

Original Article

Anti-Bacterial Activity of Natural Plants on Fish Pathogens Isolated from Different Ponds of Dhaka City

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In aquaculture, the incidence of bacterial resistance towards synthetic antibiotics has become a serious problem. Therefore, current research has been focused on detecting the presence of pathogenic bacteria in water bodies and finding a novel therapy against pathogens imparted by the natural products replacing the use of synthetic antibiotics. A total of 10 pond water samples were analysed to determine the pathogenic load employing conventional cultural and biochemical tests. Anti-bacterial activities of natural plants on fish pathogens were determined using the agar-well-diffusion method. All the water samples studied high heterotrophic bacteria counts, which were upto 10^9 cfu/ml. Among bacterial pathogens, *Pseudomonas* spp. and *Vibrio* spp. were found to be present in 6 samples and *Aeromonas* spp. in 2 samples. The *in vitro* anti-bacterial activity of the herbal plant extracts against the bacterial pathogens revealed that most of the plant extracts are effective against the pathogenic bacterial isolates.

Keywords: Aquaculture, Fish pathogens, Anti-bacterial activity, Public health

Introduction

Within the field of increasing development of agro-based economy in Bangladesh, fisheries and livestock sectors play a leading role with the annual contribution of 8% turnover in total national economy¹⁻². Around 90% of animal protein comes frequently from the fish and fish products as our daily diet²⁻³. However, contamination of fish by an array of microorganisms is not unlikely⁴⁻⁵. The overall quality of fish has been widely reported to be deteriorated by the existence of *Aeromonas* spp., *Pseudomonas* spp. and *Vibrio* spp., which not only poses health risk to the consumers but also can affect the whole economy of the country as well as the nutrition depletion of the people⁶⁻⁹. Chances of disease in fishes have correlation with their low productivity and quality; moreover, the poor environmental condition may also influence this situation¹⁰. To control such adverse condition, many synthetic drugs have been widely used worldwide; these practices led to development and selection of drug resistant bacteria¹¹⁻¹³. Moreover, antimicrobial agents used for the treatment of fish diseases are likely released in the surrounding water, which are known to cause therapeutic and environmental problems in the fields related to aquaculture¹⁴.

Considering the side-effects of synthetic drugs, a significant number of plants can be considered as the beneficial alternative for treatment of the disease as we as bringing about safety during

application into the aquaculture or environment^{8,12,15-16}. Several studies have been conducted on herbs^{12,17-20}. These reports reveal that the herbs for treatment of infection or infectious agents are the cheapest and nontoxic method to use^{8,21}.

Considering the adverse effect of using synthetic drugs, the applicability of medicinal herbs on the elimination of commonly available fish pathogens in aquatic habitat of Bangladesh has to be thoroughly assessed through checking their antibacterial activity. In Indian subcontinent, approximately 500 medicinal plant species are used for treatment of pathogenic bacteria^{9,11}. Nearly 250 species are known to be used for preparation of traditional medicines in Bangladesh². However, their bactericidal activity has merely been investigated². Based on these facts, present study portrayed total microbiological profile of pond water serving as the fish habitat, and demonstrated the anti-bacterial activity of some commonly available herbs that surround the fish habitat.

Materials and Methods

Sampling and sample processing

For the isolation of fish pathogens, 10 pond water (PW) samples were collected randomly from different locations of Dhaka City within a time frame of August to November 2014. Samples were collected aseptically early in the morning and taken in sterile polyethylene bags with ice and transported immediately to the

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laboratory followed by the standard method²². All the samples were diluted up to 10^{-5} for the isolation and quantification of pathogenic microbes. Conversely, 15 different plants were collected surrounding environment to determine the anti-bacterial activity against fish related pathogens.

Enumeration of total viable bacteria, *Pseudomonas* and *Aeromonas* species

For the estimation of total viable bacteria (TVB), pond water samples were serially diluted up to 10^{-5} . Then 0.1 ml of suspension from each dilution of the samples was spread onto nutrient agar followed by incubation at 37°C for 24 hours. For isolation of *Pseudomonas* spp. and *Aeromonas* spp., 0.1 ml of sample was spread on *Pseudomonas* agar (HiMedia, India) and *Aeromonas* agar (HiMedia, India) plates respectively, followed by incubation at 37°C for 24 hours.

Enumeration of Vibrio species

Enrichment was performed by the addition of 1 ml sample in 9 ml alkaline peptone water (APW) and incubated at 37°C for 6 hours for the isolation of *Vibrio* spp.²³⁻²⁴ afterward the samples were diluted up to 10^4 and 0.1 ml of sample was spread on thiosulphate citrate bile salt sucrose agar (TCBS). All the samples were further incubated at 37°C for 24 hours. Finally, the standard biochemical tests were performed to confirm all the isolates²⁵.

Determination of anti-bacterial activity of plants

The anti-bacterial activity of the herb samples was performed by using agar well diffusion method²⁶⁻³⁰. At first, the suspensions (with standard turbidity compared to that of the McFarland

standard of 0.5) of each of the test bacteria; i.e., *Pseudomonas* spp., *Aeromonas* spp., *Vibrio* spp., were spread evenly over the MHA using cotton swab, which in turn resulted in the uniform lawns. Wells were made in the MHA by means of a cork-borer. Each of the samples was then introduced separately in the specified well along with a positive control (gentamicin, 10 mg) and a negative control (normal saline). Presence of clear zone around the sample solution (if any) was indicative of the presence of anti-bacterial activity of the samples tested.

Results

Isolation of microorganisms from pond water samples studied

All samples were found to exhibit huge load of total viable bacteria up to 10^9 cfu/ml (Table 1). *Pseudomonas* spp. was found to be present in 6 samples within a range of 10^2 to 10^4 cfu/ml and *Aeromonas* spp. were found only in 2 samples. On the other hand *Vibrio* spp. is predominant in most of the samples within a range of 10^2 to 10^4 cfu/ml.

In vitro anti-bacterial activity of the plant samples

The majority of the plant samples were found to exhibit antibacterial activity against 3 bacterial isolates, e.g., *Pseudomonas* spp., *Aeromonas* spp. and *Vibrio* spp. (Table 2). Among the plants only the black-berried feather foil (*Phyllanthus reticulatus*) and benghal day flower (*Commelina benghalensis*) were found to inhibit the growth of all fish pathogens. However, other 10 different types of plants could inhibit the growth of *Pseudomonas* spp. and *Aeromonas* spp. On the other hand, growth of *Vibrio* spp. was inhibited by 7 plant extracts.

Table 1. Prevalence of pathogenic microbes in ponds water samples around Dhaka City

Sample	Count (cfu/ml)			
	TVBC	<i>Pseudomonas</i>	<i>Aeromonas</i>	<i>Vibrio</i>
PW-01	6.4×10^9	4.5×10^3	0	1.7×10^3
PW-02	6.0×10^9	0	0	4.0×10^2
PW-03	2.5×10^7	1.5×10^4	8.0×10^2	7.0×10^2
PW-04	3.3×10^7	1.6×10^4	1.0×10^2	4.0×10^4
PW-05	8.5×10^8	3.2×10^4	0	0
PW-06	2.4×10^7	0	0	0
PW-07	2.8×10^8	3.0×10^2	0	3.0×10^4
PW-08	4.9×10^7	2.3×10^3	0	0
PW-09	2.8×10^7	0	0	0
PW-10	1.2×10^9	0	0	0

TVBC = Total viable bacterial count

Discussion

Poor water quality and the commencement of disease in aquaculture claim one of the major environmental problems those could affect the overall sanitary as well health condition^{9,31-32}. This study was indeed designed a model with subsequent simulations to find out some strategies to combat these problems.

The presence of microbes such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Proteus* spp., *Klebsiella* spp., *Streptococcus* spp., *Staphylococcus* spp. *Vibrio* spp. etc. in water body often leads to the most diseases in fishes^{9,33}. There are many synthetic drugs and chemicals, especially the antibiotics were used to overcome the situation, but use of synthetic medicine has some adverse effect (including reduction in larval growth, sensitization reaction in fish, antibiotic resistance and side effects to the consumers) on total ecology as well as on the human health. On the contrary, medicinal herbs or plants have been used in aquaculture for treatment of fish pathogens since ancient period with least side effects^{9,34}. Moreover, use of natural extracts is more convenient for ecological system, much economical and safe as well^{9,35}. Different study on medicinal plant evidenced the presence of many growth promoting, immune stimulating, antibacterial, antiviral and antifungal compounds, and demonstrated the effectiveness of natural plants in eliminating pathogens with mitigated side effects when applied to the fish^{33,35-37}.

Present study revealed that, water samples collected from different locations were contaminated by huge quantity of viable bacteria as well as pathogens (*Pseudomonas* spp., *Aeromonas* spp. and *Vibrio* spp.), which indicate the possible outcome of diseases in fish. Encountered microorganisms might be the normal flora of fish or might be introduced from the water body of ponds which were contaminated due to intensive aquaculture or poor maintenance of sanitary conditions^{33,35}. Another facet of our study revealed the presence of significant antibacterial activity in all the herbal plant extracts used against fish pathogens. Previous studies worldwide also noticed satisfactory elimination of fish pathogens using herbal extracts^{9,33,38-39}. The susceptibility of fish pathogens to the herbal plant extracts used in this study portrayed their potentials in formulating new and more potent antibacterial drugs of natural origin with minimal adverse effects that can be applied widely in aquaculture. However, further research on plant should be carried out for demonstrating the active compound responsible for antibacterial activity which may facilitate drug design. The effect of these herbal plants on the metabolism of fish should also be checked. Finally, findings of our study suggested that for the elimination of fish pathogens in aquaculture, herbal products could be a suitable, cost effective and safe alternative of synthetic drugs whose use have many side effects and ever increasing risk of antibiotic resistance.

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