

Safe Concentration of Silver Nanoparticles in Solution for White Leg Shrimp (*Litopeaneus vannamei*) Farming

Phuong Hong Lam¹, Mai Thi Le¹, Dung My Thi Dang¹, Tin Chanh Duc Doan¹, Nguyen Phuc Cam Tu², Chien Mau Dang¹

1. Institute for Nanotechnology (INT), Vietnam National University - Ho Chi Minh City (VNUHCM), Vietnam.

2. Faculty of Fisheries, Nong Lam University - Ho Chi Minh City, Vietnam.

Received: December 02, 2019 / Accepted: December 23, 2019 / Published: February 25, 2020

Abstract: There have been many bactericidal chemicals used to limit the growth of bacteria in white leg shrimp culture. Silver nanoparticles have been used in filtering devices or mixed with the commercial feed to decrease the bacteria and fungus in shrimp farming. Adding silver nanoparticles directly to the culturing water has not been reported due to worries about bioaccumulation, polluted environment and their effect on the aquatic animals. However, studies on the effects of silver nanoparticles on cultured species have hardly been conducted. In this paper, we report the determination of the safe concentration of the silver nanoparticles for white leg shrimp by determining the acute toxicity of the used silver nanoparticles (LC_{50-48h} and LC_{50-24h}) for adult white leg shrimps (35 days old). The results showed that white leg shrimps exposed to the silver nanoparticles for 48 hours with concentrations greater than 100 ppm had high mortality (> 90%). The LC_{50-24h} and LC_{50-48h} values of the silver nanoparticles for *Litopeaneus vannamei* were determined to be 91.2 ppm and 35.5 ppm, respectively. The safety level of the silver nanoparticles recommended to be used for the white leg shrimp culture was 1.61 ppm.

Key words: silver nanoparticles, growth rate, *Penaeus vannamei*, survival rate, *Vibrio* spp.

1. Introduction

Despite the rapid development, high-density white leg shrimp farming has been facing the outbreaking pressure of diseases [1]. Therefore, many bactericides have been widely used and to control diseases regularly in white leg shrimp ponds [2]. Currently, using nanomaterials is one of the useful solutions to sterilize bacteria [3-5]. Silver Nanoparticles (AgNPs) have been widely used as an antimicrobial agent because of its bactericidal effect and improvement ability of water quality [6-16]. It was reported that AgNPs did not affect multicellular animals [17]. However, AgNPs at high concentrations could affect crustaceans because the nanoparticles could stick on shell, peel and appendages, which prevented the respiration and movement of the crustaceans [18].

Corresponding author: Tin Chanh Duc Doan, Institute for Nanotechnology (INT), Vietnam National University - Ho Chi Minh City (VNUHCM), Community 6, Linh Trung Ward, Thu Duc District, Vietnam. Email: ddctin@vnuhcm.edu.vn, ORCID id: 0000-0003-4250-9078.

Funding information: This research is funded by the South West Program of the Ministry of Science and Technology with the contract number 02/2017/HD-KHCN-TNB.DT/14-19/C04.

Safe Concentration of Silver Nanoparticles in Solution for White Leg Shrimp (*Litopenaeus vannamei*) Farming

Therefore, when using the AgNPs directly to limit bacteria in the water, it is important to determine the safe concentration of the AgNPs. Acute toxicity is the discernible adverse effect induced in an organism within a short time of exposure to a substance [19]. In the present test, acute toxicity is expressed as the median lethal concentration (LC₅₀) that is the concentration in the water which kills 50% of a test bath of shrimp within a continuous period of exposure which must be stated [19]. The safe concentration of chemicals used for laboratory animals can be determined from LC₅₀ value [20]. In this study, the safe concentration of the silver nanoparticles for white leg shrimp was determined by study on the acute toxicity of the used silver nanoparticles (LC_{50-48h} and LC_{50-24h}) for adult white leg shrimps (35 days old). The LC₅₀ value of AgNPs for *Litopenaeus vannamei* was determined after 24 hours and 48 hours of exposure. From this study, the safety level of the silver nanoparticles recommended to be used for the white leg shrimp culture was also discussed.

2. Materials and methods

2.1. Materials

The experimental water was fresh seawater with a salinity of 28 ppt. After the seawater was filtered and treated with chlorine at 1 ppm of concentration, the salinity of the seawater was adjusted to 15 ppt.

The AgNPs used in this study were synthesized from silver nitrate (AgNO₃) at Institute for Nanotechnology, Vietnam National University - Ho Chi Minh City by the chemical method. The details of preparation of the AgNPs can be found in the previous reports [21, 22]. In this study, the AgNPs were dispersed in the deionized (DI) water with Polyvinyl pyrrolidone and Ethylene glycol used as the stabilizers. The particle size measured with a Particle size analyzer (LB-550, Horiba, Japan) and Transmission Electron Microscopy (TEM) was about 5-11 nm. To achieve the concentrations of AgNPs in the test solution, the solution of AgNPs with concentration of 200 ppm was used to dilute with DI water.

The experimental shrimps were white leg shrimps without diseases. The health of the shrimps was checked carefully before they were exposed to the chemicals. The shrimps used in this experiment were PL12, purchased from a local hatchery dealer, acclimatized in the composite tank of 500 L for 40 days before carrying out experiments.

2.2. Methods

2.2.1. Preliminary experiments

There were seven (7) treatments using AgNPs at different concentrations, namely 1 ppm, 10 ppm, 25 ppm, 50 ppm, 100 ppm, 125 ppm and the control treatment without using AgNPs (0 ppm). Each treatment was repeated for three times. Experimental tanks with 20 L of volume were used with aeration and distributed by one air stone diffuser inside each experimental tank. Twenty individuals of the experimental shrimps were stocked into each tank. The external signs of the shrimps were observed and the number of the dead shrimps was recorded after 3, 6, 12, 24, 48 hours from the time of adding the AgNPs solution. The shrimps were not fed during acute toxicity trial (48 hours) to avoid contaminating the cultured water.

Safe Concentration of Silver Nanoparticles in Solution for White Leg Shrimp (*Litopeaneus vannamei*) Farming

2.2.2. Experiments

From the results of the preliminary experiments, the highest concentration of AgNPs was chosen without dead shrimps, the shrimps lived normally when they were added to clean water. The lowest concentration of AgNPs was also chosen at which 100% of shrimps died after 48 hours of treatment. From these results, the range of the AgNPs concentrations was established for the official experiments.

The official experiments were conducted with 6 treatments including 5 different concentrations of the AgNPs and one control treatment. Each treatment was repeated for three times. Twenty individuals of the experimental shrimps were stocked into separated tanks which had 40 L water. The shrimps used in the experiment were reared from post-larvae shrimps (PL12) to the age of 40 days. The shrimps were then put into the experimental tanks for one hour before adding AgNPs to adapt to the experimental conditions. The experimental conditions of all the treatments were kept with the same conditions including density, water volume and aeration. During treatment times, the shrimps were not fed. The number of dead shrimps in each tank was recorded after 1, 3, 6, 12, 24, 36, and 48 hours. The dead shrimps were removed from the tank as they were found.

2.3. Statistical analysis

The cumulative mortality rate after 24 and 48 hours in each treatment was calculated by the following equation: Mortality rate (%) = (number of the dead shrimps / total number of experimental shrimps) × 100. The regression equation between the probit of mortality value (Y-axis) and the log of tested AgNPs concentration (X-axis) at the moment of 24 and 48 hours was established to determine the LC_{50-24h} and LC_{50-48h} values of the AgNPs for *Litopeaneus vannamei*. The data obtained from the investigation was statistically analyzed by using Minitab 16 software.

The safety concentration was calculated by the equation:

$$SC \text{ (safe concentration)} = LC_{50-48h} \times 0.3 / (LC_{50-24h} / LC_{50-48h})^2 \text{ [23].}$$

3. Results

3.1. Preliminary results

Table 1. The mortality rate of white leg shrimp in the preliminary experiments

Experimental AgNPs concentration (ppm)	1	10	25	50	100	125
Mortality rate after 48 hours (%)	0	0	0	40	60	100

Safe Concentration of Silver Nanoparticles in Solution for White Leg Shrimp (*Litopenaeus vannamei*) Farming

According to the results presented in **Table 1**, the LC_0 and LC_{100} values of the AgNPs for white leg shrimp were 25 ppm and 125 ppm. Therefore, 6 tested concentrations which were chosen to determine LC_{50} of AgNPs for white leg shrimp were 25 ppm, 50 ppm, 75 ppm, 100 ppm, 125 ppm, and control treatment (0 ppm).

3.2. The acute toxicity of AgNPs for white leg shrimp

The results of testing the *Vibrio* spp. density indicated that the hepatopancreas of the shrimps was not infected. Observing the fresh of gills, swimming legs, and tail under the microscope showed that the gills were clean, the appendages were free of parasites. Therefore, the experimental results would not be affected by the above factors. The cumulative mortality rate of the experimental shrimps for each treatment was shown in **Figure 1**.

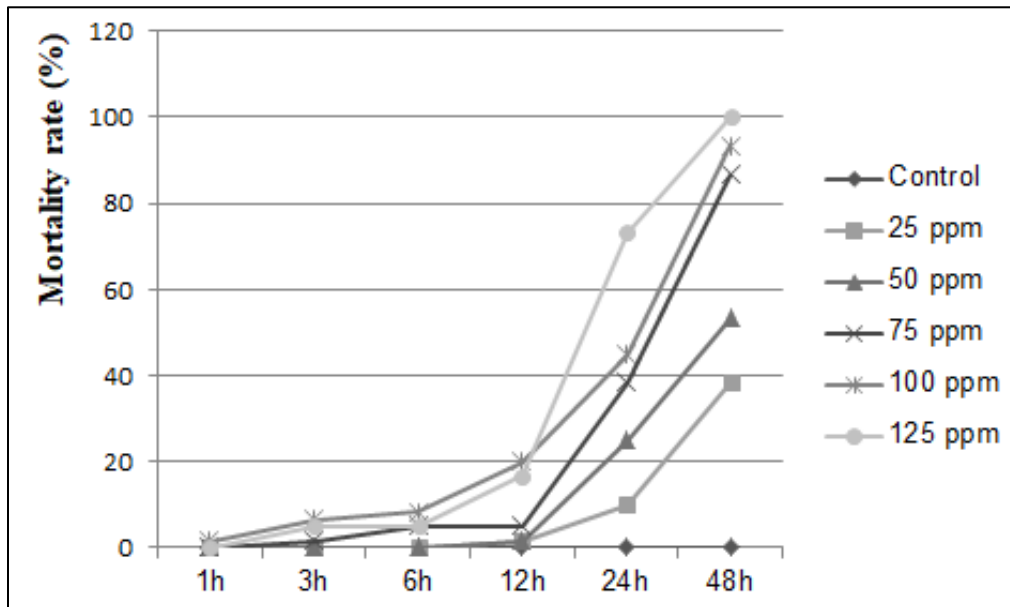


Figure 1. The cumulative mortality rate of the shrimps at different periods with tested AgNPs concentrations.

After 3 hours from the beginning of the experiments, the shrimps started to die in the treatments with very high concentrations of the AgNPs (75-125 ppm). After 24 hours, the dead shrimps were found in all the treatments (except the control treatment without using the AgNPs). The higher the concentration of silver nanoparticles was used, the higher the death rate of the experimental shrimps (after 48 hours of exposure) was found. At the concentration of 125 ppm, the mortality rate was 100%, the mortality rate was 93.3% with the concentration of 100 ppm and at the concentration of 75 ppm, the mortality rate was 86.7%.

Safe Concentration of Silver Nanoparticles in Solution for White Leg Shrimp (*Litopenaeus vannamei*) Farming

Table 2. The probit values of the cumulative mortality rate of the shrimps corresponding to the AgNPs concentrations after 24 and 48 hours.

AgNPs concentration (ppm)	Logarithm of AgNPs concentration	The number of organisms	24 h		48 h	
			Mortality rate (%)	Probability unit	Mortality rate (%)	Probability unit
0	-	20	0	-	0	-
25	1.398	20	10	3.72	41	4.77
50	1.699	20	25	4.33	53.3	5.08
75	1.875	20	38.3	4.69	86.7	6.08
100	2	20	45	4.87	93	6.48
125	2.097	20	73	5.61	100	7.33

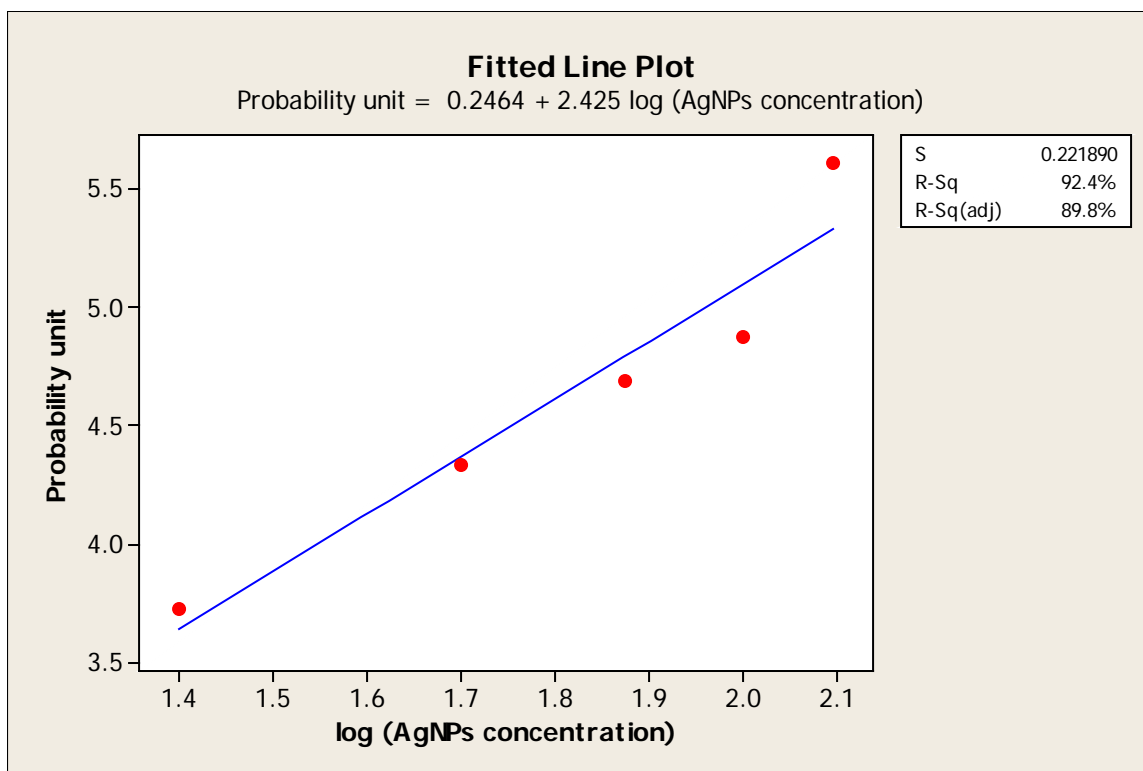


Figure 2. Regression correlation between the cumulative mortality of the shrimps and the tested AgNPs concentrations after 24 hours.

Safe Concentration of Silver Nanoparticles in Solution for White Leg Shrimp (*Litopenaeus vannamei*) Farming

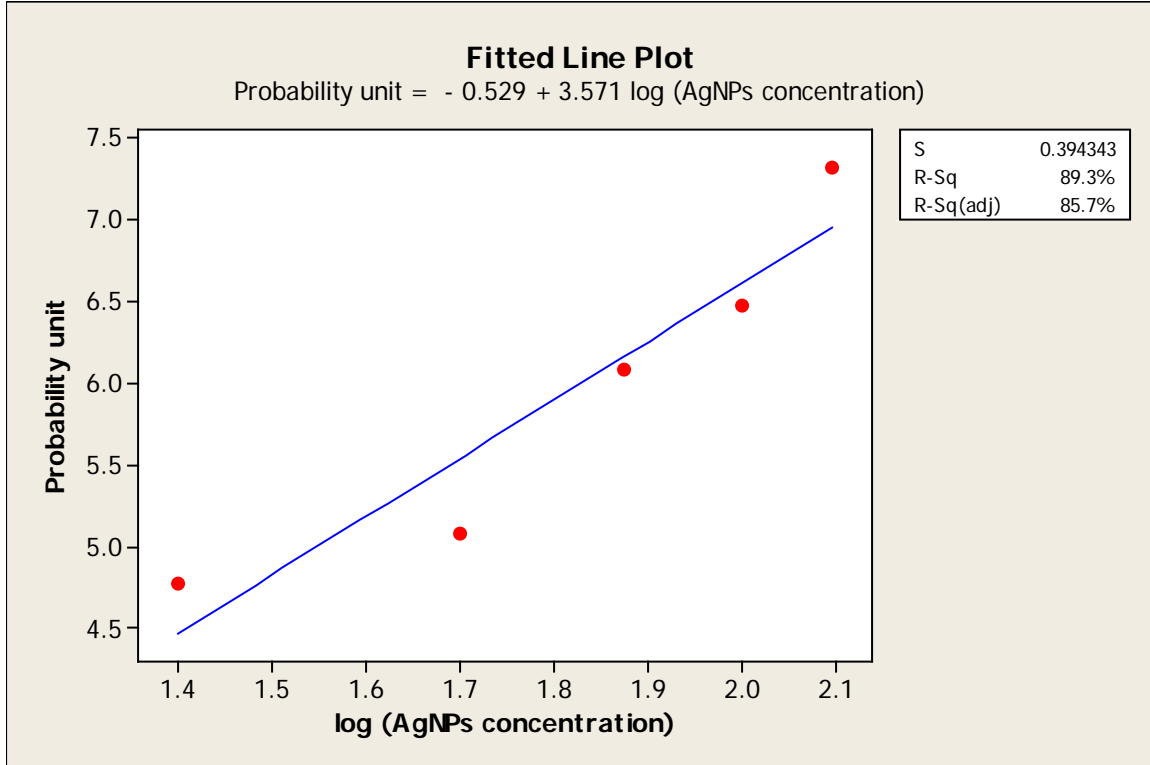


Figure 3. Regression correlation between the cumulative mortality of the shrimps and the tested AgNPs concentrations after 48 hours.

After 24 hours of exposure to the AgNPs, the regression correlation equation between the concentrations of the AgNPs and the mortality rate of the shrimps was: $Y = 0.2464 + 2.425X$. The R^2 value was 92.4, indicating a close correlation between the concentrations of the AgNPs and the mortality rate of the shrimps. After 48 hours, the correlation equation between the concentration of the AgNPs and the mortality rate of the shrimps was $Y = -0.529 + 3.571X$, with a R^2 value of 89.3 (quite close correlation between the mortality rate of the shrimps and the concentrations of the AgNPs).

From the regression correlation equation, with a probit value of 5 (corresponding to the 50% mortality rate), it was possible to calculate the LC_{50-24h} and LC_{50-48h} values of the AgNPs for white leg shrimp. They were 91.2 ppm and 35.5 ppm, respectively. Using the formula for calculation of the safe concentration, the safe concentration of AgNPs for white leg shrimp was 1.61 ppm.

4. Discussion

When using bactericidal chemicals in aquaculture farming, safety for the cultured animals need to be considered besides bactericidal efficiency, convenience of usage, cost. From the 10th to 45th day of culture, shrimps are often very sensitive to pathogens, some diseases occurring at this stage could make the death rate of nearly 100% [23]. Meanwhile, in this period, the organic matters in water begin to accumulate, thus increasing the risk of disease outbreak [24]. Therefore, using biocides at this stage is very necessary. However, the used

Safe Concentration of Silver Nanoparticles in Solution for White Leg Shrimp (*Litopenaeus vannamei*) Farming

concentration of biocides must be considered carefully to achieve the desired bactericidal effect without affecting to shrimps. In this investigation, the experimental shrimps were used at 30 days of age.

After three (3) hours of exposure to the AgNPs, the shrimps began to die in all the treatments. A higher mortality rate was recorded in the treatments using very high concentration of the AgNPs (75, 100 and 125 ppm). After 24 hours, for the treatments using AgNPs at 100 ppm and 125 ppm, the cumulative mortality rates of the shrimps were greater than 50%. And after 48 hours, the mortality rates of the shrimps in these two treatments were about 90% and 100%, respectively. The mortality rate of shrimp was directly proportional to the exposure time in all the treatments. In the control treatment, after 48 hours of the experiments, the survival rate was still 100%. The environmental factors throughout the trial were checked in all the treatments to ensure that they did not affect the results.

It was reported that the nanoparticles attached to the surface of bacterial cells and changed the character of cell membranes [17]. This is one of the bactericidal mechanisms of AgNPs. However, animal cells have a completely different protective membrane in comparison with bacterial cells [17]. They have two layers of stable, double-binding lipoproteins which are capable of electron release. Hence, they do not allow silver ions to uptake and they are not damaged. This means that AgNPs are completely harmless to humans and animals in general because the cell membrane structure is more stable and thicker than pathogenic single-cell micro-organisms such as fungi, bacteria and viruses [17]. This explains why using AgNPs at low concentrations is still safe for the main cultured animals. However, toxicity of AgNPs depends much on the size and the concentration of the used AgNPs [25]. Besides, the AgNPs concentration was reported to be directly proportional to its toxicity [26]. Several studies showed that AgNPs reduced the mitochondrial function and intracellular glutathione content, increased the intracellular ROS levels, damaged DNA and killed the cells [27]. Many genotoxicity studies have also been reported. The toxicity of AgNPs was reduced in the water containing high levels of organic carbon than in freshwater [28]. Different microorganisms also have different susceptibility to silver toxicity [29]. Moreover, nanomaterials can be harmful to crustaceans because AgNPs can accumulate under the crust, which affects the swimming, and respiratory activity of crustaceans [18]. These are the main reasons for explaining the results of this experiment. The mortality rate of shrimp was high when the shrimps were exposed to a high concentration of AgNPs from 75 ppm to 125 ppm.

Besides, when observing the appearance of the shrimps after 48 hours of the experiment, at the low concentrations of AgNPs (25 ppm, 50 ppm) with the mortality rate was about 40%, there were no abnormal signs of appearance (shell and gill of the shrimps). However, in the treatments with high AgNPs concentrations of 75 ppm, 100 ppm and 125 ppm, the shrimps showed several signs such as black scratches on the shells; swimming legs and creeping legs were corroded and had melanin stain; the tails were swollen, containing fluid inside, the gills were blackened (**Figure 4** and **Figure 5**).

Safe Concentration of Silver Nanoparticles in Solution for White Leg Shrimp (*Litopenaeus vannamei*) Farming



Figure 4. The appearance of the white leg shrimps when they were exposed to 75 ppm and 100 ppm concentrations of the AgNPs after 24 hours. (A: Black scratches on shrimp shells. B: The accessories were swollen and had stains).

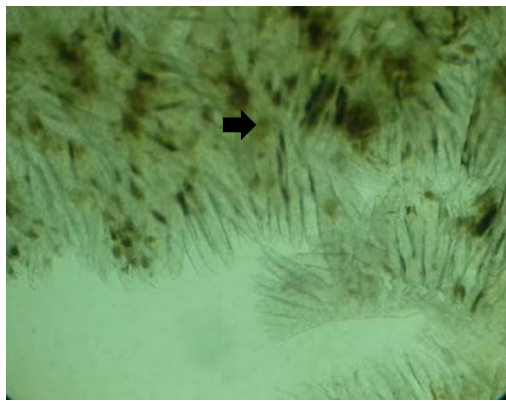


Figure 5. Shrimp gill became black (observed by an optical microscope, with an objective $\times 40$).

Discolored gill tissue showed that the gills were damaged. In common, shrimp gills are black or brown due to one of the following reasons: leaching organisms, melanization caused by bacterial necrosis, melanization reaction with the toxins in the water, ionic precipitation, due to mud or sediment in the water [2].

In this experiment, shrimp gills were blackened and shrimp shells appeared dark spots, possibly due to the melanization caused by ionic precipitation on the gills or shells. The toxic metals were absorbed and accumulated in crustaceans directly from the water through the gills or other body surfaces or from food through the gut [30]. Upon exposure to water containing metal ions, the gills of crustacean are the main intrusive sites, acting primarily as a temporary tissue system for accumulated metals [30]. Exposure to the toxin of metals or chemicals causes shrimp gills to be blackened [31, 32]. After attached to the gills, the metal ions are adsorbed by the epidermis, transported to epithelial cells, dispersed into the bloodstream, and then they go to the organs [30]. The gill of crustacean performs many functions such as ion transportation, blood osmosis, acid and base balance and ammonia secretion [30]. When the gill is injured, these functions in shrimps will be affected, leading to increasing mortality. Also, dark spots on the shell often appear on shrimps or other crustaceans. It is possibly due to the melanization reaction on the body of shrimps. In common cases, blood cells often gather in this tissue area where the damage appears, causing the melanin deposition [33]. An infective agent, a wound or a poison could stimulate this process, which explained the appearance of dark spots on shrimp shells when they were exposed to AgNPs at high concentrations [33].

Safe Concentration of Silver Nanoparticles in Solution for White Leg Shrimp (*Litopenaeus vannamei*) Farming

There have been several studies studying on the toxic effects of nanomaterials in marine organisms, especially metal nanoparticles and metal oxides because of their bactericidal application. Recently, when they have been widely applied, it is necessary to concern about the existence of these substances in the water. Therefore, there were several studies of the toxicity of nanomaterials in general and AgNPs in particular for a few underwater creatures. Almost researches were on artemia (*Daphina magna*), one of the creatures which play an important role in the food chain in natural water [3, 8, 34]. There have been few studies which evaluated the acute toxicity of AgNPs for white leg shrimp although there have been some studies on the acute toxicity of some other heavy metals on shrimps. In addition, some studies have used AgNPs to eliminate some pathogens in shrimp [10-12]. A low content of AgNPs added to food or injected into shrimp muscle did not kill shrimps and still limited pathogenic microorganisms [12]. Juarez et al. [12] evaluated the influence of the AgNPs on resistance, blood indices, and the survival rate of white leg shrimp with the concentrations of AgNPs solution which were 0.5 ppm, 5 ppm, and 20 ppm. The results showed that the shrimps injected with AgNPs solution into the body still swam normally. After 96 hours of AgNPs injection into shrimp body, the survival rate of all the concentrations was also high (> 90%), with no significant difference in comparison with the control treatment (PBS buffer solution was injected into experimental shrimp). Besides, this study also indicated that the AgNPs solution did not change the total hemocyte counts (THC) in shrimp [12]. In another study [10] AgNPs was used to limit the disease caused by *Vibrio parahaemolyticus* on white leg shrimp by mixing the AgNPs in shrimp feed. The results showed that when AgNPs were mixed into shrimp feed (with 0.1 mg AgNPs / 10 mg feed in content), after 65 days of culture, the shrimp had a survival rate of 100%. The growth in weight and the Feed Conversion Ratio (FCR) were better than the rest of the experimental groups (including the *Bacillus subtilis* supplemented group, the AgNO₃-fortified feed group with 0.1 mg / 10 mg feed, the *V. parahaemolyticus* supplemented group and the control group). Moreover, when challenged to inject *V. parahaemolyticus* with a concentration of 10⁶ CFU / ml into the abdominal muscles, the shrimps in this treatment group also had a higher survival rate and the best functional morphology [10]. In short, these above experimental results conformed to the research results that have been reported.

5. Conclusion

In conclusion, the safe concentration of the AgNPs for white leg shrimp was determined. The acute toxicity of AgNPs to adult white leg shrimp LC_{50-24h} was 91.2 ppm, and LC_{50-48h} was 35.5 ppm. The safe concentration of the AgNPs solution which does not cause death of shrimp was 1.61 ppm. The results of this study are the basis for selecting the appropriate concentration of the AgNPs solution to apply in the shrimp culture.

References

- [1]. Lightner, D.V., *A review of the diseases of cultured penaeid shrimps and prawns with emphasis on recent discoveries and developments*. Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps, 4-7 December 1984, Iloilo City, Philippines (pp. 79-103), 1985.

Safe Concentration of Silver Nanoparticles in Solution for White Leg Shrimp (*Litopenaeus vannamei*) Farming

- [2]. Verschuere, L., et al., *Probiotic bacteria as biological control agents in aquaculture*. Microbiology and molecular biology reviews : MMBR, 2000. 64(4): p. 655-671.
- [3]. Li, Q., et al., *Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications*. Water Research, 2008. 42(18): p. 4591-4602.
- [4]. Salem, W., et al., *Antibacterial activity of silver and zinc nanoparticles against Vibrio cholerae and enterotoxigenic Escherichia coli*. International Journal of Medical Microbiology, 2015. 305(1): p. 85-95.
- [5]. Luis, A.I.S., et al., *Trends in aquaculture sciences: from now to use of nanotechnology for disease control*. Reviews in Aquaculture, 2019. 11(1): p. 119-132.
- [6]. Pal, S., Y.K. Tak, and J.M. Song, *Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium Escherichia coli*. Applied and Environmental Microbiology, 2007. 73(6): p. 1712-1720.
- [7]. Wong, K.K.Y. and X. Liu, *Silver nanoparticles - the real "silver bullet" in clinical medicine?* MedChemComm, 2010. 1(2): p. 125-131.
- [8]. Kowalska-Góralaska, M., P. Ława, and M. Senze, *Impact of silver contained in the nano silver preparation on the survival of brine shrimp (Artemia salina Leach 1819) larvae*. Ecological Chemistry and Engineering, 2011. 18(3): p. 371-376.
- [9]. Franci, G., et al., *Silver Nanoparticles as Potential Antibacterial Agents*. Molecules, 2015. 20 (5): p. 8856-8874.
- [10]. Sivaramasamy, E. and W. Zhiwei, *Enhancement of Vibriosis Resistance in Litopenaeus vannamei by Supplementation of Biomastered Silver Nanoparticles by Bacillus subtilis*. Journal of Nanomedicine & Nanotechnology, 2016. 07.
- [11]. Acedo-Valdez, M.R., et al., *Antibacterial effect of biosynthesized silver nanoparticles in Pacific white shrimp Litopenaeus vannamei (Boone) infected with necrotizing hepatopancreatitis bacterium (NHP-B)*. Latin American Journal of Aquatic Research, 2017. 45: p. 421-430.
- [12]. Juarez-Moreno, K., et al., *Effect of silver nanoparticles on the metabolic rate, hematological response, and survival of juvenile white shrimp Litopenaeus vannamei*. Chemosphere, 2017. 169: p. 716-724.
- [13]. Julio Cesar Meneses Márquez, A.H.P., María del Carmen Monroy Dosta, Jorge Castro Mejía and Jaime Amadeo Bustos Martínez, *Silver nanoparticles applications (AgNPS) in aquaculture*. International Journal of Fisheries and Aquatic Studies, 2018. 6(2): p. 05-11.
- [14]. Khanh, N.V. and P.V. Cu, *Antibacterial activity of silver nanoparticles against Aeromonas spp. and Vibrio spp. isolated from aquaculture water environment in Thua Thien Hue*. Hue University Journal of Science: Agriculture and Rural Development, 2019. 128(3B): p. 12.
- [15]. Liao, C., Y. Li, and S.C. Tjong, *Bactericidal and Cytotoxic Properties of Silver Nanoparticles*. International Journal of Molecular Sciences, 2019. 20(2): p. 449.
- [16]. Ochoa-Meza, A.R., et al., *Silver nanoparticles enhance survival of white spot syndrome virus infected Penaeus vannamei shrimps by activation of its immunological system*. Fish & Shellfish Immunology, 2019. 84: p. 1083-1089.
- [17]. McShan, D., P.C. Ray, and H. Yu, *Molecular toxicity mechanism of nanosilver*. Journal of food and drug analysis, 2014. 22(1): p. 116-127.
- [18]. Exbrayat, J.-M., E. Moudilou, and E. Lapiéd, *Harmful Effects of Nanoparticles on Animals*. Journal of Nanotechnology, 2015. 2015.
- [19]. Ullah, A., et al., *Investigation of acute toxicity and LC50 value of Cu for a fish Oreochromis niloticus*. Journal of Entomology and Zoology Studies, 2016. 4(5): p. 605-607.
- [20]. J. S., et al., *Acute Toxicity Test of Four Disinfectants to Juvenile Pinctada maxima*. Insights in Aquaculture and Biotechnology, 2017. 1: p. 2-8.

Safe Concentration of Silver Nanoparticles in Solution for White Leg Shrimp (*Litopenaeus vannamei*) Farming

- [21]. Dung My Thi Dang, C.M.D., Eric Fribourg-Blanc, *Study of the formation of silver nanoparticles and silver nanoplates by chemical reduction method*. International Journal of Nanotechnology, 2015. 12(5/6/7): p. 456-465.
- [22]. Dung My Thi Dang, C.M.D., Eric Fribourg-Blanc, *Investigation of the influence of different surfactants on controlling the size of silver nanoparticles*. International Journal of Nanotechnology, 2015. 12(5-7): p. 505-514.
- [23]. De Schryver, P., T. Defoirdt, and P. Sorgeloos, *Early mortality syndrome outbreaks: a microbial management issue in shrimp farming?* PLoS pathogens, 2014. 10(4): p. e1003919-e1003919.
- [24]. Lemonnier, H., et al., *Nutrient and microbial dynamics in eutrophying shrimp ponds affected or unaffected by vibriosis*. Marine Pollution Bulletin 2010. 60(3): p. 402-411.
- [25]. Shah, A., et al., *Daphne mucronata-mediated phytosynthesis of silver nanoparticles and their novel biological applications, compatibility and toxicity studies*. Green Chemistry Letters and Reviews, 2018. 11: p. 318-333.
- [26]. Ivask, A., et al., *Size-Dependent Toxicity of Silver Nanoparticles to Bacteria, Yeast, Algae, Crustaceans and Mammalian Cells In Vitro*. PLOS ONE, 2014. 9(7): p. e102108.
- [27]. Kim, J.S., et al., *In vivo Genotoxicity of Silver Nanoparticles after 90-day Silver Nanoparticle Inhalation Exposure*. Safety and health at work, 2011. 2(1): p. 34-38.
- [28]. Blinova, I., et al., *Toxicity of two types of silver nanoparticles to aquatic crustaceans Daphnia magna and Thamnocephalus platyurus*. Environ Sci Pollut Res Int, 2013. 20(5): p. 3456-63.
- [29]. Bondarenko, O., et al., *Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review*. Arch Toxicol, 2013. 87(7): p. 1181-200.
- [30]. Henry, R.P., et al., *Multiple functions of the crustacean gill: osmotic/ionic regulation, acid-base balance, ammonia excretion, and bioaccumulation of toxic metals*. Frontiers in physiology, 2012. 3: p. 431-431.
- [31]. Johnson, S.K., *Handbook of Shrimp Diseases*. 2012, Department of Wildlife and Fisheries Sciences, Texas A&M University.
- [32]. Verma, R.S., *Acute toxicity of nickel to fresh water prawns*. Turk J Zool, 2012. 36(4): p. 534-542.
- [33]. Braga, A., et al., *Hemocytic melanization in shrimp spermatophores*. Aquaculture, 2017. 486.
- [34]. Arulvasu, et al., *Toxicity Effect of Silver Nanoparticles in Brine Shrimp Artemia*. The Scientific World Journal, 2014: p. 256919.