Effects of Eucalyptus Essence, Malachite Green and Sodium Chloride on Hatching Rate of Persian Sturgeon (*Acipenser Persicus*) Eggs

Ahmadi M., Hajimoradloo A., Ghorbani R., Chitsaz H. and Soleimani H.

Effects of Eucalyptus Essence, Malachite Green and Sodium Chloride on Hatching Rate of Persian Sturgeon (*Acipenser persicus*) Eggs

Ahmadi M., Hajimoradloo A., Ghorbani R., Chitsaz H. and Soleimani H.

Islamic Azad University, Azadshahr Branch, Azadshahr, Iran.
Department of Fisheries and Aquatic Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
Giah Essence Company, Gorgan, Iran

Abstract

This study investigation on the effects of Eucalyptus essence, Malachite green and sodium chloride treatments on the hatching success of *Acipenser persicus* eggs. Eggs were artificially fertilized, 50 counted and transferred to a static bath dip treatment in given concentrations of Eucalyptus essence Malachite green and sodium chloride for 15, 30 or 60-minute durations after 24h being incubated at 19 ± 1°C. Treatment efficacy was assessed by comparing the percent egg hatch in the treatment group to the untreated control group. Eggs treated with Eucalyptus essence at 200ppm Malachite green at 5ppm and sodium chloride at 2% recorded greater mean percent hatching compared to the controls. The highest mean percent hatching recorded in this study was in eggs treated by 200 ppm Eucalyptus essence in 60 min (up to 95% survival). Although sodium chloride gave the best performance, on the basis of safety concerns, ease of availability and cost. We recommend 2% sodium chloride treatment of *A. persicus* eggs for routine use by hatchery fish farmers to improve *A. persicus* eggs hatchability.

Keywords: *Acipenser persicus* eggs, eucalyptus essence, malachite green, sodium chloride
Introduction

Oomycete water molds are the most important fungi affecting cultured fish and considered by some to be second only to bacterial disease in terms of economic importance to aquaculture. While Oomycetes are increasingly encountered in brackish water fish, they are mainly a problem in fresh water fish.

Saprolegniasis is a continuing problem for aquatic animal culturists. Any species of fish that is intensively cultured and captured is at risk of contracting fungal disease. Genus saprolegnia in the family of Saprolegniaceae is ubiquitous in water supplies and often causes losses due to saprolegniasis in fishes. The occurrence and severity of fungal outbreaks depend on the water sources, water temperature, organic load and length of contact time.

Persian sturgeon, *Acipenser persicus*, 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 propagation 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 eggs. 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: Eucalyptus essence, Malachite green and sodium chloride.

Materials and Methods

This experiment was carried out at the Shahid Marjani's Sturgeon propagation and breeding center, Agh-ghala, Gorgan, North of Iran. Broodstock fish were caught from broodstock ponds at the fish farm and sexual riping females and males (average weight 90 ±1 kg) tested on the method of Viveen et al. (1985). To induce spawning, the selected female was injected with pituitary suspension from a sacrificed ripe fish of similar size. After 12 h, the eggs were stripped into a dry bowl and fertilized with milt from a ripe male.

After fertilization, the eggs were randomly counted into equal lots of 50-eggs and inserted inside individual 15 cm × 15 cm hatching bags made from fine meshed mosquito netting (mesh size 0.5 mm) which would prevent escape of any egg and hatched larvae. The individual hatching bags were randomly assigned in triplicate to static bath treatments of given concentrations of either Eucalyptus essence, Malachite green and sodium chloride, and a control (0 ppm) for 15 (once time), 30 (twice on 2 days consecutively), and 60-minute (three times on three days consecutively) exposure periods before being transferred to randomized compartments of the incubation tank.

Mixed river and well water supplying the incubation tank had the following characteristics: mean temperature 19 ± 1°C, total hardness as CaCO$_3$ 82.1 mg/L, pH 7.1 – 7.7, dissolved oxygen (DO) 6.6 – 7.5 and total ammonia nitrogen (TAN) 0.28 ± 0.06 mg/L. Temperature and dissolved oxygen were measured using oxygen – temperature meter (model 55, YSI, Yellow Springs Ohio, USA), pH measured by using a pH meter (Hanna Instruments, Model 8519, USA) and TAN measured using the method of Boyd and Tucker (1992) by the indophenol method. The water flow rate was maintained at 1 liter per minute with aeration provided throughout the incubation and hatching period.

To calculate the fungal colony numbers and also to obtain the complete growth of fungal colonies, the bags were incubated for a period of 48 – 72 h and 3-5 days respectively. During this period, the infected eggs by fungi were counted and separated. Hatchability (% hatch) was calculated by dividing the number of larvae by the total number of eggs per lot and multiplying by 100 (i.e. larvae/50 ×100). The data from our experiments consisted of the initial number of eggs in each the number of fry that hatched at the end of the experiment.

This experiment was done in completely randomized design by factorial analysis (with 2 factors disinfection material include; Eucalyptus essence, Malachite green and sodium chloride; 15,
30 and 60 minutes) and Duncan’s multi dimensional test for comparing of means in ($\alpha=0.05$)

**Results and Discussion**

Results showed disinfection material and exposure time had significant differences on unhatched eggs, but their interaction has significantly not difference (Table 1).

In surveying of disinfection material, the highest no hatched eggs was observed in Malachite green that it was significant than other treatments. While in Eucalyptus essence and sodium chloride, hatch ration was high and has significantly not different (Fig. 1).

In surveying of exposed period, the highest no hatched eggs ration was observed in 15 minutes that it was significant than other treatments (Figure 2).

In interaction effect, the lowest of hatched ration was significantly observed in control group with 67%. The highest hatches were Eucalyptus-60 minutes and sodium chloride-30 minutes treatments with 97% and 95%, respectively. The lowest ratio was significantly observed in control than other treatments (Fig. 3)

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unification</td>
<td>0.027</td>
<td>2</td>
<td>0.014</td>
<td>6.85</td>
<td>0.006**</td>
</tr>
<tr>
<td>Time</td>
<td>0.023</td>
<td>2</td>
<td>0.011</td>
<td>5.65</td>
<td>0.012*</td>
</tr>
<tr>
<td>Unification × Period</td>
<td>0.022</td>
<td>4</td>
<td>0.005</td>
<td>2.74</td>
<td>0.061ns</td>
</tr>
<tr>
<td>Error</td>
<td>0.036</td>
<td>18</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.108</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1:** Effect of disinfection material and exposure time on unhatched egg

**Fig. 1:** no hatched egg ration in disinfection materials

**Fig. 2:** no hatched egg ration in exposed period

**Fig. 3:** no hatched egg ration in different treatment (disinfection × period).
The model parameter statistics are given on Table 2. Mean (±SEM) percent hatch of *A. persicus* eggs subjected to varying chemical treatments and exposure times are shown in Tables 3 while Figure 4 present the predicted probability of hatch of the eggs. The full logistic regression model fits the hatch data adequately. The mean percent egg hatch in Eucalyptus essence treatment ranging from 84 to 98% was greater than in the control group. The exposure time of the eggs in the different Eucalyptus essence treatments influenced the hatch performance in the longer exposure time (60 minutes) giving a higher percent hatch compared to the shorter exposure time. Logistic analysis revealed a significant effect in egg hatch based on Eucalyptus essence treatment period (Fig. 4, Tables 2).

Logistic analysis revealed a no significant effect in egg hatch based on Malachite green treatment concentration (Fig. 4, Tables 2). The mean percent egg hatch in Malachite green treatment ranging from 74 to 90% was greater than in the control group. The exposure time of the eggs in the different Malachite green treatments influenced the hatch performance in the longer exposure time (30 and 60 minutes) giving a higher percent hatch compared to the shorter exposure time (15 minutes).

Logistic analysis revealed a no significant effect in egg hatch based on Sodium chloride treatment (Fig. 4, Tables 2). Sodium chloride treatments ranging from 78 to 98% resulted in greater percent hatch than the control group. The highest percent hatch (94.7%) was recorded at 30 min exposure period.

### Table 2: Model parameter statistics from the logistic regression of the three test chemical

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Model Parameter</th>
<th>Parameter significance (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus essence</td>
<td>0.673+0.000009547C³-0.001C²+0.027</td>
<td>$\beta_0 (0.0000), \beta_1(0.02), \beta_2(0.011), \beta_3(0.002)$</td>
</tr>
<tr>
<td>Malachite green</td>
<td>0.673+0.000002634C³-0.0001C²+0.014</td>
<td>$\beta_0 (0.0000), \beta_1(0.56), \beta_2(0.39), \beta_3(0.1)$</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.673-0.000004362C³-0.0001C²+0.007</td>
<td>$\beta_0 (0.0000), \beta_1(0.22), \beta_2(0.48), \beta_3(0.27)$</td>
</tr>
</tbody>
</table>

### Table 3: Mean (±SEM) percent hatch of *Acipenser persicus* eggs in different treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Eucalyptus</th>
<th>Malachite green</th>
<th>Sodium chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>0.673± 0.035</td>
<td>0.673± 0.035</td>
<td>0.673± 0.035</td>
</tr>
<tr>
<td>15</td>
<td>0.893± 0.024</td>
<td>0.813± 0.037</td>
<td>0.807± 0.013</td>
</tr>
<tr>
<td>30</td>
<td>0.887± 0.024</td>
<td>0.853± 0.027</td>
<td>0.947± 0.018</td>
</tr>
<tr>
<td>60</td>
<td>0.967± 0.013</td>
<td>0.847± 0.035</td>
<td>0.893± 0.029</td>
</tr>
</tbody>
</table>

Fig. 4: Predicted probability of *A. persicus* hatch exposed to Eucalyptus, Malachite green and Sodium chloride for 15, 30 and 60 min based on logistic analysis model.

Chemicals are routinely used in Iran especially in reproduction centers of for treating fungal infections of fish eggs in an effort to improve the efficiency rates. This study was intended to assess, the effect of four of the commonly used chemicals on the hatching success of *A.persicus* eggs. Since fungal infections were observed in all control egg treatments as a fluffy growth, it is probable that both chemical toxicity and fungal infection caused egg mortality resulting in the poor hatchability. In similar studies, Mousavi *et al.* (2009) reported that dosage 200 ppm Eucalyptus essence applied...
affected in control of Saprolegnia and increasing the hatching rate of rainbow trout (Oncorhynchus mykiss) eggs which are agreement with our result. Najafi et al. (2010) reported that dosage 100 ppm Eucalyptus essence controlled Rutilus frisii kutum eggs infected with Saprolegnia. In the experiment, it subjected A.persicus eggs treatment concentrations 200 ppm that they results are similar to our results. Since fungal infections were observed in all control egg treatments as a fluffy growth, it is probable that both chemical toxicity and fungal infection caused egg mortality resulting in the poor hatchability.

The best percent hatch from the sodium chloride experiment was recorded in the 1000 ppm salt solution when the catfish eggs were bathed for 30 min. Salt treatments at concentrations of more than 4000 ppm reduced the hatch rate. Improved hatch due to salt treatment at 0–5000 mg/L has also been recorded for channel catfish by Froelich and Engelhardt (1996). Phelps and Walser (1993) found that treating koi carp eggs with a salt concentration of 1000 and 2500 mg/l for 60 min exposure duration significantly (P<0.05) improved the hatch rate of the eggs compared with that of the control while a concentration of 5000 mg/l was toxic to the eggs that this results are too coinciding to us. Schnick et al. (1989) reported that a 3000 ppm sodium chloride dip effectively removes protozoa from fish egg surfaces and limits any mycelia production that may lower egg hatching. They further said that higher concentrations of up to 10,000 ppm of sodium chloride could be used to treat eggs but only as a short dip of not more than 30 s which are agreement with our result. Celada et al. (2004) on the contrary found that concentrations of 30,000 ppm of salt applied three or two times a week did not prevent fungal growth on antacid crayfish eggs indicating that to be effective, very high salt concentrations are required. in high doses. Sodium chloride on the other hand is cheap and readily available in the rural areas making its utilization practical in small-scale hatchery operations. Aghaei moghadam et al. (2011) reported that dosage 2% during 45 minutes and 1.5% during 30 minutes and bath dip treatment for 3 times had the highest effect (99%) on fungi infection that this results are too coinciding to our results.

Khodabande and Abtahi (2004) reported the highest effect in controlling of Saprolegniasis infection Cyprinus carpio egg and improved hatch rate which are agreement with our result. In the experiment, it subjected A.persicus eggs treatment concentrations 2 ppt in controlling of fungal infection.

Malachite green has been tested as a fungicide on eggs of several fish species. Effective dosages have been found to vary with the fish species tested. Liu et al. (1995) reported that doses of 25 and 75 ppm applied for 60 min every other day up to hatching time decreased hatching rates of chinese sucker (Myxocyprinus asiaticus) eggs while Marking et al. (1994) stated that concentrations of 50 and 100 ppm delayed hatching of rainbow trout eggs. Malachite green treatments of 100 and 150 ppm controlled fungi but were toxic to the eggs (Melendre et al., 2006). The concentrations of malachite green used in the present experiment were much lower and ranged from 0–20 ppm. The results show that treatments of catfish eggs with 1 ppm and 2 ppm greatly improved the percent hatch compared to the controls. Malachite green at 2 ppm treatment gave the best hatching performance with a 15 min exposure recording the highest hatching percentage (96.7%). Hogendoorn and Vismans (1980) found that higher concentrations of malachite green usually damage egg membrane and chorion. From the results of this study, formaldehyde, sodium chloride, malachite green and hydrogen peroxide produced a concentration-dependent effect on the hatching of catfish eggs over the 24-hour incubation period. Highest hatching efficiencies were recorded in C.gariepinus eggs exposed to 2 ppm of malachite green for 15 min, higher concentration of formaldehyde, sodium chloride and hydrogen peroxides also gave good results. The antifungal treatment protocols employed were simple and should be easy for rural small-scale fish farmers to apply. However several factors that would preclude the use of some of the fungicides tested in this experiment need to be pointed out (Melendre et al., 2006). In the experiment, the highest hatch rate it subjected A.persicus eggs treatment concentrations 5ppt during 30 minutes in controlling of fungal infection and had significantly not with 15 and 60 minutes,
but that they had significantly with control treatment.

Malachite green is used as the best chemical material in controlling of Saprolegnia fungi for many years, however, it cause cancer, miscreate and environment pollution. Nowadays, world follow using safe, higher output and lower environmental pollution with decreasing of chemical disinfections.

**Conclusion**

The Eucalyptus essence with 200 ppm and sodium chloride with 2% concentration can decrease mold infection rate and increase hatch through theing percent in hatcheries. Some studies have concluded that essential oils have greater antimicrobial activity than chemical components (Davidson and Parish, 1989; Gill et al., 2002). These compounds may provide an alternative for chemical therapeutics in aquaculture. Accordingly, the essential oils and Sodium chloride examined in this study have revealed to affect mold infection on Acipenser persicus eggs and increase larval yield. Finally, Essential oils and Sodium chloride need more studies for decreasing toxicological effects on eggs at high doses. In future, such informations may represent alternative therapeutic treatments in aquaculture and hatcheries.

**Acknowledgments**

The researchers would like to acknowledge Giah Essence Pharmaceutical and center of Shahid Marjani Hatchery for their support and kind assistance.

**References**


