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Effect of Growth Promoter on the Total Bacterial Load in *Anabas testudineus* Culture Ponds

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Abstract: This study was conducted to observe the total bacteria present in water and soil sample of thai koi, Anabas testudineus research ponds located behind the 1st academic building of Khulna University, Khulna, Bangladesh during August, 2012 to November, 2012. The experiment was carried out in 12 earthen ponds. The ponds were divided into four parts, three treatments named T1, T2 and T3 and control (C). Three replicates of each treatment were assigned. Water and soil were collected from the research ponds biweekly. Fish Savors were treated as half (T1), recommended (T2) and double dose (T3) in nine ponds. Bacteria were isolated from the sample by using nutrient agar media. Standard Plate Count (S.P.C) methods were done. In this present study, there was no significant difference of bacterial content among the control pond and the treatment ponds. No correlation could be established among different treatments of fish savor on the bacterial content of pond water and soil.
Key words: Anabas testudineus, bacteria count, fish savor, growth promoter; FCR.

I. INTRODUCTION

Fisheries sector contributes 4.43% to the national GDP and 22.21% to the total agricultural GDP. The country's export earnings from this sector are 2.73% in 2010-11. Fish alone is supplementing about 60% of animal protein in our daily dietary requirement [4]. The present rate of increase of fish production in Bangladesh is lesser than that of population boom. Recommended protein intake of a healthy person is 45 g/capita/day of which 15 g/capita/day animal protein is necessary. But present animal protein intake of our people is only 11 g/capita/day [3]. So it is strongly felt that all sorts of effects need to be employed to increase the fish production in all available inland water bodies to fulfill the protein demand of the people. Among the live fish thai koi *Anabas testudineus,* can play a significant role to meet the protein requirement of the populations. Climbing perch (*Anabas testudineus*) is an important fish species of Bangladesh because of its higher growth performance, easy culture technology, resistance and survivability, consumer preference, nutritional value and market demand [6]. The improvement of cultured fish is the pre-requisite of increasing total fish production because about 47.71% of total fish production comes from cultured fish and keeping foreword this aim, different companies are recently producing different kinds of feed attractants and growth promoters. There is large number of feed additives as growth promoters available to improve fish growth performance. Fish Savor is one of them. Fish Savor contains amino acids, beta-glucan and organic acid, vegetable fat and phospholipid, micronutrients and others. It is used to improve the FCR, to improve growth, to improve the disease resistant power, to remove the waste of feed, to increase the survivability and decrease the mortality etc. These may have any impact on the water quality, microbiology, phytoplankton, and zooplankton and over all the production. However, with the increasing intensification and commercialization of aquaculture production, disease is a major problem in the fish farming industry [2]. Diseases can be occurred by the transmission of bacteria. Bacteria hamper the fish production. Bacterial diseases may be aggravated by the unfavorable conditions like crowding, malnutrition and unstable temperature. The higher concentration or load of bacteria in pond water and soil may increase their load in fish body and this may exceed the acceptable limit of bacteria in fish body that may create diseases and may create various problems during export. Thus, microorganisms have major roles in pond culture, particularly with respect to productivity, nutrient cycling, and the nutrition of the cultured animals, water quality, disease control and environmental impact of the effluent **[7],** So it is important to know the effect of fish savor as a growth promoter and also a feed attractant on the total bacterial load used in *A. testudineus* culture ponds. The objective of this study was to evaluate the effect of growth promoter on the total bacterial load in *A. testudineus* culture ponds.

II. MATERIALS AND METHODS

A. Study Area

The study was conducted by collecting data from research pond complex of Fisheries and Marine Resource Technology at Khulna University, Khulna, Bangladesh. The experiment was carried out in 12 earthen ponds located behind the 1st academic building of Khulna University. All ponds were rectangular in shape with a maximum depth of 1.4 m. All the ponds were fully exposed to prevailing sunlight.

B. Study Period

The study was conducted from August, 2012 to November, 2012 (15 weeks).

C. Experimental Design

Four treatments were compared in the present study: half (T1), recommended (T2) & double (T3) doses of Fish Savor and last one, control (T4/C). Three replicates of each treatment were assigned. Fish Savor were treated as half, recommended and double dose in pond number 10 to 18 and rest 3 ponds (number 20 to 22) were treated as control(table 1). This water treatment additives & feed attractant were selected because of their availability in local market.

TABLE 1
Doses of Fish Savor

	Treatments			
	T1	T2	T3	Control
Doses of Fish Savor	Half (0.1% of Feed)	Recommended (0.2% of Feed)	Double (0.4% of Feed)	Without Fish Savor

D. Pond Preparation

Prior to the study, ponds were drained, re-excavated and left exposed to sunlight for five days. Then, all ponds were treated with agricultural lime ($CaCO_3$) at a rate of 1kg/decimal and filled with water. After three days, the ponds were fertilized with organic and inorganic fertilizer at the same rate.

E. Fry Collection

Thai climbing perch fries were collected from a well-managed nursery named Matsha Kanon at Avoynagar Upazilla of Jessore District.

F. Stocking

The ponds were stocked with fish after five days of fertilization. All fries were released in the ponds in early morning. The initial average weights of the fries were 1.91±0.08g, 1.96±0.14g, 2.05±0.14g, 1.81±0.03g, 2.06±0.31g, 2.1±0.24g and 1.97±0.13g.

G. Feeding Strategy

After acclimatization of the released fish in the pond, the *Anabas testudineus* fry were fed with ACI food. Fry were fed at the rate of 5% of total body weight of stocked fry twice daily at 09:00 am and 17:00 pm. In the early stage, starter feed was given diluting with small amount of water and later on, it was spread directly.

H. Application of growth promoter /feed attractant

Fish Savor was used as growth promoter & feed attractant. It was mixed with the feed and was applied every day.

I. Sample Collection

Water and soil sample were collected from research ponds fortnightly. Water were collected by Cotton plugged conical flax and kept in small plastic tubes. Soil were collected by spatula and kept in polythene bags. Six sampling days were: days-15, days-30, days-45, days-60, days-75 and days-90. Every sampling day 12 Water samples and 12 soil samples were collected. The samples were kept in the refrigerator bellow 5^0C until the start of analysis on the flowing day.

J. Total Bacterial Count

Microbiological analysis was done according to the Bacteriological Analytical Manual of United States Food and Drug Administration (USFDA). Total Plate Count (T.P.C) or Standard Plate Count (S.P.C) was included in that research.

K. Laboratory Work

Sterilization of equipments

Test tubes, Petri dishes, conical flask, plastic tips and other glassware were sterilized by autoclaving at a temperature of 121°C and a pressure of 15 pound per square inch for about 1 hour.

L. Media Preparation

Media is being prepared by compounding require individual ingredients or, more conveniently, by adding water to a dehydrated product which contained all the ingredients. Practically, all media were available commercially in powdered form.

1) Definite amounts of Plate Counting agar (10gm) were being accurately weighted.
2) These would take in separate volumetric flask (500ml) containing distilled water (half of the required volume).
3) Then the final volume (500ml) being adjusted by pouring additional distilled water.
4) Then the volumetric flasks keep in autoclave for sterilization. Autoclave has to be done at a temperature of 121°C for about 1 hour.
5) After autoclave the agar media have to keep in the bio-safety cabinet for 20-30min for cooling and then transferred in required number of petridishes by 25ml volume for preparing plates.

M. Sample Preparation & Dilution (Water Samples)

1ml of sample water was taken especially from the each sample and was transferred to cotton plugged bottle containing 9 ml distilled water. The sample was mixed properly by shaking the bottle carefully. Then the total amount became 10 ml. This made 1st decimal dilution (o.1). In this way four dilution tubes with the dilution strength, 10^{-1}through 10^{-4} were made.

The pour plate method requires use of 1 mL, 0.1 mL, and 0.01 mL or 0.001 mL of sample. The difficulty measuring and working with the two smaller volumes, 0.01 and 0.001 mL, require the use of sample dilutions.

N. Pour Plating

1) 1 ml of the sample of the sample homogenates from the dilution tube from each of 24 samples were transferred by pipette into each of the appropriately marked and sterile duplicate Petri dishes (size 100ml dia)
2) Approximately 20 ml of the plate count agar (kept at 45^0c in a water bath) was poured into each Petri dish within shortage possible time of original dilution.
3) The sample dilutions and agar media were mixed thoroughly and uniformly by rotating the Petri dishes clockwise and anticlockwise.
4) Then the agar media were allowed to solidify.
5) Then the solid agar media were allowed to freeze for 30 min.

O. Incubation

The Petri dishes with solidified agar were incubated at 37^0C for 48 hours in the incubator.

P. Sample Preparation & Dilution (for soil sample)

Weighing out 1.0 gram of sediment or soil and add it to a 99 ml dilution blank.

1ml of sample water was taken especially from the each sample and was transferred to cotton plugged bottle containing 9 ml distilled water. The sample were mixed properly by shaking the bottle carefully .This made 1st decimal dilution (o.1). In this way four dilution tubes with the dilution strength, 10^{-1}through 10^{-4} were made.

Q. Pour Plating & Incubation

The same procedure was followed that were used in pour plating and incubation for water samples.

R. Plate Counting

After incubation, the plates were analyzed. Analysis, in this case, means simply counting the entire colony forming units (CFU). To get reliable results with the spread plate method, only those plates were counted that produced between 30 - 300 CFUs. For ease of counting, the plates were marked into quadrants and count each quadrant separately.

1) Dividing the number of CFUs counted by the dilution factor and adjusts for the amount of sample actually plated. Reporting the number of colonies as CFU/ml. CFU/ml = Number of colonies on plate × reciprocal of dilution of sample.

2) Soil or sediment samples were reported either on a volumetric or a weight basis. After weighing, dry the spare soil sample overnight at 105°C and reweigh.

3) Estimating the number of microorganisms in total sample.

III. RESULTS

The experiment was carried out in 12 earthen ponds. Water and soil samples were collected from the research ponds to the laboratory fortnightly. The results were found from the six sampling day. Every sampling day 12 Water samples and 12 soil samples were collected. Bacteria were isolated from water and soil sample by using Plate Counting agar media. The samples were tested and total bacterial count (Standard Plate Count) found in pond water ranged from 1.75×10^4 CFU/ml to 2.47×10^4 CFU/ml. And the total number of bacteria in pond soil ranged from 1.97×10^6 CFU/g to 2.12×10^6 CFU/g.

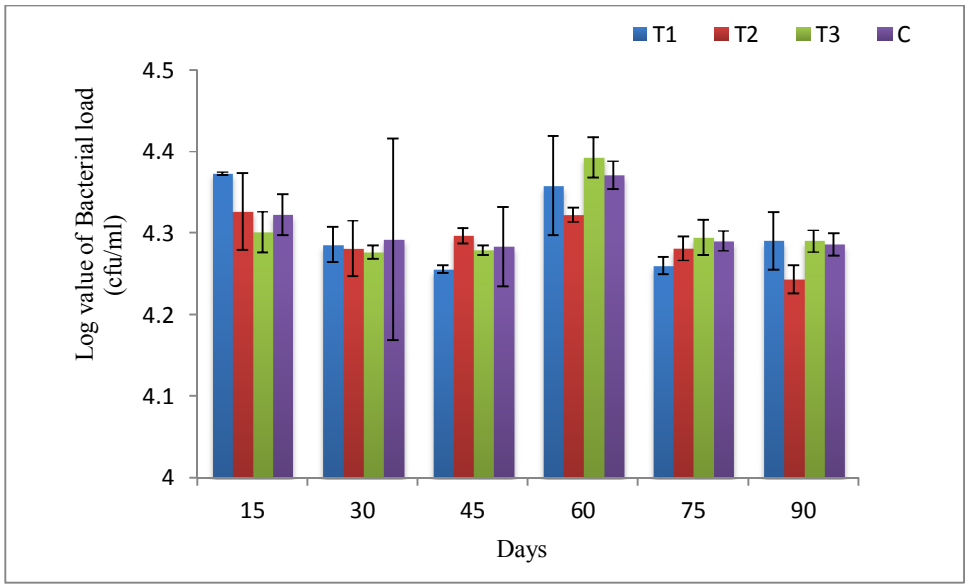

Fig. 1 Total bacterial load (cfu/ml)

The bacterial load was found that was measured in cfu/ml (Fig. 1) for water sample. The bacterial load was found that was measured in cfu/g (Fig. 2) for soil sample.

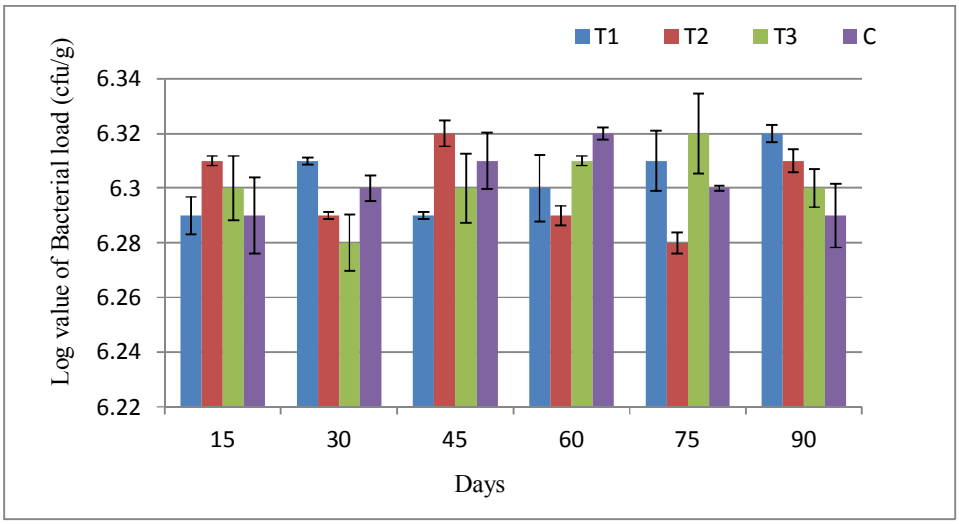

Fig. 2 Total bacterial load (cfu/g)

IV. DISCUSSION

There were differences among the ponds in bacterial content, however several experiments show different changing pattern. The microbiological status of the water in which fish culture takes place depends on a wide variety of factors influencing the environment, the most important being the organic matter content ([10],[11]). In any aquatic system, environmental parameters such as temperature, salinity, pH and dissolved oxygen play a foremost part in the (temperature 27-31ºC; salinity 14 -25 ppt, pH 7.8-8.6) distribution of bacteria [8]. In this present study different treatment showed different result may be because of different environmental parameters. Fish savor contain organic acid. A variety of organic acids applied as a spray or dips for decontamination purpose have been studied extensively and appear to constitute an effective bactericidal or bacteriostatic surface treatment which also effectively prevents the attachment microorganisms ([5],[1],[9]). This present study showed no significance changes in bacterial content among control pond and treatment pond. It may be because of other ingredients like amino acid, micronutrients etc are in Fish savor that are not responsible for reducing bacteria. However, there were some limitations in the study. Sometimes there is no bacterium in the samples means no bacterial colony was found in Petri dishes. But there was possibility to present bacterial colony. Various water depths provide various types of bacteria. There were also some problems and it may be due to sampling error. Although the samples were collected from different layer of the ponds, however it was not done perfectly. And another important thing is that the relation between growth promoter/feed attractant and bacteria in fish pond were not clearly found in other research papers From the experiment it was found that there was no sequential difference among the control pond and the treatment ponds. No correlation could be established among different treatments of fish savor (growth promoter) on the bacterial content of pond water and soil.

V. CONCLUSION

Koi fish (*Anabas testudineus)* is traditionally a popular type of fish in this region. This fish is highly popular for its high nourishing quality and prolonged freshness. Disease caused by bacteria may cause heavy mortality in koi fish. Therefore it is necessary to know the amount of bacteria in the culture pond. Although, all bacteria are not pathogenic but there are many bacteria that may cause disease. In this present study Fish savor was used in culture pond which is a commercial preparation of many ingredients with the principal one being the growth promoter. It was hypothesized that apart from growth Fish savor might affect the bacterial community of the pond water and soil. Analysis of the data, however revealed that no correlation exist among different doses of Fish savor and the bacterial content. It can thus be concluded that Fish savor has no significant effect on the bacterial content of pond water and soil. However further studies that dealing with the qualitative aspect of bacterial flora are necessary to conclusively establish this statement.

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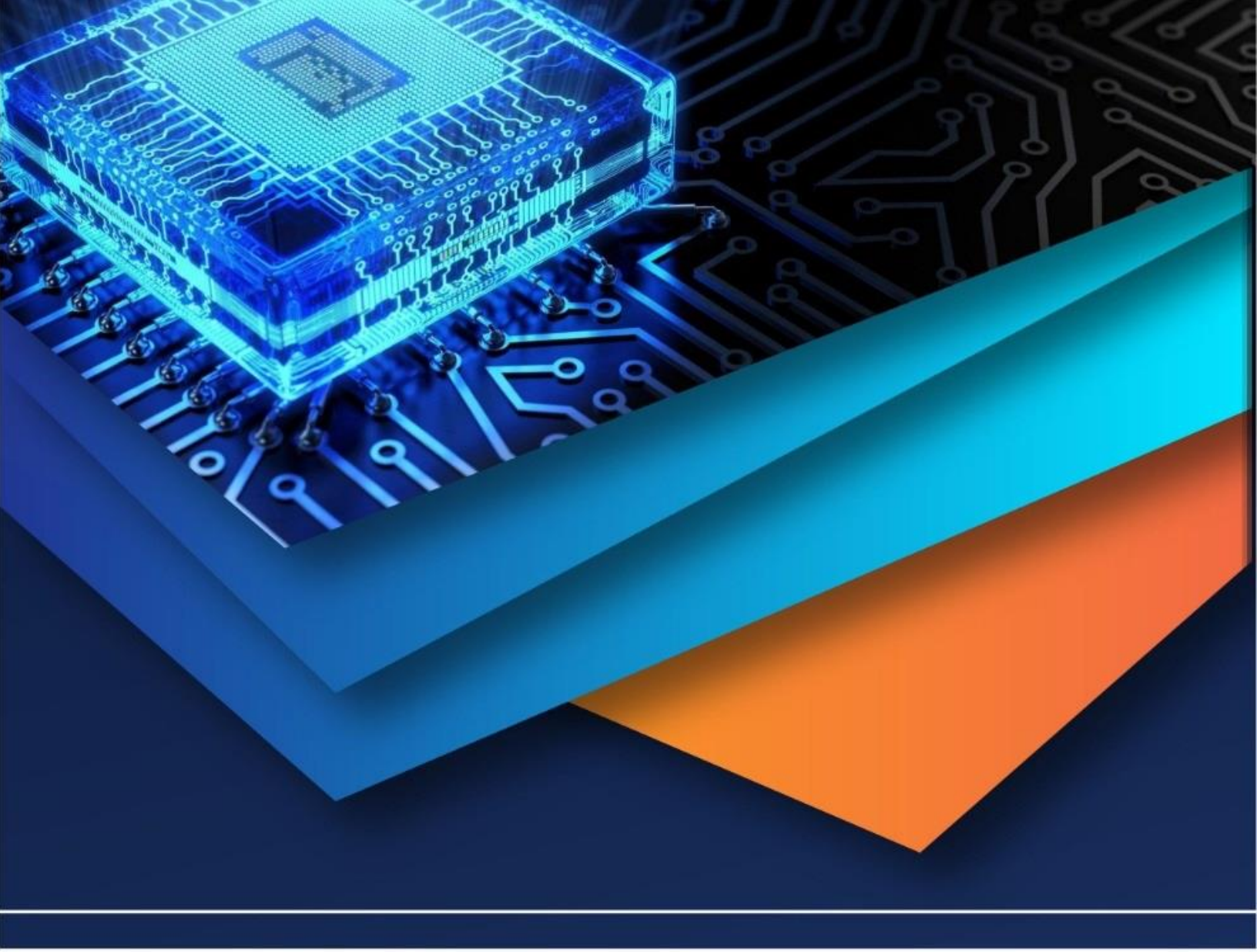

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