



Effects of disinfectants on bacterial load in a commercial fish hatchery in Mymensingh district of Bangladesh

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ABSTRACT

The study was conducted to investigate the effects of four chemical disinfectants *viz.*, salt (NaCl), lime, formalin and potassium permanganate (KMnO₄) on bacterial loads in water, eggs and fries in a commercial fish hatchery at Trishal upazila in Mymensingh district. Sampling was done in every 10 days interval for each month from March to May 2016. Hatching trays (12"×7") were disinfected using 40 ppm salt water and 20 ppm potassium permanganate. Cisterns (84 ft² each) were washed and disinfected with combination of lime (5 g ft⁻²) and salt (30 g ft⁻²) followed by application of potassium permanganate (1 ppm) and formalin (0.25 ppm). After disinfecting, bacterial load in hatching tray water reduced immediately than that of overhead tank water. Bacterial load was determined using serial dilution technique and expressed as colony forming unit (cfu mL⁻¹). The average highest bacterial load in overhead tank water was $4.89 \pm 1.71 \times 10^7$ cfu mL⁻¹ while the highest load in hatching tray water was $3.30 \pm 3.54 \times 10^6$ cfu mL⁻¹. The bacterial load of cistern water ($1.43 \pm 0.75 \times 10^3$ cfu mL⁻¹) decreased compared to tank water and gradually increased after six days of giving hormone treated feed. To prevent infection of eggs saline water was applied and lower bacterial load of $4.25 \pm 2.67 \times 10^2$ cfu mL⁻¹ was observed. The study revealed that use of chemical disinfectants in the initial stages of hatchery operation can decrease the bacterial load and thus reduces the chance of infection and diseases of eggs and fry.

Keywords: Disinfectants, tilapia, hatchery, bacterial load



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1 Introduction

Bangladesh is one of the world's leading fish producing countries with a total production of 42.76 lakh metric tons during 2017-18, where aquaculture production contributes 56.24 percent of the total fish production (DoF, 2019). Fish hatcheries have been playing an important role in the expansion of aquaculture sector through timely supply of fish seed to the farmer throughout the country. Currently there are around 926 hatcheries in Bangladesh of which 824 are private and 102 are run by the government. In 2018,

about 6,86,754 kg fish spawn was produced from these hatcheries while only 9274 kg fish seed were collected from natural sources. Currently, over 98% of fish seed is produced in hatcheries in Bangladesh (DoF, 2019). Also, the country now produces over 4 billion tilapia fry every year from over 400 tilapia hatcheries (Mohamed and Subasinghe, 2017).

In any fish hatchery, there is always the risk of introducing pathogens that can cause disease. Moreover, the diseases can come from many sources, such as newly introduced broodstocks, contaminated

equipment, birds and other animals (Mohamed and Subasinghe, 2017). They can even find their way into a hatchery during routine operational activities. A disease outbreak can cause severe financial losses and be a serious setback for a hatchery operator (Mohamed and Subasinghe, 2017). The major disease problems of fish spawn as reported included fungal infection in fertilized eggs, white spot inside the yolk sac, loss of slime, spinal deformities, enlarged head and stomach, blindness and sudden spawn mortality (Faruk and Anka, 2017). Most hatchery owners have very little understanding of health and disease issues in their system.

In hatcheries, fish eggs and spawns are maintained at high densities and they may become heavily overgrown with bacteria within hours of fertilization. The egg surface is a highly favourable substrate for bacterial growth (Hansen and Olafsen, 1989). This may not only influence egg survival rate but also create a route for pathogen transmission to the emerging larvae and between rearing units (Skjermo and Vadstein, 1999). Bacteria are deleterious to cultured fish species (Bergh et al., 1992). Also, large numbers of bacteria can present in hatchery water could have high oxygen requirements (Hansen and Olafsen, 1989).

The most important component of disease prevention and control in a hatchery is disinfection. Cleaning and disinfection procedures are necessary to avoid introducing and spreading diseases. Diseases affecting one larval tank can easily spread to other tanks through contamination (Mohamed and Subasinghe, 2017). Egg disinfection reduces the mortality and thus increases hatchery production. Disinfection is a common practice and has been widely used to reduce egg and spawn mortality and improve rearing success during the yolk sac and first feeding stages (Aydin, 2011).

A disinfectant is an agent that destroys infection producing organisms. Concentration and duration are important factors that are dependent on the conditions and procedures undertaken. A range of chemicals have been used as disinfectants in hatcheries including chlorine, iodine, formalin, phenols, iodophor sodium hypochlorite, hydrogen peroxide, ethyl alcohol, isopropyl alcohol, glutaraldehyde, lime, salt, and potassium permanganate (Wagner et al., 2008; Stuart et al., 2010; Yanong, 2012; Bowker et al., 2014; Chowdhury et al., 2015). In Bangladesh, formalin, bleaching powder, polgard, sadic, virex, timsen, emsen, bactisal, biogaurd, lenocide and some other commercial disinfectants were reported to use in aquaculture activities (Miah et al., 2016; Hossain et al., 2018; Rahman, 2019).

Microorganisms reside in the water and other aquaculture facilities may have positive or negative effects on the outcome of aquaculture operations. Positive microbial activities include elimination of toxic materials such as ammonia, nitrite, and hydrogen

sulfide, degradation of unused feed, and nutrition of fish (Akpor et al., 2014). There are also pathogenic microorganisms that cause diseases in fish. Although development of aquaculture in different aspects is notable, microorganisms are among the least known and understood elements in aquaculture facilities including fish hatcheries. Considering substantial contribution of tilapia hatcheries in supplying seeds for aquaculture, the present study aimed to determine the effects of commonly used disinfectants on bacterial loads in a commercial tilapia hatchery.

2 Materials and Methods

2.1 Description of hatchery

The study was carried out in a typical private tilapia hatchery named Biswas Agro Fisheries and Hatchery at Trishal upazila of Mymensingh district. The hatchery only 20 km away from Bangladesh Agricultural University. The hatchery had 34 cemented cisterns of 84 ft² each with a water holding capacity of 5000 L. Each cistern could carry about one million first feeding fry. There were one hundred twenty trays (12"×7") used for incubation of eggs. They also have two overhead water holding tanks. The hatchery produced about 3 core fry per year.

2.2 Applications of chemical disinfectants

Salt, lime, potassium permanganate and formalin were used in the hatchery to disinfect trays and cisterns. The disinfectants were collected from a local animal drug shop at Trishal, Mymensingh. Hatching trays were disinfected by washing with salt water at a dose of 40 ppm following final wash with 20 ppm potash. Tilapia eggs were then placed in the tray for hatching. After hatching, the hatchlings were transferred into cistern for hormone treatment for sex reversal. Before placing the hatchling, the cisterns were washed and disinfected firstly with lime (5 g ft⁻²) and salt (30 g ft⁻²) followed by application of potassium permanganate (1 ppm) and formalin (0.25 ppm). After few hours the hatchlings were released there and kept 3-6 days for hormone treatment using 17- α methyltestosterone hormone (Argent, USA) at 50 mg kg⁻¹ feed. All the incoming water from overhead tank was treated with 3 ppt salt. Since the hatchery is a well reputed typical hatchery and routinely disinfects their units using above chemicals, the treatment doses of the present study were adopted from there.

2.3 Sources of samples

The study was conducted from March to May 2016. To examine the effect of chemicals on bacterial load in

water, eggs and fish fries the samples were collected aseptically from hatching trays and cisterns both of which have been disinfected with chemicals. Water samples were also taken from overhead tank and underground well. Samples from each source were collected using 20 ml sterile glass universal on day 1, day 10 and day 20 of each month and termed as first, second and third sampling, respectively. Collected samples kept in a portable ice box immediately after collection and brought to the Fish Disease Laboratory of Faculty of Fisheries, Bangladesh Agricultural University.

2.4 Determination of colony forming unit (cfu mL⁻¹)

Bacterial counting was determined using serial dilution technique and expressed as colony forming unit (cfu mL⁻¹). Colony forming unit was determined for the bacterial suspension according to the drop count method using tryptone soya agar plates (TSA). Briefly, the bacterial suspension was diluted 10 fold seven times with distilled water. Replicate drops (20 μ L drop⁻¹) from each dilution were then placed onto a TSA plate that had been previously divided into six sections. The plates were allowed to dry before incubation at 25 °C for at least 24 h until colonies were visible and could be counted. The average number of colonies per drop was counted and cfu mL⁻¹ was determined for the bacterial suspensions using following formula:

$$\text{cfu mL}^{-1} = N_c \times D_f \times 50 \quad (1)$$

where, N_c = Average number of colonies and D_f = Dilution factor

3 Results

3.1 Bacterial load in underground and overhead tank water

The average highest bacterial load in underground water was $5.38 \pm 3.17 \times 10^4$ cfu mL⁻¹ in April while the lowest load of $1.54 \pm 0.37 \times 10^4$ cfu mL⁻¹ was found in March (Table 1). However, the second sampling in April gave the highest bacterial load (9.17×10^4 cfu mL⁻¹) and first sampling in March gave the lowest load (1.14×10^4 cfu mL⁻¹) in underground water. In the overhead tank water, the highest bacterial load of 8.5×10^7 cfu mL⁻¹ was seen in first sampling of April while it was lowest 1.03×10^7 cfu mL⁻¹ in the third sampling of April. The highest average load of $4.89 \pm 1.71 \times 10^7$ cfu mL⁻¹ was obtained in March while the lowest load of $3.94 \pm 1.97 \times 10^7$ cfu mL⁻¹ was seen in the May (Table 1).

3.2 Bacterial load in water of hatching tray

The highest bacterial load in tray water before adding eggs was 8.30×10^6 cfu mL⁻¹ and the lowest was found 1.08×10^6 cfu mL⁻¹ in the April (Table 2). The average highest bacterial load of $3.30 \pm 3.54 \times 10^6$ cfu mL⁻¹ was found in April followed by May ($1.85 \pm 1.30 \times 10^6$ cfu mL⁻¹) and March ($1.47 \pm 0.60 \times 10^6$ cfu mL⁻¹) (Table 2). Bacterial loads were found to increase in tray water after 24 hours of release eggs. The highest load of 8.90×10^8 cfu mL⁻¹ was observed in the second sampling in May and the lowest 2.5×10^8 cfu mL⁻¹ was found in third sampling of April (Table 2). The average load of $4.09 \pm 1.25 \times 10^8$ cfu mL⁻¹, $4.86 \pm 2.32 \times 10^8$ cfu mL⁻¹ and $5.59 \pm 2.50 \times 10^8$ cfu mL⁻¹ were found in March, April and May, respectively (Table 2).

3.3 Bacterial load in cistern water

Bacterial load was determined in cistern water immediately after disinfection and after 6 days of fry rearing. The average highest bacterial load of $1.43 \pm 0.75 \times 10^3$ cfu mL⁻¹ was found in April while the lowest load of $0.9 \pm 0.17 \times 10^3$ cfu mL⁻¹ was found in May from cistern water after disinfection (Table 3). The bacterial load in cistern water was found highest (2.45×10^3 cfu mL⁻¹) in second sampling of April and lowest (0.65×10^3 cfu mL⁻¹) in second sampling of March (Table 3). In the water after 6 day of fry rearing, the highest bacterial load of 7.55×10^{12} cfu mL⁻¹ was observed in third sampling of April while the lowest load of 1.37×10^{12} cfu mL⁻¹ was found in the first sampling of March. The highest average load ($5.26 \pm 1.9 \times 10^{12}$ cfu mL⁻¹) was observed in April whereas the lowest load ($1.89 \pm 1.84 \times 10^{12}$ cfu mL⁻¹) was seen in May (Table 3).

3.4 Bacterial load of eggs in hatching tray

The highest bacterial load in fish eggs was 8.0×10^2 cfu g⁻¹ in the third sampling of March and the lowest load of 1.80×10^2 cfu g⁻¹ was found in second sampling of May (Table 4). The average bacterial load was highest in March ($4.25 \pm 2.67 \times 10^2$ cfu g⁻¹) and gradually decreased in April ($3.12 \pm 2.43 \times 10^2$ cfu g⁻¹) and May ($2.8 \pm 1.18 \times 10^2$ cfu g⁻¹) (Table 4).

3.5 Bacterial load in fish fries from cistern

After first feeding, the average highest bacterial load in fries of cistern was $1.34 \pm 0.75 \times 10^4$ cfu mL⁻¹ in April while the lowest load of $0.82 \pm 0.4 \times 10^4$ cfu g⁻¹ was observed in May (Table 5). However, the second sampling in April gave the highest bacterial load (2.27×10^4 cfu g⁻¹) and the first sampling of May gave

Table 1. Bacterial load (cfu mL⁻¹) in underground water and overhead tank water

Sample sources	Period	Bacterial load (cfu mL ⁻¹)			
		1st sampling	2nd sampling	3rd sampling	Average ± SD
Underground water	March	1.14×10 ⁴	1.46×10 ⁴	2.02×10 ⁴	1.54 ± 0.37×10 ⁴
	April	5.55×10 ⁴	9.17×10 ⁴	1.41×10 ⁴	5.38±3.17×10 ⁴
	May	2.36×10 ⁴	2.19×10 ⁴	1.71×10 ⁴	2.09±0.27×10 ⁴
Overhead tank water	March	4.87×10 ⁷	7.00×10 ⁷	2.80×10 ⁷	4.89±1.71×10 ⁷
	April	8.5×10 ⁷	3.08×10 ⁷	1.03×10 ⁷	4.2±3.15×10 ⁷
	May	4.87×10 ⁷	5.75×10 ⁷	1.21×10 ⁷	3.94±1.97×10 ⁷

Table 2. Bacterial load (cfu mL⁻¹) in the water of hatching tray

Sample sources	Period	Bacterial load (cfu mL ⁻¹)			
		1st sampling	2nd sampling	3rd sampling	Average ± SD
Water before adding eggs	March	1.39 × 10 ⁶	2.25 × 10 ⁶	7.75 × 10 ⁵	1.47 ± 0.60 × 10 ⁶
	April	1.08 × 10 ⁶	5.25 × 10 ⁵	8.30 × 10 ⁶	3.30 ± 3.54 × 10 ⁶
	May	3.70 × 10 ⁶	8.0 × 10 ⁵	1.06 × 10 ⁶	1.85 ± 1.30 × 10 ⁶
Water after 24h of egg release	March	3.43 × 10 ⁸	5.85 × 10 ⁸	3.00 × 10 ⁸	4.09 ± 1.25 × 10 ⁸
	April	4.00 × 10 ⁸	8.03 × 10 ⁸	2.55 × 10 ⁸	4.86 ± 2.32 × 10 ⁸
	May	2.85 × 10 ⁸	8.90 × 10 ⁸	5.03 × 10 ⁸	5.59 ± 2.50 × 10 ⁸

Table 3. Bacterial load (cfu mL⁻¹) in cistern water

Sample sources	Period	Bacterial load (cfu L ⁻¹)			
		1st sampling	2nd sampling	3rd sampling	Average ± SD
After chemical wash	March	1.05×10 ³	0.7×10 ³	2.27×10 ³	1.34 ± 0.67×10 ³
	April	1.2×10 ³	2.45×10 ³	0.65×10 ³	1.43 ± 0.75×10 ³
	May	0.8×10 ³	1.15×10 ³	0.75×10 ³	0.9 0± 0.17×10 ³
After 6 d of fry release	March	1.37×10 ¹²	1.52×10 ¹²	7.53×10 ¹²	3.47 ± 2.87×10 ¹²
	April	5.25×10 ¹²	3.0×10 ¹²	7.55×10 ¹²	5.26 ± 1.90×10 ¹²
	May	2.12×10 ¹²	1.67×10 ¹²	1.90×10 ¹²	1.8 ± 1.84×10 ¹²

Table 4. Bacterial load (cfu g⁻¹) in the fish eggs

Period	Bacterial load (cfu g ⁻¹)			
	1st sampling	2nd sampling	3rd sampling	Average ± SD
March	1.95×10 ²	2.8×10 ²	8.0×10 ²	4.25±2.67×10 ²
April	3.42×10 ²	2.5×10 ²	5.95×10 ²	3.12±2.43×10 ²
May	2.20×10 ²	1.80×10 ²	4.50×10 ²	2.83±1.18×10 ²

Table 5. Bacterial load (cfu g⁻¹) in fish fries of cistern

Sample sources	Period	Bacterial load (cfu g ⁻¹)			
		1st sampling	2nd sampling	3rd sampling	Average ± SD
Fry from cistern [†]	March	0.9 × 10 ⁴	1.95 × 10 ⁴	0.32 × 10 ⁴	1.06 ± 0.67 × 10 ⁴
	April	1.3 × 10 ⁴	2.27 × 10 ⁴	0.45 × 10 ⁴	1.34 ± 0.75 × 10 ⁴
	May	0.3 × 10 ⁴	1.4 × 10 ⁴	0.77 × 10 ⁴	0.82 ± 0.45 × 10 ⁴
6 d hormone treated fries	March	2.25 × 10 ⁵	1.12 × 10 ⁵	5.33 × 10 ⁵	2.9 ± 1.78 × 10 ⁵
	April	4.05 × 10 ⁵	5.55 × 10 ⁵	5.85 × 10 ⁵	5.15 ± 0.79 × 10 ⁵
	May	1.45 × 10 ⁵	4.61 × 10 ⁵	7.2 × 10 ⁵	4.42 ± 2.35 × 10 ⁵

[†] After first feeding

the lowest load (0.3×10^4 cfu g⁻¹) in fries after first feeding (Table 5). After adding hormone treated feed on 6th day, the highest load of 7.2×10^5 cfu g⁻¹ was observed in the third sampling of May and the lowest load of 1.12×10^5 cfu g⁻¹ was seen in the second sampling of March (Table 5). The average load found in March, April and May were $2.9 \pm 1.78 \times 10^5$ cfu g⁻¹, $5.15 \pm 0.79 \times 10^5$ cfu g⁻¹ and $4.4 \pm 2.35 \times 10^5$ cfu g⁻¹, respectively (Table 5).

4 Discussion

Disinfectants used in fish hatcheries prevent eggs and fries to get infection. The present study was conducted to examine combined effects of four chemical disinfectants including lime, salt, potassium permanganate and formalin on bacterial load in hatchery water, eggs and fish fries. These chemicals are commonly used for cleaning and disinfecting fish hatchery units in Bangladesh (Brow and Brooks, 2002; Faruk and Anka, 2017).

Bacterial load was determined in ground water and compared with discarded water in different parts of hatchery proper. Variations were observed in bacterial load in water at different stages of hatchery operations. The highest bacterial load in underground water was observed in the second sampling of April which was 9.17×10^4 cfu mL⁻¹. However, rapid increase of bacterial load was seen in the overhead tank which was 8.5×10^7 cfu mL⁻¹ in April. The mean highest load of $3.94 \pm 1.97 \times 10^7$ cfu mL⁻¹ in overhead tank which was 3 folds higher the bacterial load of ground water. That rapid increase occurred may be due to increase of temperature in the environment which influences the bacterial proliferation. During this experiment temperature of the ground water was recorded as 21.9 °C when the environmental temperature was 29.5 °C. Temperature of the surrounding environment may be the cause of bacterial proliferation. Somehow it can be reduced by using some salt during cleaning the overhead tank. Haider (2015) found bacterial load in overhead tank of hatcheries was 2.57×10^5 – 3.76×10^5 cfu mL⁻¹ which seems quite

lower than the bacterial load found in the present study. This might be due to different conditions and water sources.

The highest bacterial load in water from hatching tray after disinfection was observed $3.3 \pm 3.54 \times 10^6$ cfu mL⁻¹ whereas the highest load of overhead tank was $4.89 \pm 1.71 \times 10^7$ cfu mL⁻¹. The sudden decrease of bacterial load could be identified due to use of salt during washing the tray and the use of saline in the tray. Saline was used in the hatching tray water to prevent the fungal infection of eggs and reduce mortality rate. After 24 hours of egg rearing bacterial load gradually increased in tray water. The increase bacterial load may be occurred due to the presence of dead egg shells as a nutrient rich media for microorganisms. Zahura et al. (2004) reported that the use of salt with lime effectively reduces the microbial infection by reducing microorganisms although it was not mention the bacterial fluctuation occurred due to disinfectant. Komar et al. (2004) found that water treatment did not have a significant effect on bacterial count, it only reduces the number of bacteria from the sources. The author further reported that the bacterial counts did not exhibits a pattern, it is highly probable that initially the bacteria present in the hatching tray started blooming using the available nutrients in the tray with eggs and water.

Bacterial load in cistern water was rapidly decreased than the load at overhead tank due to use of chemicals such as salt, lime, formalin, potassium permanganate to disinfect the cistern. Haque et al. (2014) found that fish reared with oxytetracycline treated feed gradually decreased the bacterial load in the aquarium water, gills, intestine and skin of their experimental fish. After antibiotic treatment bacterial load of water was 1.40×10^3 cfu mL⁻¹ in laboratory condition. They further reported that use of oxytetracycline successfully reduced bacterial load in aquarium water and organ throughout the experimental period. Uddi and Kader (2005) reported a variety of chemicals were used in hatcheries for increased and controlled production of seed in hatcheries, improvement of survival rates and control of pathogen.

Chloramphenicol, erythromycin, oxytetracycline, pre-furan were found to be widely used to control all types of bacteria while formalin and malachite green used as anti fungal agents (Uddi and Kader, 2005).

The highest bacterial load of $5.26 \pm 1.9 \times 10^{12}$ cfu mL⁻¹ was found in cistern water after 6-day hormone treatment of fry where the initial load was $1.43 \pm 0.75 \times 10^3$ cfu mL⁻¹. Only hormone (17- α methyl testosterone) mixed feed without any antibiotics was given to the fry. Thus, decomposition of excess feed and feces of fry might be responsible for rapid increase of bacterial load in cistern water. Boyd (2017) reported that bacteria are the primary organisms of decay in aquaculture systems. If fresh organic matter like feed is applied to water initially with low in organic matter concentration and bacterial activity, bacteria will rapidly respond to this food and increase in number as they decompose the substrate.

The highest value of bacterial load of 8.0×10^2 cfu g⁻¹ was found in the eggs collected from the hatching tray where saline water used to prevent infection of microorganisms. Nickum (2014) reported that disinfectants mainly formalin, hydrogen peroxide, iodine, ozone, copper sulfate, potassium permanganate etc. were used to remove fungus and other disease agents that can affect hatching. Austin (2006) found that higher number of bacteria in fish eggs ranged from 10^3 - 10^6 and the adhesion and colonization of the bacteria occurs within a few hours of fertilization.

Variations were seen in bacterial load in fries. The highest bacterial load of hormone treated fry was $5.15 \pm 0.79 \times 10^5$ cfu g⁻¹. During this period no antibiotics or drugs were given with feed. So a rapid increase of bacterial load was observed in the present study. The highest bacterial load of $1.34 \pm 0.75 \times 10^4$ cfu g⁻¹ was found after first feeding which increased to $5.15 \pm 0.79 \times 10^5$ cfu g⁻¹ after 6 day hormone treated fry. Haque et al. (2014) observed that the use of oxytetracycline with feed decreased the bacterial load in aquarium water, gill, intestine and skin of fish, whereas the bacterial content remain unchanged or little increased in the control group. Moshtaghi et al. (2014) reported that the expose of sturgeon fries in copper sulfate and potassium permanganate solution decreased bacterial load in gill, intestine, and skin including surrounding water. These compounds have disinfecting effect on bacterial load of gill, skin and surrounding water.

5 Conclusion

Fish hatcheries play a vital role in timely supply of quality seed throughout the country to sustain aquaculture production. Early development stage of fish eggs and fries are more susceptible to infectious diseases. The present study revealed that use of disinfectants in the initial stages of hatchery operation

can decrease the bacterial load and thus reduce the chances of occurrence of infection and diseases in eggs and fries. Further study should include determination of the effect disinfectants on both qualitative and quantitative bacterial floras in fish hatcheries.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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