Hatching and survival rate of milkfish (*Chanos chanos*) larvae treated with nanosilver

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Abstract. This study was aimed to know the effect of hatching and survival rate of milkfish larvae (*Chanos chanos*) treated with nanosilver. Three concentrations of nanosilver such as 0.25 ppm, 0.5 ppm and 0.75 ppm were applied to culture media at least an hour before stocking. Newly hatched larvae were stocked at the density of 50 pcs $l^{-1,}$ and it was reared for 25 days in 5 m^3 of concrete tank. Result of the study found that no significant effect ($p>0.05$) of the treatments given on hatching rate and survival of milkfish larvae. However, larvae treated with nanosilver tend to gain higher hatching rate (65.5±4.1 - 86.6±19.0%) compared to control one (65.3±7.2%). Further, larvae survival it was ranged of 24.2±0.9-26.3±0.1% by the end of the trial. Nanosilver treatments has potential to reduce the total bacteria and *Vibrio* concentration into the media and need further study primarily on dosage and frequency used.

1 Introduction

Nanotechnology is experiencing rapid development for the production and application of particles at sizes 1–100 nm [1, 2]. Metal nanoparticles such as silver (Ag), gold (Au), and copper (Cu) have begun to be widely used in the fields of biology, optics, and magnetic properties. Nanosilver is one of the most widely used nanomaterials in commercial products due to its antibacterial, antifungal, and antiviral properties [3-9]. Nanosilver has the ability to fight bacteria through several mechanisms such as avoiding bacterial resistance, and this ability is different from the use of antibiotics [10].

Several studies have shown that the physical and chemical properties of nanosilver help increase the efficiency of silver materials, especially in disease control [11]. In the aquaculture industry, nanotechnology has not been widely applied and is only used in improving the quality of ingredients in food formulations, antifouling coatings, antibacterials for seafood tanks and packaging, and for environmental remediation systems [12, 13].

Early studies on the use of nanosilver in aquaculture are focused on shrimp infected by white spot virus (WSSV) and *Vibrio parahaemolyticus* bacteria, as triggers for *Early Death Syndrome* [14]. Another study is reported that nanosilver is used as a water disinfection agent to avoid the presence of pathogenic bacteria and viruses with significant results [15]. The disinfection function from nanosilver has the potential to replace water system purification that only relies on physical filtration. The utilization of nanosilver as a water disinfection material before milkfish egg stocking is an alternative to increase egg hatchability and survival of milkfish larvae.

2 Method

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2.1 Materials and tools

The materials used in this test were: nanosilver solution produced by the LAN (Laser and Advance Nanotechnology) laboratory- Department of Physics Diponegoro University at ~~with~~ a concentration of 50 ppm, seawater salinity of 30 ppt, milkfish eggs, natural feed in the form of *Nannochloropsis oculata* and rotifers (*Branchionus plicatilis*), larvae feed with 30% of protein content.

The tools in this test were larvae rearing tank with a volume of 5 m^3 settled alongside several aeration points about 1 m apart connected to a root blower. Other equipment includes 100 mL beaker glass, bucket, dipper, seed measuring device (millimeter block), and tank basal cleaning. Water quality was measured with a DO (dissolved oxygen) meter, pH meter, refractometer, and thermometer. The presence of total bacteria and *Vibrio* in rearing media was analyzed in the Laboratory of Aquatic Animal Health Management- Main Center of Brackish Water Aquaculture Jepara (BBPBAP Jepara)- Ministry of Marine Affairs and Fisheries.

2.2 Larval Rearing and Treatment

The rearing of milkfish larvae was carried out in a cement tank with a capacity of 5 m^3. The cement tank was filled with seawater at a salinity of 30 ppt. The eggs were obtained from broodstock at the Milkfish Hatchery Unit, Main Center of Brackish Water Aquaculture Jepara. Before the eggs stocking, each tank was given nanosilver with different concentrations as treatment, namely: 0.25 ppm; 0.5 ppm; 0.75 ppm, and also control treatment, each with double replications. Milkfish eggs were stocked in the afternoon (~ 16.00) at a density of 50 L^{-1}. Live food for milkfish larvae such as *N. oculata* and rotifers (*Branchionus plicatillis*) were given on the d-1 until harvest with a frequency of two times a day, in the morning and evening. Rotifers were applied with a density of 10 ind mL^{-1} and increased to 25 ind mL^{-1} along with the age of larvae rearing. Larvae feed which has been mashed was given *ad libitum* from the age of d-10. The frequency of artificial feeding was 4 times a day, namely: at 07.00 am; 10.00 am; 13.00 pm, and 17.00 pm.

Water quality management was carried out by siphoning out of unconsumed feed and any other debris in the tank (> d-14) and water renewal. After 10 days of larva rearing, daily water was exchanged totally until harvest. Harvesting was done at the age of d-21 or more and conducted in the morning to avoid larvae stress.

2.3 Total Bacteria and Vibrio Determination

The total number of bacteria and Vibrio in the culture media of milkfish larvae were counted as biological parameters in the use of nanosilver. The total number of bacteria and Vibrio were counted on the first day of the rearing period and on the tenth day of the rearing period. Water samples were collected for each treatment. Testing was conducted in the Laboratory of Aquatic Animal Health Management- Main Center of Brackish Water Aquaculture Jepara (BBPBAP Jepara)- Ministry of Marine Affairs and Fisheries. Bacterial counting was carried out using the Total Bacterial Count (TBC) and Total Vibrio Count (TVC) methods. Bacterial and Vibrio colonies were counted in a petri dish after incubation. The results of the calculations between treatments were then compared.

2.4 Data Analysis

The test data included hatching rate, survival rate, larvae length, total bacteria, and *Vibrio* at age d-1; d-10, and d-20 or at harvest time. The data were presented with the mean ± standard deviation and then analyzed with (ANOVA) with SPSS vs. 25 at the 95% confidence level ($p<0.05$).

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3 Results and Discussions

3.1 Results

3.1.1 Hatching rate

The addition of nanosilver at a dose of 0.5 ppm obtained the highest hatching rate value of 86.6 ± 19.0%, while decreased at a dose of 0.75 ppm (81.8 ± 2.8%), a dose of 0.25 ppm (65.5 ± 4.1%) and the lowest was in the control treatment at 65.3±7.2% (Figure 1). However, the effect of treatment on the tested parameters did not show any significant difference ($p>0.05$).

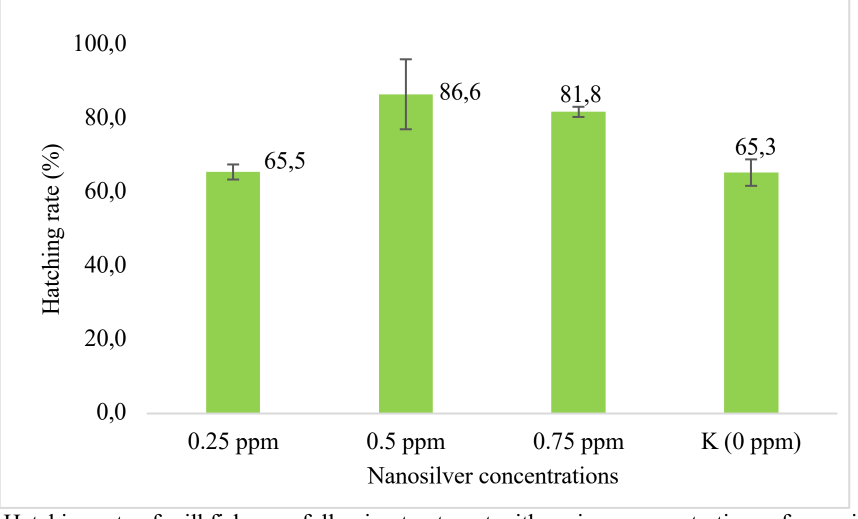

Fig. 1. Hatching rate of milkfish eggs following treatment with various concentrations of nanosilver

3.1.2 Survival Rate of Larvae

The application of nanosilver with different doses did not affect the survival of milkfish larvae (Figure 2). The test showed that the highest survival rate was without the use of nanosilver (control treatment) with value 27.4 ± 1.0% and then followed sequentially by nanosilver dose of 0.25 ppm (26.3 ± 0.1%), 0.75 ppm (26.2 ± 1.0%) and 0.5 ppm (24.2 ± 0.9%).

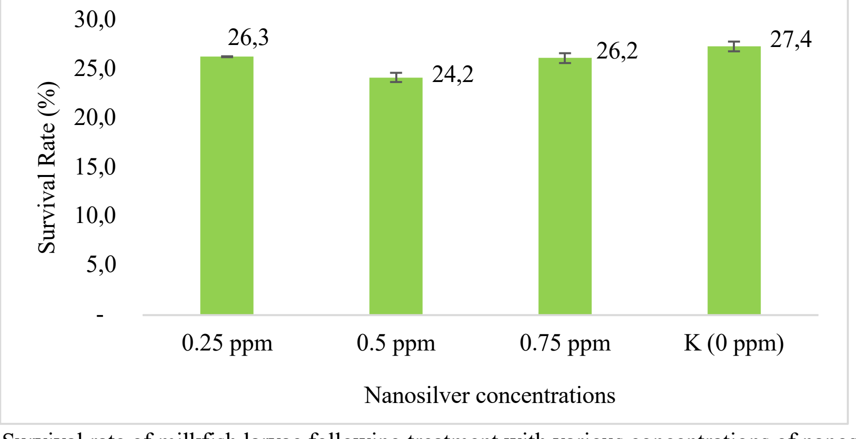

Fig. 2. Survival rate of milkfish larvae following treatment with various concentrations of nanosilver

3.1.3 Larvae Length

Milkfish larvae length at the end of the test did not show any difference ($p<0.05$). Periodic observation of larvae length, starting from the first 10 days until harvest showed the same trend (Figure 3).

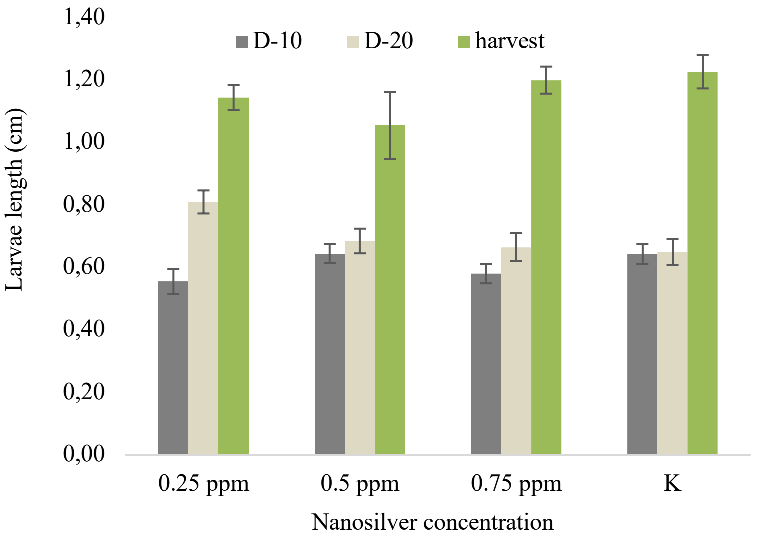

Fig. 3. Length of milkfish larvae following treatment with various concentrations of nanosilver

3.1.4 Total Bacteria and Vibrio

The application of nanosilver in larvae-rearing media was capable of reducing the bacterial density up to the first 10 days of rearing, compared to the control treatment (Figure 4). The same result was also shown in the number of *Vibrio* bacteria (Figure 5).

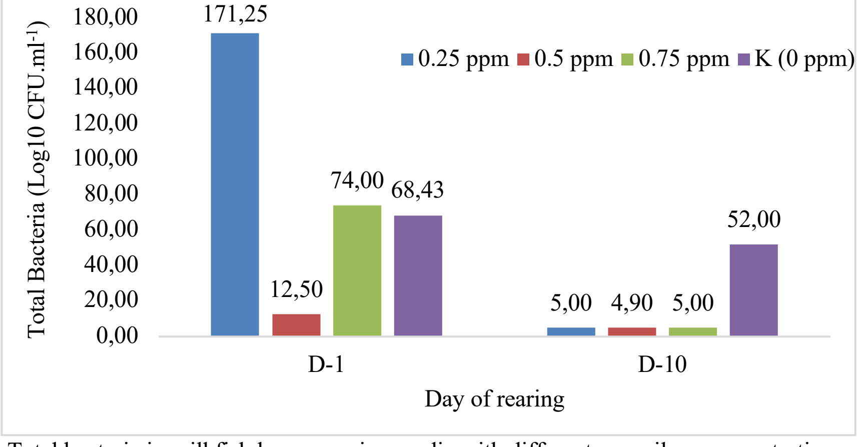

Fig. 4. Total bacteria in milkfish larvae rearing media with different nanosilver concentration

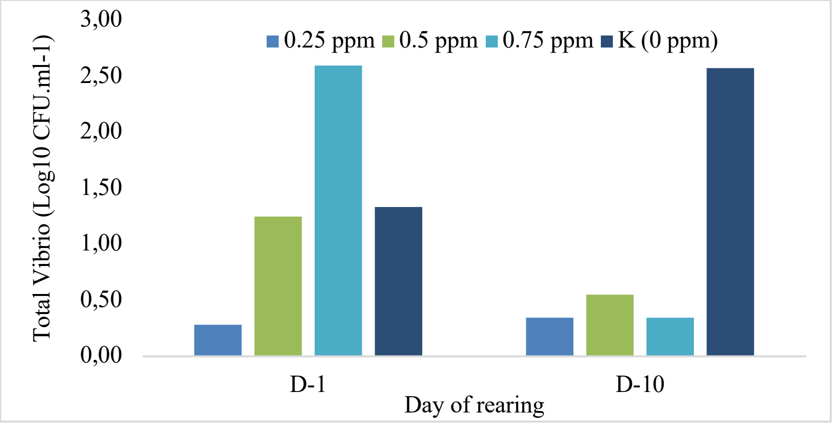

Fig. 5. Total Vibrio in milkfish larvae rearing with different nanosilver concentrations

3.2 Discussions

The use of nanosilver in aquaculture is widely applied to water and feed media to reduce water treatment costs, disease, and environmental pollution control [12]. Effective use and safety for consumers [14], evaluation of environmental risks [16], and their impact on biodiversity [17] need to be considered.

The results showed that the effects of nanosilver treatment did not significantly effective (p>0.05) on the hatching and survival rate of milkfish larvae. However, the application of nanosilver showed a tendency to produce a higher hatching rate (65.50-86.58%) compared to the control treatment (61.20%). These results indicated that nanosilver as a disinfectant in larvae rearing media did not affect the embryonic development of stocked eggs. This result is in line with the study that states the addition of silver nanoparticles to circulating media for rainbouw trout fertilization has increased the survival rate of its eggs at 11.24% calculated from the fertilization phase until hatching [18].

The test results show that the application of nanosilver did not affect the survival rate of milkfish larvae, but this needs to be tested further. Several studies have evaluated the toxic effects of nanomaterials on marine organisms, particularly metal NP oxides [19]. The study conducted determine the toxicity of nanosilver to the juvenile vannamei shrimp (*Litopenaeus vannamei*) injected with silver nanoparticles has not affected survival rate and reached 98.7% [14].

Another benefit of nanosilver is disease control due to its bactericidal effect [20], as reported in the purification of water samples from *E. coli* and *V. cholearae* [21]. In line with the tests that have been carried out, it shows a decrease in the population of bacteria and Vibrio until the 10th day. On the contrary, there is an increase in the population of control treatment. The frequency and period of nanosilver addition need to be further studied, especially for milkfish larvae rearing.

4 Conclusions

The test results showed that there was no effect (p>0.05) of nanosilver treatment on the hatching and survival rate of milkfish larvae. However, the treatment of nanosilver showed a tendency to produce a higher hatching rate (65.50-86.58%) compared to the control treatment (61.20%). The effect of nanosilver treatment could reduce the population of total bacteria and *Vibrio* until the 10th day.

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