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Impact of Vermicompost Manure on Microbial Population in Carp Rearing Pond

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Abstract

Effect of vermicompost, poultry manure, cow dung and pig manure used to monitor on microbial population in carp rearing pond water of Indian carp f*viz. Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. The fingerlings stocked @ 30 fish per pond in the ratio of 3 : 4 : 3. The bacterial strains isolated in ponds water treated with different manures were seven gram negative (*A. hydrophilla, E. coli, E. aerogens, Shigella* sp., *K. oxytoca, P. aeruginosa, P. fluorescens*) *and* three gram positive (*M. luteus, S. aureus and Streptococcus* sp.). The average counts of heterotrophic pathogenic bacteria in poultry manure founded to be maximum in decreasing order followed by pig manure, cow dung, vermicompost, vermicompost and control, respectively. However, bacteria, *E. aerogens, P. fluorescens, P. aeruginosa, Shigella* sp., *K. oxytoca* and *Streptococcus* sp. found absent in vermicompost treatments. All the three species gained maximum growth in vermicompost followed by cow dung > poultry manure and pig manure.

Keyword: Heterotrophic, Manures, Major Carp, Microbial, Pathogenic

1. Introduction

More than half of the world population depends upon fish as a source of animal protein. Fish flesh contains all the essential amino acid and minerals i.e. iodine, phosphorus, potassium, iron, copper and vitamin A and D in desirable concentrations[1]. It serves as valuable ingredient to a healthy diet because of its low carbohydrate and unsaturated fat contents. It is often recommended by doctors to heart patients since it is an excellent source of Omega 3 fatty acid. So the inclusion of fish in our diet can make a valuable contribution to any diet that contains mainly of cereals, starchy roots and sugar for the healthy growth[2]. Freshwater fish in Indian ponds commonly suffer from bacterial diseases such as various kinds of skin ulcerations, albinoderma, erythroderma, furunculosis, and vertical-scale disease, primarily caused by *Aeromonas* sp. and *Pseudomonas* sp. Among the various practices, depending upon the variable inputs, semi-intensive carp culture practices in rural aquaculture involve utilization of various organic manures for plankton production. These manures are either directly utilized by the fish or they enrich the aquatic ecosystem with autotrophic (plankton) and heterotrophic microbial communities. Nearly half of the fish currently consumed as food worldwide are raised in fish pond rather than caught in the wild. In most of the situations, cultured fish remain healthy even in the continuous presence of pathogens. However, when environmental stresses occur and the balance shifts in favor of the disease, the characteristic pathogens flourish. Due to the outbreak of disease in aquaculture industry, use of antibiotic has led to the development of drug-resistant strains resulting in reduction of natural defense mechanism in the aqua cultural animals. To overcome these short comings, we have come to develop a newer technology through the use of vermicompost as pond fertilizer, which not only keeps the watery environment congenial for growth, but also lower the incidence of developing the pathogenic organism. Vermicompost is a product of vermi-biotechnology that is frequently used in agro-ecosystems as organic manure. The advantage of use of vermicompost as organic manure is the quick availability of nutrients in 'ready-to-uptake' forms[3]. So far, the information regarding efficacy of vermicompost as manure in aquaculture pond is scanty. In view of the above points in the present investigation we evaluated the effect of different manures on the bacterial population of treated fish ponds.

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2. Materials and Methods

A series of experiments carried out using earthen ponds with the size of 20 ft × 22 ft × 10 ft. The ponds were cleaned with lime @ 200 kg/ha/yr and filled with inland ground water obtain from deep tube well and allowed to stabilize for about 15 days. Six different treatments with four replications were maintained in ponds. The combination of different fish *C. catla*, *L. rohita* and *C. mrigala* species in 3 : 4 : 3 ratios were selected. To fertilize the ponds, semi dried pig manure @ 4,000 kg/ha/yr (T_2), poultry manure @ 6,000 kg/ha/yr (T_3), cow dung @10,000 kg/ha/yr (T_4), vermicompost @ 10,000 kg/ha/yr (T_5), vermicompost @ 15,000 kg/ha/yr (T_6) and control (T_1) were applied at 25% initial and remaining split doses given at fortnightly in ponds according to the experiment. In the present investigation, effect of the above six treatments (T_1, T_2, T_3, T_4, T_5 and T_6) on the bacterial population in fish pond used for culture of major Indian carps was investigated.

2.1 Estimation of Microbial Population from Manures Treated Fish Ponds

For microbial analysis, water and sediment samples were collected from the treated ponds after 30 days in sterilized glass bottles and processed within 6 hrs of collection. The analysis of bacterial samples was done in the laboratory.

2.2 Culture of Microbes

The water and soil samples enumerated in nutrient agar by serial dilution of the sample followed by the conventional spread plate method. These samples spreaded over the nutrient agar (NA) medium under aseptic conditions. The plates incubated in B.O.D at 30 ± 2°C for 18–24 hours. Growth on nutrient agar plate observed in all treatments after 18–24 hours. Pure colonies of bacteria obtained by further sub-culturing single colonies on nutrient agar plates. The samples kept under laboratory conditions for isolation and the identification of bacterial strains: Isolated pure colonies of microbes were subjected to a number of important biochemical tests (Krieg and Holt (1984)). The identification of microbes done with the help of computer program, PIBWin. The primary tests such as Gram staining, catalase, oxidase and growth in nutrient broth tests for identification of bacteria done as Hans Christian Gram method. Primary identification tests identified the bacteria to a generic level. Further tests such as Malonate utilization, Methyl red and Voges-Proskauer, Indole test, Urea agar base, Urea agar base, Citrate test, Endo agar, LB Agar (Luria bertani agar), Nitrate reduction test, Glucose broth, Sucrose broth, Triple sugar iron medium, Starch hydrolysis, Tributrin and Carbohydrate utilization tests carried out to identify the species of the particular bacteria. The results of various primary and secondary biochemical tests fed in the computer program PIBWin. On the basis of results of various tests, an identification score given to each bacterial isolate by the program.

2.3 Tertiary Test for the Confirmation of Microbe

The confirmation test of these bacteria done with the help of selective media used for culturing that particular bacterium. Specific media provide nutrients that enhance the growth and predominance of a particular bacterial species and prevent the growth of other bacterial species. Growth of bacteria on specific medium taken as a confirmation of the identification of isolated bacterium. Various specific medium used for confirmation of isolated bacteria such as Eosin Methylene Blue (EMB) agar, Antibiotic assay medium C (M 555, Himedia), Rimler-Shott medium base (M576 Himedia), Hugh Leifson glucose medium, Blood, Luria and Pseudomonas agar base for *Micrococcus luteus, Aeromonas hydrophil, Staphylococcus aureus, Enterobacter aerogens, Pseudomonas* sp, *K. oxytoca* and *E. coli* bacterial species (Table 1).

2.4 Determination of Colony Forming Unit (cfu/ml) of Isolated Microbes

Colony Forming Unit (cfu/ml) used to identify the number of viable microorganisms in a fixed amount of liquid. The assumption is that each viable bacterial cell is separate from all others and will develop into a single discrete colony. Thus, the number of colonies should give the number of bacteria that can grow under the incubation conditions employed. The bacterial sample diluted by factors of 10 and plated on agar. After incubation, the number of colonies on a dilution plate showing between 30 and 300 colonies determined. A plate having 30–300 colonies chosen because this range is considered statistically significant. Determination of cfu of bacteria involved the serial dilution method. On the following day number of colonies on each NA plates was counted and colony forming unit (cfu) recorded by using formula:- CFU/ml = No. of colonies × Dilution factor.

3. Results

Ten bacterial strains isolated and identified from different organic manures treated fish ponds. Seven gram negative (*A. hydrophila, E. coli, E. aerogens, P. fluorescens, P. aeruginosa, Shigella* sp., *K. oxytoca*) and three grams positive (*M. luteus, S. aureus, S.* sp.) bacterial strains identified (Table 1). The pathogenic heterotrophic *A. hydrophila, E. coli, M. luteus, S. aureus* bacteria presented in treatment control, pig manure, poultry manure, cow dung, vermicompost @ 10,000 and vermicompost @ 15,000 kg/ha/yr. The bacteria *E. aerogens, P. fluorescens, P. aeruginosa, Shigella* sp., *K. oxytoca* and *Streptococcus* sp. found absent in vermicompost @ 10,000 and vermicompost @ 15,000 kg/ha/yr. Only ten species of bacteria were identified and characterized under the study condition. The following species of bacteria were isolated and identified from water and sediment samples are presented as:-

3.1 Aeromonas hydrophila

Aeromonas hydrophila Gram negative, single short rod shaped aerobic bacteria. This was positive for catalase and oxidase. The results of secondary tests revealed that this bacterium was positive for glucose fermentation, nitrate reduction, glucose acid, maltose, mannitol, glycerol, fructose, galactose, sucrose, lactose, starch, and grew well at 37°C. However, the bacterium found negative for urease, Simmon citrate, inositol, TSI and H_2S production. The bacterium showed good growth in Vogus-Proskauer medium at 37°C. Based on these tests, identification score assigned to this isolate by the PIBWin Programme was 0.99954 (Table 2).

3.2 Escherichia coli

Escherichia coli Gram negative, aerobic, fermentive, rod shaped and observed positive for catalase, glucose fermentation, nitrate, glucose acid, maltose, mannitol, glycerol, inositol and growth at 37°C. The bacterium showed negative results for urease, starch and voges-proskauer test. The identification score assigned to the bacterium was 0.99987 (Table 2).

3.3 Enterobacter aerogens

Enterobacter aerogens Gram negative, aerobic, rod shaped and positive for catalase, glucose fermentation, nitrate, glucose acid, maltose, mannitol, glycerol, inositol, and showed growth at 37°C. Negative results for urease, starch and Voges- proskauer tests observed. Based on above mentioned tests the identification score assigned to this bacterium by PIBW in was 0.95528 (Table 2).

3.4 Shigella sp.

Shigella sp. anaerobic, gram negative, rod shaped and positive for catalase, mannitol, sorbitol, glucose acid, nitrate-nitrite, enrlich indole tests. The bacterium negative for urease, oxidase, Simmon citrate, starch hydrolysis, adonital, xylose, sucrose, malonate, Arginine dihydrolase, maltose, lactose, cellobiose, glycerol, inosital, Vogus Proskauer 37°C (Table 3). The bacterium showed positive growth at 37°C, methyl red at 37°C. Based on the above mentioned tests an identification score of 0.99701 assigned to the bacterium.

3.5 Klebseilla oxytoca

The results of primary tests of this isolate revealed that this bacterium gram negative, aerobic, rod shaped, catalase positive. The results of secondary tests revealed that the bacterium positive for sucrose, mannitol, galactose, inositol, adonitol, maltose, fructose, lactose, sorbitol, indole, starch, D (+) xylose, Erhlich indole, urea and glycerol. However, the bacterium negative for arginine dehydrogenase, Simmon citrate, nitrate reduction and motility test. The bacterium showed good growth in methyl red and did not grow in Vogus-Proskauer broth at 37°C. Based on these tests, identification score assigned to this isolate by the PIBWin Programme was 0.96612 (Table 3).

3.6 Pseudomonas fluorescens

Pseudomonas fluorescens aerobic, non fermentive, Gram negative, rod shaped and positive for catalase, oxidase, Simmon citrate, xylose, glucose, fructose, arginine dihydrolase, 10% glucose, lactose, glycerol. The bacterium showed growth at 37°C *P. fluorescens* negative for urease, nitrate reducation, malonate, maltose, glucose fermentation, starch hydrolysis, Ehrlich indole, adonitol, glucose acid, sucrose, sorbitol, lactose and cellobiose. Based on these tests, identification score assigned to this bacterium by the PIBWin Programme was 0.99565 (Table 2).

3.7 Pseudomonas aeruginosa

Pseudomonas aeruginosa aerobic, non fermentive, gram negative, rod shaped and positive for catalase, oxidase and Simmon citrate tests. The bacterium was negative

Table 1. Heterotrophic pathogenic microbial strains isolated from ponds treated with different manures

Microbe	T_1	T_2	T_3	T_4	T_5	T_6
Gram negative						
Aeromonas hydrophila	+	+	+	+	+	+
Escherichia coli	+	+	+	+	+	+
Enterobacter aerogens	+	+	+	+	–	–
Pseudomonas fluorescens	+	+	+	+	–	–
Pseudomonas aeruginosa	+	+	+	+	–	–
***Shigella* sp.**	+	+	+	+	–	–
Klebsiella oxytoca	+	+	+	+	–	–
Gram positive						
***Streptococcus* sp.**	+	+	+	+	–	–
Micrococcus luteus	+	+	+	+	+	+
Staphylococcus aureus	+	+	+	+	+	+

T_1 = Control; T_2 = Pig manure @ 4,000 kg/ha/yr; T_3 = Poultry manure @ 6,000 kg/ha/yr; T_4 = Cow dung @ 10,000 kg/ha/yr; T_5 = Vermicompost @ 10,000 kg/ha/yr and T_6 = Vermicompost @ 15, 000 kg/ha/yr
+: Presence of bacteria; –: Absence of bacteria

Table 2. Biochemical characterization of Gram negative microbial strains isolated from ponds treated with different manures

Biochemical tests	Microbe Isolate	Microbe Isolate	Microbe Isolate	Microbe Isolate
Gram reaction	–	–	–	–
Shape	Rods	Rods	Rods	**Rods**
Colour of colony	White	White	White	**Cream**
Aerobic	+	+	+	+
Anaerobic	–	+	–	–
Catalase	+	+	+	+
Oxidase	+	–	–	+
Glucose Fermentation	+	+	+	–
Urease	–	–	–	–
Simmon citrate	–	–	+	+
Starch hydrolysis	–	–	–	–
Ehrlich indole	+	+	–	–
Nitrate–Nitrite	+	+	+	–
Adonitol	–	–	+	–
Xylose	–	+	+	+
Glucose acid	+	+	+	–
Sucrose	+	–	+	–
Sorbitol	–	+	+	–
Malonate	–	–	+	–
Arginine dihyrolase	+	–	–	+
Lactose	–	+	+	–
Maltose	+	+	+	–

Biochemical tests	Microbe Isolate	Microbe Isolate	Microbe Isolate	Microbe Isolate
Mannitol	+	+	+	+
Cellobiose	–	–	+	–
Glycerol	+	+	+	+
Inositol	–	–	+	+
Methyl red 37°C	+	+	–	–
Voges Proskauer 37°C	–	–	–	–
Growth at 37°C	+	+	+	+
Bacterial identified	***Aeromonas hydrophila***	***Escherichia coli***	***Enterobacter aerogenes***	***Pseudomonas fluorescens***
ID score	0.99219	0.99987	0.95521	**0.99595**
ID Model score	1.00000	1.00000	1.00000	**1.00000**

+: For good growth; –: for no growth

Table 3. Biochemical characterization of Gram negative microbial strains isolated from ponds treated with different manures

Biochemical tests	Microbe Isolate	Microbe Isolate	Microbe Isolate
Gram reaction	–	–	–
Shape	Rods	Rods	Rod
Colour of colony	Cream	Cream	Orange
Aerobic	+	+	–
Anaerobic	–	–	+
Catalase	+	+	+
Oxidase	–	+	–
Glucose fermentation	–	–	
Urease	+	+	–
Simmon citrate	+	+	–
Starch hydrolysis	+	–	–
Enrlich indole	+	–	+
Nitrate-Nitrite	+	+	+
Adonital	+	–	–
Xylose	+	+	
Glucose acid	+	–	+
Sucrose	+	–	–
Sorbitol	+	–	+
Malonate	+	+	
Arginine dihydrolase	–	+	–
Lactose	+	–	–
Maltose	+	+	–
Mannitol	+	+	+
Cellobiose	+	–	–
Glycerol	+	+	–
Inosital	+	–	–
Methyl red 37°C	+	*	+

Voges Proskauer 37°C	–	*	–
Growth at 37°C	+	+	+
Bacterial identification	***Klebsiella oxytoca***	***Pseudomonas aeruginosa***	***Shigella spp.***
ID score	0.96612	0.99753	0.99701
ID Model score	1.00000	1.00000	1.00105

+: For good growth; –: for no growth; *: Absence of test

Table 4. Biochemical characterization of Gram positive microbial strains isolated from ponds treated with different manures

Biochemical Tests	Microbial Isolate	Microbial Isolate	Microbial Isolate
Gram reaction	+	+	+
Shape	Cocci in clusters	Cocci in clusters	Cocci in chains
Colour of colony	Yellow-orange	White-cream or Yellow-orange	White – cream
Aerobic	+	+	–
Anaerobic	–	+	–
Catalase	+	+	–
Oxidase	+	–	–
Glucose Fermentation	–	+	+
Urease	+	+	–
Simmon citrate	+	–	–
Starch hydrolysis	–	–	+
Ehrlich indole	–	–	–
Nitrate–Nitrite	–	+	–
Adonitol	–	–	+
Xylose	–	–	+
Sucrose	–	+	–
Sorbitol	–	+	+
Arginine dihyrolase	–	+	–
Lactose	–	+	+
Maltose	–	+	+
Mannitol	–	+	+
Cellobiose	–	–	+
Glycerol	–	+	+
Inositol	–	–	–
Tryptophan	–	+	–
Glucose	+	+	+
Fructose	+	+	+
Insuline	–	–	–
Voges Proskauer 37°C	+	+	+
Galactose	–	+	+
Bacterial identified	***Micrococcus luteus***	***Staphylococcus aureus***	***Streptococcus* sp.**
ID score	0.99620	0.90954	0.99964

+: For good growth; –: for no growth

Table 5. Growth of isolated microbial strains on specific medium

Selective Media	Confirmation of bacteria
Rimler-Shott medium base	*Aeromonas hydrophila*
Luria agar base	*Escherichia coli*
EMB medium	*Enterobacter aerogens*
Blood agar	*Klebsiella oxytoca*
Pseudomonas agar F Base	*Pseudomonas fluorescence*
Antibiotic Assay Medium No. 38	*Pseudomonas aeruginosa*
Hugh Lesion glucose medium	*Micrococcus luteus*
Antibiotic Assay Medium. C	*Staphylococcus aureus*
Blood agar	*Streptococcus* sp.

for urease, indole, adonitol, glucose acid, sucrose, sorbitol, lactose and cellobiose (Table 3). The bacterium showed positive growth at 37°C. Based on the above-mentioned tests an identification score of 0.99573 was assigned to the bacterium. Growth on Antibiotic assay medium C confirmed the identification of bacterium.

3.8 Micrococcus luteus

Micrococcus luteus bacterium Gram positive, arobic and positive for catalase, oxidase, glucose, fructose, urease, Simmon citrate and Vogus-Proskauer. However, the bacterium was negative for maltose, mannitol, adonitol, xylose, lactose, nitrate. insuline and galactose. The bacterium showed good growth in Vogus-Proskauer medium but did not grow in methyl red at 37°C. Based on these tests, identification score assigned to this isolate by the PIBWin Programme was 0.99620 (Table 4).

3.9 Staphylococcus aureus

Staphylococcus aureus bacterium Gram positive, arobic and positive for lactose, maltose, mannitole, sucrose, sorbitol, glucose, fructose, Vogus-Proskauer, glycerol, galactose and arginine dihydrolase. However, the bacterium was negative for starch, Ehrlich Indole, Simmon citrate and inositol. The bacterium showed good growth in Vogus-Proskauer medium. Based on these tests, identification score assigned to this isolate by the PIBWin Programme was 0.90954 (Table 4).

3.10 Streptococcus sp.

Streptococcus sp. Gram positive, anaerobic, fermantive, positive for starch, adonitol, xylose, sorbitol, lactose, maltose, mannitol, cellobiose, glycerol, glucose, fructose and Voges-proskauer and glactose tests. This bacterium showed negative result for catalase, oxidase, urease, Simmon citrate, Enrlich indole, nitrate, sucrose, arginine dihydrolase, inositol, and tryptophan and inuline tests. Identification score assigned to this bacterium was 0.99964 (Table 4). Growth on Blood agar confirmed the identification of bacterium.

The pathogenic heterotrophic *A. hydrophila, E. coli, M. luteus, S. aureus* bacteria presented in treatment control, pig manure @ 4,000, poultry manure @ 6,000, cow dung @ 10,000, vermicompost @ 10,000 and vermicompost @ 15,000 kg/ha/yr. But pathogenic heterotrophic *E. aerogens, P. fluorescens, P. aeruginosa, Shigella* sp., *K. oxytoca, Streptococcus* sp. bacteria absent in vermicompost @ 10,000 and vermicompost @ 15,000 kg/ha/yr. Results of enumeration of heterotrophic bacterial populations showed a highly variable result among the six treatments. The average counts of heterotrophic bacteria in vermicompost @ 15,000 kg/ha/yr 1.94×10^7 cfus/ml, vermicompost @ 10,000 kg/ha/yr 1.89×10^7 cfus/ml, cow dung 3.2×10^7 cfus/ml, poultry manure 4.0×10^8 cfus/ml and pig manure 3.6×10^8 cfus/ml was higher than the control 1.87×10^8 cfus/ml in (Table 6). A marked difference in the mean counts of gram negative, *A. hydrophila, E. coli, E. aerogens, P. fluorescens, P. aeruginosa, Shigella* sp., *K. oxytoca* and gram negative, *M. luteus, S. aureus, Streptococcus* sp. bacteria was also observed among the treatments (Table 6). Highest counts for both grams positive and gram negative bacteria were observed in the poultry manure followed in decreasing order pig manure, cowdung, vermicompost, vermicompost and control treatments. Bacteria, *E. aerogens, P. fluorescens, P. aeruginosa, Shigella* sp., *K. oxytoca* and *Streptococcus* sp. were absent in T_5 and T_6 treatments from the water of ponds.

Table 6. Viable counts of isolated heterotrophic pathogenic microbial strains from ponds treated with different manures

Inoculated bacteria	Viable counts (cfu/ml) in treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
Gram negative						
Aeromonas hydrophila	1.8×10^6	2.30×10^6	3.01×10^7	1.80×10^{11}	1.45×10^8	1.50×10^9
Escherichia coli	1.31×10^7	2.10×10^8	2.30×10^8	1.5×10^7	1.31×10^7	1.44×10^7
Enterobacter aerogens	1.73×10^7	2.7×10^7	2.80×10^7	1.73×10^7	–	–
***Shigella* sp.**	1.85×10^7	1.9×10^7	2.2×10^7	2.21×10^4	–	–
Klebsiella oxytoca	1.87×10^8	2.09×10^8	2.00×10^8	1.87×10^8	–	–
Pseudomonas fluorescens	1.66×10^7	2.10×10^9	2.70×10^{10}	1.60×10^8	–	–
Pseudomonas aeruginosa	1.71×10^8	2.25×10^9	2.80×10^9	1.71×10^8	–	–
Gram positive						
Micrococcus luteus	1.51×10^7	2.7×10^7	3.3×10^7	1.75×10^7	1.51×10^7	1.90×10^7
Staphylococcus aureus	1.89×10^7	2.9×10^7	3.6×10^7	2.00×10^7	1.89×10^7	1.94×10^7
***Streptococcus* sp.**	1.7×10^6	3.6×10^8	4.0×10^8	3.2×10^7	–	–

T_1= Control; T_2 = Pig manure @ 4,000 kg/ha/yr; T_3 = Poultry manure @ 6,000 kg/ha/yr; T_4 = Cow dung @ 10,000 kg/ha/yr; T_5 = Vermicompost @ 10,000 kg/ha/yr and T_6 = Vermicompost @ 15, 000 kg/ha/yr.

4. Discussion

In the present investigation seven-gram negative and three gram positive heterotrophic pathogenic bacterial strains isolated from different manure treated fish ponds. The number of gram negative bacterial isolates correlated with studies of various scientists reported that manures treated water samples micro flora of aquatic animals consists mainly of gram-negative aerobic, obligate anaerobic and facultative anaerobic bacteria, the composition of which may change with environmental stresses[4], Diet[5], and fish age[6]. The isolated bacterial strains belonged to *Aeromonas* sp., *Pseudomonas* sp., *Shigella* sp. and members of the family Entero bacteriaceae. These bacteria dominate microflora of freshwater species[7]. Ravikumar *et al.* (2010) isolated ten bacteria from diseased ornamental fish, five gram negative bacteria, *Escherichia coli., Klebsiella pneumoniae* ,*Shigella flexneri, Proteus myxofaciens* and *Vibrio* sp., five gram positive bacteria, *Streptococcus pyogens., Staphylococcus aureus, Staphylococcus epidermis, Bacillus cereus* and *Clostridium* sp. Palavesam *et al.*[8] identified eight bacterial genera in the tissue sample and they were: *Vibrio* sp., *Corynebacterium sp*., *Bacillus* sp., *Flexibacter* sp., *Aeromonas* sp., *Pseudomonas* sp., *Streptococcus* sp. and *Serratia* sp. from *Epinephelus tauvina.* The abundance of heterotrophic pathogenic bacteria in the pond sediments did not differ from one system to another in the present study. This implies that the sediment in all fish ponds in the experiment, regardless of the farming system, contained the optimal amount of essential nutrients necessary for rapid growth of heterotrophic pathogenic bacteria. Jana and De[9] obtained similar results in the sediment of traditional and manure treated ponds. According to Jinyi *et al.*[10] because of the sedimentation of applied manure and pond mud in both manure-applied and controlled ponds, the amount of bacteria in the water column decreases between the bottom of the pond and the surface layer of water with the continuous release of microorganism from the sediments. Similar results were obtained in our study. Greater abundance of seven gram negative (*A. hydrophila, E. coli, E. aerogens, P. fluorescens, P. aeruginosa, Shigella* sp., *K. oxytoca*) and three gram positive (*M. luteus, S. aureus, Streptococcus* sp.) heterotrophic pathogenic bacterial strains were identified in the water and sediments of vermicompost @ 15,000 kg/ha/yr, vermicompost @ 10,000 kg/ha/yr, cow dung @ 10,000 kg/ha/yr, poultry manure @ 6,000 kg/ha/yr and pig manure @ 4,000 kg/ha/yr, compared to the control treatment, indicate their sewage character. Very high counts of heterotrophic pathogenic bacteria in ponds manured with animal excreta have been reported by many authors Cloete *et al.*[11]; Jinyi *et al.*[12];

Jinyi *et al.*[10]; Hamza *et al.*[13]. The control treatment, however, significantly reduced the population of total heterotrophic pathogenic bacteria in both water and sediment, compared to the manure treatments. The water quality was also influenced by the management conditions. Similar results observed by Blackburn and Henriksen[14]; Jana and Barat[15]; Mei *et al.*[16]; Yao and Zhaoyang[17]. In an earlier experiment, a direct significant ($P \leq 0.05$) observed between the weight gain of Indian major carps and the amount of zooplankton present in tanks under different doses of organic manuring, Jha *et al.*[18]. These results were corroborating with the finding of Jana and Chakrabarti[19], Ludwig[20]. All aquaculture production systems must provide a suitable environment to promote the growth of aquatic crops. Although application of organic manure does not directly cause bacterial diseases in fish, the significantly greater abundance of heterotrophic pathogenic bacteria in the water and sediments of the manured treatments vermicompost @ 15,000 kg/ha/yr, vermicompost @ 10,000 kg/ha/yr, cow dung @ 10,000 kg/ha/yr, poultry manure @ 6,000 kg/ha/yr and pig manure @ 4,000 kg/ha/yr could lead to diseases. Should fish resistance to disease be low, the possibility of occurrence of bacterial disease is higher in these treatments. Therefore, proper pond management should be observed to prevent any chance of bacterial disease. Though it has been established that high fish yield in culture systems can be achieved by higher abundance of plankton through organic manuring, practical alternatives to pond manuring are needed because manuring may reduce water quality. Intensive stocking of Indian major carp ponds in India requires a standard water quality to be maintained throughout, so that fish growth is not adversely affected. In view of the financial constraints of marginal farmers who cannot afford modern aeration or waste-treatment equipments, raising of Indian major carp larvae in ponds fed exogenously with zooplankton is of considerable significance because not only would such feeding support high rates of survival and production, it would also maintain greater abundance of zooplankton in the system and better water quality with lower concentrations of heterotrophic pathogenic bacteria in the system.

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