Effect of nanosilver particles on hatchability of rainbow trout (*Oncorhynchus mykiss*) egg and survival of the produced larvae

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Abstract

Effect of nanosilver particles was studied on the hatchability of rainbow trout (*Oncorhynchus mykiss*) egg and survival of the produced larvae at about 12ºC. In the first experiment the water-based nanosilver particles was used at concentrations of 0.5, 1, 2 and 4 mgL^{-1} for 30 minutes per day starting 24 hour post egg incubation until the hatching time. The mean percentage of hatchability reached in 27.6±0.2, 38.2±0.1, 41.6±0.4 and 48.6±1.5 in troughs treated with 0.5, 1, 2 and 4 mgL^{-1} nanocid, respectively compared with 64.7±0.2 % for trough treated with malachite green at 2 mgL^{-1} as positive control ($P<0.05$) and 5.1± 0.2 % for trough without treatment (untreated group) ($P<0.0.5$). In the second experiment the effect of the nanosilver particles was evaluated in the form of nanosilver dressing i.e. coating nanocid on the surface of the incubator troughs. The mean percentage of hatchability was 69.4±0.1% compared with 52.5%±0.1 recorded for normal control (trough without nanosilver dressing) ($P<0.05$). When growing the produced larvae in the same troughs until one g in body weight, there was an insignificant increase in the weight of larvae kept in nanosilver trough (mean weight 1.12±0.09 g) compared to control group (mean weight 1.03±0.02g) ($P>0.05$). These data suggest a possible application of nanosilver particles in aquaculture sector particularly using incubator troughs of trout containing nanosilver materials.

Key words: Silver nanoparticles, Rainbow trout egg, Hatchability, Survival

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Introduction

Nanotechnology, the science of producing and utilizing nano-sized particles is growing rapidly and in the last few years it begun to evolve in to a valuable science. In this view nanosilver particles have received more attention because of their antimicrobial properties and their applications to a wide variety of healthcare products including drug delivery systems, therapeutics, biosensors, environmental remediation and dressing materials (Kong et al.,2000 ; Aitken et al.,2006; Lansdown, 2006).

The small size of nanoparticles of metallic silver in solid or colloidal state allows for a high antimicrobial effect than silver salts (Lansdown, 2006; Atiyeh et al., 2007; Choi et al., 2008). The nanosilver particles can inhibit growth and multiplication of the bacteria and fungi by binding to their DNA, suppress their respiration by poisoning the respiratory enzymes and component of electron transport system, and alter the microbial membrane function by binding to bacterial surface (Wright et al., 1994; Percival et al., 2005).

A relatively new field of nano-aquaculture can incorporate nano technology in fish farming for increasing production, diagnosing any disease, cleaning fish ponds, developing a system of mass vaccination of fish using nanocapsules containing DNA nanovaccination and fastening the growth of fish through iron nanoparticles consumption in fish feed (Wong et al., 1992; O'Hagan, 1998; Janes et al., 2001; Alan, 2006; Rajesh Kumar et al., 2008).

Despite the broad applications of nanoparticles in both human and terrestrial animal veterinary medicines, minimum works have been done in aquatic medicine so far. The occurrence of fungal and bacterial infections particularly during hatching period of trout egg and growing of the produced larvae is still one of the main obstacles encountered with the trout industry worldwide. Particularly such problem has been elaborated since the use of the most effective aquaculture antifungal, malachite green has been inhibited several years ago. Therefore, the investigation to find new environmentally friendly chemical substances is a present necessity.

The objective of this study was to assess the effect of nanosilver particles in the form of water soluble suspension and also as a dressing material coated on the egg incubator/troughs on the hatchability of rainbow trout (*Oncorhynchus mykiss*) egg and survival of the produced larvae.

Matherials and mehthods

Nanosilver

The nanocid P-series powder products of NanoNasb Pars Company, Tehran, Iran are comprised of titanium dioxide coated with 1-10% nanosilver particles and used as an additive in industrial products such as polymers, glue, ceramic and paints. In this study, the nanocid powder coated on the surface of the polyethylene trough (200 x50 x 40 cm) was used. This trough has a unique specification possessing silver ion on the surface.

Also, the water soluble colloidal nanosilver particles (4000 mgL^{-1} stock solution) with average size of 5 nm containing Tween prepared in distilled water and brown in color were used.

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Experimental design

Brood stock selection and spawning

Apparently healthy brood rainbow trout were used in this experiment. The ripen brood fish were spawned after anesthetizing them with clove oil essence at 100 mg L^{-1}. The fertilization of eggs was performed according to the routine produced method described for trout eggs.

Trial 1

Six troughs each containing 14700 fertilized eggs (each trough containing three trays and each tray containing 4900 eggs (350 g) were used. The dead eggs were removed carefully 24 hour post-incubation and the remaining eggs were left untouched until the eyed-eggs stage. Four troughs were used as treatments and treated with nanosilver particles at concentrations of 0.5, 1.0 and 2 and 4 mgL^{-1} for 30 minutes per day until the eyed-egg stage. The dead eggs were removed carefully at eyed-egg stage, and the removal of dead eggs was continued daily until hatching time. One trough was treated with malachite green at 2 mgL^{-1} for 20 minutes per day as the positive control and one trough without any treatment as the untreated group (Table 1). The experiment was repeated for the second time and the percentage of hatched eggs was then calculated at the end of hatching period as follow:

% hatched eggs= (number of hatched eggs/number of initial eggs) x 100

Trial 2

One nanosilver trough containing four trays and each tray containing 6300 eggs (totally 25200 fertilized eggs) was used as the treatment. Two normal troughs similar to those in trial 1 were used as control groups. The dead eggs were removed carefully 24 hours post-incubation and the remaining eggs were left untouched until the eyed egg stage. The dead eggs were then removed carefully daily until hatching time. The experiment was repeated for the second time and, the percentage of hatched eggs was then calculated at the end of hatching period as follow: Percent of hatched eggs= (number of hatched eggs/number of initial eggs) x 100

The hatched larvae were then grown in the same troughs until one g in body weight. The larvae were first fed at 4.8% of their body weight on a commercial diet of SFT00 up to 0.5 g body weight and then at 4.4% of their body weight on SFT0 and SFT1 diets up to 1 g weight. The percentage of produced larvae was calculated as follow: Percentage of produced larvae= (number of survived larvae at the end of the experiment/number of initial larvae) x 100. Also, the biometry of larvae was preformed every 10-15 interval days. Daily mortality was also recorded.

Water quality

Water quality parameters consisting of dissolved oxygen, temperature, pH, ammonia, nitrite, carbon dioxide and hardness were 8±0.4 mgL^{-1}, 11.5±0.5°C, 7.5±0.1, <0.01±0.05 mgL^{-1},< 0.1±0.1mgL^{-1}, ca 9mgL^{-1} and 170±1.2 mgL^{-1}, respectively.

Other hatchability parameters including inlet water flow (7.5-8 $L.min^{-1}$ for the eggs and 45-50 $L.min^{-1}$ for the larvae), protection of light during hatching period and no manipulation of eggs were performed during the experiment.

Statistics

The statistical analysis of the obtained data was carried out using SPSS version 14

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software and T-test, Kruskal-Wallis and Mann-Whitnney test at P<0.05 as the confidence.

Results

The results of hatchability of eggs treated with nanosilver particles are shown in Table 1. The mean survival of eggs up to eyed-stage and treated with nanosilver particles at concentrations of 0.5, 1, 2 and 4 mg/L were 91.8±1.6, 91.1±0.3, 94.1±0.1, 94.5±0.1%, respectively, while those of positive (malachite green) and non-treatment group were 96±0.09% and 89.36±0.1 %, respectively. Statistical analysis showed that no significant difference was seen among the treated groups and also between untreated group and all nanosilver treated eggs up to eyed-egg stage (P>0.05).

Table 1: Survival of fertilized rainbow trout eggs treated with different concentrations of nanosilver at about12ºC. The data are shown as Means ± SE, based on two replications

Treatment (mgL^{-1})	Mean No. of initial fertilized eggs ± SE	Mean No. of survived eggs up to eyed-egg stage ± SE (%)	Mean No. of survived eggs from eyed-egg stage up to hatching time ± SE (%)
Nanosilver (0.5)	14029 ± 1.6	12881±2.2 (91.8±1.6)	3877±1.9 (27.6±0.2)
Nanosilver (1)	14043 ± 1.0	12797±1.9 (91.12±0.3)	5372±2.1 (38.25±0.1)
Nanosilver (2)	14001 ± 0.9	13175±1.6 (94.1±0.1)	5823±1.8 (41.6±0.4)
Nanosilver (4)	13987 ± 1.8	13217±2.4 (94.49±0.1)	6810±1.1 (48.6±1.5)
Malachite green (2)	14015± 2.5	13455±2.0 (96±0.09)	9080±2.2 (64.7±0.2)
Untreated group	14001± 2.3	12515±2.4 (89.36±0.1)	719±2.1 (5.1±0.2)
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In addition, eggs treated with malachite green (positive control) insignificantly resulted in a higher survival rate compared with both untreated and treated groups (P>0.05). Also, the mean survival of eggs from eyed-egg stage up to hatching period were 27.6±0.2, 38.25±0.1, 41.6±0.4 and 48.6±1.5 percent at concentrations of 0.5, 1, 2 and 4 mgL^{-1} nanosilver particles, respectively, while those of malachite green (positive control) and untreated group were 64.7±0.2 and 5.1±0.2 percent, respectively (Table 1) (Fig. 1). Statistically, there was significant difference between untreated and treated groups (P<0.05). Also, there was a significant difference between positive control and all nanosilver treated groups (P<0.05).

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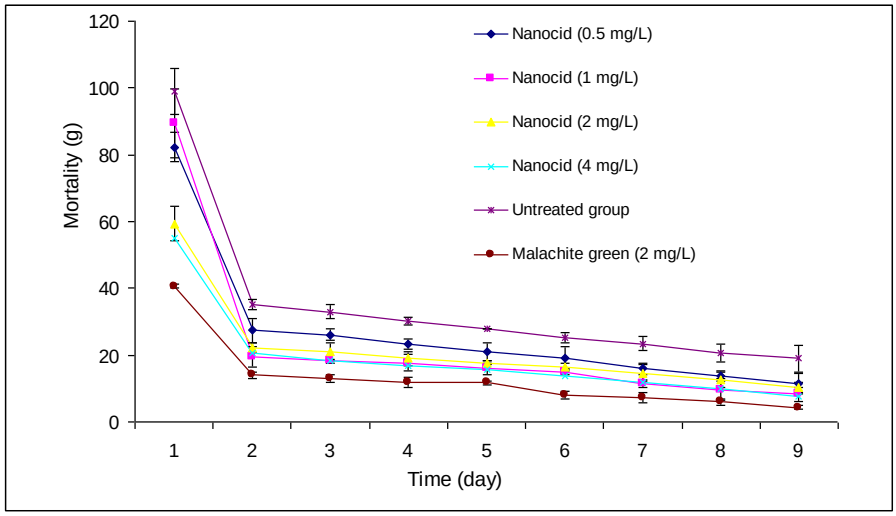

Figure 1: Mean mortality pattern of fertilized eggs of rainbow trout treated with different concentrations of nanosilver particles for 30 minutes per day until eyed-egg stage at about 12ºC

Table 2: Survival of the fertilized rainbow trout eggs incubated in nanosilver coated and normal troughs at about12 ºC. The data are shown as Means ± SE, based on two replications

Treatment	Mean No. of initial fertilized eggs ± SE (g)	Mean No of survived eggs up to eyed-egg stage ± SE (%)	Mean No of survived eggs up to hatching time ± SE (%)
Nanosilver trough	24365±1.9 (1728±1.5)	22503±1.7 (92.4±0.2)	16920±2.1 (69.4±0.1)
Normal trough	27606±2.0 (1958±1.6)	22360±2.2 (81±0.3)	11627±1.9 (52.5±0.1)

Eggs treated with 2 and 4 mgl $^{-1}$ nanosilver resulted significantly in higher survival than treated groups at 0.5 mgl $^{-1}$ nanosilver particles ($P<0.05$), while no significant difference was seen between eggs treated at 0.5 and 1 mgl $^{-1}$ nanosilver particles ($P>0.05$). Also, no significant difference was seen between eggs treated with 1 and 2 mgl $^{-1}$ nanosilver particles ($P>0.05$).The results of survival of eggs incubated in nanosilver trough are shown in Table 2.

The mean survival rate of eggs up to eyed-egg stage in nanosilver trough and normal trough was 92.4±0.2 and 81±0.3 percent, respectively which showed a significant difference ($P<0.05$). Also, the mean survival rate of eggs from the eyed-egg stage up to hatching time was 69.4±0.1 and 52.5±0.1 percent for nanosilver trough and normal trough, respectively which showed a significant difference ($P<0.05$). In addition the pattern of mortality of eggs from eyed-egg stage up to hatching time is shown in Figure 2. Based on this figure, the mean mortality of eggs incubated in nanosilver trough was significantly lower than the mean mortality of eggs incubated in normal trough during 7 days after eyed-egg stage ($P<0.05$).

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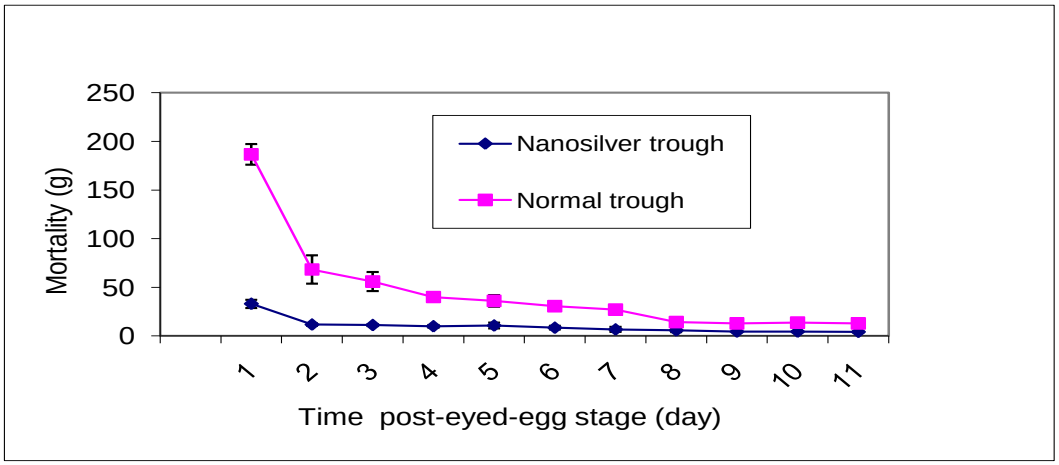

Figure 2: Mean mortality of rainbow trout egg incubated in nanosilver trough compared with normal trough at about 12 ºC

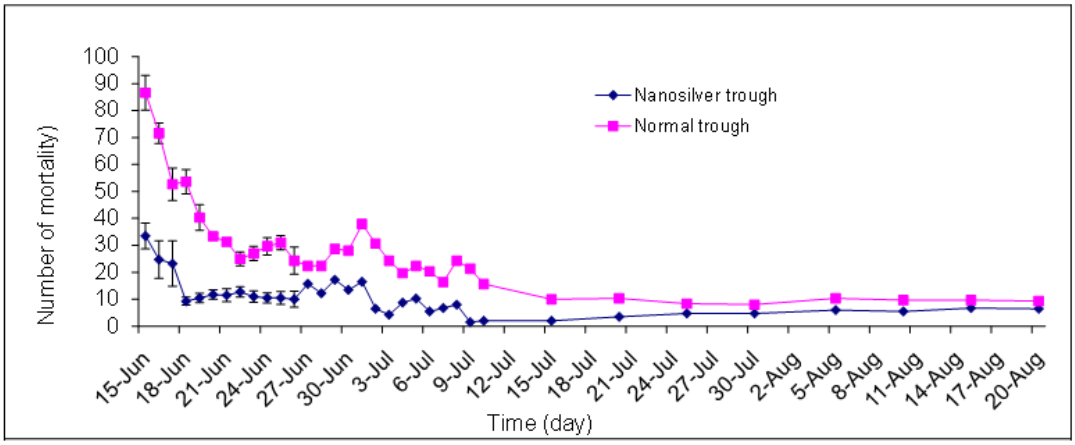

Figure 3: Mortality progress of rainbow trout larvae grown in the nanosilver trough compared with normal troughs

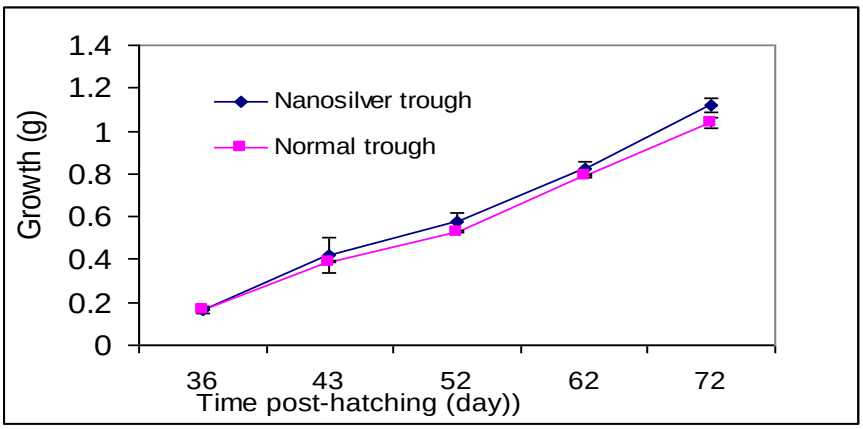

Figure 4: Growth of produced rainbow trout larvae grown in nanosilver trough at about 12°C

The pattern of mortality rate for the produced larvae is shown in Figure 3. According to this figure the mean mortality of larvae grown in nanosilver trough was significantly lower than the normal trough during almost 30 days post-hatching period ($P<0.05$).At the end of growing period (up to one g weight), the

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mean survival of grown larvae in nanosilver trough was 91.8±1.5 percent while that of normal trough was 87.3 ± 0.1 percent ($P<0.05$). Also, the larval biometry (mean total weight) resulted in 1.12±0.09 g and 1.03±0.02 g for nanosilver trough and normal trough, respectively ($P>0.05$) (Figure 4).

Discussion

The activity of colloid nanosilver particles against a wide range of gram positive/ negative bacteria, viruses and fungi have been reported by a number of researchers including Panacek et al. (2006), Ju-Nam and Lead (2008), Zhang et al. (2008), Weir et al. (2008), Pallab Sanpui et al. (2008), Rai et al. (2009), Fayaz et al. (2009), Tai et al. (2009) and Barrena et al. (2009).

The mechanism behind the antimicrobial activity of nanosilver particles is basically due to interaction with sulfur containing proteins in bacterial cell wall as well as with phosphorus containing compounds like DNA and finally cell lysis and death occurs as a result of disrupting membrane permeability, deactivating cellular enzymes, impairment of energy cycle and DNA replication (Feng et al., 2000; Jeon et al., 2003; Sondi and Salopek-Sondi, 2004; Morones et al., 2005; Song et al., 2006).

Two major kinds of nanotechnology products are defined as nanomaterials fixed on a substrate i.e. coated devices and free nanoparticles (Piotrowska et al. 2009). In this study we used both forms of nanosilver particles to investigate their antimicrobial effects on the survival of rainbow trout eggs and the produced larvae. In the first experiment the nanosilver particles at used concentrations could inhibit growth and multiplication of potential pathogenic micro organisms particularly *Saprolegnia* so that the percentage of egg survival and its hatchability were significantly higher than untreated control ($P<0.05$). However such antimicrobial effect was less than malachite green after eyed-egg stage. Also, the use of nanosilver particles coated on the trough in the second experiment resulted in a higher rate of egg survival and hatchability indicative of its more efficient antimicrobial effects compared with the untreated control group.

Nanosilver particles coated devices are characterized with clear antimicrobial properties since they release silver ions gradually, thus provide a sustained supply of silver ions at the coating interface for prolonged prevention of bacterial growth (Furno et al., 2004; Roe et al., 2008; Chen and Schluesener ,2008). Accordingly the higher rate of trout egg survival and hatchability obtained in the present study may be due to the continuous release of silver ions from the polymeric silver coated trough providing suitable antimicrobial activity. Moreover it should be noted that nanosilver particles chemistry including their size distribution, morphology, surface area, charge, surface modifications, chemical composition (purity) have important role in their possible mechanisms of antimicrobial action (Singh et al.,2009). Nanosilver particles tend to aggregate in aqueous solutions because of their hydrophobic properties. Different stabilizers are being used to prevent their agglomeration and to obtain isolated nanosilver particles to produce more efficient antimicrobial effects (Hyung et al., 2007). The nanosilver particles used in this study were

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in the form of silver ion with about 5 nm in size containing some Tween probably as a surfactant and according to the manufacturer description no more substance was added. Development of new nanoparticle surface engineering strategies should lead to more environmentally friendly nanoparticles. Biogenic synthesis of nanomaterials is an important aspect of current nanotechnology research, for instance use of fungus *Trichoderma viride* for the extracellular biosynthesis of silver nitrate particles from silver nitrate solution has been reported by Fayaz et al. (2009). It was observed that the aqueous silver (Ag+) ions, when exposed to filtrate of *T. viride* reduced in solution, thereby resulted in formation of extremely stable nanosilver particles.

In conclusion, this study suggests an application of nanosilver particles with their unique chemical and physical properties particularly in the form of nanosilver dressing troughs as a disinfectant in fish farming. Also, nanosilver particles may have a priority to current antibiotic/antifungal chemicals as according to Rai et al. (2009), the microorganisms are unlikely to develop resistance against silver nanoparticles, compared to antibiotics due to their large surface area in contact with and broad range of targets in the microbes. However, there are some questions, which need to be addressed, such as the exact mechanism of interaction of silver nanoparticles on the fungal and bacterial cells and how the surface area of nanoparticles influences its killing activity.

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