

# Application of nanosilver-coated zeolite as water filter media for fungal disinfection of rainbow trout (*Oncorhynchus mykiss*) eggs

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**Abstract** This study investigated the indirect use of silver nanoparticles (AgNPs) for reduction of fungal infections during incubation period of fertilized rainbow trout eggs. Different concentrations of nanosilver-coated zeolite (0.5, 1, and 1.5 % AgNPs) were compared with unmodified zeolite as water filter media in semi-recirculation systems. For testing the effect of AgNPs on reduction of fungal infection, fertilized eggs were transferred in incubators receiving water from filters coated with nanosilver. The eggs in each incubator were inoculated with *Saprolegnia*-infected trout eggs. Any dead or infected eggs and embryos were periodically removed, while the performance of the filters was assessed by calculating the survival rates from fertilization up to completion of the yolk-sac absorption stage. The results showed that the filters containing 0.5 % AgNPs increased the survival rate by 4.56 % from fertilization to the swim up stage compared to the control ( $p < 0.05$ ). Also, the additional application of activated carbon (as absorbent media) along with AgNP-coated media in filters caused an increase of about 11.24 % in the survival rate for the larval stage ( $p < 0.05$ ). In contrast to the control group with about 6 % fungal infection, no infections were observed during the incubation period in the incubators containing nanosilver-coated filters. Therefore, the final results confirmed that the indirect use of AgNPs in the aforementioned filters were significantly effective for preventing fungal infections in semi-recirculation systems for rainbow trout, making them a candidate

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for replacing the chemical fungicides currently used during egg incubation in hatchery systems.

**Keywords** Aquaculture · Silver nanoparticles · Coating · Water filter · Incubator · Rainbow trout · *Saprolegnia* · Recirculating system

## Introduction

Nanotechnology is a novel technology, and many promising applications are starting to appear in the area of agriculture sciences, especially aquaculture, where the antimicrobial properties of certain nanomaterials are of particular interest (Li et al. 2008). For example, the effectiveness of nano-metallic particles for the disinfection of potable water has been reported in the order of silver (Ag), copper (Cu), zinc (Zn), titanium (Ti), and cobalt (Co) (Nangmenyi and Economy 2009). However, there has not yet been any report as far as it is known on the indirect use of nanomaterials for the disinfection of water for the aquaculture industry.

Water is one of the transmittal routes of many microorganisms that cause diseases in aquatic organisms; thus, many disease protection methods are based on water disinfection. The main cause of economic loss in aquaculture is diseased fish, followed by oomycete (water molds) infections (Meyer 1991). Therefore, reducing fish diseases is crucial to the future success of the aquaculture industry, and in this regard, nanotechnology may offer some new solutions. Saprolegniasis caused by water molds, such as *Saprolegnia*, *Achlya*, and *Aphanomyces*, is one of the major diseases affecting fish eggs and fish populations, making its control of great economical importance in commercial fish farming, especially during embryonic incubation period. As the incubation period of rainbow trout and other salmonids fertilized eggs takes several weeks, fungal attacks can result in extensive egg mortality unless they are prevented or controlled by regular fungicide treatments (Niska et al. 2009). However, the use of disinfectant chemicals can have unfavorable effects on the cultured organisms and surrounding environment. For example, malachite green is very effective to control fungal infections in fish and fish eggs, yet it is known to be carcinogenic, mutagenic, and teratogenic (Meyer and Jorgenson 1983; Pottinger and Day 1999); therefore, its application is limited to the treatment of unedible fish. Formalin is one of few registered aquatic fungicides, yet it is not completely effective for controlling fungal infections in fish or fish eggs (Bruno and Wood 1999), and there are also concerns about its effect on both the environment and the personnel handling it (Fitzpatrick et al. 1995). The use of other fungicides, such as ozone, hydrogen peroxide, sodium chloride, iodophor, and copper is not widespread (Bruno and Wood 1999; Forneris et al. 2003; Rach et al. 1998; Schreier et al. 1996). Thus, new methods need to be developed for controlling fungal infections in aquaculture.

Recently, various inorganic antibacterial and antifungal materials containing silver have been developed, and some of them are already in commercial use (Hansel et al. 1998; Johari et al. 2014b; Kawahara et al. 2000; Palenik and Setcos 1996; Shahverdi et al. 2007; Wang et al. 2007; Yamamoto et al. 1996). Silver is well known to have a wide antibacterial spectrum and be relatively safety (Cho et al. 2005; Mohan et al. 2007; Oya 1996; TIC 1998). One of the antimicrobial silver species that has yet received little attention is nanometer-sized silver particles (AgNPs) (Cho et al. 2005; Mohan et al. 2007; Shahverdi

et al. 2007). Some efforts have examined the direct use of colloidal silver nanoparticles as an antibacterial and antifungal medication in the incubation systems of rainbow trout (Soltani et al. 2009 and Soltani et al. 2011). Although colloidal nanosilver is known to have antimicrobial properties, when added directly to water for disinfection, however, this approach might cause disastrous impact on the environment and organisms (Asghari et al. 2012; Johari et al. 2013a, b; Johari et al. 2014a, c; Salari Joo et al. 2013). Notwithstanding, one of the special properties of silver nanoparticles is that they can be easily mixed or used to coat other substances, making them a good candidate for the indirect treatment of water via coated water filters (Jain and Pradeep 2005; Lv et al. 2009; Nangmenyi and Economy 2009; Oyanedel-Craver and Smith 2008; Phong et al. 2009). If the coating process is performed in an exact manner, the release of silver from the coating can be gradual and minimal, plus the antimicrobial activity will be long-term (Lv et al. 2009; Soloviev and Gedanken 2011).

Accordingly, to investigate the indirect use of silver nanoparticles as an antifungal medication in aquaculture, this study applied silver nanoparticles coated on natural zeolite, to the water filtration system of rainbow trout egg incubators to control and reduce fungal infections caused by *Saprolegnia* Sp.

## Materials and methods

### Preparation and characterization of filter media

Natural zeolite was obtained from Afrand Tooska Co. (Iran) to use in the base of filter media. The zeolite granules were washed with distilled water and graded using standard sieves. Zeolite grains sized between 2 and 4 mm were then coated with silver nanoparticles at nominal concentrations of 0.5, 1 and 1.5 % by Pars Nano Nasb Co. Ltd. (Tehran, Iran). All the coatings were performed using the sol–gel dip coating technique (Jeon et al. 2003; Gil et al. 2006).

To determine the crystalline phase of the nanosilver-coated zeolite, X-ray diffraction (XRD) was performed using a Philips X'Pert-MPD X-ray Diffraction System (Netherlands) (Tube: Cu  $\text{k}\alpha$ ,  $\lambda$ : 1.54056 Å, Step Size: 0.02°/s, Voltage: 40 kV, Current: 40 mA). Plus, an X-ray fluorescence (XRF) chemical analysis of the nanosilver-coated zeolite was also performed using a Philips PW2404 XRF Spectrometer (Netherlands).

Scanning Electron Microscope (SEM) photographs of the coated and uncoated zeolite were recorded using a Philips XL30 SEM (Netherlands) after sputter-coating a thin gold layer using a Philips SCD005 cool sputter coater. The particle size of the silver nanoparticles coated on the zeolite was determined based on a selection of ten of the best taken SEM photographs using Axio Vision digital image processing software (Release 4.8.2.0, Carl Zeiss Micro Imaging GmbH, Germany). For this purpose, more than 50 particles were measured in each SEM photograph and the mean  $\pm$  SD was calculated.

### Recirculation systems description and operation

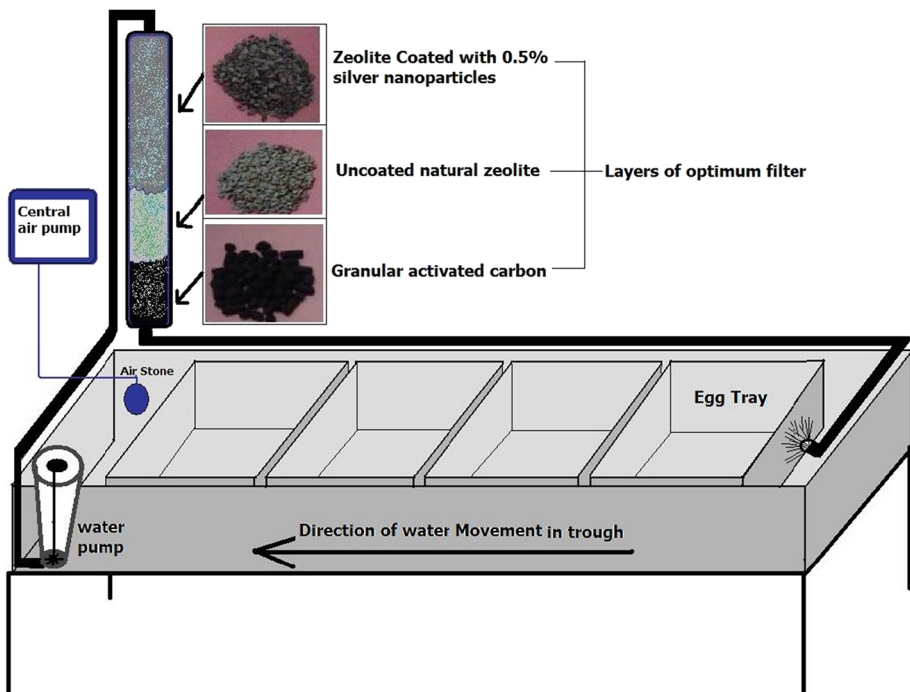
The tap water was dechlorinated by adding 1 mg/l sodium thiosulfate, followed by continuous vigorous aeration for at least 48 h in 1000-l reservoir tanks. The dechlorinated tap water used as the water source and various chemical characteristics, including the ammonium, sulfide, magnesium, total hardness, potassium, calcium hardness, and chloride, was all measured using a Palintest photometer (Model: 8000, UK), and the sodium was

measured using a Philips atomic absorption spectrophotometer (Model: PU9400X). The means of these chemical values for the dechlorinated tap water are shown in Table 1.

In total, 21 separate semi-recirculation systems were used in this study, and a schematic diagram of one of those recirculating systems is shown in Fig. 1. Each system consisted of a standard fiberglass California type trout trough (220 × 45 × 17 cm) that was filled with 100 l of dechlorinated tap water and equipped with a water recirculating system. The water was pumped using a single centrifugal pump (5 l per minute) from the end part of the trough to a cylindrical filter column (80 cm height × 8 cm diameter). These filter columns are commercially available in the market with the name “in line filter housing.” After passing through the filter media, the water was returned to the opposite side of the trough. The water aeration was performed using a spherical diffuser stone (5 cm diameter) that was connected to a central air pump. In each incubation system, 50 % of the water was exchanged with fresh oxygenated water every day.

**Table 1** Chemical characteristics of dechlorinated tap water used in incubation systems

Parameter	NH <sub>4</sub> <sup>+</sup>	S <sup>2-</sup>	Mg <sup>2+</sup>	Cl <sup>-</sup>	Na <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Calcium hardness	Total hardness
mg/L	0.1	0.00	39	2.4	13.8	13.8	3.9	26	150



**Fig. 1** Schematic diagram of one of the recirculating systems used in this study. The incubation system used a combination of 0.5 % AgNP-coated zeolite, plus activated carbon and uncoated zeolite as the absorbent filter media (0.5Ag-AFM)

## Filters

A total of seven filter types were evaluated in this study, six of them were treated with silver nanoparticles, plus one as the control. Filters consisting of 400 g uncoated natural zeolite were used as the control. The first group of filters included treated filter media, consisting of 400 g natural zeolite coated with 0.5, 1, or 1.5 % of silver nanoparticles. Meanwhile, the second group of filters included a nanosilver-coated media layer plus an absorbent filter media (AFM) layer. AFM consisting of a layer of 200 g natural zeolite along with a layer of 200 g granular activated carbon. The details of all the filters are summarized in Table 2, and each was tested in triplicate.

To measure the release of silver ions or silver nanoparticles from the filters into the water, sampling was performed from each trough on days 5, 15, and 30 after egg fertilization. The water samples were acidified with HNO<sub>3</sub> to reduce the pH to less than 2 and then placed in light-shielded glass vessels at 4 °C. Prior to the measurements, the water samples were digested using 69 % HNO<sub>3</sub> and the total silver concentrations measured using a Philips model PU9400X atomic absorption spectrophotometer (ASTM, 1991).

## Fertilization and incubation of rainbow trout eggs

Milt and egg pools were obtained from 12 different males (1 year old) and 12 different females (2 years old), respectively, by artificial spawning and then fertilized using a conventional method for rainbow trout. Sixty minutes after fertilization (completion time for water uptake), 500 fertilized eggs were transferred to each incubation tray (2000 eggs per trough) supplied with recirculated water ( $10 \pm 0.6$  °C). The water temperature and chemistry were routinely monitored and maintained constant over the entire incubation period. During the incubation period, the troughs were covered with dark blue plastic sheets to protect the eggs and larvae from light. Any dead eggs or embryos were periodically removed, and the survival rates expressed as the percentage of the initial number of eggs 24 h after fertilization (during the 24 h following fertilization, any white eggs were

**Table 2** Summary of filters used in this study

Filter name (notation)	Main filter media (MFM)	Nominal percentage of nanosilver-coated on MFM	Measured percentage of nanosilver in MFM by XRF	Absorbent filter media (AFM)
0Ag-O	Uncoated natural zeolite (400 g)	0	0	None
0.5Ag-O	Nano-Silver coated natural zeolite (400 g)	0.5	0.216	Natural zeolite (200 g)+
1Ag-O		1	0.627	
1.5Ag-O		1.5	1.05	
0.5Ag-AFM	Granular activated carbon (200 g)	0.5	0.216	
1Ag-AFM		1	0.627	
1.5Ag-AFM		1.5	1.05	

removed and considered as unfertilized eggs, and thus not included in the survival rate calculations).

The eggs were checked every day, and those showing fungal infection were removed and recorded. Also, survival from 24 h post-fertilization to the eyed-stage (eyeing rate, 150 °C days), survival from 24 h post-fertilization to hatching (hatching rate, 300 °C days), survival from 24 h post-fertilization to the completion of yolk-sac resorption (YSR survival rate, 550 °C days), and survival from hatching to yolk-sac resorption were all monitored. The occurrence of noticeable morphological malformations (spinal cord torsion, head, or caudal fin malformations) at YSR was expressed as the percentage of malformation. The weight of all the normal larvae at YSR (55 days post-fertilization) was also measured. All the animals were treated humanely as regards the alleviation of suffering, and all the laboratory procedures involving the animals were reviewed and approved by an Animal Care and Use Committee in accordance with the Animal Welfare Act and Interagency Research Animal Committee guidelines (Nickum et al. 2004).

### **Inoculation of *Saprolegnia* Sp. into incubation systems**

A pure stock of *Saprolegnia* Sp., previously purified and characterized by the Department of Aquatic Animal Health, Veterinary Medicine Faculty of the University of Tehran (Shahbazian et al. 2010), was cultured on a glucose-yeast extract agar (GYA) and stored at 4 °C until use. Subcultures were created by culturing the *Saprolegnia* Sp. in a glucose-yeast extract broth at 20 °C for 4 days. Approximately 10 ml of the subculture inoculum was then diluted in 90 ml of sterilized distilled water and used to inoculate trout eggs that were placed in several 250-ml Erlenmeyer flasks (100 eggs per flask) and covered with Parafilm (modified method of Gieseke et al. 2005). A white fungal growth appeared on the trout eggs after 4 days. Fifty infected eggs were then placed in the entry of water into the each trough to inoculate the water of each rainbow trout recirculating system (100 l). The inoculation was started on the third day after fertilization and then repeated every 4 days until the eggs hatched.

### **Statistics**

In all cases, the standard deviations (STDV) and standard errors of the means (SEM) were calculated. All the statistical analyses were performed using SPSS Statistics 17.0 software, and all the data tested for normality (Kolmogorov–Smirnov test). The data were analyzed using a one-way analysis of variance (ANOVA), and the significant means compared using Duncan's test.  $p < 0.05$  was considered statistically significant.

## **Results**

### **Characterization of filter media**

The XRF results revealed the chemical composition of the natural zeolite coated with different percentages of silver nanoparticles. As seen in Table 3, the presence of silver was evident in the AgNP-coated zeolite. For the nominal 0, 0.5, 1, and 1.5 % AgNP-coated zeolite, the actual percentages of silver were 0, 0.216, 0.627, and 1.05 %, respectively;

**Table 3** Chemical composition of natural zeolite coated with different nominal concentrations of silver nanoparticles determined using XRF analysis

Nominal silver concentration (%)	Chemical composition determined by XRF (% wt.)												
	L.O.I	Na <sub>2</sub> O	MgO	Al <sub>2</sub> O <sub>3</sub>	SiO <sub>2</sub>	P <sub>2</sub> O <sub>5</sub>	SO <sub>3</sub>	K <sub>2</sub> O	CaO	TiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	Sr	Ag
0	14.11	1.54	1.11	10.46	67.86	0.02	0.02	1.80	1.85	0.16	0.91	0.11	0
0.5	13.96	1.42	1.11	10.29	67.92	0.02	0.02	1.89	1.90	0.17	0.92	0.11	0.216
1	12.58	1.22	1.01	10.40	68.93	0.02	0.02	1.92	1.99	0.18	0.93	0.11	0.627
1.5	13.04	1.28	1.09	10.33	68.23	0.02	0.02	1.84	1.84	0.15	0.94	0.11	1.05

L.O.I Loss on Ignition

based on this, the actual percentages of nanoparticles incorporated in the natural zeolite were less than the nominal percentages.

An X-ray diffractogram of the AgNP-coated and uncoated natural zeolite is shown in Fig. 2. The XRD results indicated that clinoptilolite was the main phase present in the zeolite samples in this study. As seen in Fig. 2, there was no distinct difference in the XRD patterns between the AgNP-coated and uncoated zeolite samples.

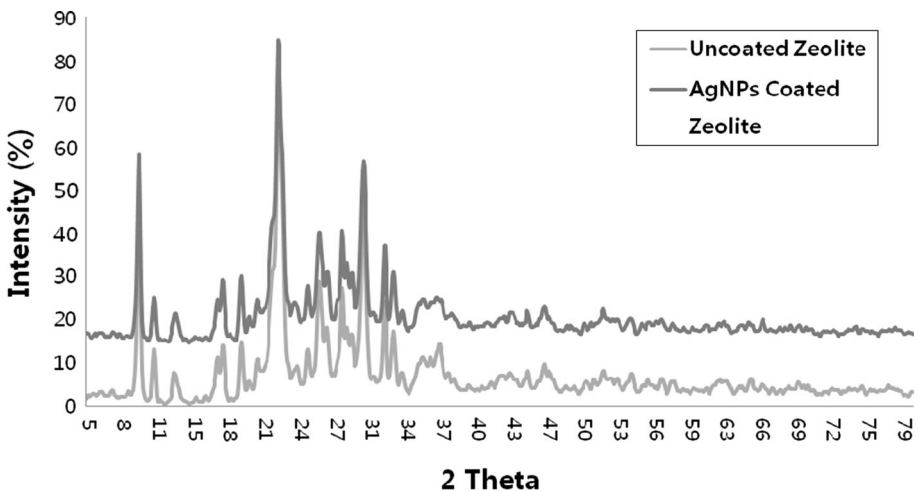
Scanning Electron Microscope (SEM) photographs of the AgNP-coated and uncoated natural zeolite are shown in Fig. 3. As seen in this figure, spherical-shaped silver nanoparticles uniformly coated the surface of the zeolite. A data analysis of the SEM photographs revealed that the mean particle size of the silver particles on the surface of the zeolite was  $90.26 \pm 45.51$  nm.

### Egg incubation and larval production efficiency

In all the trials, the water quality parameters were within the acceptable tolerance ranges for rainbow trout during the incubation period. The means and standard deviations for the dissolved oxygen, temperature, and pH during the experiment were  $8.91 \pm 0.30$  mg/l,  $10 \pm 0.6$  °C, and  $8.28 \pm 0.04$ , respectively.

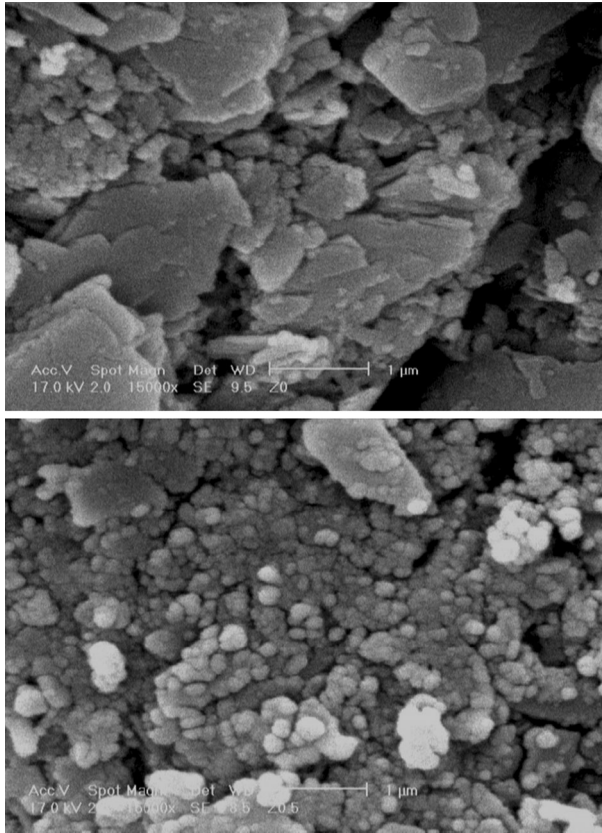
An equal number of eggs (2000) per treatment were used at the beginning of the experiment, but losses were observed only in some treatments during 24 h post-fertilization; so that although the numbers of eggs in 0Ag–O, 0.5Ag–O, 0.5Ag–AFM, and 1Ag–AFM treatments showed a negligible reduction after 24 h post-fertilization (<0.5 %), these numbers reduced from 2000 eggs to 198, 415, and 637 eggs in 1.5Ag–O, 1Ag–O, and 1.5Ag–AFM respectively.

During the incubation, fungal infection was only observed in the control groups (in about 6 % of the initial eggs) and just before the eyed-egg stage. The fungal colonies appeared as white to brown cotton-like growths on the egg mass and caused nearby eggs to adhere together, resulting in the death of these eggs. After the eyed-egg stage and in spite of continued inoculation with *Saprolegnia*, no fungal infection was observed in any of the AgNP-treated groups, including the live and dead eggs.



**Fig. 2** X-ray diffractograms of uncoated and silver nanoparticle-coated natural zeolite

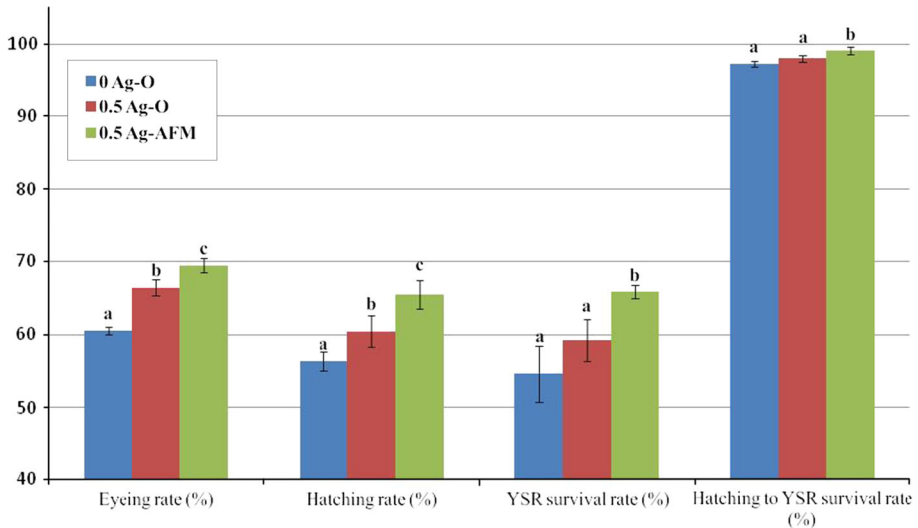




**Fig. 3** SEM photographs of uncoated (*above*) and silver nanoparticle-coated (*below*) natural zeolite. The scale bars are as indicated in the pictures (1  $\mu\text{m}$ )

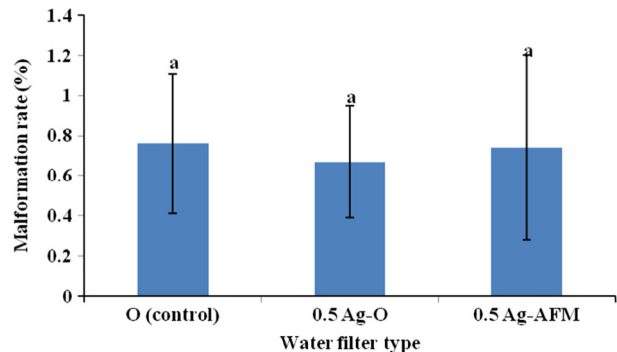
Notwithstanding, it is important to note that, despite the lack of fungal infection with the AgNP treatments, all the eggs treated with the 1 and 1.5 % AgNP-coated zeolite (1Ag-O and 1.5Ag-O) died during the early stages of embryonic development. In particular, the total silver concentrations measured in the water for these two groups showed a high release of silver compounds from the filters into the water. Moreover, when using the AFM layer along with a nanosilver-coated media layer in these two groups, a negligible portion of eggs reached the eyed-egg stage (2.5 % with 1Ag-AFM and 0.5 % with 1.5Ag-AFM), while none of them reached the hatching stage.

In contrast, the 0.5 % AgNP-coated zeolite (0.5Ag-O) treatment resulted in higher eyeing and hatching rates compare to those for the uncoated zeolite (0Ag-O or control) treatment (Fig. 4,  $p < 0.05$ ). Furthermore, the 0.5Ag-AFM, which used both AFM and 0.5 % AgNP-coated zeolite, resulted in the highest eyeing and hatching rates when comparable to those with the 0.5Ag-O and 0Ag-O filters (Fig. 4,  $p < 0.05$ ). The survival from 24 h post-fertilization to the completion of yolk-sac resorption (YSR survival rate) as well as survival from hatching to yolk-sac resorption was also significantly higher for the 0.5Ag-AFM group when compared to that for the 0.5Ag-O and 0Ag-O (control) groups (Fig. 4,  $p < 0.05$ ).



**Fig. 4** Survival rates from 24 h post-fertilization to eyeing (eyeing rate), from 24 h post-fertilization to hatching (hatching rate), from 24 h post-fertilization to completion of yolk-sac resorption (YSR survival rate), and from hatching to completion of yolk-sac resorption with 0Ag-O, 0.5Ag-O, and 0.5Ag-AFM (mean  $\pm$  SD). Values with *different superscripts* (a, b, c) are significantly different (ANOVA,  $P < 0.05$ )

**Fig. 5** Malformation rates (spinal cord torsion, head, or caudal fin malformations) calculated after completion of yolk-sac resorption (YSR) with 0Ag-O, 0.5Ag-O, and 0.5Ag-AFM (mean  $\pm$  SD). There was no significant difference in the malformation rates (ANOVA,  $P > 0.05$ )

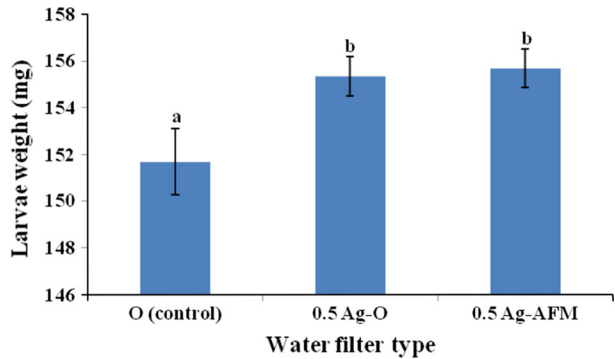


The differences in the larval malformation with the different treatments in this study were not significant and always lower than 0.8 % (Fig. 5,  $p > 0.05$ ). Also, although the weight of the larvae on the 55th day post-fertilization was significantly higher for the 0.5Ag-O and 0.5Ag-AFM groups compared to the controls (Fig. 6, ANOVA,  $p < 0.05$ ), this difference was not remarkable (only a slight increase of about 4 mg).

### Release of silver compounds from filters

The total silver concentrations released from the different filters into the water on days 5, 15, and 30 after fertilization are summarized in Table 4. No silver concentration was detected in the water of the control incubators that did not use AgNP-coated media (0Ag-O). For the 0.5Ag-O group, silver was detected at concentrations of less than about

**Fig. 6** Weights of all normal larvae measured after completion of yolk-sac resorption (YSR) with 0Ag-O, 0.5Ag-O, and 0.5Ag-AFM (mean ± SD). Values with different superscripts (a, b) are significantly different (ANOVA,  $P < 0.05$ )



**Table 4** Total silver concentrations in water of incubation systems on 5th, 15th, and 30th day after fertilization

Filter name	Total Ag concentration (mg/L, mean ± SD)		
	5th dpf	15th dpf	30th dpf
0Ag-O	0	0	0
0.5Ag-O	0.040 ± 0.03 <sup>a,A</sup>	0.035 ± 0.07 <sup>a,A</sup>	0.047 ± 0.08 <sup>a,A</sup>
0.5Ag-AFM	0.003 ± 0.01 <sup>a,B</sup>	0.013 ± 0.05 <sup>b,B</sup>	0.015 ± 0.03 <sup>b,B</sup>
1Ag-O	0.183 ± 0.20 <sup>C</sup>	–	–
1Ag-AFM	0.199 ± 0.12 <sup>C</sup>	–	–
1.5Ag-O	0.318 ± 0.31 <sup>D</sup>	–	–
1.5Ag-AFM	0.345 ± 0.19 <sup>D</sup>	–	–

dpf days post-fertilization

<sup>a,b</sup> means within a row with different superscripts are significantly different ( $P < 0.05$ )

<sup>A,B,C,D</sup> means within a column with different superscripts are significantly different ( $P < 0.05$ )

0.05 mg/l, yet there was no significant difference between the sampling days ( $p < 0.05$ ). For the 0.5Ag-AFM group, despite the application of the AFM, silver was detected on the 5th day post-fertilization, although the concentration was significantly lower than the silver released with the 0.5Ag-O (0.003,  $p < 0.05$ ). In addition, the concentration of silver detected in the 0.5Ag-AFM group was significantly lower on the 5th day than on the 15th and 30th day ( $p < 0.05$ ).

With the 1 and 1.5 % AgNP-coated media treatments, high concentration of silver was detected in the water on the 5th day, and even the AFM were unable to decrease the silver concentrations in these groups (Table 4). It should also be noted that, due to the high mortality rate of eggs in these groups during the first few days, there were no remaining samples to measure the silver concentration on day 15 and 30.

## Discussion

The results from different characterization methods of filter media revealed that coating of silver nanoparticles were performed correctly. No peak related to crystalline silver was identified in the AgNP-coated zeolite, indicating that the silver nanoparticles coating the

surface of the zeolite samples were amorphous. X-ray amorphous silver nanoparticles have already been reported by Liu et al. (2001), who suggested that the very small AgNP crystals seemed to be structureless, making them undetectable by XRD.

According to the results of egg incubation, it seemed that water filters containing silver nanoparticles have the ability to control and inhibit fungal infections during the incubation period of rainbow trout. While the ability to sterilize drinking water using filters containing silver nanoparticles has already been proven (Jain and Pradeep 2005; Lv et al. 2009; Nangmenyi and Economy 2009; Oyanedel-Craver and Smith 2008; Phong et al. 2009), this is the first application of these filters in aquaculture.

The mechanisms of the antimicrobial activity of silver nanoparticles have previously been reported (Kim et al. 2007; Morones et al. 2005; Rai et al. 2009) and include: (1) changing and damaging the membrane structure of a microorganism, which increases its permeability and disrupts its transportation functions, resulting in cell death, (2) penetration of a microorganism and interaction with phosphorus and sulfur-containing compounds, such as DNA and proteins, (3) loss of the replication ability of the DNA, (4) inactivation of certain enzymes, (5) attacking the respiratory chain, (6) generating hydrogen peroxide and free radicals, and (7) the release of silver ions from the nanoparticles, the antimicrobial activity of which is well known (Feng et al. 2000; Song et al. 2006; Yamanaka et al. 2005; Yoshihiro 2002). Among the above-mentioned mechanisms, two antifungal mechanisms are suggested to explain the behavior of the AgNP-coated zeolite: (1) The fungi are directly killed by the silver ions released from the filters (fungicidal effect) and (2) by passing through the AgNP-coated zeolite, the fungi are contaminated with silver ions, yet still survive; however, they cannot grow into colonies on the surface of the eggs, as the silver ions affect their replication and growth ability (fungistatic effect).

As mentioned in the results, notwithstanding the lack of fungal infection with the AgNP treatments, all the eggs treated with 1Ag–O and 1.5Ag–O died during the early stages of embryonic development. Moreover, according to the results, increasing the percentage of nanosilver coating on the filter media significantly increased the release of silver compounds into the water. The high concentration of silver compounds in the water appeared to be the reason for the egg mortality with the 1 and 1.5 % AgNP treatments. Yet, it is well known that silver compounds are toxic and lethal for different life stages of rainbow trout (Buhl and Hamilton 1991; Davies et al. 1978; Grosell et al. 2000; Hogstrand et al. 1996; Nebeker et al. 1983; Asghari et al. 2012; Johari et al. 2013a, b). During the embryonic stage, the egg's chorion is a protective barrier against environmental stressors and many toxic chemicals (Rombough 1985). In the case of chronic exposure to silver, it has been shown that about 85 % of the total silver content can be absorbed by the chorion, while the rest is absorbed by the embryo (Guadagnolo et al. 2000). However, at high silver concentrations, even the protective properties of the chorion are unable to prevent the toxic effects of silver. Therefore, while it is believed that the antimicrobial activity of nanosilver-coated filters is due to the gradual release of ionic silver from the filters (Lv et al. 2009; Nangmenyi and Economy 2009), this study showed that, if the released amount exceeds a certain level, it can have toxic effects on aquatic organisms. In fact, this technology is a double-edged sword, and one should carefully select a special dose of silver nanoparticles in filter media, which while not toxic to fish, but kill the pathogenic microorganisms. Consequently, since the quality and quantity of the silver coating showed a substantial impact on the filter performance during the incubation period, more studies are needed to find better techniques for the stable coating of silver nanoparticles on the surface of different filter media to reduce the release of silver compounds from filters.

It seems that, unlike the 1 and 1.5 % silver nanoparticle-coated filters that were lethal for trout eggs, the filter media coated with 0.5 % AgNPs increased the propagation efficiency during the incubation of the fish eggs and larva; this increase appeared to be due to the prevention effects of the silver nanoparticles against fungal infections in the incubation system. Also, the use of the absorbent filters along with the filters containing silver nanoparticles significantly increased the propagation efficiency. In this study, the aim of using the absorbent filters was absorption of the silver compounds released from the AgNP-coated media. However, the zeolite and activated carbon filters had some effect in this regard, and the increased propagation efficiency with the 0.5Ag-AFM could be partly due to absorption of the silver compounds by the AFM. Furthermore, some of the increase may have been due to the absorption of other harmful compounds by the AFM, as activated carbon and natural zeolite are both known as good absorbents of ammonia and chemical compounds, such as drugs and malachite green, in aquaculture (Dryden and Weatherley 1987a, b, 1989; Bergero et al. 1994; Aitcheson et al. 2000, 2001; Wang and Ariyanto 2007; Jegatheesan et al. 2009). Further studies are needed to develop better AFM with a longer and higher capacity for silver absorption.

## Conclusion

The results of the present study revealed that the application of 0.5 % nanosilver filters was significantly effective in preventing fungal infections and resulted in a higher propagation output. This new generation of antifungal water filters may also have the potential for widespread use in water treatment applications in the aquaculture industry for other aquatic animals and against other microorganisms. More studies might be needed to examine possible undesirable effects on the cultured fish, such as the bioaccumulation, changes in expression of genes related to silver toxicity, food consumption and growth rate.

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