

Disinfection Efficiency of Three Anti-Fungal Agents (Nanosil, Chloramine-T and Hydrogen Peroxide) on Persian Sturgeon (*Acipenser persicus*, Borodin 1897) Larvae

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Abstract

Due to the high mortality of eggs and larvae of Persian sturgeon caused by fungal infection, prevention could be a more effective tool for fish health management. In this respect, our study was conducted to compare the performance of the three types of antifungal agents (Nanosil, Chloramine-T and hydrogen peroxide) against fungal infection of Persian sturgeon larvae. For this purpose, five treatment groups with three repetitions including: T₁: 80 mg/L Nanosil, T₂: 40 mg/L Nanosil, T₃: 40 mg/L hydrogen peroxide, T₄: 15 mg/L Chloramine-T, T₅: 20 mg/L Chloramine-T were designed. Also, an antifungal free group was considered as control. Antifungal treatments were conducted for a period of 18 days as 15min bath per day. According to our results, no significant differences were found in physico-chemical parameters of aquarium water (i.e. pH, dissolved oxygen concentration and temperature) before and after experiment. T₁ (80 mg/L Nanosil) had better effect on decreasing of fungal infection than the other treatments, followed by T₃ (40 mg/L hydrogen peroxide).

Keywords: Disinfection, Fungal infection, Nanosil, Chloramine-T, Hydrogen peroxide, Persian sturgeon

1. Introduction

Special attention to health principals and prevention of occurrence of diseases are important in aquaculture, in order to sustain and increase fish production (Shiu_nan, 1996). On the other hand, excessive use of drugs in

aquaculture has several adverse impacts including pathogen resistance and residues pollution of natural soil and water bodies in relation to fish rearing facilities. Moreover, accumulation of drugs in fish tissues is dangerous for humans and may cause allergy, pathogen resistance and renal and hepatic diseases (Azari Takami, 1997). Fungi belong to the *Saprolegnia* order or to other orders that inhabit aquatic bodies such as the water supply of fish hatcheries mostly cause infections to fish (Marking et al., 1994). In inappropriate condition of rearing, fish become more sensitive to fungal attacks. The fungal infections affect all stages of the life cycle and some of their effects are dermal and visceral infections and fish poisoning arising from consumption of infected food. In acute situations, fungal infection cause high mortality in fish hatcheries (Nooruzi et al. 2002). Grimaldi (1971) has reported high fish mortality (approximately 20-50 tones) in northern lakes of Italy due to the Branchiomycosis disease. In fish larvae, fungal pathogens invade gills and fins. Fungal infections caused mortality rate of 10-15% in rainbow trout fry, which had absorbed yolk resources and were in mixing feeding situation (Puya, 2004). Persian sturgeon, *Acipenser persicus*, Bordin 1897 is a valuable sturgeon species that has been considered for biological conservation programs in the southern basin of the Caspian Sea (Kiabi et al., 1999). Numerous larvae are produced annually by artificial reproduction in order to restore natural reserves. In this respect, health management particularly the control of fungal infection is necessary to obtain sustainable production in sturgeon aquaculture. Currently, different antifungal agents such as malachite green and formalin are applied for the disinfection of culture water of Persian sturgeon larvae. However, because these materials have detrimental impacts on fish health and environment, the application of these drugs has been restricted. The present study was conducted in order to test three antifungal agents: Nanosil (a substance containing silver), Chloramine-T and Hydrogen peroxide.

2. Materials and Methods

The experiment was carried out at the Dr Dadman International Sturgeon Research Institute, Rasht, Iran. Five treatment groups with three repetitions including: T₁: 80 mg/L Nanosil, T₂: 40 mg/L Nanosil, T₃: 40 mg/L hydrogen peroxide, T₄: 15 mg/L Chloramine-T, T₅: 20 mg/L Chloramine T were designed. Also, an antifungal free experiment was considered as control. Antifungal treatments were conducted for a period of 18 days as 15min antifungal bath per day. In our experiment, each of the 18 aquariums of the same capacity (20 L) was stocked with 140 sturgeon larvae. For each aquarium, oxygen was provided by an air-stone connected to air pump. For dosage calculation of the antifungal drug, the water volume (L) of each aquarium was multiplied at the forecasted dosage per L. For example, for T₂: 20 L × 40 mg/L = 800 mg Nanosil. The physico-chemical parameters of the water including pH and dissolved oxygen (D.O.) were measured one time two days by pH-meter and oxygen-meter, respectively. The temperature was measured by a thermometer.

The fungal infection of larvae was estimated as follows: 1 g of larvae was sampled and placed in sterile glass bottle with closed plug. Afterwards, the larvae were washed 3-5 times with distilled water and then the obtained suspension composed of water and fungus was diluted ten-fold with distilled water in sterile glass pipes. After that, 0.5 mL of diluted suspension was incubated in culture mediums i.e. Sabro Dextrose Agar (SDA) and corn meal containing Chloramphenicol and Gentamisin. To calculate the fungal colony numbers and also to obtain the complete growth of fungal colonies, the inoculated plates were incubated for a period of 48-72 h and 3-5 days at 25 °C, respectively. After this period, the colonies were counted and the CFU (Colony-Forming Unit) values were calculated on the basis of the mL of water and the g of larvae. For CFU calculation, the arithmetical average of two fungal counting was multiplied at the dilution rate (Industrial Standard and Research Organization of Iran, 1981). The SPSS software was used for data analysis. All data were normal according to Kolmogorov Smirnov test. One-way analysis of variance (ANOVA) was employed to compare the means. When significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify which means were different.

3. Results

3.1 Physico-chemical parameters of water during the experimental period

The range of the physico-chemical parameters of water for the control group were as follow: (Desolved oxygen (D.O. (mg/L): 7.03-8.5; Temperature (°C): 16.03-18.03; pH: 7.2-7.98). No significant differences were found for pH (Figure 1), D.O. (Figure 2) and temperature (Figure 3) before and after the use of antifungal drugs.

3.2 Fungal numbers/g of larvae during the first week of the experiment

The fungal numbers/g larvae (CFU/g) showed a significant differences before and after the application of antifungal drugs ($P < 0.05$) (Figure 4). The CFU/g values were significantly lower compared to the controls. In this regard, T₁, T₃ and T₅ had a better effect on reducing fungal numbers than the other experimental treatments.

3.3 Fungal numbers/g of larvae during the second week of the experiment

Apart from T₁, CFU/g showed no significant differences before and after the application of the antifungal drugs ($P>0.05$) (Figure 5). The CFU/g values of the control were higher compared to the other treatments. Accordingly T₁ had a better effect on reducing the fungal number compared to the other experimental treatments.

3.4 Fungal numbers/g of larvae during the third week of the experiment

Exception T₁, there are significant differences in CFU/g values before and after applying of antifungal drugs ($P<0.05$) (Figure 6). In this regard, T₁ had better effect on decreasing of fungal number than other experimental treatments.

3.5 The effects of antifungal drugs on CFU/g for entire experimental period

CFU/g showed significant differences before and after the application of the antifungal agents ($P<0.05$) (Figure 7). T₁ had better effect on reducing fungal numbers compared to the other experimental treatments, followed by T₃.

4. Discussion

Applying suitable and efficient antifungal agents without toxicity is important for fish health and particularly for sturgeons that are ecologically and commercially valuable species. Fungus belongs to the *Saprolegnia* order or to other orders may cause serious economic losses to fish farmers (Marking et al., 1994; Gaikowski et al., 1998). Nevertheless, few studies have been conducted on fungal infections in sturgeons. In this study, no significant differences were found in the physico-chemical parameters of the aquarium water (pH, D.O. and temperature) before and after the use of the antifungal agents. Therefore, the recorded stability of these parameters prevents stressful situations for the Persian sturgeon larvae. Nevertheless, the concentration of the D.O. increased after the application of hydrogen peroxide which could be due to its dissolution to water and O₂ (Dawson et al.). However, this increase was not statistically significant. It seems that water quality of hatchery and the existence of organic material in the water affect the D.O. concentration during the dissolution of hydrogen peroxide. In agreement with our results, Vahabzadeh et al. (2003) and Mirvaghefi et al. (2005) observed the elevation of D.O. concentration after the application of hydrogen peroxide. Noori et al. (2010) reported that Nanosil and hydrogen peroxide increased D.O. concentration of the water, but this increase was lower for Nanosil. This showed that hydrogen peroxide had a higher efficiency compared to Nanosil in the production of D.O. Our results demonstrated that T₁ (40 mg/L Nanosil), T₃ (40 mg/L hydrogen peroxide) and T₅ (20 mg/L Choramine T) had a better effect on reducing fungal infection than the other experimental treatments during the first week of experiment. However, the antifungal impact of hydrogen peroxide and Choramine T decreased gradually during the second and the third week of the experiment, while Nanosil maintained its efficiency. This could be attributed to the gradual resistance of the fungi to hydrogen peroxide and Choramine T. Thus, it seems that fungi had lower resistance to Nanosil, since Nanosil had a longer impact on reducing fungal colonies. Nevertheless, since fungal resistance is unavoidable, it is suggested that any antifungal agent be used for short period of time or various drugs be used during larval rearing in the hatchery.

According to our results, T₁ had a better effect on decreasing fungal infection compared to the rest of the experimental treatments during the entire experimental period, followed by T₃ (40 mg/l hydrogen peroxide). In Iran, the data on antifungal impacts of Nanosil is rare. Azari Takami et al. (2008) demonstrated that Nanosil (100 mg/L) had a better effect against bacterial and fungal infection of rainbow trout, *O. mykiss* eggs compared to malachite green, as hatching and eyeing rates were higher. Moreover, Puya et al. (2004) showed that hydrogen peroxide (100 mg/L as 30 min bath) every two days had better disinfection impact on rainbow trout larvae than malachite green. Noori et al. (2010) demonstrated that Nanosil (350 mg/L) and hydrogen peroxide (750 mg/L) were efficient on the control of fungal infection of common carp, *C. carpio* eggs. Those doses increased the hatching rate and had not adverse effects on egg survival. Generally, the working doses for the control of fungal infections depend on species and water temperature (Marking et al., 1994). On the other hand, several factors including acute and chronic stress, changes in water temperature, handling, injuries, over stocking and primary pathogens intensified *Saprolegnia* infection in channel catfish (*I. punctatus*) (Durborow et al., 2003). Hydrogen peroxide (as 30 min bath with a dose of 25 mg/L) healed grey mullet, *M. cephalus* affected by *Amyloodinium* fungi (Montgomery-Brock et al., 2000).

In conclusion, the present results showed that Nanosil (80 mg/L) had a better effect on reducing fungal infection of Persian sturgeon larvae than other antifungal agents. Hydrogen peroxide at a dose of 40 mg/L was also efficient. Hydrogen peroxide is the active part of Nanosil which is dissolved readily to water and D.O. without adverse impact on the natural environment.

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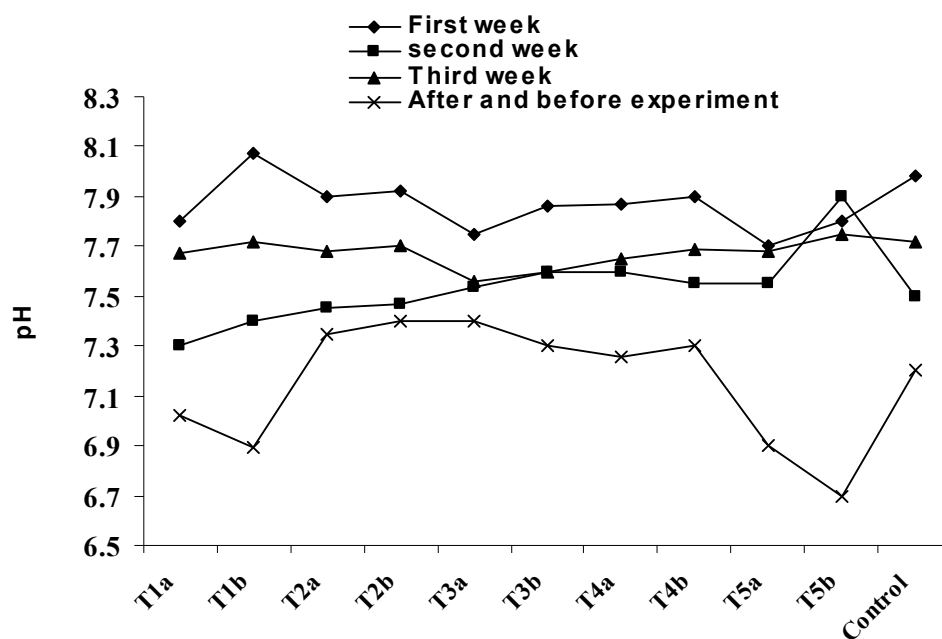


Figure 1. pH values of the water before (a) and after (b) the use of antifungal agents

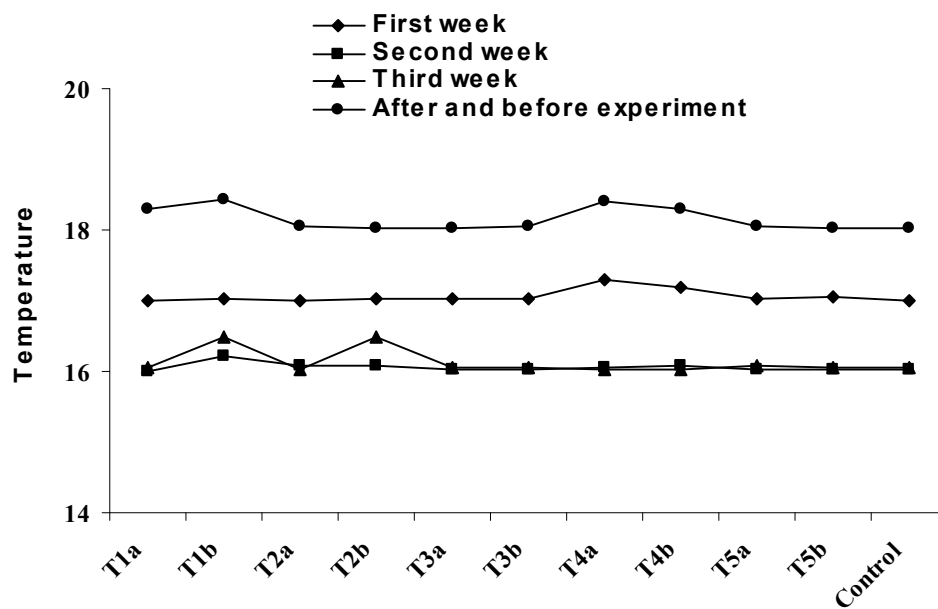


Figure 2. Water temperature before (a) and after (b) the use of the antifungal agents

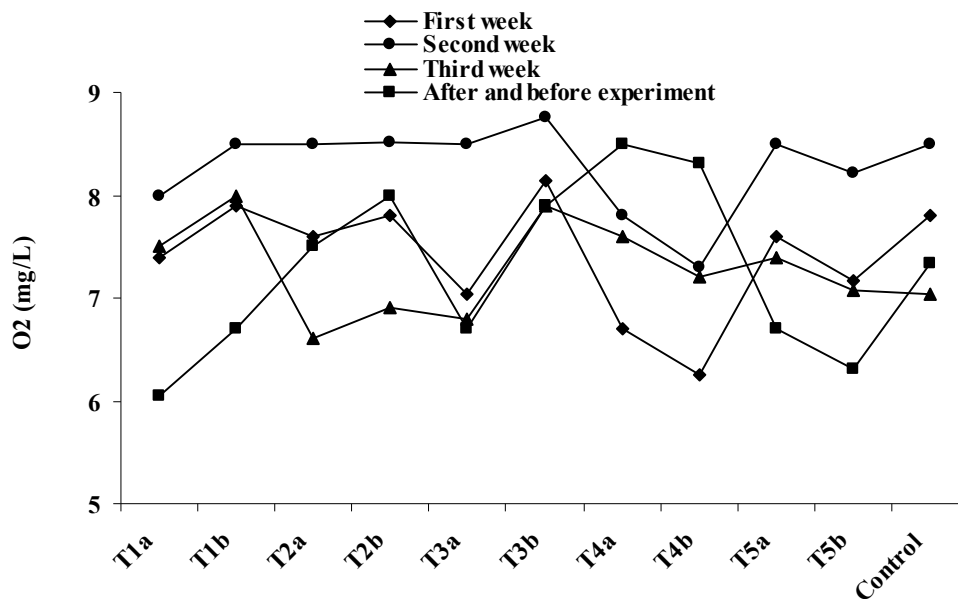


Figure 3. D. O. concentration of water before (a) and after (b) the use of the antifungal agents

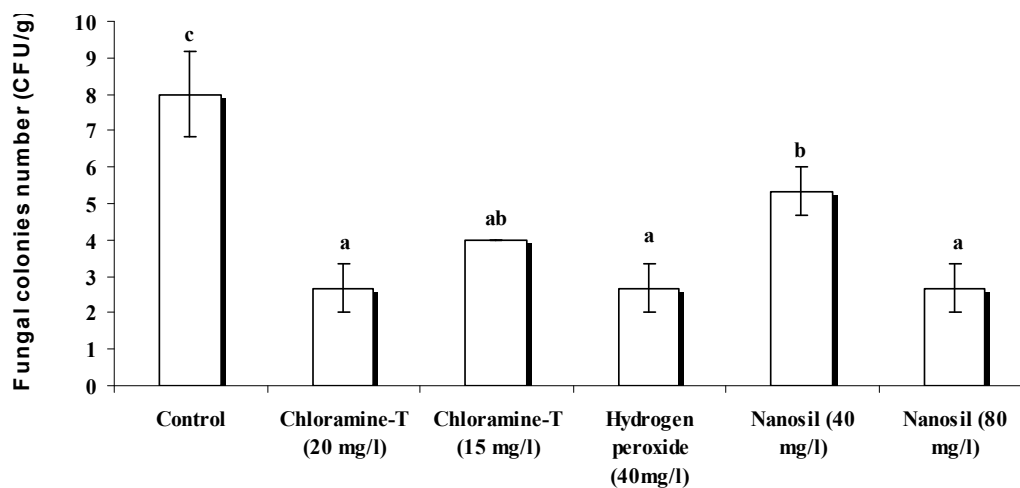


Figure 4. Fungal colonies numbers (CFU/g) of Persian sturgeon larvae after treatment of water antifungal agents during the first week of the experiment

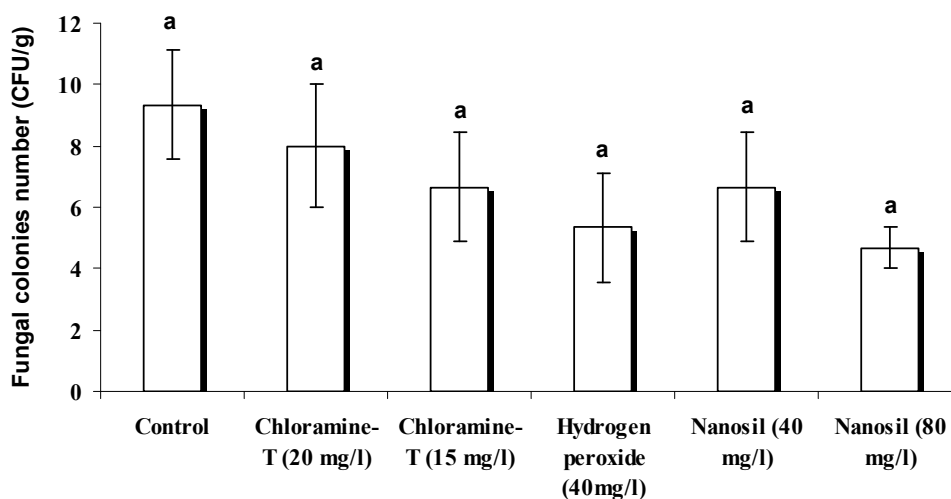


Figure 5. Fungal colonies numbers (CFU/g) of Persian sturgeon larvae after treatment of water with antifungal agents during the second week of the experiment

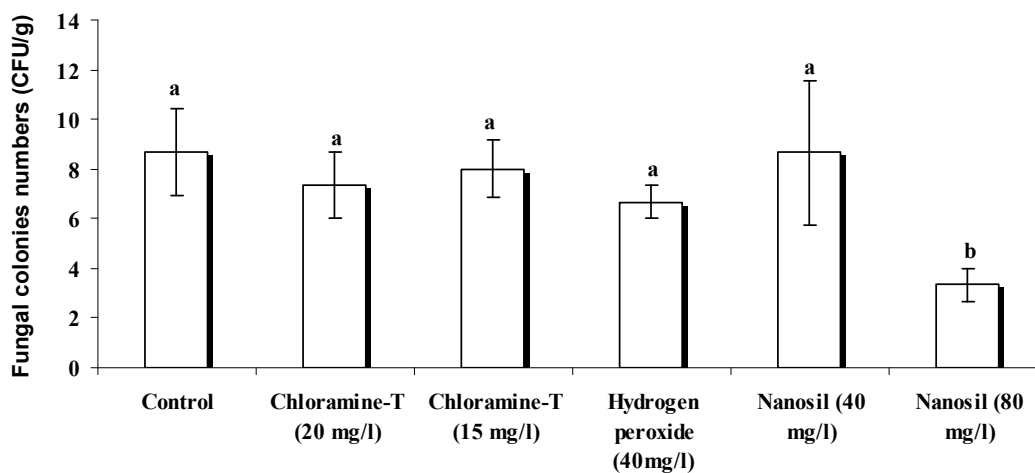


Figure 6. Fungal colonies numbers (CFU/g) of Persian sturgeon larvae after treatment of water with antifungal agents during the third week of the experiment

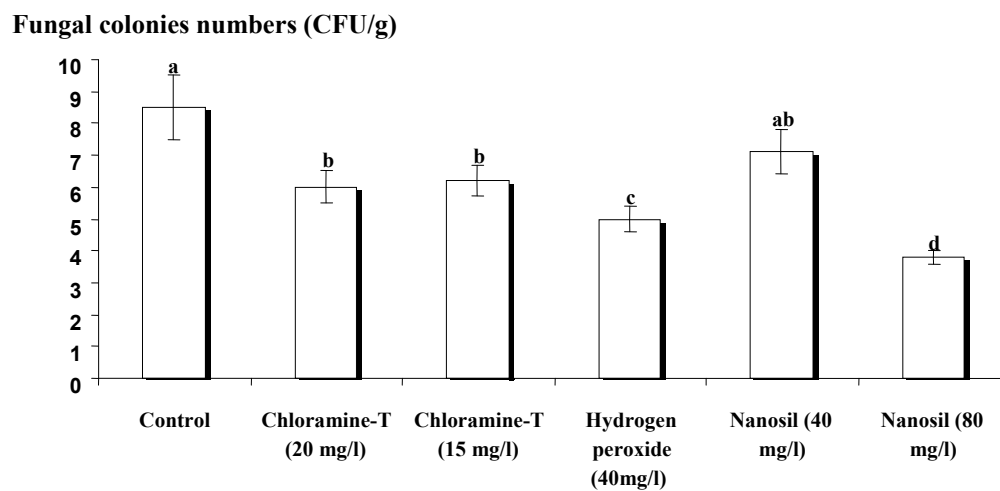


Figure 7. Fungal colonies numbers (CFU/g) of Persian sturgeon larvae after treatment of water with antifungal agents over the course of the experiment