Toxicity of chlorpyrifos insecticide on Asian red-tailed catfish (Hemibagrus nemurus) and its degradation potency using activated carbon

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Abstract. The chlorpyrifos is an insecticide with high toxicity and persistence in nature. The activated carbon has the potency to degrade some insecticides through the adsorption process. The research aimed to determine the lethal toxicity (LC50-96 hours) of the chlorpyrifos insecticide on red tail fingerling. The fish with 2.5±0.2 cm in total length and 0.18 ± 0.002 g in body weight were used. The concentrations of the chlorpyrifos as a treatment were 0.0, 1.0, 1.4, 1.9, 2.7, 3.8, 5.6, and 7.5 mg/l. Three kinds of experiments were set up, namely, without activated carbon (as a control), coconut shell activated carbon, and wood activated carbon. Each treatment consisted of three replicates. The probit analysis performed to obtain the LC50-96 hours' value. The result showed that the LC50-96 hours of Asian catfish was 4.44 mg/l (without activated carbon), 4.84 mg/l (coconut shell activated carbon), and 8.52 mg/l (wood activated carbon). The effectiveness of activated carbon to reduce the toxic of the chlorpyrifos found on the wood activated carbon (91.9%) then followed by the coconut shell activated carbon was 9.0%. Thus the implementation of the wood activated carbon is fruitful in terms of reducing the chlorpyrifos toxic.

1. Introduction

The aquaculture of Asian red-tailed catfish (Hemibagrus nemurus) in Indonesia has mostly been cultured in the ponds and the floating net cages. The water source for aquaculture activity in Indonesia is mainly river, lake, and reservoir. However, the water source has been frequently threatened by many kinds of pollutants such as household, industrial and agricultural activities. For instance, an insecticide is one of the pollutants that derived from the agricultural activity, which frequently discharges into the surface water source via rain or surface runoff. Such pollutant harms aquatic organism, including fish. The negative impact of pesticide pollution is not only lethal to fish but also sub-lethal, which will affect growth and reproduction.

Insecticides should be the last alternative to control pest organisms, but in the field, reality shows that the use of insecticides is a real option. Almost all kinds of insecticide have a broad spectrum that has toxic chemical compounds as a source of pollution for fish and the environment [1].

Chlorpyrifos white crystalline shaped organophosphate insecticide that has a pungent odor will kill various types of aquatic organisms when it enters into the surface water body [2]. Spraying is the application of chlorpyrifos, in part or all of the insecticides will fall to the surface water. This matter is a source of pollution potential for aquatic environments. Organophosphate insecticide is one type of an insecticide that most commonly use. Data states, using it in the West Sumatera province dominated up to 99.8% [3]. Organophosphate insecticides work by blocking enzyme acetylcholinesterase. The
accumulation build-up acetylcholine led to chaos on a system's initial nerve impulses to the muscle cell, become muscle spasms, occurs paralysis, and then organisms will die [4].

The fingerling Asian catfish gill, kidney tissue, and physiological blood will damage due to the effect of agriculture pesticides [5]. Organophosphate insecticides parathion 25% harms survival and have changes of gill and heart and tissue histology on GiFT tilapias. The concentration of that insecticides 0.0005 ml\(^{-1}\) resulted 6.67% of survival rate.

The aims of this study were to determining the lethal toxicity (LC50-96 hours) of the chlorpyrifos insecticide on Asian red-tailed catfish fingerling and testing two kinds of activated carbon from coconut shell and wood concerning reducing its toxicity.

2. Materials and methods
The research was carried out from April to September in 2018 in the toxicology laboratory at Research and Development Station for Freshwater Aquaculture Environmental Technology and Toxicology, Cibalagung, Bogor, West Java, Indonesia.

2.1. Fish, test solution of chlorpyrifos insecticide and test facilities
Asian red-tailed catfish fingerling with a length of 2.5 ± 0.2 cm and a weight of 0.18 ± 0.002 g were used in this study. Fish introduced into the aquarium with a size of 80 x 50 x 40 in the laboratory for ten days in terms of fish adaptation. Aquades diluted the chlorpyrifos as a test solution. Natural solvents (acetone) were added for the homogeneity of the answer to the specified level. The basic test facilities consist of 21 glass aquariums, each measuring 40 x 20 x 20 cm, which are equipped with runoff pipes from PVC to facilitate the replacement of the test solution during testing. Each aquarium fitted with a replacement solution container with a size of 68 x 25 x 35 cm.

This research was conducted with a toxicity test method in a laboratory, which was technically and procedurally standardized [6]. This method was applied before and after the carbonation process test in 2 stages. The first stage was a preliminary test to determine the lethal concentration of the upper and the lower threshold of chlorpyrifos insecticide for Asian catfish fingerling. Next step was preparing six levels magnitude of insecticide concentration between two lethal thresholds mentioned, by geometric sequences based on the formula:

\[
\log (N / n) = K \log (a / n) \quad \text{................................. (1)}
\]
\[
a / n = b / a = c / b = d / c = e / d = f / e = g / f = N / g \quad \text{............................. (2)}
\]

Where:

\[
N = \text{Upper threshold concentration}
\]
\[
n = \text{Lower threshold concentration}
\]
\[
K = \text{Amount of concentration tested}
\]
\[
a, b, c, d, e, f, \text{and } g = \text{the concentration tested with the value as the smallest concentration.}
\]

As a control, water media is used without insecticides or other ingredients. For each test concentration above three test aquariums were used, each of which contained 10 test fish in 10 liters of the test solution. This advanced test is carried out for 96 hours. The death of the test fish was observed and recorded cumulatively after an exposure time of 24, 48, 72, and 96 hours. Test fish is declared dead if the movement of the operculum has stopped entirely. Dead fish are immediately taken from the aquarium so as not to pollute the test solution. The physical and chemical characteristics of the test solution must meet the criteria for fish toxicity testing, therefore during the tests, an examination of temperature, pH, dissolved oxygen, free carbon dioxide, total ammonia, nitrite, and nitrate was carried out.
The test data were analyzed by Probit Analysis Program Version 1.5, to obtain the Median Lethal Concentration (LC50) value, which is the concentration of insecticidal pesticides which killed 50% of the test fish at certain exposure times (24, 48, 72 and 96 hours), with an interval value of 95% confidence limit.

2.2. Activated carbon preparation
The material used for activated carbon were coconut shell and wood. The material was cleaned and cut off into slices with a 3cm diameter. 2.5 – 3.5 kg materials were put in a container and added 100 ml H$_3$PO$_4$ 20% then diluted by water until submerged. Stirred well and stored for 24 hours. The materials washed with water to remove dust and drained. A combustion in the furnace with a temperature of 900°C was conducted to get activated carbon. Furthermore, this material soaked in a solution of phosphoric acid for 24 hours. The phosphoric acid serves as cleaning the stick to a surface carbon and retaining heat propagator. After drained material is activated by the water vapor heat flow at a temperature of 900°C for 60 minutes in an electric furnace equipped with a thermocouple as temperature control, the product is assumed as pure activated carbon (100%).

2.3. Potential degradation
Activated carbon is solid carbon, which has a reasonably high surface area ranging from 100 - 2000 m$^2$/g. This condition is because these substances have very complex pores that range from micro sizes below 20 A, meso sizes between 20-50 A and macro sizes of more than 500 A the activated carbon is an extensive adsorption material, so that has the potential to reduce the toxicity of insecticide through the adsorption process.

3. Results and discussion
Preliminary test results revealed that the upper threshold concentration (N) and lower threshold concentration (n) in chlorpyrifos insecticide ranged from 1 to 10 mg/L. From the range with equations 1 and 2, there are 6 concentrations for the follow-up test whose magnitude is between the lethal concentration of the lower threshold and the threshold, namely 0, 1.4, 1.9, 2.7, 3.8, 5.4 and 7.5 mg/L. After 24, 48, 72, and 96 hours of exposure, the cumulative fingerling mortality was obtained for each chlorpyrifos concentration (Table 1).

Table 1. The mortality of Asian red-tailed catfish fingerling on lethal toxicity test of chlorpyrifos insecticide during the observation time.

| Test concentration (mg/L) | Number of fish (fish) | Mortality (%) / exposure period (h) |
|--------------------------|-----------------------|------------------------------------|
|                          |                       | 24   | 48   | 72   | 96   |
| 0                        | 30                    | 0    | 0    | 0    | 0    |
| 1.4                      | 30                    | 0    | 0    | 0    | 0    |
| 1.9                      | 30                    | 0    | 0    | 0    | 3.33 |
| 2.7                      | 30                    | 0    | 0    | 6.67 | 16.67|
| 3.8                      | 30                    | 0    | 6.67 | 13.33| 26.67|
| 5.4                      | 30                    | 20   | 23.33| 46.67| 56.67|
| 7.5                      | 30                    | 93.33| 100  | 100  | 100  |
The data in Table 1 showed that the mortality of Asian red-tailed catfish fingerling increased with increasing chlorpyrifos concentration and lead to the duration of the exposure period. Mortality reached 100% at a concentration of 7.5 mg/L in 48 hours, while at a concentration of 5.4 mg/L was only 56.67% at the exposure period of 96 hours. This condition is presumably because the most sensitive bioactivity of the activated ingredient of chlorpyrifos in Asian red-tailed catfish occurs in the range of concentrations of 3.8 - 7.5 mg/L so that any increase in concentration will have a significant impact on increasing mortality.

Chlorpyrifos belongs to the organophosphate insecticide that works to inhibit cholinesterase enzyme (AchE) and has the potential to disrupt species behavior, which is likely to be an effort to survive [7]. The death of fish due to exposure to insecticides is caused by the inclusion of insecticides through gills. Furthermore, it will enter the blood vessels, and interfere with the work of nerve regulating enzymes and cause the nerves to work without being controlled so that it will cause death in fish [4, 8]

The regression equation is used to describe the relationship between chlorpyrifos concentration and the percentage of Asian red-tailed catfish fish mortality. Based on the regression equation, it turned out that linear regression and logarithms had robust correlations. Logarithm model, the response of living things, shows the relationship of fish growth characteristics, where the higher the concentration of chlorpyrifos insecticides, the higher the percentage of fish mortality (Figure 1).

![Figure 1. Linear regression of chlorpyrifos concentration on mortality of Asian red-tailed catfish fingerling](image)

It is seen that there was a definite positive correlation between the concentration value of chlorpyrifos insecticides and the percentage of fingerling mortality, as evidenced by the value $R^2 = 0.9831$ (close to 1). So the higher the concentration of chlorpyrifos given, the higher the percentage of fingerling mortality. On the contrary, the lower concentration of chlorpyrifos insecticide given, the lower the percentage of death of fingerling. There was a positive correlation between the concentration of pollutants (chlorpyrifos) and the death percentage of test animals [9]. The relationship of this positive correlation is caused by the toxicity of the chlorpyrifos insecticide at a certain concentration level, which can kill the
fish. The concentration of chlorpyrifos insecticide 0.0005 ml/L resulted in a low tilapias survival rate, which only 6.67%, whereas without chlorpyrifos was 100% [10].

The results of fish mortality test analysis for each test dose at each time of observation with the Probit analysis revealed that lethal toxicity values (LC50) of chlorpyrifos insecticide on Asian red-tailed catfish fingerling presented in Table 2.

**Table 2. Lethal toxicity test of chlorpyrifos insecticide on Asian red-tailed catfish fingerling**

| Exposure time (h) | LC50 (mg/L) | 95% Confidence limit | Estimated probit line |
|-------------------|-------------|----------------------|----------------------|
| 24                | 6.08        | 5.72 – 6.47          | $Y = 16.50X - 7.93$  |
| 48                | 5.67        | 4.43 – 7.16          | $Y = 12.36X - 4.31$  |
| 72                | 5.01        | 4.59 – 5.49          | $Y = 7.81X - 0.46$   |
| 96                | 4.44        | 4.01 – 4.95          | $Y = 5.72X + 1.30$   |

In contrast to the mortality of Asian catfish, the LC50 value of chlorpyrifos insecticides is getting smaller with increasing concentration and increasing exposure time (Table 2). The LC50-96 hours of chlorpyrifos against the Asian catfish fish seed is 4.44 (4.01 - 4.95) mg/L with an estimated probit line $Y = 5.72 X + 1.30$. According to the rank of lethal toxicity of chlorpyrifos included in group B, which is highly toxic pesticides with a value of LC50-96 hours 1-10 mg/L [11].

The values of LC50-96 hours of chlorpyrifos insecticide on carp were equal to 0.03 mg/L and for tilapia by 0.08 mg/L [12]. The difference in toxicity value due to the effects of pollutants on organisms depending on species, sex, and stage of the fish life cycle [13]. The longer the exposure time will cause higher toxicity [14].

Organophosphate insecticides work to inhibit cholinesterase (AchE) enzymes and have the potential to disrupt species behavior, which is likely to be an attempt to survive [7]. Symptoms of organophosphate insecticide poisoning develop during or after exposure that takes place in minutes to hours, depending on the method of exposure [15]. Increased production of mucus in the gills and skin is a reaction of fish bodies to avoid the adverse effects of pollutants that enter the body. But excessive mucus production can threaten fish life because it will inhibit the process of exchanging oxygen and CO2 in gills [16].

### 3.1. Potential degradation

The potential for degradation will certainly be significantly influenced by the type of material and the adsorption time, which can be seen from the increase in the value of LC50-96 hours (Figure 2).
Figure 2. The mortality of Asian red-tailed catfish on the exposure to the different concentration levels of chlorpyrifos at different activated carbon (Description: A) coconut shell and B) wood)

Table 3 shows the degradation test of wood activated carbon on chlorpyrifos insecticide, and there was an increase in the value of LC50-96 hours for the activated carbon in Asian red-tailed catfish.
Table 3. Chlorpyrifos lethal toxicity (LC50-96 hours) degradation on Asian red-tailed catfish through the adsorption process using activated carbon.

| Activated carbon | LC50-96 hours (mg/L) | 95% Confidence limit | Estimated probit line |
|------------------|----------------------|----------------------|-----------------------|
| Control          | 4.44                 | 4.01 – 4.95          | Y = 5.72 X + 1.30     |
| Coconut shell    | 4.84                 | 4.45 – 5.27          | Y = 8.06 X + 1.09     |
| Wood             | 8.52                 | 7.42 – 10.36         | Y = 4.15 X – 0.66     |

The LC50-96 hour values of chlorpyrifos insecticide on Asian red-tailed catfish were different between before and after the adsorption process where the value increases. This condition happens because activated carbon can adsorb chemicals dissolved in water. The toxicity of chlorpyrifos to Asian red-tailed catfish can be reduced through the adsorption process using activated carbon, where the potential for reduction can be seen from the increase in the value of LC50-96 hours. From the two types of activated carbon used, the material derived from coconut shell could increase the LC50-96 hours of chlorpyrifos to Asian red-tailed catfish from 4.44 mg/L to 4.84 mg/L or increase by 9.1%. On the contrary, carbon activated wood products can increase the value to 8.52 mg/L (91.9% increase) (Figure 3). This matter shows that wood activated carbon was able to reduce the toxicity of chlorpyrifos to Asian red-tailed catfish up to 91.9%, where this potential is 10 times better than activated carbon from coconut shells. Active carbon was very effective in reducing (95%), infiltrating (73%), and loading reduction (99%) of chlorpyrifos toxicity while reducing, infiltrating, and loading reduction by composites were 18, 67, and 77%, respectively [17].

Figure 3. LC50 (mg/L) values by using wood and coconut shell activated carbon based on observation time.

4. Conclusion
The result showed that the LC50-96 hours of Asian catfish was 4.44 mg/L (without activated carbon), 4.84 mg/L (coconut shell activated carbon), and 8.52 mg/L (wood activated carbon). The effectiveness of activated carbon to reducing the toxic of the chlorpyrifos was found on the wood activated carbon (91.9%) then followed by the coconut shell activated carbon was 9.0%. Thus the implementation of the wood activated carbon is beneficial in terms of reducing the chlorpyrifos toxic in the water.
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