At each of the phases showed that the highest concentration of treatment with a concentration of 2 ppm experienced phase changes and the fastest hatching. Osmotic pressure between the egg and the maintenance medium is higher, the metabolic energy needed for homeostasis will increase [12]. The entry of water into the egg is caused by differences in osmolarity and imbibition of proteins on the surface of the yolk [13]. When fish eggs are removed and fertilized, the permeability of plasma membranes plays an important role in overcoming changes in external conditions by regulating ions and water across the plasma membrane. According to [14], egg death can be caused by an embryo that is unable to maintain its osmotic control. [15] said that the unbalanced state of intracellular fluid would lead to plasmolysis. Plasmolysis will result in shrinkage due to the discharge of fluid from the egg to the outside, which in turn can cause egg death.

In addition, according to [16], the ability to detoxify or excrete toxicity correlates with the concentration of organophosphate, which causes a decrease in egg hatchability. The lower the organophosphate concentration, the higher the hatchability of the eggs and vice versa if the concentration of organophosphate is higher, the decreasing hatchability of the silver rasbora fish eggs. Organophosphate is able to inhibit the action of the enzyme acetylcholinesterase (AChE), which causes the disruption of acetylcholine in delivering impulse stimulation from pre-synapse to post-synapse (neurotransmitter) so that the work of the muscles becomes disrupted. Muscle work is not directed to cause symptoms of poisoning that affect the entire body [17].

4. Conclusion
Organophosphate pesticides can affect embryo development and the hatchability of silver rasbora fish eggs. The higher the concentration of organophosphate, the faster the hatching of eggs and hatchability of the eggs decreases. A dose of 0.5 ppm from organophosphate pesticides can affect embryo development and hatchability of silver rasbora fish eggs.

5. References
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