Effects of potassium permanganate treatment on fungal Infection, eyed eggs, hatching percentage and larval deformities of Rainbow Trout in West Mazandaran conditions.

Banavreh, A.[1]*, Abtahi, B.[2] ,Mirzakhani, M.K.[3]
1,3- M.Sc. In Fisheries, Faculty of Marine Scinces, Tarbiat Modarres University. Noor. Mazandaran, a_banavreh@yahoo.com 2-Assist. Prof. Of Fisheries Dept, Faculty of Marine Scinces, Tarbiat Modarres University, Noor Mazandaran.

Abstract
Fungal infection may decrease hatching rate of salmonid eggs. Historically culturists to control fungus of eggs used Malachite Green. However, the U.S. Food and Drug Administration (FDA) withdrew its approval in 1991 because of its teratogenicity. In Iran, it is still in use for aquaculture practices. Potassium Permanganate could be considered as the alternative choice within salmonid farms, taking into account that E.P.A. Potassium Permanganate knew it as oxidant and detoxifier. In this study Potassium Permanganate (50 and 100 mg/l in 15 min bath) Was compared with Malachite Green (1.5 mg/l in 45 min bath) and natural control (without drugs).
Each treatment group consisted of 3 replicates, which covered 1000 Rainbow Trout eggs. After one week, fungal infection, eyed eggs and hatching percentage, and larval deformities rate were determined. The results indicated the low fungal infection rate in Malachite Green treated group in comparison with natural control ($p<0.05$), although the former and lather revealed no significant difference with Potassium Permanganate treated groups. Comparing with other treatments, Potassium Permanganate (100 mg/l), showed an increment in rate of eyed eggs ($p<0.05$).

Keywords: Potassium Permanganate, Malachite Green, Fungal infection, Rainbow Trout, Mazandaran.

Introduction:

Saprolegniasis is a kind of fungal disease of fish and their eggs cause by saprolegniacea (Noga, 2000). These fungies live in freshwater but some of them can grow in brackish water up to 2.8 ppt, (Azari, 1997).

Handling fish, fluctuation of temperature, presence of parasites and increasing the organic matter, increase the chance of affection of this disease (Bruno and Wood., 1994). Dead eggs are a very good medium for developing the saprolegnia fungi. A saprolegnia zoospore can grow on dead egg and produce a mass of mycelium. These myceliums surround live eggs and suffocate them so another mass of eggs died and invaded directly by the fungi. This cycle continues until all the eggs will die. Fungal growth on dead eggs produces too many zoospores. These zoospores invade another masses of eggs and kill them.

Malachite green use to control this disease. But its harmful effects i.e. carcinogenic and teratogenic effects and slow decomposition in the nature using of this drug was prohibited by F.D.A (Food And Drug Administration) (Marking *et al*., 1994).

Potassium permanganate is used for ectoparasites, bacteria, fungal disinfections on the skin and gill of fishes. It is accepted by E.P.A (Environment Protection Agency) as an oxidant. It decreases BOD of water by oxidize the organic matter and the amount of oxygen will be increase by using potassium permanganate (Noga, 2000). Marking (1994) examined two concentrations 50 and 100 mg/l of potassium permanganate for prohibition of fungal infection on Rainbow Trout eggs and find out that although it decreased fungal infection of Rainbow Trout eggs but rate of hatching didn't increase.

Shafizadeh (2002) examined the effect of potassium permanganate concentrations in prevention of infection in Persian sturgeon eggs (*Acipenser persicus*) and showed that potassium permanganate at 5.37 mg/l can prevent fungal disease. Malachite green is used in most breeding and cultivation farms of Iran and research and recommendation of some new ant fungal drugs is needed.

Methods

This research had been taken in Shahid Bahonar breeding and cultivation farm in Kalardasht from 11/13/2003 to 2/10/2004. The Fertilized eggs were remained in a container (in a dark place) to absorb water. Then the amount of 1000 Rainbow Trout eggs were selected by graduated jar and counting voluminally and were placed in a traph (each traph consist of three trays and each treatment consist three replicates). Drug treatment began 48 hour after fertilization. Potassium permanganate treatment by two concentration of 50 and 100 mg/l for 15 min were implemented every other day until 4 day before hatching. Malachite green was treated by 1.5 mg/l (usual concentration in farms) every other day until 4 day before hatching too. We had also a natural control, received no treatment.

Physico-chemical characters of hatchery water were determined by suitable eqiupments. Measured parameters are temprature, pH, dissolved oxygen, conductivity and hardness.

To determine the fertilization rate, the eggs were entered into stocard solution seven days after blastopor formation (Barnes *et al*.,1998).

Determining factors in this research were as fallows:

A) Fungal infection:

Amount of infection from fertilization to eyed egg stage, number of infection eggs, infection masses and number of eggs in each mass were determined (Barnes *et al*., 1998).

$$I = \frac{\text{Number of infected eggs}}{\text{Total number of eggs}} \times 100$$

B) Rate of eyed eggs (Arndt *et al*.,2001):

$$E = \frac{\text{Number of eyed eggs}}{\text{Total number of eggs- Primitive mortality}} \times 100$$

C) Hatching rate (Arndt *et al*.,2001):

$$H = \frac{\text{Number of hatched eggs}}{\text{Number of eyed eggs}} \times 100$$

D) Deformity rate:

Finally, deformited larvae were determined 6 days after hatching. They were as fallow:

Fry deformities included joined eggs, curved and bent spines and deformed yolk sacs.

$$M = \frac{\text{Deformed larvae}}{\text{Hatching eggs}} \times 100$$

The SPSS software and one-way ANOVA were used to analysis the data and comparision of averages were taken by DUNCAN test.

Results:

Physico-chemical parameters during incubation of eggs (November-December) are shown in table 1.

Table 1: Physico-chemicals parameters during incubation of eggs.

Parameter	Max	Min	Medium
Dissolved oxygen (D.O)	8.4	7.5	8.05
Temprature (T°C)	11	8	8.8
PH	8	7.9	7.95
Hardness (mg/l)	112	142	132
Conductivity µs/cm	353	312	336

Infection, infected mass and the number of eggs in each mass in eyed egg stage were shown in Table 2.

Table 2: Infection, infected mass and the number of eggs in each mass

Treatments	Number of infected mass	Percent of infection	Average number of eggs in each mass
Potassium permanganate (50 mg/l)	15	6.3 ±1.9 ab	4.2
Potassium permanganate (100 mg/l)	16	7±0.66ab	4.45
Malachite green	2	1.1±1.5a	5.4
Control	23	9.7±1.9b	4.3

Results by Duncan test shown significant difference between malachite green and natural control treatment but there is no significant difference between potassium permanganate and other treatments.

The result of average percent of eyed egg in different treatments was shown in table 3.

Table 3: Average percent of eyed egg in different treatments

Treatment	Percent of eyed egg
Potassium permanganate (50 mg/l)	67.6±3.4b
Potassium permanganate (100 mg/l)	73.9± 0.86 a
Malachite green	62.2±2.9b
Control	64.6±0.3b

Duncan test indicated that the percentage of eyed egg increase in potassium permanganate by 100 mg/l concentration treatment and have significant difference in the other treatments, but there is no significant difference in the other treatments.

Hatching period of eggs takes place 43 days and average percent of hatching in different treatments has shown in table 4.

Table 4: average hatching percent of treatments

Treatment	Percent of hatching
Potassium permanganate (50 mg/l)	98±0.96a
Potassium permanganate (100 mg/l)	98.5±0.45a
Malachite green	98.2±1.1a
Control	98.3±0.24a

Result of Duncan test indicate hatching percent have no significant difference in different treatments.

At least, average percent of deformed larvae was determined 1 week after hatching that was shown in table 5. Deformed Rainbow Trout larvae were shown in figure 2.

Table 5: average percent of deformed larvae 1 week after hatching

Treatment	percent of deformity
Potassium permanganate (50 mg/l)	0.09±0.085a
Potassium permanganate (100 mg/l)	0.13±0.13a
Malachite green	0.2±0.08a
Control	0.1±0.14a

Although malachite green had most deformity percent, no significant difference was shown between treatments in Duncan test.

Discussion:

Increasing aquatic fungal (*Saprolegniacea*) cause problems on farmed fish eggs (Schreier *et al*., 1996). However the sensitively of eggs to fungal disease depends on some things such as water quality, flow rate of water and density of eggs in incubators. Dead eggs and organic matter in aquaculture are suitable substances for growing fungal on them. One way to control of Saprolegniasis is using chemical drugs. But should be noticed that chemical drugs always be can't used as the some way because their efficacy and toxicity varies by existing of organic matter and physico-chemical quality parameters of water (Rach *et al*., 1998).

Chemotrophy by common drugs is a usual way to treatment this disease especially in eggs of fish and malachite green is one of the most important chemicals for treatment and prevention of disease. Although it is widely used for its suitable effects in Iran but it is prohibited in some countries because of its teratogenic, carcinogenic, toxic effects and environmental pollution (Marking *et al*.,1994).

Permanganate is an active oxidant ion against parasites that kills them by oxidizing their cellular walls. Another reports indicate that magnate dioxide construct protein complex on the outer surface that caused brown color and producing this protein complex effect on respiratory structure of parasites and kill them (Anonymous, 1994). It used as a aquatic anti-fungal but it isn't used anymore now. Potassium permanganate is toxic in high pH because magnate dioxide sediment on fish gills. So it can't use in salt water effectively. It shouldn't be also used combined with formalin (Noga, 2000). During treatments with potassium permanganate in 15 min bath dissolved oxygen and pH were measured every 5, 10 and 15 min and no significant difference was indicated.

Results of potassium permanganate treatments for Rainbow trout eggs indicate that it has weaker effect than malachite green to prevent fungal infection but the rate of eyed egg in potassium permanganate treatments by 100 mg/l are more than all other treatments and have significant difference by other ones and it's because of increasing bacteria density in eyed egg stage and so potassium permanganate increased the amount of eyed eggs by killing the bacteria on the eggs surface (Barnes *et al*., 2000). The rate of hatching and the rate of deformities have no significant difference between treatments.

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