Median Lethal (LC50-48) with Azadirectha Biopestisides in Post Larva Tiger Shrimp (Penaeus monodon) in Low Salinity

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Abstract. Abstract. The agricultural system in Indonesia is now more advanced by combining agriculture and fisheries on the same land, so research like this is carried out. This study aims to investigate the lethal concentration (LC50-48 hours) of the active ingredient biopesticide Azadirectha on tiger shrimp at the post-larvae stage, and to analyze the effect of Azadirectha biopesticide on the behavior of tiger prawn post larvae. The concentration of biopesticides used in the main test was determined from the results of 3 preliminary tests with an upper threshold value of 90,000 ppm and a lower threshold value of 30,000 ppm. Five biopesticide concentrations were used for the main test, i.e. 37,372 ppm, 46,555 ppm, 57,994 ppm, 72,244 ppm, 89,994 ppm, and control. The data analysis technique for LC 50 uses probit analysis. The results obtained by the lethal concentration (LC50-48 hours) with Azadirectha biopesticide on mortality of tiger shrimp post-larvae were 54,719.57 ppm, the effect of biopesticides on post larvae behavior of tiger prawns at a concentration of 40,000 ppm only stayed at the bottom and even died while with a maximum biopesticide. At the concentration of 37,372 ppm, the shrimp showed active behavior, making it a safe concentration limit for the shrimp to sustain a living.

1. Introduction

Tiger prawns (Penaeus monodon) are the main commodity of brackish water, which makes a significant contribution to increasing local revenue in South Sulawesi. The failure of tiger prawn culture and in hatchery units is generally caused by disease. Shrimp disease can be caused by infectors, environment, nutrition, density factors, and mismanagement at both the hatchery and cultivation levels. The mechanism of disease development generally depends on the environment and the organism's defense system. The bad environment provides an opportunity for pathogenic infectors to develop. In the aquaculture sector, the presence of pathogenic bacteria is greatly feared by many fish, shrimp, and shellfish farmers. Because these microorganisms can threaten and even cause mass death in fish and shrimp. This of course will be very detrimental to the fisheries sector and can also threaten human health [1].

One of the optimizations of the potential of irrigated rice fields and increasing farmers' income is by engineering the land with appropriate technology. The way that can be done is by changing the
agricultural strategy from a monoculture system to a diversified agricultural system, for example by applying the cultivation of saltwater cultivation technology.

To keep the shrimp safe from mixing with harmful pesticides and to be environmentally friendly, the use of chemical pesticides has switched to using biopesticides. In general, biopesticide is defined as a pesticide whose basic ingredients are plants. When applied, biopesticides will repel pests immediately and after the pest dies, the residue will disappear in nature. *Azadirachta indica* is a plant source of pesticides (biopesticides) that can be used for environmentally friendly pest control. One of the natural ingredients that can be used to treat vibriosis attack is neem (*Azadirachta indica*). Neem plants grow a lot in the land tropical and sub-tropical climates. Neem is a plant multifunctional, therefore this plant is also known as the Wonderful tree [2].

The term biopesticide consists of three syllables, bio, pest, and sida. Bio means life. Pest means pests or disturbing organisms that can be disease or even cause death. Sida means murder. So biopesticides can be defined as all biological materials, whether in the form of plants, animals, microbes, or protozoa that can be used to destroy pests and disease-causing humans, animals, and plants. In Indonesian terms, experts in this field often refer to it as biological control agents. The advantages of biopesticides have a good impact on the environment with the same function, and biopesticides can also be added with an active ingredient called *Azadirachta* to improve the quality of these pesticides. Therefore, there is nothing wrong with farmers switching to biopesticides [3].

*Azadirachta indica* is a plant source of pesticides (biopesticides) that can be used for environmentally friendly pest control because its use as a biopesticide does not cause harmful impacts of pollution. Azadirachtin, which is contained in neem seeds and leaves, is effective as an insecticide. Neem seeds that are 3-8 months old have the highest azadirachthin content which acts as a repellent, which causes insect pests to be reluctant to approach the substance [4].

2. Materials and methods

2.1. Description.

The term pesticide only appeared after the development of the agrochemical industry in Europe and America which produced synthetic chemical fertilizers and pesticides. Although actually poisonous substances work very dangerous and dangerous directly, but among traditional farmers it is called medicine. Then pesticide products with active ingredients derived from living organisms, termed biopesticides.

Biopesticides are also termed biorational pesticides. That is, it does not result in the total eradication of the existing pest population and other organisms that are not the target of treatment. The United States environmental protection agency (US-EPA) has divided them into three broad groups. This sorting has become a reference for many other institutions in the world, including the World Agriculture and Food Agency. (Figure 1).
2.2. Method

This research was conducted from February - April 2020. The research was conducted at the Research Institute for Coastal Aquaculture and Fisheries Extension, Maros. This study used PL 14 tiger prawn post larvae. In this study, first, the water salinity of tiger prawns was acclimatized to 10 ppt using formula [1].

The bio pesticides used are of the weak category. Based on the active ingredients, neem seeds and leaves contain azadirachtin, salanin, meliantriol, nimbin, and nimbidin, which are secondary metabolites of the neem plant. Neem plant active compounds do not kill pests quickly but interfere with pests during the feeding process, growth, reproductive power, molting, inhibit marriage and sexual communication, decrease egg hatchability, and inhibit chitin formation. Bio pesticide production of Balingtan has been tested with a concentration of 10,000 ppm which is sufficient to repel pests in rice plants (Indonesian Agricultural and Environmental Research Institute, Pati-Semarang).

This research is quantitative research with experimental methods. The research design used was a completely randomized design. This research was conducted in 2 stages, namely the preliminary test to find the concentration range used for the main test and the main test wherefrom the results of preliminary test 1 to preliminary test 3 can be determined the threshold for the concentration of biopesticides that can kill 100% post tiger shrimp larvae that is 90,000 ppm and the lower threshold of biopesticide concentration that does not exist lethal post-larvae of tiger prawns, namely 30,000 ppm, then proceed with determining the concentration of biopesticides for the main test using formula [2].

Acclimatization of test animals

Prior to testing, the test animals with shrimp were acclimatized for 5 days. This is so that the test animals can adapt to different environmental conditions from the original conditions. At the acclimatization stage, the mortality of test animals should not be more than 10%. This is based on the requirements of test animals in healthy conditions [5]. The formula used to dilute seawater into brackish water is:

\[ V_1 \times N_1 = V_2 \times N_2 \] (1)

Acclimatization is carried out in an aquarium filled with sea water, which salinity is lowered every day, aerated continuously. During the acclimatization stage, the test animals were fed every morning and evening. The food given is natural feed and artificial feed. Natural feed, namely cultured artemia, and artificial feed, namely fine pellets.

The stage of giving biopesticides

Each jar is filled with 2 liters of water with a salinity of 10 ppt. Each jar is given the code K, A, B, C, D, E with (three) 3 replicated. The jar that has been filled with brackish water is inserted into the tiger shrimp post. Furthermore, Azadirachta biopesticide was given according to the concentration to be tested.

Preliminary test

A preliminary test was performed to determine the upper threshold concentration (LC100-48hr) and the lower threshold concentration (LC0-48hr). The test animals used in the preliminary test were 50 in each container with a water volume of 2 liters. In the preliminary test, the upper threshold concentration (N) and the lower threshold concentration (n) were determined. the upper threshold
concentration (N) is the lowest concentration of the toxicant that causes the whole test animal to die at 48 hours, while the lower threshold concentration (n) is the highest concentration of the non-fatal toxicant in the test animals at the exposure time of 48 hours. After the preliminary test was carried out, the highest and lowest concentrations were determined for the main test. The concentration interval for contamination (k = 5) was determined using the equation [6].

**Main Test**

The concentration of biopesticides used for the main test was determined from the results of 3 preliminary tests with a threshold value of 90,000 ppm and a lower threshold value of 30,000 ppm. The formula used to determine the concentration of biopesticide in the main test is:

\[ \log \frac{N}{n} = k \log \frac{a}{n} \]  \hspace{1cm} (2)

\[ \frac{a}{n} = \frac{b}{a} = \frac{c}{b} = \frac{d}{c} = \frac{N}{e} \]  \hspace{1cm} (3)

Information: N: The highest concentration
n: The lowest concentration
k: The number of concentrations tested
a, b, c, d and e: Concentrations between the lowest concentration and the highest concentration

**Observation of the behavior and survival of the test animals**

Behavior observations were carried out within 15, 30, 60, 120, 240, 480, 960, 1440 and 2880 minutes [5] Observation of the viability of the test organisms and to see the behavior of the test animals after contamination and to find out how big the mortality rate of the test organisms at certain exposure times [6]

**Statistic analysis.**

Determination of LC-50 using probit analysis. Probit analysis is an analysis that can predict lethal concentrations that can kill 50% of the test animals (post tiger shrimp larvae). The relationship between the logarithmic value of the test concentration and the probit value of the percentage of animal mortality is a linear function of \( y = a + bx \). The value of LC50- 48 hours is obtained from the antilog test value of m. The value of m is the value of x in the equation and the value of y is the probability of mortality of 50%.

3. **Results and Discussion**

The results of measurements of the physicochemical variables of water on the maintenance media during the study are presented in Table 1.
Table 1. Range of measurement results for physical and chemical parameters on the test medium

| COD | Parameter | Salinity | DO (ppm) | Temperatur(℃) | pH | NH₃ | NO₂ | Alkalinity |
|-----|------------|----------|----------|---------------|----|-----|-----|------------|
| K   |            | 9 - 10 ppt | 4.05 – 5.30 | 28 - 31.7 | 6.74 – 7.45 | 0.0156 | 0.0019 | 88         |
| A   |            | 9 - 10 ppt | 4.46 – 5.00 | 28.9 – 31.3 | 6.78 – 7.34 | 0.0181 | 0.0061 | 88         |
| B   |            | 9 - 10 ppt | 4.60 – 5.21 | 28.7 – 31.1 | 6.45 – 7.41 | 0.0576 | 0.0061 | 88         |
| C   |            | 9 - 10 ppt | 4.54 – 5.18 | 27.9 – 31.3 | 6.41 – 7.75 | 0.1749 | 0.0353 | 98         |
| D   |            | 9 - 10 ppt | 4.66 – 5.13 | 27.4 – 31.4 | 6.51 – 7.38 | 0.2379 | 0.2036 | 100        |
| E   |            | 9 - 10 ppt | 4.45 – 4.86 | 28.8 – 31.4 | 6.58 – 6.98 | 0.4148 | 1.1023 | 102        |

Probit on the graph indicates the percentage transformation of post larvae mortality of tiger prawns while linear (probit) is a straight line that describes the relationship between variables. The results of the probit analysis are presented in Figure 2.

Figure 2. Curve linear regression equation between logarithm of biopesticide concentration and probit% mortality of tiger shrimp post larvae.
Figure 3. Post larva mortality of tiger prawns in the main test.

3.1. Preliminary Test 1
The test results showed that with a concentration of up to 1000 ppm post tiger prawn larvae did not experience death. Meanwhile, concentrations of 10,000 ppm and above post larvae experience death. Post larvae of tiger prawns are still actively moving up to a biopesticide concentration of 10,000 ppm. Shrimp response with a concentration of 100 ppm to 10,000 ppm of shrimp remained active in swimming. While the response of shrimp with a concentration of 100,000 ppm and 100,000 ppm of shrimp is just at the bottom are they limp. Preliminary tests conducted on the effect of biopesticides on mortality of tiger shrimp post larvae showed that the higher the concentration of biopesticides given, the higher the mortality of tiger prawns post larvae for 48 hours. The mortality of post larvae of tiger prawns is suspected because the shrimp's body is exposed to many of biopesticides. This is consistent with the statement of Sprague [7] which states that the effect of pollutants on organisms depends on the concentration and duration of exposure to the pollutants.

3.2. Preliminary Test 2
The test results showed that with a concentration of up to 20,000 ppm post tiger prawn larvae did not experience death. Meanwhile, concentrations of 40,000 ppm and above post larval experience death. Post larvae of tiger shrimp are still actively moving up to a concentration of 20,000 ppm of biopesticide. The response of shrimp at a biopesticide concentration of 5000 ppm to 20,000 ppm was still active in swimming. Meanwhile, the biopesticide concentration of 40,000 ppm to 80,000 ppm the response of the shrimp is no longer active; it just stays on the bottom. From the observations it can be concluded that the greater the provision of high biopesticide concentrations is very influential on mortality and post larva behavior of tiger prawns. The length of time exposed to biopesticides exposed to the body of the tiger prawns will result in the test animals being silent and even resulting in death. The death of tiger prawns occurs because they are continuously in the body of tiger prawns to absorb toxins and this is very disturbing to the life of tiger prawns. The result is damage to skin tissue which can lead to secondary infection [8].
3.3. Preliminary Test 3
The preliminary test results are shown in Table 4.3. The test results showed that with a concentration of up to 30,000 ppm post tiger prawn larvae did not experience death. Meanwhile, concentrations of 50,000 ppm and above post larvae experience death. Post larvae of tiger shrimp are still actively moving up to a biopesticide concentration of 30,000 ppm. The response of the test animals at a biopesticide concentration of 10,000 ppm and 30,000 ppm was seen to be active, while starting with a biopesticide concentration of 50,000 ppm the shrimp were seen to be inactive and only stayed on the bottom. From the observations during the preliminary test 1 to 3 it can be concluded that the post response of tiger prawn larvae begins to be inactive from a concentration of 40,000 ppm. From the mortality value obtained through this preliminary test, then a lower threshold that does not cause death in test animals is determined for 48 hours and an upper limit that causes 100% death in test animals for 48 hours [9].

3.4. Main Test
The test results showed that with a concentration of 40,000 ppm and above, the post larvae experienced death. Post larvae of tiger prawns are not actively moving, their behavior is only at the bottom. Following is Figure 4.

![Figure 4. Post larva mortality of tiger prawns in the main test](image)

a. Mortality
From the results of the observations made by giving biopesticides, there was an effect on the tested animals. At a biopesticide concentration of 37,372 ppm the mortality rate reached 2% to 4%, At a biopesticide concentration of 46,555 ppm the mortality rate reached 20% to 30%, At a biopesticide concentration of 57,994 ppm the mortality rate reached 56% to 60%, At a biopesticide concentration of 72,244 ppm the mortality rate reached 76% to 84%. At a biopesticide concentration of 89,995 ppm, there were 100% deaths. The increase in the number of mortality is in line with the increase in the amount of exposure to the concentration of biopesticides given and the length of time. In the control treatment, the test animals did not experience death because the test animals were not exposed to biopesticides. The results of the 3 main tests and the main test can be concluded that the post larvae of tiger prawns experience a 100% mortality rate with a concentration of more than 90,000 ppm. According to Effendi [6], the longer the exposure time of a toxicant to the following test animals, the higher the
dose when exposed will have an effect on the test animal, one of which is death. The death of tested animals in the acute toxicity test or definitive test is caused by the entry of biopesticide exposure into the body's tissues [10]. The higher the concentration of biopesticides dissolved in the test animals, the lower the survival rate of the tested animals because high concentrations of pesticides cause damage to the gills and organs associated with the gills. This results in disruption of the respiration process and ultimately leads to death [11]. The process of mortality begins with changes in behavior such as from normal movements to erratic movements and finally the fish die [12]. Post larvae mortality of tiger prawns is thought to be because the shrimp's body absorbs water containing a lot of biopesticides which causes cell breakdown and interacts with proteins and semi-permeable membranes [13]. In addition, death can also be caused by difficulty in breathing due to damage to the epithelium of the gills which results in damage to gill function [13, 14].

b. Behavior

From the observation that the concentration of biopesticide 37,372 ppm remains active, which means that at this concentration the shrimp can still receive biopesticide, while the concentration of biopesticides is 46,555 ppm, 57,994 ppm, a concentration of 72,244 ppm, 89,995 ppm looks just at the bottom. The results of 3 preliminary tests and main tests can be concluded that post larvae of tiger prawns experience active behavior when given a biopesticide concentration of less than 40,000 ppm, while post larvae of tiger shrimp experience silent behavior and even die when given a concentration of more than 40,000 ppm. Some post larvae of tiger prawns experience molting at concentrations of less than 40,000 ppm as long as they are exposed to biopesticides as their growth is ready to change hormones for molting. The molting event in shrimp is considered a normal process. In simple terms, molting can be said as the process of changing the shells when the shrimp are in their growth period. In this phase, the size of the shrimp meat increases while the outer shell does not increase in size, so that for adjustment the shrimp will release the old shell and form a new shell. Post larvae of tiger prawns given more biopesticide doses can be seen by looking at their behavior such as loss of balance, uncontrolled movements followed by changes in body posture that are not normal, just sitting at the bottom and paralyzed. This behavior is thought to be an adaptation pattern to minimize the toxic biochemical processes that have been absorbed into the body of tiger shrimp post larvae, so that they can survive or slow down the lethal effects. As research has been carried out, post larvae of tiger prawns appear to be active when given biopesticides with the active ingredient azadirachta, but if the concentration is too excessive, the shrimp's life will be disrupted and cause the shrimp's death. According to Martini [15], tested animals that are exposed to toxic or pollutant power can be seen from their movements which are paralyzed, still and then die. Clinically, animals contaminated with poison showed symptoms of stress when compared to controls, characterized by decreased appetite, less stable movements, and tended to be at the bottom.

Biopesticides

From the observation, the biopesticide used with a biopesticide concentration of 37,372 ppm is still safe for tiger shrimp post larvae, while biopesticide concentrations above 40,000 ppm will disrupt the grow of tiger shrimp post larvae. From the observations, the color of *Azadirachta* biopesticide has a greenish brown color, so that the more concentrations of biopesticides are given, the more dark brown the water is. Neem leaves (*Azadirachta*) contain the active ingredient tannins which can produce pigments from the neem plant. The following is the color of the water at each concentration in the main test (Figure 5).
4. Conclusion.

1. The lethal concentration (LC50) with biopesticide with the active ingredient *Azadirachta* on mortality of tiger shrimp post larvae is 54,719.57 ppm. From the results of observations with a biopesticide concentration of 30,000 ppm, there was no mortality of tiger shrimp post larvae and postal response of active tiger prawns.

2. The effect of biopesticides on post larvae of tiger prawns with a concentration of 40,000 ppm of biopesticide post larvae of tiger prawns experiences a behavior that is only silent on the bottom and even dead, while with a maximum concentration of biopesticide 37,372 ppm post tiger prawn larvae experience active behavior.

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