



Efficacy of some disinfection methods on the microbial load of eggs and equipment of tilapia hatchery

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Abstract

In fish hatcheries, high densities of eggs are maintained in incubators with microbial loads that vary from those in the natural habitat. Moreover, the accumulation of organic debris on tools used in the hatchery facility assists the spread of infectious diseases. This study was designed to evaluate the effect of different iodophor concentrations (10, 50, 100 and 150 ppm) for different contact times (5, 10 and 15 min) on total bacterial count (TBC) and total fungal count (TFC) and hence on the hatchability percentage of tilapia eggs. In addition, the effect of common salt (NaCl), sunlight exposure, 1% Halamid®, 1% Virkon S® and 3% Aquazix® for contact times (5, 10 and 15 min) in reducing TBC and TFC on hatchery equipment (buckets, nets and dishes) was also evaluated. Iodophor treatment at 10 ppm for 15 min resulted in significant reduction of both TBC and TFC on tilapia eggs by $\geq 4 \log_{10}$ reductions but failed to achieve complete disinfection of egg surface. Conversely, 50 ppm or higher iodophor concentrations did not display antimicrobial activity after all tested contact times. Hatchability percentage was insignificantly different between 0 ppm (90%) and 10 ppm (94%) of iodophor. However, 10 ppm iodophor was significantly different ($P < 0.05$) from 50 ppm (85%) and 100 ppm (65%). At 150 ppm, there was no hatchability. After application of different disinfectants, limited to general bactericidal activity of $\geq 4 \log_{10}$ reduction was observed in common salt (NaCl 150 g /7 liters) after 15 min in dishes only, sunlight exposure after 10 min in dishes only and 3% Aquazix after 15 min in all tools. Significant and 100% log reduction of both TBC and TFC was observed in 1% Halamid® after 10 min and 1% VirkonS® after 15 min.

(Keywords: disinfection, fish, eggs, hatchability, equipment, iodophor and halamid)

Introduction

Egypt ranked 8th position in leading aquaculture producing countries in the world with about 987 thousand tonnes according to FAO (2011). The development and expansion of a large number of tilapia hatcheries in Egypt supported the development aquaculture sector Saleh, (2007). The mass-scale business of tilapia fry production includes the broodstock management, artificial egg incubation and larval rearing (Macintosh and Little, 1995). Bacterial infections are major causes of both egg losses and deformed fish larvae (Harboe et al. 1994). These losses are not attributed to obligate pathogenic bacteria, but rather to proliferation of opportunistic bacteria in the environment of intensive egg incubation (Skjermo and Vadstein 1999). The control of bacteria on fish eggs includes the application of suitable surface chemicals disinfectants on fish eggs (Atanasov et al. 2011). Iodophors link free iodine with a carrier to maintain the reactive nature of iodine (McDonnell & Russell, 1999). Iodophors are effective for pathogen-specific egg disinfection (Yanong and Erlacher-Reid, 2012), however little information about its activity for tilapia eggs disinfection is known.

Good sanitary measurements play an important role in removing the organic debris. All

equipment, surfaces and vehicles must be cleaned and disinfected within the aquaculture facility (Yanong and Erlacher-Reid, 2012). The choice of a suitable disinfectant is a key element of disinfection. Sunlight can be an effective disinfectant if given sufficient time and intensity (Danner and Merrill, 2006). "Chloramine T" or Halamid® is a disinfectant that attacks microbes through a process of oxidation, therefore they cannot build up a resistance to it. In addition, chloramine T is highly stable and remains active over an extended period of time (Edrisi et al., 2012). Virkon S® (DuPont, USA) is effective at inactivating both enveloped and non-enveloped viruses (Eleraky et al., 2002). Aquazix® is a disinfectant based on hydrogen peroxide which has received attention for its control of several fish pathogens and is recommended as a general disinfectant in aquaculture (Avendano-Herrera et al., 2006).

This study was designed to assess the effect of iodophor on total microbial load and on the hatchability percentage of tilapia eggs. In addition, the effect of common salt (NaCl), sunlight exposure, Halamid®, Virkon S® and Aquazix® in reducing total microbial load on hatchery tools will also be evaluated.

Materials and Methods

Egg disinfection

The experiment was carried out in a tilapia hatchery at Al-Fayoum Province. The effect of different concentrations of iodophor on total microbial load and on hatchability percentage was performed according to Subasinghe and Sommerville (1985) with some modifications. Different concentrations of Iodophor were prepared using distilled water (10 ppm, 50 ppm, 100 ppm and 150 ppm). The prepared disinfectant concentrations were maintained in plastic containers at a temperature of $27 \pm 1^\circ \text{C}$ for one hour before the treatment to raise its temperature to the egg incubation temperature. Tilapia eggs were removed from the mouth of brooding females within 15 hr of spawning and were held in 5 funnels, each containing 100 eggs. The eggs were exposed to the disinfectant concentrations 10, 50, 100, 150 ppm placed in containers respectively and the last container was left without disinfectant as a control. At 5, 10 and 15 min contact time, one gram egg sample was removed and homogenized in a screw capped vial containing 1 ml sterile normal saline according to Jantrakajorn and Wongtavatchai (2015) then ten-fold serial dilutions were prepared. The total viable bacterial counts were estimated by spread plate method on plate count agar medium (Oxoid) and total viable fungal counts were estimated by spread plate method on Sabouraud's dextrose agar (Oxoid) using methods described by APHA *et al.* (1998). After 15 min, nets containing eggs were removed and washed by gentle agitation in distilled water. Then the eggs were incubated in their funnels with a closed recirculatory system. Temperature, pH, dissolved oxygen, ammonia and nitrite were monitored throughout the experiment (Table 1). The number of hatched fry was counted within 12 hr of hatching. The percentage hatchability of fertile eggs was calculated for each treatment.

Equipment disinfection

Different disinfectants were used to reduce microbial contamination on buckets, nets and dishes (3 units per each) used in the hatchery. Disinfectants were diluted according to the manufacturer instructions. Dilution was made with tap water; 1% Halamid® (Axcence, France), 1% Virkon S (Dupont, USA) and 3% Aquazix (BBZix, Spain). We also evaluated the effect of sunlight exposure and NaCl (150 g / 7 liters).

Before application of each disinfectant, tools were swabbed with sterile cotton swabs

moistened with sterile normal saline and sample were obtained from an outlined squares using wire template 10x10cm, according to Collins *et al.* (1991). Swabs were kept in sterile test tubes containing 5 ml sterile physiological saline. Test tubes were transferred to laboratory in an ice box as soon as possible and ten-fold serial dilutions were prepared. Duplicate plate count agar plates (Oxoid) and duplicate Sabouraud's dextrose agar (Oxoid) plates containing 0.05 mg of chloramphenicol/ml were inoculated each with 100 μl from each dilution and total bacterial and total fungal counts were determined APHA *et al.* (1998). After application of each disinfectant, the tools were swabbed again as previously mentioned at 5, 10 and 15 min contact times and total bacterial and total fungal counts, respectively. According to European Committee for Standardization (CEN), EN 13697-2001, bactericidal activity was defined as $\geq 4 \log_{10}$ reduction of organisms attached to a surface under examination.

Statistical analysis

Differences in total microbial counts in relation to the type and concentration of disinfectant and the time of contact were analyzed by SPSS version 20 (SPSS Inc., Chicago, IL, USA). Pearson Chi-Square, Two-way analysis of variance (ANOVA) and LSD test for post hoc comparison were used. The level of significance was set at $P < 0.05$.

Results

Effect of iodophor on total microbial count and hatchability percentage of tilapia eggs

Table (2) showed total microbial count for eggs before and after exposure to different concentrations of iodophor (10 ppm, 50 ppm, 100 ppm and 150 ppm). Total bacterial count (TBC) for eggs before treatment was $(7.18 \pm 0.86) \log_{10}$ colony forming unit (CFU) / g. Iodophor concentration at 10 ppm significantly ($P < 0.05$) reduced TBC to $(3.31 \pm 1.30) \log_{10}$ CFU / g after 15 min, while at 5 or 10 min contact time, insignificant reduction was recorded. At 50, 100 and 150 ppm, there was no significant difference in TBC after all tested contact times.

Total fungal count (TFC) for eggs before treatment was $(5.63 \pm 1.87) \log_{10}$ CFU / g. Log reduction in TFC was significantly ($P < 0.05$) higher at 10 ppm than 50, 100 and 150 ppm. Iodophor concentration at 10 ppm significantly reduced TFC to $(0.69 \pm 0.00) \log_{10}$ CFU / g after 15 min, while at 5 or 10 min contact time, insignificant reduction was

recorded. At 50, 100 and 150 ppm, there was no significant difference in TFC after all tested contact times. Hatchability percentage was insignificantly different between 0 ppm (90%) and 10 ppm (94%). However, 10 ppm iodophor was significantly different ($P < 0.05$) from 50 ppm (85%) and 100 ppm (65%). At 150 ppm, there was no hatchability.

Effect of some disinfectants on total microbial count of hatchery equipment

Table (3) illustrates mean \pm SD of TBC (\log_{10} CFU / cm^2) before and after exposure to different disinfectants for different contact times on some hatchery tools. Before disinfection, the average TBC was around 8.79 ± 1.76 , 8.89 ± 1.83 and $8.81 \pm 1.83 \log_{10}$ CFU / cm^2 for buckets, dishes and nets, respectively. After application of different disinfection methods, bactericidal activity of $\geq 4 \log_{10}$ reduction was observed in common salt (NaCl 150 g / 7 liters) after 15 min in dishes only, sunlight exposure after 10 min in dishes only, 1% Halamid[®] after 5 min in all tools with 100% log reduction after 10 min in all tools, 1% Virkon s[®] after 10 min in all tools with 100% log reduction after 15 min in all tools, and 3% Aquazix after 15 min in all tools. Significant log reduction ($P < 0.05$) was recorded for 1% Halamid[®] after 10 min followed by 1% Virkon s[®] after 15 min. Table (4) illustrates mean \pm SD of TFC (\log_{10} CFU / cm^2) before and after exposure to different disinfectants for different contact times on some hatchery tools. Before disinfection, the average TFC was around 4.43 ± 0.78 , 4.63 ± 0.83 and $4.71 \pm 0.85 \log_{10}$ CFU / cm^2 for buckets, dishes and nets, respectively. After application of different disinfection methods, significant fungicidal activity of $\geq 4 \log_{10}$ reduction ($P < 0.05$) was observed in 1% Halamid[®] and 1% Virkon s[®] after 10 min in all tools with 100% log reduction. After 15 min, all disinfectants showed 100% log reduction of TFC in all tools.

Discussion

In fish hatcheries, high densities of eggs are maintained in incubators with microbial loads that vary from those in the natural habitat. Disinfection of eggs aimed at eliminating or greatly diminishing pathogen transmission to different fish farms (Wagner et al. 2008). Iodine has proved itself as an efficient disinfectant against multiple fish pathogens (Wagner et al., 2010) and at the same time, it has showed low toxicity to fish eggs (Stuart et al., 2010). The

results of this study showed that iodophor treatment at 10 ppm for 15 min significantly reduced TBC and TFC of tilapia eggs by $\geq 4 \log_{10}$ reductions but failed to achieve complete disinfection of egg surface. Conversely, 50 ppm or higher iodophor concentrations did not display marked activity after all tested contact times. Similar results were reported by (Tendencia, 2001) who recorded that (2.5–20 mg /L) iodine, the lower concentrations of iodine was to be effective in reducing the total bacterial load of grouper eggs, *Epinephelus coioides*, at both the cleavage and eyed stages. Also, Varner-Jeffreys et al. (2007) recorded that the minimal recommended concentration of active iodine for limiting fungus development is about 10 mg dm^{-3} . On the contrary, (Stuart et al. 2010, Overton et al. 2010, Katharios et al. 2007, Peck et al. 2004) recorded that the most commonly used concentrations, which were thought to be efficient disinfectants, were 50-100 mg dm^{-3} .

The effect of iodophore disinfection on hatchability percentage revealed insignificant improved hatchability ($P > 0.05$) at 10 ppm level. Though, the improvement at 10 ppm level was significantly higher than that at 50 and 100 ppm level ($P < 0.05$). In contrast, at 150 ppm the hatchability was nil. These results were different from (Subasinghe and Sommerville, 1985) who reported that hatchability improvement at 50 and 100 ppm level was significantly higher than that at 10 ppm level. Meanwhile, we agree with (Subasinghe and Sommerville, 1985) who observed no hatchability at iodine concentrations higher than 100 ppm. It can be concluded that although hatchability percentage between control and 10 ppm has no significance different but the significant decrease in total bacterial count and total fungal count recommend the use of disinfectant to prevent the vertical transmission of pathogens from the parent stock to the progeny and to prevent horizontal transmission from the egg facility to the rearing facility.

Sanitation is the backbone of fish biosecurity system in modern aquaculture. The accumulation of organic debris in the aquaculture facility assists the spread of a number of infectious diseases. In this study, the effect of some disinfection methods on hatchery tools was evaluated. Common salt, sun exposure and Aquazix though displayed limited to general antimicrobial action, failed to completely remove surface microbial loads. Significant and 100% \log_{10} reduction of heterotrophic viable

bacterial and fungal counts, from the surface of buckets, nets and dishes used in the hatchery facility, was reached by the use of 1% Halamid® (Chloramine T) for 10 min and 1% Virkon S® for 15 min. These results agreed with (Güngör and Yurudu, 2015) who reported that after the application of Chloramine T, the heterotrophic bacterial counts on the surfaces were decreased. Log reduction in total bacterial count after application of 1% virkon S on buckets, dishes and nets was > 4 log after 10 min and 100% log reduction was achieved after 15 min. This result agreed with Eleraky *et al.* (2002) who reported that manufacturer's guidelines for Virkon S® recommend a 1.0% solution for disinfecting equipment with a 10 min contact time. After application of Aquazix, the count was reduced after disinfection to be (4.38 - 4.61) log₁₀ CFU / cm² after 15 min. Aquazix resulted in > 4 log₁₀ reduction in the cultivable heterotrophic bacteria on buckets, dishes and nets after 15 min. This result is in accordance with Leung *et al.* (2012) who observed that after five minutes of exposure to recommended concentrations of hydrogen peroxide (0.017% to 8.75%) during clinical disinfection, microbial cells remain intact and alive.

In conclusion, for disinfection of tilapia eggs, iodophor treatment at 10 ppm for 15 min significantly reduced TBC and TFC by ≥ 4 log₁₀ reductions but failed to achieve complete disinfection. Although hatchability percentage between control and 10 ppm has no significant difference but the significant decrease in TBC and TFC recommend the use of disinfectant for the health of fish larvae after hatching. For disinfection of hatchery tools, 1% Halamid® for 10 min and 1% Virkon S® for 15 min were the most effective disinfectants with 100% log₁₀ reduction for both TBC and TFC.

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Table (1) Mean ± SD of water quality parameters and the total microbial countsdetermined during the experiment

parameters	Mean ± SD
Temperature ° C	28.47 ± 0.29
pH	7.60 ± 0.32
Dissolved oxygen mg/l	5.07 ± 0.21
Unionized Ammonia mg/l	0.19 ± 0.07
nitrite- nitrogen (No ₂ -N ₂) mg/l	0.05 ± 0.03
Total bacterial count / ml	5.97 ± 0.07
Total fungal count / ml	5.47 ± 0.15

Table (2) Mean ± SD of total microbial count (log₁₀ CFU/ g)of tilapia eggs after exposure to different concentrations of iodophor

Total bacterial count				
Concentration	Contact time			
	0 min (Control)	5 min	10 min	15 min
10 ppm*	7.18 ± 0.86	5.56 ± 1.42	5.48 ± 1.36	3.31 ± 1.30*
50 ppm	7.18 ± 0.86	6.75 ± 0.83	6.68 ± 0.82	6.62 ± 0.84
100 ppm	7.18 ± 0.86	7.05 ± 0.78	7.01 ± 0.79	6.96 ± 0.77
150 ppm	7.18± 0.86	7.09 ± 0.81	7.06 ± 0.81	7.02 ± 0.79
Total fungal count				
	Contact time			
	0 min (Control)	5 min	10 min	15 min
10 ppm *	5.63 ± 1.87	4.89 ± 1.52	1.00 ± 0.00	0.69 ± 0.00*
50 ppm	5.63 ± 1.87	5.06 ± 1.63	4.39 ± 1.69	4.07 ± 0.00
100 ppm	5.63 ± 1.87	5.32 ± 1.71	5.23 ± 1.69	5.20 ± 1.67
150 ppm	5.63 ± 1.87	5.33 ± 1.79	5.29 ± 1.75	5.25 ± 1.72

* Values are significantly different at(P < 0.05).

Table (3) Mean ± SD of TBC (log₁₀ CFU /cm²) after exposure todifferent disinfectants for different contact times on some hatchery tools

Type of disinfectant	Contact time											
	0 min			5 min			10 min			15 min		
	buckets	Dishes	nets	buckets	dishes	nets	buckets	dishes	Nets	buckets	dishes	nets
Salt	8.79 ±1.76	8.89 ±1.83	8.81 ±1.83	5.74 ±0.80	5.81 ±0.87	5.74 ±0.91	5.68 ±0.83	5.64 ±0.86	5.66 ±0.88	5.55 ±0.96	4.52 ±0.99	5.57 ±0.87
Sunlight exposure	8.79 ± 1.79	8.86 ±1.82	8.92 ±1.84	5.79 ±0.84	5.76 ±0.90	5.78 ±0.86	5.74 ±0.86	4.70 ±0.88	5.72 ±0.86	5.66 ±0.88	4.55 ±0.88	5.64 ±0.89
Halamid**	8.77 ± 1.80	8.54 ±2.33	8.82 ±1.86	4.64 ±0.83	4.66 ±0.85	4.57 ±0.84	0.00 ±0.00*	0.00 ±0.00*	0.00 ±0.00*	0.00 ±0.00*	0.00 ±0.00*	0.00 ±0.00*
Virkon S*	8.82 ± 1.83	8.85 ±1.85	8.87 ±1.87	5.75 ±0.81	5.82 ± 0.88	4.74 ±0.88	4.61 ±0.86	4.57 ±0.79	4.58 ±0.86	0.00 ±0.00*	0.00 ±0.00*	0.00 ±0.00*
Aquazix	8.84 ±1.83	8.82 ±1.86	8.87 ±1.82	5.69 ±0.81	5.76 ±0.90	5.80 ±0.89	5.58 ±0.79	4.58 ±0.79	5.63 ±0.90	4.48 ±0.90	4.38 ±0.79	4.61 ±0.94

* Values are significantly different at P (< 0.05)

** More significant

Table (4) Mean ± SD of TFC (log₁₀ CFU / cm²) after exposure to different disinfectants for different contact times on some hatchery tools

Type of disinfectant	Contact time											
	0 min			5 min			10 min			15 min		
	buckets	Dishes	nets	buckets	dishes	nets	buckets	Dishes	nets	Buckets	dishes	nets
Salt	4.43 ±0.78	4.63 ±0.83	4.71 ±0.85	3.64 ±0.30	3.67 ±0.28	4.67 ±0.86	2.53 ±0.29	2.43 ±0.00	3.60 ±0.87	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Sunlight exposure	4.46 ±0.77	4.39 ±0.85	4.91 ±0.88	3.83 ±0.44	3.71 ±0.24	4.76 ±0.87	3.62 ±0.32	3.60 ±0.19	4.70 ±0.82	0.00 ±0.00	0.00 ±0.00	0.00 ±0.69
Halamid*	4.58	4.42	4.77	3.44	2.47	4.65	0.00	0.00	0.00	0.00	0.00	0.00

	±0.74	±0.81	±0.80	±0.33	±0.00	±0.81	±0.00*	±0.00*	±0.00*	±0.00	±0.00	±0.00
Virkon S*	4.58	4.38	4.87	3.93	3.04	4.77	0.00	0.00	0.00	0.00	0.00	0.00
	±0.75	±0.67	±0.86	±0.38	±0.00	±0.84	±0.00*	±0.00*	±0.00*	±0.00	±0.00	±0.00
Aquazix	4.49	4.40	4.89	3.86	2.69	4.80	3.30	2.60	3.63	0.00	0.00	0.00
	±0.75	±0.77	±0.87	±0.41	±0.00	±0.88	±0.40	±0.86	±0.90	±0.00	±0.00	±0.00

* Values are significantly different at (P < 0.05)

الملخص العربي

كفاءة بعض طرق التطهير على الحمل الميكروبي للبيض و أدوات مفرخ البلطي

تامر إسماعيل- سماح لبن- ندى القباني- مهجة بدوي

قسم الصحة والرعاية البيطرية - كلية الطب البيطري - جامعة القاهرة

أجريت هذه الدراسة لتقييم تأثير استخدام الأيودوفور على نسبة الفقس والعد الميكروبي لبيض البلطي و كذلك تقييم تأثير استخدام بعض أنواع التطهير على بعض الأدوات المستخدمة في المفرخ للتقليل من انتشار الميكروبات. أوضحت نتائج استخدام الأيودوفور لتطهير البيض ان استخدامه بتركيز 10 جزء في المليون لمدة 15 دقيقة أدى الي انخفاض معنوي في العد الكلي الفطري والبكتيري عن التركيزات 50, 100, 150 جزء في المليون ولكن فشل في التطهير الكامل للبيض. لم يكن هناك فرق معنوي في نسبة الفقس بين عدم استخدام الأيودوفور و استخدامه بتركيز 10 جزء في المليون لمدة 15 دقيقة إلا أن الانخفاض المعنوي في العد الكلي الفطري والبكتيري وصى باستخدام الأيودوفور لصحة يرقات الأسماك بعد الفقس. أظهرت نتائج استخدام المطهرات المختلفة لتطهير الأدوات المستخدمة في المفرخ أن الهلاميد 1% و فيركون 1S% كانا أكثر المطهرات فاعلية من الملح (150 جم / 7 لتر) وتعرض المعدات لأشعة الشمس وأكوازيكس 3% فقد أدى استخدام الهلاميد 1% و فيركون 1S% الي انخفاض معنوي في العد الكلي الفطري والبكتيري بعد 5 دقائق للهلاميد، وبعد 10 دقيقة للفيركون S ونجحا في التطهير الكامل للأدوات بعد 10 دقائق للهلاميد، وبعد 15 دقيقة للفيركون S.