Expression of heat shock protein 70 (hsp70) in liver and kidney organ of silver rasbora (*rasbora argyrotaenia*) exposed by sublethal organophosphate pesticides

A N Fadilah¹, I Sulmartiwi², L Lutfiyah³*

¹Program study Aquaculture, Faculty of Fisheries and Marine, PSDKU Universitas Airlangga in Banyuwangi.
²Departmen of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, 60115 Indonesia.

Corresponding Author* : ninatullutfiyah@fpk.unair.ac.id

Abstract. *Heat Shock Protein* 70 (HSP70) is a stress protein that can appear in all cell types. HSP70 test is useful as a biomarker (biological response marker) that can be used as a stress marker in fish. Liver and kidney tissue, including types of organs that play an important role in metabolic processes in fish, make it possible for these tissues to respond to the presence of HSP70. This research aims to determine the effect of sublethal exposure to organophosphate pesticides on the expression of HSP70 in the liver and kidneys organ of Silver Rasbora, and to determine the comparison of HSP70 expression between the liver and kidneys organ of Silver Rasbora that exposed to sublethal organophosphate pesticides. This research was conducted in November 2020 - February 2021 using a completely randomized design (RAK) method consisting of 5 treatments and 4 replications. Each treatment consisted of a concentration of 0.001 ppm, 0.005 ppm, 0.01 ppm, 0.05 ppm and control treatment of 0 ppm. Silver Rasbora were acclimatized and maintained for 7 days and subjected to pesticide exposure treatment for the last 96 hours. The main parameters in this research were the expression of HSP70 in the liver and kidneys of Silver Rasbora and the supporting parameters observed were survival rate, temperature, pH, ammonia, and dissolved oxygen (DO). The main parameters were analyzed using the one-factor ANOVA test and Duncan's advanced test. Meanwhile, to determine the average difference between liver and fish kidneys, the independent sample T test was used. Supporting parameters were analyzed descriptively using tables and graphs. The results showed that exposure to sublethal organophosphate pesticides affected the appearance of HSP70 expression in the liver and kidneys of Silver Rasbora. The HSP70 value in both organs increased with increasing pesticide concentration. The liver organ has an average HSP70 value higher than that of the kidneys organ.
1. Introduction

The use of pesticides in agriculture only functions as much as 90%, the rest has polluted the surrounding environment, especially fish [1]. Organophosphate is an active neurotoxin, it doesn't even require any conversion to inhibit the enzyme acetylcholinesterase [2]. 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 that is not directed to cause symptoms of poisoning that affect the entire body [3].

HSP 70 is a group of HSPs found in abundance in organisms from bacteria to mammals, and their expression is markedly induced in response to environmental stressors, such as heat shock, UV and radiation, and chemical exposure [4]. The liver is the center of metabolism [5], The kidney organ acts as the main hematopoietic organ which also functions in the osmoregulation process [5]. Based on the above explanation, it is necessary to conduct research on sublethal exposure to organophosphate pesticides in stingrays, so that the expression of HSP70 in the liver and kidneys exposed to sublethal organophosphate pesticides can be used as a reference for detecting stress levels in fish.

2. Material and Method

2.1 Research methods and Design

The research method used 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; 0.50; 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 ppm, 0.5 ppm, 1 ppm, 1.5 ppm and 2 ppm with 4 replications. The research container was a 5-liter jar equipped 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

The main parameter in this study was the expression of Heat Shock Protein 70 (HSP70) in the liver and kidneys of silver rasbora fish (Rasbora argyrotaenia) exposed to sublethal organophosphate pesticides. Supporting parameters observed are survival rate (SR) and water quality data.
3. Result and Discussion

**Table 1.** Value HSP 70 in Kidney and Liver Organ of Silver rasbora fish

| Treatment | Liver          |           | Kidney         |           |
|-----------|----------------|-----------|----------------|-----------|
| P0        | 295.28± 15.26  |           | 202.09± 9.22   |           |
| P1        | 424.46± 48.96  |           | 204.11± 39.93  |           |
| P2        | 486.25 b ± 59.61|           | 242.44 a ± 12.09|           |
| P3        | 623.74 c ± 44.05|           | 355.70 b ± 61.62|           |
| P4        | 731.59 d ± 42.47|           | 398.35 b ± 83.12|           |

Note: Different superscripts in the same column show significant differences (p<0.05).

The results showed that the sublethal concentration of organophosphate pesticides had an effect on the HSP70 values of the liver and kidneys of silver rasbora fish. The highest average value of HSP70 in the liver was in treatment P4 with an average value of 731.59 ng/ml and the lowest average value in treatment P0 was 295.28 ng/ml. While in the kidney, the highest average value of HSP70 was in treatment P4 with an average value of 398.35 ng/ml and the lowest average value in treatment P0 was 202.09 ng/ml.

**Picture 1.** Grafik Survival Rate Silver Rasbora Fish

The survival rate of silver rasbora fish observed at 96 hours after exposure to sublethal doses of organophosphate pesticides showed that stingrays had a high survival rate of 100% in all treatments.

**Table 2.** Water Quality

| Parameter   | Value  |
|-------------|--------|
| Temperature | 27-28  |
| pH          | 7-8    |
| DO (ppm)    | 5-8    |
| Amonia (ppm)| 0-0.25 |
The expression of HSP70 in fish is a sensitive indicator of the cellular response to stressor exposure in the aquatic environment. HSP70 acts as a cell balancer when the internal and external conditions of the fish's body are unstable or experiencing stress due to stressors [6]. Organophosphate pesticides have a toxic action that is to inhibit the bond between acetyl and acetylcholinesterase (AchE). Organophosphates that enter the fish body will penetrate into the cells then the phosphorylated group of the organophosphate will bind to the acetylcholinesterase ester group by means of covalent bonds to form the organophosphate acetylcholinesterase complex, causing the accumulation of acetylcholine [1]. Accumulation of acetylcholine at the receptor can cause the diffusion of oxygen into the blood capillaries to be disrupted, resulting in a nervous imbalance and disrupting all cell activities. Cells that receive a stress response will directly form the HSP70 gene, but if this stress signal continues continuously then HSP70 will also continue to be produced so that the impact can cause damage to cells such as edema, hyperplasia, fusion, cell swelling and necrosis [7].

4. Conclusion
The HSP70 value in both organs increased with increasing pesticide concentration. The liver organ has an average HSP70 value higher than that of the kidneys organ.

5. Reference
[1] Shoaib, N., Siddiqui, P. J. A., & Ali, A. (2012). Pakistan Journal of Zoology, 44(2), 569–572.
[2] Setyawati, I., Wiratmini, N. L., & Wiryatno, J. (2011). JB, 15(2), 44–48.
[3] Richendrfer, H., & Creton, R. (2015). NeuroToxicology, 49, 50–58.
[4] Yamashita, M., Yabu, T and Ojima, N. 2010. Stress Protein HSP70 in Fish. Aqua-BioScience Monographs Vol. 3, No. 4, pp. 111–141
[5] Faheem, M., N. Jahan and K. P. Lone. 2016. J ANIM SCI, 26 (2) : 514-522.
[6] Rajeshkumar, S. and N. Munuswamy. 2011. CHEMOSHERE, 83: 415-421.
[7] Padrilah, S. N., S. A. Ahmad., N. A. Yasid., M. K. Sabullah., H. M. Daud., A. Khalid and M. Y. Shukor. 2017. ENVIRON SCI POLLUT RES, 24: 22510-22523.

6. Unknowledgement
The authors extend their gratefully acknowledge to Faculty of Fisheries and Marine Universitas Airlangga during college.