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The Effects of Water Quality Additives on Bacterial Load and Production in Thai Climbing Perch (*Anabas testudineus* Bloch, 1792) Culture Pond

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Abstract
This research was carried out to investigate the effects of commercial water quality additives named aqua cleaner and deotop on bacterial load in Thai climbing perch (*Anabas testudineus*) culture pond as well as to observe the effects of the additives on the production of climbing perch. The study was conducted under four treatments that were designated as T_1(5ml/decimal aqua cleaner and 61g/decimal deotop,T_2(10ml/decimal aqua cleaner and 122g/decimal deotop),T_3(20ml/decimal aqua cleaner and 244gm/decimal deotop),T_4(control without any additives) and each of them with three replicates.T_4([(4.99±0.03)×10^4cfu/ml water], [(5.49±0.09)×10^6cfu/g soil]) and T_3([(3.80±0.58) ×10^4cfu/ml water], [(4.99±0.23) ×10^6cfu/g soil]) revealed the highest and lowest average bacterial load in both water and soil of the culture pond respectively whereas the highest production of perch was found in T_2(10.23 kg/decimal) followed by T_1(8.44 kg/decimal), T_3(8.39 kg/decimal), and T_4(7.71 kg/decimal).The result from this study suggests that the additives supplemented at a certain dose could decrease the excess bacterial load at a level results in production enhancement.

Keywords: Water quality additives, Thai climbing perch, Bacterial load, Production.

1. Introduction
Fish is one of the major sources of animal protein through all over the world especially in Bangladesh about 60% of animal protein in the daily dietary requirement comes from different types of fishes [1, 2, 3]. Recommended protein intake of a healthy person is 45 g/capita/day of which 15 g/capita/day animal protein is necessary whereas the present animal protein intake of our people is only 11 g/capita/day [4]. With the rapidly increasing population, it is necessary to enhance the fish production to ensure the minimum animal protein intake. Therefore, it is very important to update the culture techniques as well as to culture fish species that have highly nutritional value. Thai climbing perch contain about 20.22±1.94% protein that is greater than many others fish species [5]. They are known as the name of Koi in Bangladesh, India, Pakistan and also available in Nepal, China, Myanmar, Thailand, Cambodia, Philippines, Indonesia, Singapore, and Sri Lanka. In the recent time they are becoming highly demanded cultivable fish due to their high nutrition, test, faster growth, shorter culture period and having ability to withstand harsh environmental condition [6].

The improvement in fish cultivation is the pre-requisite to increase total fish production because about nearly 53%of the total fish production comes from cultured fisheries and the rest are from captured fishes [7]. Keeping foreword this aim different companies are recently producing different kinds of water quality additives named deotop, aqua cleaner plus normally used to improve water quality in culture pond. So recently fish farmers are using those chemicals in their farm to have a better output. Those chemicals have beneficial effects on water quality in aquaculture ponds include elevated decomposition of organic substances, reduced nitrogen and phosphorus concentrations, higher dissolved oxygen, control of ammonia, nitrite and hydrogen sulfide, and lower disease occurrence [8]. Moreover, those additives have the possibility to affect microbial composition directly by killing or enhancing selection of potential opportunistic fish pathogens and providing conditions for non-opportunistic and harmless bacteria as a result the survival rate, growth rate as well as the production rate are ultimately enhanced [9].

Bacteria have a great influence in aquatic system. They usually contribute to eliminate the toxic materials such as ammonia, nitrite, and hydrogen sulfide, degradation of uneaten feed,

and nutrition of aquatic animals such as shrimp, fish, and production of aqua-farmer. These and other functions make microorganisms including bacteria key players in the health and sustainability of aquaculture. Yet, bacteria are among the least known and understood elements in aquaculture. From another point of view they have a couple of negative impact in aquatic system mainly enhancement of biological oxygen demand (BOD), different harmful diseases, dissolve oxygen reduction in water and so on [10]. Huge researches had been conducted through the world based on Thai climbing perch but researches with the effects of water quality additives on their growth performance as well as effects of those additives on bacterial load of the culture environment are hardly exist. Thus the output of the present research will be able to provide some ideas of the role of those additives on Thai climbing perch production.

2. Materials and Methods

2.1. Study Area and Experimental Design

This study was conducted by collecting data from research pond complex Fisheries and Marine Resource Technology Discipline at Khulna University, Khulna, Bangladesh (Figure 1). The experiment was covered 12 earthen ponds where all ponds were 1 decimal in size, 1.4 m in depth and all of them were rectangular in shape. All ponds were conducted under almost the same environmental factor like equal expose of sunlight as much as possible to avoid the extraneous effect on the experiment. All ponds were divided into 4 treatments and each treatment contained 3 replicates like- Treatment-one or T_1 (ponds one to three); Treatment-two or T_2 (ponds four to six); Treatment-three or T_3 (ponds s to nine); Treatment-four orT_4 (ponds 10 to 12). The treatments consisted of three levels at an initial dose of aqua cleaner & deotop such as half dose (5ml+61gm)/decimal for T_1, recommended dose (10ml+122gm)/decimal for T_2, double dose (20ml+244gm)/decimal for T3 and control without any dose (T_4), and were conducted every after 15 days.

2.2. Pond Preparation, Fry Stocking and Feeding

The research ponds were dried before the stocking of fry. After pond water had been pumped out the ponds were exposed to sunlight for a period of about 20 days. Then liming was done for each pond at the rate of 180g/decimal. After seven days of liming ponds were filled up with water up to 1.4 m. After that, the research ponds were well fenced by nylon net with the support of bamboo sticks. Then the fry of average 4g weight were stocked in the research ponds at a stocking density 500 fry in each pond (1 decimal). At the beginning of stocking, the feeding rate was 10% of their body weight that gradually decreased to 0% where feeding frequency was two times per day.

2.3. Sampling and Data Collection

At an interval of 15 days about 50 fish were randomly caught with the help of fishing net from each pond and their weight was measured with balance. All the data recorded in a note book and spread sheet and finally calculated the average weight of fishes according to the treatment at every sampling day. The following formulas were used to determine the survival rate and production under each treatment.

- Survival rate = Initial number of fish–final number of fish / Initial number of fish × 100
- Production = No. of fish caught × average weight at harvesting

In addition at each sampling period, the water and soil from each pond were also collected and total bacterial load in both of them were counted in Biochemistry and Molecular Genetics Lab, Khulna University, Khulna.

2.3.1. Standard Plate Count (S.P.C) for Bacteria

In case of water sample, at first a definite amount of nutrient agar (11.75gm) was accurately weighted. Then it was taken in volumetric flask (500ml) containing distilled water and autoclaved at a temperature of 121 ^{0}C and pressure of 15 Ibs/sq inch for 20 minutes (ICMSF). Four dilution tubes with dilution strength were marked, 10^{-1} to 10^{-4}. Then with a sterile pipette 1.0 ml from stock solution was mixed with 9 ml of sterile water in the dilution tube marked 10^{-1} for tenfold (1 :10) dilution and by following serial dilutions further diluted up to 10^{-4} times. A new, sterile pipette was used between each dilution and to mix the dilution tubes thoroughly each time. Then 1.0 ml of each dilution was transferred on sterile Petri dish (In duplicate). About 20 ml of autoclaved agar was poured and mixed thoroughly by rotating the Petri dish, first in one direction and then in the opposite direction. The writing on the plate bottoms was faced up and incubated them at 370 ^{0}C for 48 hours. After incubation, the plates or Petri dish were analyzed. Analysis, in this case, means simply counting the entire colony forming units (cfu). The assumption that we have to make for this procedure is that each cfu originated from one individual microorganism. To get reliable results with the spread plate method, only those plates that have between 30 - 300 cfu were counted. As I counted colonies, marking them off with a Sharpie to avoid repeated counting. The number of cfu of bacteria in the sample is calculated with the help of the following formula.

- cfu/ml = counts/ (dilution factor × amount of sample plated]

But in case of soil sample, just after the media preparation a stock solution of soil was prepared by adding 1.0 g of soil with 100ml sterile water in a sterile conical flask. Then the similar procedure was followed as like as water sample.

2.4. Data Handling Technique

Microsoft excel 2010 was used to determine mean, standard deviation as well as to build figure and SPSS 17 was used to determine coefficient of correlation. Coefficient of correlation was done at 5% significance level.

Fig 1: Map of Khulna University and location of the present study site

3. Result and Discussion

Different water quality additives which were used in the present experiment exhibited considerable effects on the production and bacterial load in the cultured pond. T_2 (10.23kg/decimal) revealed the highest production value followed by T_1(8.44 kg/decimal), T_3(8.39 kg/decimal), T_4(7.71 kg/decimal) (Table1-2).There was a poorly positive correlation between additives and the production (r=0.41).The additives play an important role to elevate decomposition of organic substances, reduce nitrogen and phosphorus concentrations, increase dissolved oxygen, control of ammonia, nitrite and hydrogen sulfide, lower disease occurrence and ultimately to enhance production. A couple of research previously conducted also showed that water quality additives improve fish production rate by means of different ways including enhance fish growth, improve feed efficiency through the production of exo-enzymes, modulation of intestinal micro flora, enhance immunity, antagonism to pathogens and improve water quality [11]

Table 1: Fortnightly growth rate of Thai climbing perch (*A. testudineus*) by average weight (g) under different treatments during the study period

Treatment	Average Weight (g/individual)						
	29 July (Weight at Stocking)	14 August	29 August	14 September	29 September	14 October	29 October (Weight at Harvesting)
T_1	4.00±0	9.15±0.25	12.19±0.05	14.02±0.81	16.01±0.13	18.74±0.47	19.4±0.17
T_2	4.00±0	11.09±0.26	14.05±0.54	16.2±0.22	19.52±0.52	21.93±0.51	22.74±0.40
T_3	4.00±0	9.11±0.22	12.26±0.25	14.4±0.28	15.89±0.72	18.37±0.95	19.06±0.54
T_4	4.00±0	8.77±0.48	11.29±0.43	13.01±0.99	15.88±0.33	16.98±0.21	17.92±0.98

Table 2: Growth, survival rate and production of Thai climbing perch (*A. testudineus*) at each treatment through the culture period

Treatment	Cultured Period (days)	Pond Area (decimal)	Stocking Density (number/decimal)	Average weight at stocking (g/individual)	Average weight at harvesting (g/individual)	Survival Rate (%)	Production (kg/decimal)
T_1	90	1	500	4	19.4	87	8.44
T_2	90	1	500	4	22.74	90	10.23
T_3	90	1	500	4	19.06	88	8.39
T_4	90	1	500	4	17.92	86	7.71

In water sample the maximum average cfu of bacteria were found in T4 [(4.99±0.03)×104cfu/ml] where no additives were used whereas minimum average cfu were found in T3 [(3.80±0.58) ×104cfu/ml] where double dose of additives were used (Figure 2). In soil the maximum and minimum average values were found in T4 [(5.49±0.09)×106cfu/g] and T3 [(4.99±0.23) ×106cfu/g] respectively (Figure 3). The values of average bacterial load found in water and soil of fish cultured pond in different previously conducted experiments are almost similar with the findings of the present research [12, 13, 14]. Average bacterial load in both water and soil showed moderately negative correlation with dose of the additives(r= -.87, r= -.60 respectively).

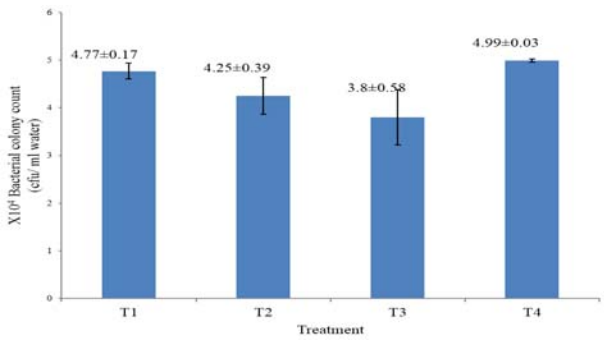

Fig 2: Average bacterial load in water sample (cfu/ml water) in different treatments through the study period

The additives reduce the bacterial load in water and soil because those contain different chemicals namely causticthio-sulphate, secondary alkene sulphonate, EDTA, methailium, zeolite, calcium oxide minerals and others sulfide compounds that acts a germicides [15]. In addition, the present experiment revealed a highly negative correlation between bacterial load and production of fish. This is due to the facts that excess load of bacteria in water and soil increase the biological oxygen demand (BOD) as well as to reduce dissolve oxygen (DO) in the aquatic system that are also hindrance for fish growth. A research conducted by Steinke *et al.* (1990) revealed that excess bacterial load leads to higher decomposition process resulting reduction of DO as it is highly consumed by this process and he also mentioned that the continuous degradation of the organic matter under low dissolved oxygen conditions results in the reduction of inorganic substances and thus often lead to high concentration of nitrite, ammonia, ferrous ion (Fe2+), H2S and methane that are very much toxic for fish. He concluded that this oxygen depletion hamper the production [16]. The findings of another researcher also showed that excess rate of mineralization process are the main reason for DO depletion in aquatic system resulting lower production of fish. In some research experiments it was also found that several fish species reveal a better production in recirculation culture system where it is possible to control the over bacterial load through recirculation system [17].

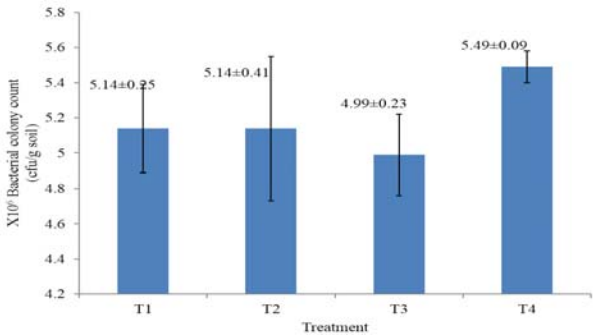

Fig 3: Average bacterial load in soil sample (cfu/g soil) in different treatments through the study period

4. Conclusion

The result obtained from this experiment clearly reveal that the commercial water quality additives named aqua cleaner and deotop have a positive effect on the production of Thai climbing perch. In addition those additives play an important role to reduce the excess microbial load in both the soil and water results in the improvement of water quality of the culture pond that is prerequisite to get a better output in fish culture. It is possible to get about 3kg more Thai claiming perch production per decimal by using those additives at a recommended dose in culture ponds. Thus those additives will be able to contribute in the fish production enhancement.

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