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Efficacy of Medication Therapy to Control of *Saprolegniasis* on Rainbow Trout (*Oncorhynchus mykiss*) Eggs

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Abstract: This study was conducted to investigate and comprise two new disinfectant (Gentian Violet (GV) and Huwa-San (HS)) drug solutions for applied to daily operations of breeding centers and to evaluate the proper level of them in incubation period of rainbow trout eggs. In the first and second experiments, fish eggs were treated with GV solutions at levels of 1.5; 2.5; 3.5 mg/l; and HS at levels of 3; 5; 7 mg/l in addition to negative control (without drug) and positive control (malachite green (MG)) (2 mg/l) with 4 replicates and 500 fertilized eggs in each replicate. After incubation of the fertilized eggs, the eggs hatchability was calculated. Each experiment was analyzed as a completely randomized design. The results showed that the negative control had the higher un-hatched eggs rate in both experiments (GV: 94.50% and HS: 94.75%) ($P<0.05$). The MG (2 mg/l) had no significant difference with treatment 3.5 mg/l GV ($P>0.05$). However the amount of 7 mg/l HS had significance no equality ability with MG (2 mg/l). The results indicated that the relative success in the treatment of rainbow trout with GV (3.5 mg/l) preparations, it seems be truly adequate substitute for MG.

Key words: Saprolegniasis · Gentian Violet · Huwa-San · Rainbow Trout Eggs

INTRODUCTION

In hatcheries, aquatic fungi of the order Saprolegniales often cause serious damage to the incubating eggs, if no effective prophylactic and therapeutic treatments are applied to the eggs. *Saprolegniasis* is integument mycoses disease of fish and its eggs [1]. The disease is caused mainly by the fungi of Saprolegniasis family and mainly Saprolegnia and *Ikea* genus involved in disease causing. The disease most commonly occurs in fish farming enterprises and workshops. The eggs, offspring and breeder especially after laying are susceptible to current disease. Saprolegnia attack fish eggs in stripping equipment and usually grown and fix on the dead eggs, then can invade the healthy nearby eggs [2]. Contaminated fish eggs (especially salmon) are considered an important fish reproduction problem. The problem is much felt whatever the incubation period was last. Various materials have been proposed for the treatment of contaminated eggs that Malachite Green (MG), aniline derivatives, is one of the components [3]. The MG is anti-microbial [4], carcinogenic [5] and mutagenic [6, 7] with extreme care and attention to use. Also, has gradually been replaced in the treatment of superficial fungal infections. Because of it persist for a long time and may pass via the food chain to human consumptions. Other compounds including formaldehyde, sodium chloride, potassium permanganate, hydrogen peroxide [8], nano-silver particles [7] had showed that have low efficacy. Application of materials which had minor effects on salmon eggs survival and hatch as well as had proper power to parity with the MG in terms of price, availability, performance and mentioned injuries is mandatory. Therefore, this study was conducted to investigate and comprise two new disinfectant (Gentian Violet (GV) and Huwa-San (HS)) drug solutions for applied to the daily operations of breeding centers and to evaluate the proper level of them in incubation period of rainbow trout eggs.

MATERIALS AND METHODS

Description of System: The system is located in the city of Nahavand (Hamedan-Iran). Total roofed area of 2000 m and two pool set 20 pcs (40 pools totally) with a holding capacity of 30 tons of biomass rainbow trout and the incoming water supply from deep wells with a flow rate maximum activity of 24 liters per second.

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Table 1: The chemical characteristics of flood water in the system

Parameters	T (°C)	Tu (NTU)	TH	pH	EC (µS/cm)	CO_2	CO_3	HCO_3	O_2	Nitrite	Nitrate	NH_4	Ca	Mg
Inner	12.9	7.0	158.0	7.58	292.0	-	12.0	134.0	9.0	0.012	0.86	0.19	44.0	11.0
Outer	12.1	10.0	150.0	7.63	299.0	2.5	-	170.0	7.6	0.011	0.80	0.30	46.0	8.0

T: Temperature; Tu: Turbidity; TH: Total Hardness; EC: Electrical Conductivity. The unit of parameters (except pH and mentioned parameters) is ppm.

The system is designed in such a way that the output of the water pool was transferred inside the complex network of fine mechanical filter (drum filter) or a 60 micron mesh screen and after physical refining the elimination of solid was out from system and is transferred to a sedimentation pond that is built outside of the site. Also, the biological treatment process was set in the bio-filter and after oxygenation and pass under ultraviolet lamps re-enter to the pools set again. Table 1 is shows the quality of inner and outer water in system.

Samples Collection: To drug therapy the rainbow trout eggs were used as substrate in this study. After examination of breeders' health history, selection of male and female breeders, mature breeders were selected and green eggs were collected from breeders. The operation breeders eggs collection was made after anesthetize of fishes by pink clove (4 mg/l) administration. Eggs and sperms were obtained, dried fertilization were made by mixing ova and sperm and passed water absorption. To obtained fertilized eggs female 336 pieces and male 106 pieces were used.

Processing Operation and Treatments: In this study, two separated experiments were designed. In the first experiment, a total of 2223 fertilized fish eggs were treated with GV solution at levels of 1.5; 2.5; 3.5 mg/l; negative control (without drug administration) and positive control (MG) (2 mg/l) with 4 replicates and 500 fertilized eggs in each replicate. In the second experiment, treatments were various levels of HS at levels of 3; 5; 7 mg/l; negative control (without drug administration) and positive control (MG) (2 mg/l) with same above condition. The distributions of treatments are shown at Tables 2 and 3. After removing the dead eggs in the first 24 hours of incubation, the total amount of un-fertilized eggs for each treatment was calculated (Table 4). The treatments were daily applied for 20 minutes. Medicinal operations were applied after 36 hours of incubation until the stage of eyed eggs.

Determination of Eggs Hatchability: After incubation of the fertilized eggs, dead eggs were collected and removed for 24 days. Casualties were not collected until the eyed stage and then slowly and carefully dead eggs were daily counted. Eggs washes and shocks times were considered as 18 days after fertilization as well as eyeing time was considers as 19 and 20 days after fertilization. After eyeing the possibility were provided to isolate the infected and dead eggs. Finally, the hatched rate was obtained using the following formula:

Table 2: Number of eggs in each replicate in Gentian Violet therapy

	1	2	3	4
NC	6900	6860	6780	6850
1.5 mg/L	6840	6990	6730	6890
2.5 mg/L	6940	6830	6790	6740
3.5 mg/L	6990	6750	6730	6780
MG (2 mg/L)	6880	6860	6920	6650

NC: Negative Control; MG: Malachite Green

Table 3: Number of eggs in each replicate in Hawa-San therapy

	1	2	3	4
NC	6870	6830	6850	6780
3 mg/L	6910	6890	6870	6730
5 mg/L	6830	6850	6870	6790
7 mg/L	6900	6920	6740	6730
MG (2 mg/L)	6870	6850	6910	6640

NC: Negative Control; MG: Malachite Green

Table 4: The total number of un-fertilized eggs in both experiments

Experiment 1	NC	1.5 mg/L	2.5 mg/L	3.5 mg/L	MG (2 mg/L)
	115	114	117	120	111
Experiment 2	NC	3 mg/L	5 mg/L	7 mg/L	MG (2 mg/L)
	125	110	111	119	118

NC: Negative Control; MG: Malachite Green

Hatched eggs= total eggs-the number of dead eggs until hatching

Percentage of hatched eggs = (number of hatched eggs/primary number of wasted eggs) × 100

Statically Analysis: All data were analyzed for normal distribution using the NORMAL option of the UNIVARIATE procedure of GLM procedure of SAS [9]. Each experiment was analyzed as a completely randomized design. The data were subjected to analyses of variance (one-way ANOVA), at the $P<0.05$ confidence level using Duncan's multiple range test [9].

Table 5: The rate of un-hatched eggs treated with Gentian Violet and Huwa-San

Gentian Violet		Huwa-San	
NC	94.50[a]	NC	94.75[a]
1.5 mg/L	43.00[b]	3 mg/L	58.00[b]
2.5 mg/L	37.50[b]	5 mg/L	52.00[c]
3.5 mg/L	32.00[c]	7 mg/L	47.00[d]
MG (2 mg/L)	29.25[c]	MG (2 mg/L)	27.75[e]
SEM	5.54	SEM	5.04

NC: Negative Control; MG: Malachite Green. Means with different superscript letters in same column are significantly different (P<0.05). SEM: standard error of means.

RESULTS AND DISCUSSION

The rate of un-hatched eggs influenced by various treatments in both experiments is showed in Table 5. The results showed that the negative control had higher un-hatched eggs rate in both experiments and the results were close together (GV: 94.50% and HS: 94.75%) (P<0.05). Because of presence of various infections which attack and impress hatchability, it appears that without egg medicinal therapy hatched eggs was dramatically reduces. The results of current study are supporting this finding. The results, also, indicated that MG had higher efficiency rather than that of other treatments to increase hatched eggs in both experiments (GV: 29.25% and HS: 27.75%) (P<0.05). The MG has been extensively used as an arasiticide [10, 11], fungicide [12-14], antiprotozoan [15, 16] and against other infection [17] in fish farming throughout the world. It was found to be the most effective fungicide among 49 compounds tested against an oomycete fungus [18]. It has prevented the growth of *Haliphthoros* on rock lobster [19] and Ful-2 on salmon [20]. Moreover, *Saprolegniasis* has been effectively controlled by MG in salmons [21], channel catfish [22] and rainbow trout [23]. Eggs of *Cyprinus carpio* and tench have been treated prophylactically to prevent fungal infection [24, 25]. Therefore, along with above mentioned studies powerfulness of MG as a multi-agent against wide range of infection diseases was approved in rainbow trout eggs in this study. Then, the high rate of hatched eggs could be expected. Also, Table 5 shows that the rate of un-hatched eggs was significantly reduced when drug therapy dosage was increased (P<0.05). The explanation of this observation is likely being the increase of medicinal therapy effectiveness to omit infection agents. However, MG (2 mg/l) had no significantly differences with treatment 3.5 mg/l GV (P>0.05). According to many adverse effects of MG (carcinogenesis, mutagenesis, chromosomal fractures, teratogenicity), serious public health hazards and potential environmental problems, it seems that 3.5 mg/l GV could be a good substitute for MG (2 mg/l) to remove of *Saprolegniasis* effects on egg hatchability. Though the former must be cleared by the Food and Drug Administration before it can be currently used to treat fish eggs. But, in the second experiment, the amount of 7 mg/l HS had significantly no equality ability with MG (2 mg/l). It seems that higher dosage was needed. Generally, comparing different studies is difficult because systematic data on effectiveness of the other fungicide sources are limited.

CONCLUSION

This study is effective example of a direct application of other fungicide (Gentian Violet and Huwa-San) for fish production. The results demonstrated that medicinal therapy of rainbow trout (*Oncorhynchus mykiss*) eggs with fungicide is necessary and would help to control of *Saprolegniasis* with increase in hatched eggs. The Gentian Violet at level 3.5 mg/l seems had competition ability with malachite green (2 mg/l). However, its usefulness in aquatic toxicology should be further evaluated.

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