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TOXICITY EVALUATION OF IODINE DISINFECTANT INCLUSION ON MORTALITY AND GROWTH OF *Artemia franciscana* NAUPLII

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Abstract

Artemia franciscana nauplii is a source of nutrients and pathogen vectors due to the non-selective nature of the filter feeder. Prevention of pathogens is done by adding iodine disinfectant to suppress the emergence of bacteria. One way to find out if a substance is toxic is by testing the toxicity and lethal concentration (LC50) on *A. franciscana* nauplii. The aim of this study was to evaluate the disinfectant effect of iodine and determine the lethal concentration of iodine in 50% of *A. franciscana* nauplii (LC50). Brine Shrimp Lethality Test (BSLT) method using *Artemia* as test animal. The results of the toxicity test with several doses of iodine showed that the toxic threshold value for 96 hours was 20.31 ppm. The results were supported by the presence of stunted growth at a dose of 100 ppm by measuring the length and weight. The conclusion of this study is that the administration of iodine at several concentrations is toxic to *A. franciscana* nauplii if it exceeds 20.31 ppm and affects the growth of *A. franciscana* nauplii.

***Keywords**: Artemia franciscana, Growth, Iodine, Nauplii, Toxicity*

Abstrak

Naupli Artemia franciscana menjadi sumber nutrisi dan vektor patogen dikarenakan adanya sifat non selective filter feeder. Pencegahan patogen dilakukan dengan penambahan disinfektan iodin untuk menekan munculnya bakteri. Salah satu cara mengetahui suatu bahan bersifat toksik dengan uji toksisitas dan tingkat lethal concentration (LC_{50}) pada naupli A. franciscana. Tujuan penelitian ini adalah mengevaluasi efek disinfektan iodin dan menentukan konsentrasi iodin yang mematikan 50% naupli A. franciscana (LC_{50}). Metode Brine Shrimp Lethality Test (BSLT) dengan menggunakan Artemia sebagai hewan uji. Hasil penelitian uji toksisitas dengan beberapa dosis iodin didapatkan nilai ambang batas toksik selama 96 jam adalah 20,31 ppm. Hasil didukung adanya pertumbuhan terhambat pada dosis 100 ppm dengan mengukur panjang dan berat. Kesimpulan penelitian ini adalah pemberian dosis iodin pada beberapa konsentrasi bersifat toksik pada naupli A. franciscana apabila melebihi 20,31 ppm dan berpengaruh terhadap pertumbuhan naupli A. franciscana.

***Kata Kunci**: Artemia franciscana, Iodin, Naupli, Pertumbuhan, Toksisitas*

1. INTRODUCTION

Artemia franciscana nauplii is often used as natural food for shrimp and fish larvae due to its high nutritional content (Hiola *et al*., 2014). These nutrients are obtained from the characteristics of *Artemia* as a non-selective filter feeder, which means it does not select food and consumes anything that enters its mouth (Mohebbi *et al*., 2015). In addition to the nutrients present in *A. franciscana* as feed for shrimp larvae, this species can also be a vector for the spread of pathogens in shrimp larvae (Haq *et al*., 2012). Alternative prevention of pathogens can be done by giving disinfectants to suppress the emergence of bacteria, viruses, or other diseases, such as iodine. It is possible that adding iodine to *Artemia* with increasing iodine levels can reduce bacterial levels (Hawkyard *et al*., 2011).

The use of iodine disinfectant in aquaculture can be toxic if used in large quantities and have negative effects on the environment. The presence of these chemicals can also affect the health of aquatic organisms and can cause death (Yun *et al*., 2020). One way to find out if a material is toxic is by using a toxicity test. The principle of the test is to determine the toxic effect of a substance given in a short time and in a certain dose (Jelita *et al*., 2020). In addition to knowing the toxic effect of a substance, a toxicity test also needs to be carried out to determine the level of mortality or lethal concentration (LC_{50}) in *A. franciscana* nauplii after being given an iodine disinfectant. The aim of this study was to evaluate the effect of exposure to iodine disinfectant and determine the lethal concentration of iodine in 50% of *A. franciscana* nauplii (LC_{50}).

2. RESEARCH METHOD

2.1 Research Time and Place

This research was conducted in April-July 2022. A toxicity test of iodine disinfectant on *A. franciscana* nauplii was conducted at the Aquaculture and Anatomy Laboratory of the Faculty of Fisheries and Marine, Airlangga University, Surabaya. Measurement of the length and weight growth of *A. franciscana* nauplii was carried out at the Laboratory of Microbiology and Fish Diseases, Faculty of Fisheries and Marine, Airlangga University, Surabaya.

2.2 Water Preparation

Water preparation in this study started with sterilize filtered seawater in a culture bath that is given aeration and given chlorine at a dose of 20 ppm for one day. Then, 10 ppm sodium thiosulfate was added with an interval of a few minutes to neutralize (Trisnabatin *et al*., 2021).

2.3 *Artemia franciscana*

A. franciscana was obtained from commercial *Artemia* cysts from Great Salt Lake, USA. Cyst density 2 g/L in a glass bottle filled with seawater and given strong aeration and bright lighting. The cysts are hatched by the decapsulation process. The decapsulation process involves hydration, i.e., removal of the cyst from the chocolate shell in a chlorine solution. The hydrated cysts were drained and put into the decapsulation solution for 15 minutes (for 2 grams of cysts dissolving 10 mL of 10% NaOCl and 0.3 grams of NaOH in 17.34 mL of seawater), aeration was given, and the temperature was kept constant at 40˚C so as not to kill the embryo. Then the cysts were washed with clean water in a 120 micron planktonet filter and neutralized using a sodium thiosulfate solution until the chlorine smell disappeared (Widodo *et al*., 2016). More than 80% of cysts hatch during overnight decapsulation. Unhatched cysts are shaped like deposited coffee beans (Kumar and Babu, 2015). After the decapsulation process, the *Artemia* will become instar nauplii with the next process, namely washing and harvesting for further treatment (Ludevese-Pascual *et al*., 2020).

2.4 Iodine Disinfectant

Iodine disinfectant is obtained from commercial iodine in the form of liquid with a concentration of 10%. 10% iodine has the widest spectrum for antimicrobial activity of antiseptics, including spores, bacteria, fungi, and viruses (Lachapelle *et al*., 2013).

2.5 Toxicity Test

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The research method used is the Brine Shrimp Lethality Test (BSLT) which is one of the bioassay methods using Artemia as a test animal (Surya, 2018). A toxicity test was carried out with iodine disinfectant concentrations of 0.1 ppm, 1 ppm, 10 ppm, and 100 ppm. 200 mL of seawater was filled in a 500 mL measuring cup, and 10 newly hatched *A. franciscana* nauplii were added according to the concentration of the iodine immersion treatment, and each treatment was given three replications. Toxicity testing was carried out by observing the mortality of *Artemia* for 96 hours by counting the number of deaths of *A. franciscana* nauplii (LC_{50}) tested.

2.6 Mortality Rate of *A. franciscana* Nauplii

A toxicity test can be determined by measuring the effect of exposure to iodine disinfectant on the growth of *A. franciscana* nauplii, and the mortality rate under conditions of different doses of iodine. Mortality rates were calculated for 96 hours for various doses of iodine. The percentage of mortality rate is calculated by following the formula (Arulvasu *et al.*, 2014):

$$\text{Mortality (\%)} = \frac{\text{Number of dead } \textit{Artemia} \text{ nauplii}}{\text{Number of live } \textit{Artemia} \text{ nauplii}} \times 100$$

2.7 Length Measurement

Individual length growth of *Artemia* sp. was seen with a microscope equipped with a micrometer, while the individual length of *Artemia* sp. was calculated using the formula from Effendie (1979); Putra *et al*. (2018) as follows:

$$L = L_t - L_o$$

Description: L : Length growth
L_t : Length at the end of maintenance
L_o : Length at the start of maintenance

2.8 Weight Measurement

Weight growth is the difference between the average weight of the *Artemia* nauplii at the beginning of the observation and the average weight at the end of the observation and is calculated based on the formula from Effendie (1979); El-Dahhar *et al*. (2022) as follows:

$$W = W_t - W_0$$

Description : W : Weight growth
W_t : Weight at the end maintenance
W_o : Weight at the end maintenance

3. RESULTS AND DISCUSSION

The results of the research conducted for the toxicity test of 0.1 ppm iodine; 1 ppm; 10 ppm; 100 ppm in *A. franciscana* nauplii using the BSLT method, the LC_{50} value for 96 hours was 20.31 ppm (Table 1).

Table 1. Probit analysis and LC_{50} calculation

ppm	Log (ppm) (x)	Probit (y)	% Dead	Mortality	Total	LC_{50} (ppm)
0,1	-1	0	0%	0	30	
1	0	3,87	13%	4	30	20,31
10	1	4,92	47%	14	30	
100	2	5,52	70%	21	30	

A toxicity test is a test used to see the pharmacological activity of a compound that occurs in a short time after giving a certain dose. The principle of the test is that the bioactive components present in the materials used are always toxic if given in high doses and become drugs at low doses (Jelita *et al*., 2020). Iodine is a disinfectant that can be lethal to Gram-positive and Gram-negative bacteria, fungi, and protozoa with increased exposure time (Lachapelle *et al*., 2013). The use of iodine disinfectant in aquaculture can be toxic if used in large quantities. This toxicity test usually uses *Artemia* nauplii test animals because the skin is thin and sensitive to the environment. Foreign substances or compounds that exist in the environment will be absorbed into the body by diffusion and directly affect their lives (Yun *et al*., 2020).

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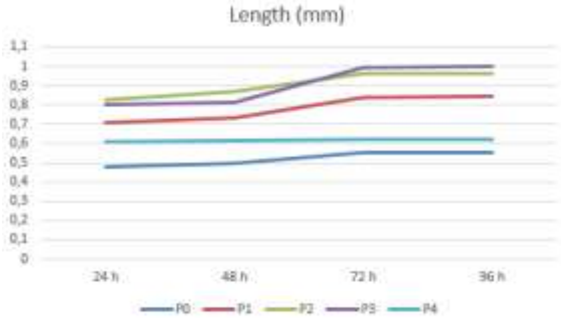
A toxicity test on *A. franciscana* nauplii was carried out with iodine doses of 0.1 ppm, 1 ppm, 10 ppm, and 100 ppm for 96 hours or 4 days. After being exposed to disinfectant with a certain dose level, it gives an LC_{50} result of 20.31 ppm, so iodine can be said to be toxic at doses of more than 20.31 ppm. This is also in line with the research of Pangastuti *et al*. (2009), who gave iodine treatment to shrimp nauplii at a dose of 20 ppm to reduce the number of bacteria present in shrimp nauplii. Shrimp nauplii and *Artemia* nauplii have the same characteristics and are still included in the crustacean class, so that the LC_{50} result of 20.31 ppm is not much different from the 20 ppm iodine dose used in shrimp nauplii.

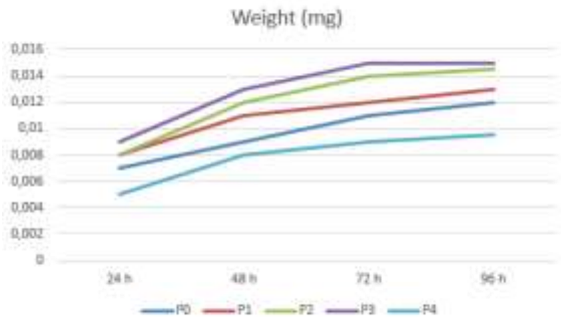
Based on the research that has been done, the toxicity test can affect the physical performance of *A. franciscana* nauplii by showing stunted growth (Figure 1) and can be known by measuring the length and weight of *A. franciscana* nauplii (Figure 2).

Figure 1. *A. franciscana* nauplii which were treated with different doses of iodine immersion for 96 hours.

Description: P0= *A. franciscana* nauplii before being treated with iodine; P1= 0.1 ppm iodine dose; P2= 1 ppm iodine dose; P3= 10 ppm iodine dose; P4 = 100 ppm iodine dose. All images were viewed with a 100x magnification microscope.



(A)



(B)

Figure 2. A. Length of *A. franciscana* nauplii during toxicity test. **B.** Weight of *A. franciscana* nauplii during toxicity test. P0= nauplii *A. franciscana* before being given iodine treatment; P1= nauplii *A. franciscana* after being given 0.1 ppm iodine dose (b); P2= nauplii *A. franciscana* after being given 1 ppm iodine dose; P3= nauplii *A. franciscana* after being given 10 ppm iodine dose; P4 = nauplii *A. franciscana* after being given 100 ppm iodine dose.

The dose level of iodine given to *A. franciscana* nauplii also affected the growth of the naupli. The growth of nauplii can be known by measuring the length, weight, and

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morphology of the nauplii can be seen. Based on the results of the toxicity test of exposure to iodine disinfectant on *A. franciscana* nauplii, it was shown that the nauplii developed well from treatment P0 to P3, whereas in treatment P4 experienced stunted growth due to long data. Treatment of P0 *A. franciscana* nauplii grew optimally and had a length of 0.476 mm for 24 hours and 0.553 mm for 96 hours. The next treatment, P1 *A. franciscana* nauplii, had a length of 0.71 mm for 24 hours and 0.842 mm for 96 hours. Furthermore, P2 *A. franciscana* nauplii has a length of 0.826 mm for 24 hours and 0.966 mm for 96 hours. Then, at P3 *A. franciscana* nauplii has a length of 0.801 mm for 24 hours and 0.999 mm for 96 hours. The last treatment, P4 *A. franciscana* nauplii, had a length of 0.608 mm for 24 hours and 0.623 mm for 96 hours. The varying length of *A. franciscana* nauplii was caused by the presence of different doses of iodine in each treatment. The length of *Artemia* nauplii that just hatched and swam freely for a period of 24 hours has a length of about 0.4 mm (Das *et al*., 2012), which means that it is still in harmony when added with iodine disinfectant for toxicity testing, but in the toxicity test for 96 hours the results showed that P4 treatment with a dose of 100 ppm iodine experienced stunted growth because it only had a length of 0.623 mm. The normal length of the *A. franciscana* nauplii for 96 hours or 4 days usually ranges from 0.77 mm in length (Azirah *et al*., 2015), so the iodine dose treatment can be said to be toxic.

The length growth of *A. franciscana* nauplii also affected the weight of the nauplii. The weight of *A. franciscana* nauplii from various treatments (Figure 3) showed that there were variations between treatments for 96 hours. Toxicity test for 24 hours resulted in a weight of P0 *A. franciscana* nauplii 0.007 mg; P1 weighs 0.008 mg; P2 weighs 0.008 mg; P3 weighs 0.009 mg, and on P4 it weighs 0.005 mg for 24 hours. These different results indicate that there are differences in the dose of iodine during the toxicity test. Treatment P4 had weight loss compared to other treatments. This is because the dose of iodine used is 100 ppm. Generally, *A. franciscana* developed into nauplii for 24 hours and weighed 0.015 mg (Tombinawa *et al*., 2016), so the P4 treatment experienced stunted growth. At the same time, the toxicity test for up to 96 hours has a varied weight. The different weight growth rates were due to the food digested by the *A. franciscana* nauplii. Growth will occur when there is an excess of amino acid (protein) input from food. Materials derived from food will be used by the body for basic metabolism, movement, production of sexual organs, and maintenance of body parts to replace unused cells (Djunaedi, 2015). The administration of iodine disinfectant with different doses affects the length and weight because iodine automatically enters the nauplii's body and affects the nutritional content of the *A. franciscana* nauplii, thus also affecting the weight of the *A. franciscana* nauplii.

The growth of *A. franciscana* nauplii was also affected by water quality during toxicity testing (Table 2).

Table 2. Water quality during toxicity test on *A. franciscana* nauplii.

Dose	DO (mg/L)	Temperature (°C)	Salinitas (ppt)	pH	NH_4 (mg/L)
0,1 ppm	5,5	28,1	40	6,89	0
1 ppm	5,3	28,3	40	6,71	0
10 ppm	5,28	28,1	40	6,55	0
100 ppm	4,9	27,9	40	6,49	0,5

The growth of *A. franciscana* nauplii is also influenced by external factors, such as the environment. The results of the toxicity test of *A. franciscana* nauplii given iodine disinfectant showed that the water quality varied (Table 2) and was still within the safe threshold for *Artemia* cultivation. *Artemia* can live in a wide salinity range. *Artemia* salinity levels between 30-35 ppt, can live in fresh water for 5 hours before finally dying. The optimal temperature for cyst hatching and growth is 25-30°C. In addition to temperature and salinity, the survival of *Artemia* depends on oxygen levels, pH, and ammonia levels. pH ranges from 7-8.5. pH below 5 or higher than 10 can kill *Artemia*. Minimal light is required in the hatching

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process. Ammonia levels of 2 mg/L can be life-threatening for *Artemia*. The optimal dissolved oxygen content is >3 mg/L. Environmental conditions in this stable range can minimize mortality in *Artemia* cultivation (Mohebbi *et al*., 2016).

5. CONCLUSIONS

The administration of different doses of iodine to *A. franciscana* nauplii showed an LC_{50} value of 20.31 ppm, which means that the substance is toxic to *Artemia* if it exceeds that dose and affects the growth of *A. franciscana* nauplii.

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