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Effect of Chlorination on Bacterial Load in Brackishwater Shrimp Culture Pond

MS Khatun[1], Md. Motiur Rahman[2], M Ariful Islam[3] and Ajhar Ali[4]
[1,2,3] Bangladesh Fisheries Research Institute, Shrimp Research Station, Bagerhat, Bangladesh
[4]Bangladesh Fisheries Research Institute, Brackishwater Station, Khulna, Bangladesh

Abstract: *An experiment was done to know the effect of chlorination on bacterial load in shrimp culture pond. The experiment was done under 12 brackishwater shrimp culture ponds of 1000 m^2 each. Chlorination was done monthly @ 9ppm, 6 ppm, 3ppm and no chlorination (control). The load of total heterotrophic bacteria (THB) and total coliform (pathogenic bacteria) was checked before and after two hours and two following successive days after every application of chlorine.*
The highest percentage in reduction on of total coliform load after 2 hours and two days was observed in T_3 where the highest dose of chlorination was done. THB load was decreased in water and soil after 2 hours where chlorination was done but the load was slightly increased in control where no chlorination was done(table 3). The load of THB in water after 2 days was increased in T_1,T_2 and T_3 but decreased in T_4.

Key words: Chlorination,Bacteria,Shrimp

1. INTRODUCTION

Throughout the world aquaculture is the fastest growing food producing sector [1]. Shrimp (or prawn) culture is widespread throughout the tropical world. Disease outbreaks are considered to be a significant constraint to the development of the aquaculture sector, with a global estimate of disease losses in the range of several billion US$ per year [2].
Pathogens remains in water body naturally but disease occur when their load is increases. If we manage low pathogen load throughout the culture period, therefore we can prevent disease. Chlorine is commonly practiced in brackish water aquaculture systems for disinfection and oxidation. But indiscriminate use leads to increase in cost, inhibit phytoplankton production. Chlorine is used in aquaculture medium for disinfection and oxidation of organic matter of source water. Disinfection refers to the selective destruction of disease causing organisms. Chlorination will also successfully eliminate toxic plankton (dinoflagellates) and aquatic animal pests and competitors that come in with the water supply.

Poduska and Hershey [3] developed a mathematical model to explain virus inactivation by chlorination, based on the principles of first-order, irreversible chemical reaction kinetics. Chlorination in aquaculture is used to include attempts to destroy disease organisms, control phytoplankton abundance, and improve water quality in ponds stocked with fish or shrimp [4]. Sukumar *et al.* [5] studied the impact of chlorination on water quality of shrimp farms and revealed that even though chlorination helped in preventing horizontal propagation of diseases, it had an adverse effect on the development of algae in shrimp ponds and on the survival of the other biota of the receiving water bodies. Browdy *et al* [6] developed a bio-secure shrimp culture system and reported destruction of at least 99% of the bacteria and yeast present in the filtered seawater by chlorination. Husnah and Chang [7] conducted an experiment to determine the chlorine demand and the efficacy of three chlorine doses to disinfect indigenous bacteria in the sediment of a shrimp (*P. monodon* Fabricius) pond and reported that chlorine at a dose of 1200 and 2400 mg/l inactivated 100% bacteria within 2 days of contact time. With high chlorine dose, chlorination was effective to inactivate bacteria only to a depth of 2.1 cm.

2. METHODOLOGY

2.1 Experimental design

The experiment was conducted in 12 shrimp culture ponds of 1000 m^2 each following the design as given in Table 1.

Table 1: Design of the Experiment

Treatments (T)	Dose of Chlorination	Application frequency	Replications
T_1	No chlorination	Monthly (3 times)	3
T_2	3 ppm		
T_3	6 ppm		
T_4	9 ppm		

2.2 Pond preparation
The ponds were prepared by treating soil with lime (Quick lime:dolomite 3:1) @ 250 kg/ha and then filled with tidal water up to a depth of 1.0 m. After that, all animalcules were killed and removed from the pond. The buffering capacity of water of the ponds was strengthened by applying dolomite @ 15 ppm. The pond water was fertilized with urea, TSP, and MoP @ 2.5 ppm, 3.0 ppm 1.0 ppm, respectively.

2.3 Stocking and management
After production of sufficient plankton, required quantity of PCR tested PL20 was stocked @ 5/m^2 stocking density to all experimental pond on March, 2013. The stocked shrimps were fed with commercial feed (Saudi-Bangla shrimp feed). Feed was applied by spreading and adjusted fortnightly after sampling with cast net. To maintain undisturbed ecology of the ponds, no water was exchanged. Only the evaporated water was replenished with the water of the adjacent canal. Health of the stocked shrimps was checked almost daily through check tray

2.4 Bacterial count
The load of total heterotrophic bacteria (THB) of both water and soil was checked before and after two hours and two following successive days after every application of chlorine. Total heterotrophic bacteria were cultured in nutrient agar media by using pour plate technique method. Viable colony was counted using a colony counter.

For THB count the colony forming unit (cfu)/ml can be calculated using the following formula

cfu/ml = (No. of colonies× Dilution Factor)/ Volume of culture plate

Total coliform of culture water was checked before and after two hours and two following successive days after every application of chlorine.The term "total coliforms" refers to a large group of Gram-negative, rod-shaped bacteria that share several characteristics. They are provisionally identified by the production of acid and gas from the fermentation of lactose. Total heterotrophic bacteria were cultured in MacConkey broth media by using most probable count (MPN) method. The numbar of total coliform was counted by using the chart of MPN index and 95 per cent confidence limits for various combinations of positive results when five tubes are used per dilution (10 ml, 1.0 ml, 0.1 ml portions of sample)

2.5 Data analysis
Statistical analyses were done using Microsoft Office Excel and SPSS (Statistical Package for Social Science) software.

3. RESULTS AND DISCUSSION
Total heterotrophic bacteria and pathogenic bacterial (total coliform) load were tested before and after chlorination. THB count in water and soil is shown in table 2 and their change is shown in table 3.THB load was decreased in water and soil after 2 hours where chlorination was done but the load was slightly increased in control where no chlorination was done(table 3). The load of THB in water after 2 days was increased in T_1,T_2 and T_3 but decreased in T_4.

Table 2: Total heterotrophic bacterial load in different treatments

Dose of chlorination	THB count in water(CFU/ml) x10^4			THB count in soil(CFU/ml) x10^5		
	Before chlorination	After 2 hr.	After 2 days	Before chlorination	After 2 hr.	After 2 days
No chlorination(T_1)	9.5±0.28	10±0.53	11.2±0.54	28±0.65	28±0.44	30±0.72
3 ppm (T_2)	12.3±0.80	10±0.22	23±0.62	23.6±0.48	14.8±0.39	21±0.63
6 ppm (T_3)	42±0.52	9.8±0.30	51.2±0.46	55±0.81	16±0.33	29±0.70
9 ppm (T_4)	43±0.49	11±0.61	22±0.51	26±0.52	19±48	24±0.42

Table 3. Change in THB load of water and soil after chlorination at different doses

Dose of chlorination	THB in water			THB in soil		
	Before chlorination (CFU/ml) x10^4	Change (%)		Before chlorination (CFU/ml) x10^5	Change (%)	
		After 2 hr.	After 2 days		After 2 hr.	After 2 days
No chlorination	9.5±0.28	+5.26	+17.89	28±0.65	00	+7.14
3 ppm	12.3±0.80	-23	+86.99	23±0.48	-37.28	-11.01
6 ppm	42±0.52	-76.67	+21.90	55±0.81	-70.90	-47.27
9 ppm	43±0.49	-74.41	-48.83	26±0.52	26.92	-7.69

Total coliform load in water is shown in table 4 and their change is shown in table 5. Total coliform load was decreased after 2 hours and two days where chlorination was done but the load was slightly increased in control where no chlorination was done(table 3). This is similar to the findings of Sukumar *et al* [5] who found that chlorination helped in preventing horizontal propagation of diseases.

Table 4: Total coliform load in different treatments

Dose of chlorination	Total coliform in water(MPN/100ml)		
	Before chlorination	After 2 hr.	After 2days
No chlorination	240	240	260
3 ppm	300	80	170
6 ppm	110	34	60
9 ppm	300	33	50

Table 5. Change in Total coliform load in different treatments after chlorination at different doses

Dose of chlorination	Total coliform in water(MPN/100ml)		
	Before chlorination	Change (%)	Change (%)
		After 2 hr.	After 2 days
No chlorination	240	00	+8.33
3 ppm	300	-73.33	-43.33
6 ppm	110	-69	-45.45
9 ppm	300	-89	-83.33

The highest percentage in reduction on of total coliform load after 2 hours and two days was observed in T_3 where the highest dose of chlorination was done.

4. CONCLUSION

From the present study it is stated that, chlorination reduces pathogenic bacterial load higher than THB load. Therefore, the use of chlorination in shrimp culture pond can reduce pathogenic bacterial load and can reduce the risk of disaese.

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