BANGLADESH FISHERIES RESEARCH INSTITUTE
FINAL REPORT
ON

DETERMINATION ON EFFICACY OF SELECTIVE CHEMICALS AND DRUGS USED IN AQUACULTURE IN BANGLADESH (2010-2013)

BY

Dr. Momtaz Begum
Sr. Scientific Officer

FRESH WATER STATION ,MYMENSINGH 2201

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EXECUTIVE SUMMARY

Three different experiments were conducted in the Bangladesh Fisheries Research Institute at different durations in between September 2010 to April 2013. The main objective was to determine the effectiveness of commonly used main three drugs/chemicals. These drugs were (i) water treatment chemicals, *viz* Timsen ,Geofersh, Geoprime, Geotox (ii) Oxygen Releaser drug , *viz,*. Oxygold, Oxymax, Oxyflow, Oxymax,Oxymore and (iii) Antibiotics, *viz,*. Aquamycine, Oxysentine and Renamycine These three chemicals were tested with the same objective , to determine the effective dose, pond productivity, fish production ,histopathplogical change and create awareness among the fish farmer & enterpreneurs. From the findings it could be suggested that 1.5 times higher dose than recommended dose of each drug ,water treatment compound (Timsen, Geo prime, Zeofresh, Geotox) , (Oxygen releaser drug), Antibiotics (Aquamycine, Oxysentine and Renamycine) have been optimized for increasing growth, disease resistance and biochemical activities that should be required for healthy environment. The overall results indicate that Histopathological study did not show any negative changes on the organ of fishes.

The First research activities were conducted to determination the efficacy of common selected water treatment drugs for three months. Experiment was designed into four treatment **(**T_1 - Timsen, T_2 -Geofersh, T_3 -Geoprime, and T_4 –Geotox, Recommended dose of the company was used for treatment, with three replications. After 5 days of liming each of the following drugs Timsen, Geofresh, Geoprime, Geotox was used for each pond and fingerlings of Monosex male tilapia, (ABW: 6.25 ± 0.27 gm), were stocked in all the ponds with same stocking density of 100 fish/pond. Feeding fingerling was maintained twice daily in the morning & evening with commercial Saudi-Bangla feed at the rate of 10% of the body weight in the first week. For second week daily ration was adjusted at the rate of 5% of the body weight. Data on water quality parameters, survivability, and growth were recorded before and after using of drugs. Essential water quality parameters were recorded weekly and growth, survivability were recorded after ten days interval. Histopathological studies were done one month's interval. It was observed that after using drugs in all treatments the value in case of Timsen pH (8.12±0.58), alkalinity (119.40±9.67), nitrate (0.28±0.04) and phosphate (1.00±0.19), dissolved oxygen (4.56 ± 0.29), were maintained. Ammonia became reduced due to use of drugs. During observation of physical water quality parameters transparency (31.27±3.06) became increased and temperature (28.25±3.38) became lower than control water body. Net production of Gift Tilapia (1955.6 4± 24.28 kg/ha) also provided higher in Timsen treated pond than other treated ponds.

Histopathological study did not show any negative changes on the organ of fishes. Among all the drug, Timsen was the best in all aspects (improve tilapia fish health and water quality) .Other three drugs (Geotox, Geo-fresh, and Geo-prime) resulted more or less same result.

The second experiments were conducted, in two ways one in laboratory condition and another one in experimental ponds of the Freshwater Station .First one were conducted in 24 plastic buckets in the laboratory condition and, the buckets were filled up with 30L of tap water. Five selective drugs (oxygen releaser) were tested and each had three replications. Before using drugs, initial concentration of DO was recorded, after that oxygold, oxymore, oxymax, oxyflow and oxylife were used in different doses. Data were recorded at 30min., 60 min., 120 min., 180 min., and 240 min .interval for each drug .It was observed that concentration of dissolved oxygen was initially increased and later gradually decreased in successive hours. In second phase, an experiment was conducted in the earthen ponds of Bangladesh Fisheries Research Institute (BFRI), Mymensingh from the period of August - October 2011. Twelve ponds having an area of 40.00 m^2 each were prepared through liming @ 250 kg/ha and mustard oil cake @ 500 kg/ha, then ponds were filled with underground water up to a depth of 1 m and inorganic fertilizers of TSP and urea were applied @ 35 kg/h with 3:1 ratio. After 5 days of fertilization, Tilapia, *O. niloticus* fry (ABW: 3.33 gm, ABL: 5.53±0.83 cm), were stocked in the ponds with same stocking patterns 100 fish/pond with three different doses of drugs (T-1 Recommended dose, T-2 1.5 times lower than recommended dose, T-3 1.5 times higher than recommended dose). It was found that in all the cases oxygen concentration was increased significantly. Among all the treatments, T-3 (1.5 times higher than recommended dose) was the best in all aspects. Histopathological studies did not show any remarkable changes of the selective organ of fishes.

In third research, the feeding trail with antibiotic mixing feed was carried out a static indoor rearing system of Bangladesh Fisheries Research Institute (BFRI), Mymensingh consisting of a series of rectangular cistern (2500L each) for 8 weeks. The same aged uniform size of fish fry were randomly distributed into groups of 100fish (averaging 2.18±0.83 gm in weight) per cistern .Three selective antibiotics were tested and each had three replications. The fish were individually weighed at the starting of the experiment and also by weekly fish weight (fish sampling) was done to adjust the daily feed ration for the following week. The same experiment was repeated in the ponds for more perfection. Before using antibiotics all water quality parameters were recorded and for histopathological study, 10 fish were randomly sacrificed for analysis.

The finding of the above two trails it was observed that after using antibiotics in all treatments the value of pH (8.12±0.58), alkalinity (119.40±9.67), nitrate (0.28±0.04) and phosphate (1.00±0.19), dissolved oxygen (4.56 ± 0.29), were suitable range and good for fish health and fish culture. Ammonia became reduced due to use of drugs. Fish production also provided higher in Renamycin treated pond than other treated ponds. Histopathological study did not show any negative changes on the organ of fishes. Among all the antibiotics, Renamycin was the best in all aspects (improve fish health and water quality) .Other two drugs (Oxycentine and Aquamycine) resulted more or less same result.

However more elaborate studies are necessary to observe the effects of drugs and others with more bio-chemical parameters before making comments.

CHAPTER 1
GENERAL INTRODUCTION

Background & Justification

Fish culture in Bangladesh is improving gradually towards commercial practice where stocking densities is increasing and commercial feeds are being used. Management of water quality and maintenance of culture environment is becoming difficult, and thus the cultured species are becoming more susceptible to diseases. Farmers are now interested to use chemicals in feed, to maintain water quality, combat disease and increase production.

With the intensification of aquaculture, different farms are trying to introduce chemotherapeutics, feed additives and growth enhancers etc. in order to increase production and combat disease outbreaks. Different pesticide companies are advocating for their chemicals and biological products as remedy to diseases and as growth enhancer. Unfortunately, at present there is no complete information on the use of chemicals in aquaculture practices in Bangladesh (Faruk *et al.* 2005).

In aquaculture, chemicals are used mainly in the treatment and prophylaxis of disease problems, which constitute the largest single cause of economic losses. On the other hand, in recent time various chemicals/biological products are used in aquaculture as feed additives and water treatment compounds for high fish production. The chemicals are different forms of limes, fertilizers, and various commercial forms of growth and water productivity enhancer products However, the increasing use of chemicals in aquaculture has lead to widespread public concern . The present study have been undertaken to know the efficacy of drugs on aquaculture in Bangladesh.

CHAPTER 11

REVIEW OF LITERATURE

A number of diversified studies had been carried out about the use of aqua-drugs and chemicals on aquatic animal health management. These papers contain a lot of information about aqua-drugs and chemicals. The following information, relevant to the present study was briefly reviewed.

Fish and fisheries play a significant role in the economy of Bangladesh in terms of animal protein supply, foreign currency earning, employment and poverty alleviation. This sector contributes 4.43% to gross domestic product (GDP), 2.70% of export earning and 58% of the total protein supply in the diet of the people of Bangladesh (DoF, 2011).

Several of these aspects have been well documented (Anderson and Levin, 1999; Tendencia and De la Pena. 2001).
Different Pharmaceuticals companies are advocating for their chemicals and biological products as remedy to diseases, water quality treatment and as growth enhancer. Unfortunately, at present there is no complete information on the use of chemicals in aquaculture practices in Bangladesh (Faruk *et al.,* 2005).

The most prevalent diseases were tail and fin rot, epizootic ulcerative syndrome, nutritional disease, red spot and gill rot. Forty six percent farmers used combination of lime and potassium permanganate, 22.4% farmer used only lime and 10% farmer used lime and salt together in response to particular disease problem was mentioned by Faruk *et al*. (2004).

Alam (2011) studied the efficacy and performances of three most commonly used Eon Animal Health Products. Three selected aqua drugs were JV Zeolite, Oxymax and Bioaqua-50. Ammonia (mg/l), nitrite (mg/l), dissolved oxygen (mg/l), pH, hardness (mg/l) and alkalinity (mg/l) were measured by using testing kits. Improvement of the parameters was recorded after each hour for three hours.

A range of chemicals including antibiotics were used in aquaculture for fish health management and disease treatment. JVzeolite, Geotox, Green zeolite, Orgavit aqua, Fish vitaplus, AQ grow-G, Oxy flow, Oxy max and O_2-marine were the most widely used aqua-drugs and chemicals in Bangladesh. Major active ingredients of these antibiotics were oxytetracycline, sulphadiazine,

chlorotetracycline, sulphamethoxazole, amoxicillin and co-trimoxazole was stated by Faruk *et al*. (2008).

Swann (2009) described the suitable ranges of water quality parameters may be kept by using water quality treatment drugs for aquaculture water temperature suitable for warm water species would be 24 to 32°C, dissolved oxygen content of water would be 5 mg/l, pH would be 6.5 to 9.0, alkalinity would be at least 20 mg/l for recirculation system, nitrite-nitrogen would be 0.03 to 0.06 mg/l and nitrate-nitrogen would be 0.0 to 3.0 mg/l. It was found that ammonia, nitrite, alkalinity, dissolved oxygen, hardness and pH ranged from 0.7 to 4.0 mg/l, 0 to 0.2 mg/l, 115 to 180 mg/l, 3.0 to 4.0, 100 mg/l and 7.3 to 8.2 during the study period after used of drug. The studies suggested that the water quality parameters varied with different dose and different time intervals.

Davis *et al.* (2009) described that feeds used for fish growth had some negative impact on water quality because feeds were also source of pollutant, which ultimately caused water quality deterioration and disease outbreak.

Quick lime and slaked lime both had a very high pH and in addition to increased alkalinity, could have a sterilizing effect against disease. A range of chemotherapeutics were used to control fish disease was reviewed by Chinabut and Lilley (1992).
Islam (2009) studied survival rates (%) were 70.4, 80.75, and 72.25% for native koi, Thai koi and other two hybrids respectively. He found that the hybrids resembled the native koi in skin color with high growth performances like Thai koi.

Ahmed and Rab (1995) suggested that addition of lime to ponds during the culture period decreased the severity of EUS outbreaks.

Tamuli and Shanbhogue (1996) reviewed the efficacy of some commonly available chemicals in the treatment of anchor worm (*Lernaea maelraensis*) infection in India. The authors used potassium permanganate, formalin and sodium chloride bathing treatment twice a day over five consecutive days at 30 ppm $KMn0_4$ for 20 minutes was found to be 100% effective in killing adult and embedded larval parasites.

Liu *et al.* (1996) studied on the efficacies of formalin, potassium permanganate, sodium chloride and copper sulphate as prophylactic treatments for saprolegniosis. Formalin (25 mg/L) was

effective as both for a prophylactic and post infective treatments. Sodium chloride at 5000 mg/L was effective in preventing saprolegniosis.

Singh and Singh (1997) obtained seven isolates of *Edwardsiella tarda* and showed that all the isolates were resistant to colistin and gentamicin but sensitive to ciprofloxacin. chloramphenicol, nalidixic acid, nitrofurantioin, ofloxacin and streptomycin.

GESAMP (1997) found that for soil and water treatments, alum (aluminium sulfate) at the rate of 10-20 mg/L, gypsum at concentrations of 250-1000 mg/L, lime at dose of 100-8000 Kg/ha, geolite at a dose of 100-500 Kg/ha. Antibacterial agent amoxicillin, nitrofuran, macrolides active against gram-positive bacteria. Used of sulphonamides to control diseases such as furunculosis, enteric red mouth disease and vibriosis.

Rahman and Chowdhury (1999) conducted trials of chemotherapy to control the ulcer disease affecting catfish. The best result was obtained by a successive bath in 1-2% NaCl suspension and subsequent oral treatment with commercial oxytetracycline at a dose of 75 mg/Kg body weight of fish for 5 days.

Brown and Brooks (2002) reviewed that 52% farmers in Bangladesh used potassium per-manganate, while 40% used lime, 11% used salt as a disease treatment. A few farmers used other treatments such as disinfectants, banana leaves, fertilizer, and alum and water exchange.
Liu *et al*. (2004) found that norfioxacin, gentamicin, tobramycin, ciproifloxacin, tetracycline, tetacfylin, polyrnyxin etc., have a significant bacteriostatic effect on *Pseudomonas* sp.

Tafalla (1999) suggested the use of oxytetracycline (OTC) was one of the most frequently used antibiotics in aquaculture, although negative side-effects were reported in some cases. Although cell viability did not decrease after in vitro exposure, head kidney macrophage respiratory burst and phagocytosis were inhibited by the in vitro treatment, and were dose-dependent.
Khan *et al.* (2011) mentioned that traditional chemicals in fish health management included lime, salt, potassium permanganate, sumithion, melathion, formalin and bleaching powder in

Mymensingh region. The authors also concluded that twenty eight pharmaceutical companies were producing and marketing aqua-drugs and chemicals in Mymensingh region. These include Polgurd plus, Deletix, Timsen, Vectisol, Virex, Renamycin, Aquamycine and Oxy-Dox-F.

Hossain *et al*. (2009) reported that severe necrosis of hepatocytes, pyknosis, vacuoles, fat droplets and hemorrhage were observed in small indigenous species during December and January. Ahmed *et al*. (2009) In this study gill had no remarkable change due to treated water quality treatment drugs.

Liver had highly necrotic hepatocytes, pyknotic and inflammatory cell during the months of December and January (Roy *et al*. 2006). Samsuzzaman.*et.*a*l* 2011 disagreed to the author study, he reported histopathologically in the control treatments at both the BAU ponds and farmer's level ponds, skin, muscle, liver, kidney and gill of fish had almost normal structure the present experiment did not show any remarkable change to the different organ of fish, However, in the chemical treated ones, the above mentioned investigated organs of fishes had remarkable pathological changes like necrosis, hemorrhage, vacuum, pyknosis, necrosis, hypertrophy and partial loss of some parts. It was observed that loss of epidermis, necrosis, vacuum, haemorrhage and pyknosis were found in the skin and muscle layer of aqua-drugs and chemical treated fishes which may be occurred other chemicals and drugs except water quality treatment drugs. Islam (2009) studied survival rates (%) were 70.4, 80.75, and 72.25% for native koi, Thai koi and other two hybrids respectively.

There are problems associated with the use of chemicals. With the expansion of aquaculture in Bangladesh, there has been increasing trend in using chemicals in aquatic animal health management. Commonly used chemicals in Bangladesh aquaculture are lime, rotenone, various forms of inorganic and organic fertilizers, phostoxin, salt, dipterex, antimicrobials, potassium permanganate, copper sulphate, formalin, sumithion, melathion etc. (Phillips, 1996; Hasan and Ahmed, 2002; Brown and Brooks, 2002; DoF, 2002 and Faruk *et al.*, 2005).
Indiscriminate use of aqua-drugs and chemicals often lead to problems like drug resistance, tissue residues, adverse effect on species biodiversity, etc, which ultimately affect the cultured species, human and environment. Several of these aspects have been well documented (Anderson and Levin, 1999; Tendencia and De la Pena, 2001).

Water quality i.e. the physico-chemical and biological characteristics of water, plays an important role in plankton productivity as well as the biology of the cultured organisms and finally yields. Water quality determines the species optimal for culture under different environments (Dhawan and Karu, 2002).

The physico-chemical attributes of a water body are principle determinants of fish growth rates and development (Jhingran, 1991). Good water quality in fish ponds is essential for survival and adequate growth (Burford, 1997).

CHAPTER-111
GENERAL METHOLOGY

Farmer's uses different chemicals and biological products rather than most commonly used lime and fertilizers, in a limited scale. Large numbers of chemicals and biological products are now being introduced by different agrochemical agencies/pesticide companies. The categories of products are as follows:

- Water treatment compounds
- Oxygen releaser
- Antibiotics

Three to five drugs/chemicals in each category were selected and tested in the laboratory and pond condition. The selected drugs/chemicals are as follows:

Category	Name of Drugs/chemicals
• **Water treatment compounds**	Timsen, Geotox, Geoprime, Geo-Fresh
• **Oxygen releaser**	Oxygold, Oxymore, Oxylife, Oxyflow and Oxymax
• **Antibiotics**	Aquamycin, Captox and Oxysentin

A total of three experiments were conducted during the present study in ponds of Bangladesh Fisheries Research Institute (BFRI), Mymensingh. This chapter describes the general methodologies that were followed for each of the experiment.

Experimental Site and Pond preparation

Each experiment was carried out in earthen ponds at the hatchery comlpex of Bangladesh Fisheries Research Institute (BFRI), Mymensingh. The ponds were rectangular in shape with an area of 80m^2 each having an average depth of 1.5m, well exposed to sun light, and were free from aquatic vegetation. The ponds had inlet and outlet facilities, and connected to a deep tube-well using flexible plastic pipe for water supply. At the stage of preparation, all selected experimental ponds were dried up, bottom was ploughed and kept exposed to sunlight for three days. Then ponds were prepared through liming @ 250 kg/ha and gradually filled-up with

underground water up to a depth of 1 m. After filling up the ponds with water, drugs/chemicals were applied at selective doses according to the design for each experiment .

Water quality monitoring

Throughout the experimental period, the water quality parameters were recorded weekly. Water quality measurement and sample collection were before and after using of drugs. Transparency (cm), water temperature (^oC), pH and dissolved oxygen (mgl^{-1}), Ammonia-nitrogen (mgl^{-1}), Nitrate-nitrogen (mgl^{-1}), and Phosphate-phosphorous (mgl^{-1}) were measured every week before and after using

Methods used for water quality analysis

During the study period, water temperature was recorded with a Celsius thermometer. Transparency was measured with a Secchi disc of 20 cm diameter. pH of the water samples was measured by a direct reading digital pH meter (Jenway, model 3020 CORNING 445 pH meter) and dissolved oxygen was also measured by using a digital DO meter (YSI, model 58) on the spot. The concentration of nitrate-nitrogen (NO_3-N) was determined by HACH kit (DR-2010, a direct reading spectrophotometer) using NitraVer-6 and NitriVer-3 powder pillow. Ammonia-nitrogen was also determined by the HACH kit with Rochelle salt and Nessler reagent. The same HACH kit and Phosver-3 powder pillow was used to determine phosphate-phosphorus (PO_4-P).

Collection and Selection of fishes for research

Monosex male GIFT tilapia and Thai koi was collected from BFRI, Mymensingh. After 3 days of using drugs fingerlings of monosrx male tilapia, were stocked in all the ponds with more or less same stocking density of 100 fish/pond. Before starting the experiment the fish were acclimatized to the experimental conditions for one week.

Feeding

Feeding of fingerling was maintained twice daily in the morning & evening with commercial Saudi-Bangla feed at the rate of 10% of the body weight in the first week. For second week daily ration was adjusted at the rate of 5% of the body weight. Essential water quality parameters were recorded weekly and growth of fish was recorded after 10 days interval.

Ponds selection

This experiment was conducted by using twelve ponds having an area of 40.00 m^2 each & average depth was 1.2 m

Description of selected drugs

Water treatment compounds **:** Timsen, Geotox, Geoprime, Geo-Fresh

Oxygen releaser **:** Oxygold, Oxymore, Oxylife, Oxyflow and Oxymax

Antibiotics **:** Aquamycin, Captox and Oxysentin

Water treatment Drugs : Timsen, Geotox, Geoprime, Geo-Fresh

Name of drugs/Company	Before Stocking	After Stocking
Timsen(n- alkyl dimethylbenzo ammonium chloride=40%.: Stabilized urea=60%) Eon Animal Health	100g/acre (Water depth: 3-4 ft)	100g/acre (Water depth: 3-4 ft)
Zeo-prime ($Si0_2$, Al_2O_3, Fe_2O_3, CaO, MgO, TiO_2, K_2O,$Na0_2$ MnO_2) SK-F Animal Health	18—24 kg/acre	60g/acre (Water depth: 3-4 ft)
Geotox(SiO2, Al2O3, Fe2O3 CaO, MgO) (Novartis. Animal Health)	20-25kg/acre (Water depth: 3-6 ft)	10-20 kg/acre (Water depth: 3-6 ft) **every** 30-40 days interval
Zeo-Fresh(Sio2, Al2O3, Fe2O3, CaO, MgO, LoI, K2O) (Square pharmaceutical Ltd.)	24 kg/acre (Water depth: 3-6ft)	10 kg/acre (Water depth: 3-6ft), every 4 weeks interval.

Oxygen Releaser Drug : Oxygold,Oxylife, Oxyflow,Oxymax,Oxymore

Name of drugs/Company	Preventive	Treatment
Oxygold, (Sodium per- carbonate 90%) Fish-Tech.	250-500 gm / Acre.	750-1000 gm / Acre.
Oxylife (Oxygen Precursor Prebiotics,Detoxificants) Square Pharm.Ltd.	400 gm / Acre, twice a week..	500 gm / Acre, every alternate day.
Oxyflow (H_2O_2- Hydrogen per-oxide) Novartis Animal Health.	250-500 gm / Acre.	500gm/ Acre
Oxymore(Sodium carbonate per oxi- hydred 90%) SK-F	250-500 gm / Acre.	700 gm / Acre.
Oxymax, (Calcium per- oxidnate 80%) Eon Animal Health)	250-500 gm / Acre.	500-600 gm / Acre.

Antibiotics : **Aquamycin, Oxysentis, Renamycin**

Name of drugs/Company	Preventive	Treatment
Aquamycin(Fish Tech)	400-500 gm/ton feed	1-1.5 kg/ton feed, 5 days
Oxysentin(Oxytetracycline HCI BP) Novatis Animal Health.	50 gm/100gm feed, 10 days	100-200 gm/100kgfeed, 5-7 days
Renamycin (Reneta)	60 gm/100kg feed, 3-5 days or 1gm/2 lit. water, 3-5 days	1 gm/lit water, 5-7 days or 100gm/100kg feed, 5-7 days

Water quality monitoring

Throughout the experimental period, the water quality parameters were recorded weekly. Water quality measurement and sample collection were before and after using of drugs. Transparency (cm), water temperature (^oC), pH and dissolved oxygen (mgl^{-1}), Ammonia-nitrogen (mgl^{-1}), Nitrate-nitrogen (mgl^{-1}), and Phosphate-phosphorous (mgl^{-1}) were measured every week before and after using of drugs.

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Water Quality Testing Kits

Plate3.5. Hardness Test Kits

Plate3.6 Dissolved Oxygen Test Kits

Plate3.7. pH Test Kits

Plate3.8 Alkalinity Test Kits

Plate3.9 Nitrite Test Kits

Plate3.10. Ammonia Test Kits

Fish Sampling and harvesting

Sampling was done after 10 days interval by using a seine net to observe the growth of fish to adjust the feeding rate. Small and rather inadequate sample 10-15 fish were taken to make some rough assessment of growth trends, even knowing that such samples might not present the actual growth situation. Growth of fish in each sampling was measured by using a digital electronic

balance (Denver-XP-3000; precision=0.1 gm). The sampled fish were handled very carefully as the species are very susceptible to handling stress. To determine survival and growth of fish sampling of fish was done after 10 days interval.

Fishes were completely harvested after finishing the research. Primarily, the partial harvesting of fishes was performed by repeated netting, using a seine net. Final harvesting was done by de-watering the ponds using pump. During harvesting all fishes of each pond were collected and weighed individually to assess the survival rate and production.

Analysis of Plankton

Collection and preservation of plankton samples

Plankton samples from each of the experimental ponds were collected weekly for the 1st experiment and fortnightly for others. Ten liters of water samples were collected from different places and depth of the ponds and passed through plankton net to get a 50 ml filtered sample. The samples were then preserved immediately with 5% buffered formalin in a sealed plastic bottle.

Enumeration and identification of plankton

Plankton was counted using a Sedgewick-Rafter counting chamber cell (S-R cell). One ml sub-sample was transferred to the counting chamber of the S-R cell (providing 1000 fields) and all cells or colony forming units occurring in 10 randomly chosen fields were counted using a compound binocular microscope.

Analysis of Physical, chemical and production data

The following equations were used to determine the growth parameters,

a) Weight gain (g):

Weight gain = Mean final weight − Mean initial weight

b) Percent weight gain (%):

$$\% \text{ Weight gain} = \frac{\text{Mean final weight} - \text{Mean initial weight}}{\text{Mean initial weight}} \times 100$$

c) Average daily gain (g):

$$\text{ADG (g)} = \frac{\text{Mean final weight} - \text{Mean initial weight}}{T_2 - T_1}$$

d) Specific growth rate (% per day)**:**

$$\text{SGR (\% per day)} = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1} \times 100$$

Where,

W_1 = Initial live body weight (g) at time T_1 (day)

W_2 = Final live body weight (g) at time T_2 (day)

Histopathological procedure

For histopathological study, monthly sample was collected from various organs such as skin, muscle, gill, liver and kidney by a sharp scalpel and forceps. Skin-muscle were collected from the place between anterior part of dorsal fin and lateral line and by removing operculum, gills samples were collected. For liver and kidney, fishes were dissected and then portions of liver and kidney were collected. All collected samples were fixed in 10 % natural buffered formalin. The amount of fixative was 10 times to bulk of tissue fixed. The sizes of the samples were 1 cm^3. The preserved samples were taken out and trimmed by scalpel. Trimmed samples were placed separately in perforated plastic holders and covered by perforated steel covers. Labeling was made with dark pencil (2B) on perforated plastic holders. The samples were then arranged in a steel rack and processed through an automatic tissue processor (SHADON, Citadel 1000) for dehydration, clearing and infiltration. Alcoholic series of higher concentration, xylene and paraffin (3 series) ere used in the processor maintaining at various time schedules as mention below

Table 1: Time schedule in the automatic tissue processor

Container	Chemicals	Times (hour)	Process
01	50% Alcohol	1	Dehydration
02	80% Alcohol	2	
03	100% Alcohol	2	
04	100% Alcohol	2	
05	100% Alcohol	2	
06	100% Alcohol	2	
07	100% Alcohol	2	
08	Xylene	2	Clearing
09	Xylene	1	
10	Molten wax	1	Infiltration
11	Molten wax	2	
12	Molten wax	2	

The samples were then embedded with melted wax, steel mold and perforated plastic holder. Proper care was taken for the placement and orientation of skin-muscle and gill in steel molds during the embedding. After embedding, the paraffin blocks were placed on table to become hard. Then the blocks were placed in a deep freeze for half an hour and after that steel molds were separated from the paraffin blocks. Trimming was done from the side and surface of the block by scalpel and a microtome machine (Leica JUNG RM 2035). Embedded blocks were then placed in the deep freeze for 30 minutes before final sectioning. After having sections, the ribbon of sections was placed on a water bath (Electro thermal, paraffin-section, mounting bath) at 40˚ C. A suitable section was selected and separated from ribbon, which was finally picked up over a glass slide. To fix the section, the prepared slide was placed on a hot plate (37˚ C) for overnight. The sections were then cleared with xylene, rehydrated with alcoholic series and stained with haematoxylin and eosin stains proceeding through various chemicals of different concentrations and time schedules as mentioned in Table

Table 2: Staining procedure followed during the experiment.

SL. No.	Process	Solution	Times (min)
01	Clearing	Xylene	2
02		Xylene	2
03	Rehydration	100% alcohol	2
04		100% alcohol	2
05		95% alcohol	2
06		70% alcohol	2
07		Running tap water	2
08	Stain	Haemotoxylene	10
09	Reduce stain	Running tap water	2
10	Counter stain	Eosin	12
11	Dehydration	70% alcohol	3 dips
12		95% alcohol	3 dips
13		100% alcohol	2 dips
14		100% alcohol	2 dips
15	Clearing	Xylene	2
16		Xylene	2

After staining the sections were mounted with Canada balsam and covered by coverslip. The prepared slides were left on clean platform to hold the cover slips permanently and then examined under a compound microscope. Photomicrographs of the stained sections were done by using a photomicroscope. Comparisons of structure and pathology of organs were made among treatments.

Data analysis For statistical analyses of data , a one –way ANOVA was carried out *STATGRAPHICS Version -7* statistical package for the PC.

CHAPTER 1V

Determination on efficacy of selected water treatment drugs used in aquaculture in Bangladesh"

Description of study area (Water treatment compounds)

The research was carried out for 90 days (September- November, 2010) to find out the determination on efficacy of selected common water treatment chemicals and drugs in Aquaculture in Bangladesh.

Experiment Design:

The results of Timsen, Geoprime**,** Geotox, and Zeofersh on aquaculture activities were tested in the earthen ponds of Bangladesh Fisheries Research Institute (BFRI), Mymensingh from the period of September- November, 2010. Experiment was designed into four treatment **(**T_1 - Timsen, T_2 -Zeofersh, T_3 -Geoprime, and T_4 –Geotox, Recommended dose of the company), with three replications.

Ponds preparation

Twelve ponds were prepared through liming @ 250 kg/ha after drying of pond. After 5 days of liming each of the following drugs Timsen, Geofresh, Geoprime, Geotox was used for each ond. The ponds were filled with underground water up to a depth of 1m.

Plate 4.1: Pond drying

Water quality parameters

Water quality parameters represented pond productivity. Productive pond provided higher fish production. Less productive pond are not suitable for aquaculture. For ideal fish culture pH range between 6.5 and 8.5, Total alkalinity value should be above 100 mg/l, DO should be above 4 mg/l, Ammonia should be less than .01mg/l. Water quality parameters of three treatments have been presented in the following table.

Table: 4.1 Water quality parameters of three treatments before and after use of drugs

Variables	Drugs							
	Timsen		Geo-fresh		Geo-prime		Geo-tox	
	Before Treat-ment	After Treat-Ment	Before Treat-ment	After Trea-ment	Before Treat-ment	After Trea-ment	Befoe Treat-ment	After Treat-ment
Transparency (cm)	28.40 ±2.16	31.27 ±3.06	27.73 ±1.75	29.53 ±1.88	27.47 ±2.39	29.20 ±2.76	28.67 ±2.23	30.07 ±2.52
Temperature (°C)	28.41 ±3.36	28.25 ±3.38	28.48 ±2.77	28.38 ±2.73	27.78 ±2.09	27.71 ±2.07	28.29 ±2.98	28.27 ±2.96
DO (mg l^{-1})	3.72 ±0.33	4.56 ±0.29	3.44 ±0.28	4.14 ±0.29	3.30 ±0.16	4.07 ±0.17	3.33 ±0.26	4.06 ±0.26
pH	6.93 ±0.25	8.12 ±0.58	6.62 ±0.34	7.71 ±0.31	6.49 ±0.19	7.64 ±0.14	6.42 ±0.17	7.56 ±0.18
Alkalinity (mg l^{-1})	95.00 ± 8.84	119.40 ±9.67	94.07 ±6.56	115.73 ±11.13	93.33 ±8.01	115.3 ±6.97	92.47 ±6.32	110.3 ±9.19
Nitrate-nitrogen (mg l^{-1})	0.12 ± 0.05	0.28 ±0.04	0.11 ±0.04	0.18 ±0.03	0.12 ±0.04	0.19 ±0.04	0.13 ±0.05	0.21 ±0.03
NH_3-N (mg l^{-1})	0.19 ±0.04	0.01 ±0.01	0.17 ±0.04	0.04 ±0.02	0.19 ±0.04	0.05 ±0.03	0.19 ±0.03	0.06 ±0.03
PO_4-P (mg l^{-1})	0.61 ±0.21	1.00 ±0.19	0.37 ±0.09	0.58 ±0.12	0.44 ±0.10	0.62 ±0.08	0.44 ±0.11	0.65 ±0.13

After using drugs Transparency (cm), pH, Alkalinity (mg l^{-1}), Nitrate (mg l^{-1}), Ammonia (mg l^{-1}), DO (mg l^{-1}), Phosphate (mg l^{-1}) were significantly changed in case of all treated pond. Actually after using drugs above parameters reached optimum level for aquaculture. Only temperature was no significant due to after using drugs. * Symbol provided significance level 5%.

Physical Parameters

Temperature (^{o}C)

The mean values of water temperature were before using of drugs 28.41 ± 3.36 ^{o}C, 28.48 ± 2.77^{o}C 27.78 ± 2.09 ^{o}C and 28.29 ± 2.98^{o}C in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments, respectively. The mean values of water temperature were recorded after using of drugs 28.25 ± 3.38^{o}C, 28.38 ± 2.73^{o}C, 27.71 ± 2.07 ^{o}C, and 28.27 ± 2.96^{o}C in Timsen, Geo-fresh, and Geo-prime and Geo-tox treatments, respectively.

Transparency (cm)

The mean (± SD) values of transparency were before using of drugs 28.40 ± 2.16cm, 27.73±1.75 cm, 27.47 ± 2.39 cm and 28.67 ± 2.23 in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments, respectively. The mean (± SD) values of transparency were recorded after using of drugs 31.27 ± 3.06cm, 29.53 ± 1.88 cm, 29.20 ± 2.76cm, and 30.07 ± 2.52 cm in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments, respectively.

Chemical Parameters

pH (Hydrogen ion concentration)

The mean (± SD) values of pH were before using of drugs 6.93 ± 0.25, 6.62 ± 0.34, 6.49 ± 0.19 and 6.42 ± 0.17 in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments, respectively. The mean (± SD) values of pH were recorded after using of drugs 8.12 ± 0.58, 7.71 ± 0.31, 7.64 ± 0.14and 7.56 ± 0.18 in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments, respectively

Total Alkalinity (mg l^{-1})

The mean (± SD) values of total alkalinity were 95.00 ± 8.84 mg l^{-1}94.07 ± 6.56mg l^{-1}, 93.33 ± 8.01 and 110.33 ± 9.19mg l^{-1} in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments, respectively. The mean (± SD) values of total alkalinity were recorded 119.40 ± 9.67mg l^{-} 115.73 ± 11.13mg l^{-1}, 115.33 ± 6.97 mg l^{-1} and 110.33 ± 9.19mgl^{-1} in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments, respectively

Dissolved oxygen (mg l^{-1})

The mean values of DO were before using of drugs is 3.72 ± 0.33 mg l^{-1}, 3.44 ± 0.28 mg l^{-1}, 3.30 ± 0.16 mg l^{-}and 3.33 ± 0.26mgl^{-} in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments,

respectively. The mean values of DO were after using of drugs is 4.56 ± 0.29 mg l^{-1}, 4.14 ± 0.29 mg l^{-1} , 4.07 ± 0.17 mg $l^{-}$and 4.06 ± 0.26mgl^{-} in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments, respectively.

Table 4.2: Tilapia Production table showing production and survival% of different drugs treated pond.

Treatment	Drugs				Level of significance
	Timsen	Geo-fresh	Geo-prime	Geo-tox	
Harvest number.	85.00 ± 1.73	77.00 ± 2.00	75.67± 1.53	79.00± 1.00	NS
% Survival	85.00 ± 1.73	77.00 ± 2.00	75.67 ± 1.53	79.00 ± 1.00	NS
Total weight (gm)	7728.8^{a} ± 101.61	5860.83^{a} ± 198.48	5668.40^{b} ± 257.17	2289.36^{a} ±319.02	*
% weight gain	1472.80^{a} ± 28.93	1247.84^{b} ± 32.28	1230.40^{b} ± 28.14	1215.52^{b} ± 9.98	*
Specific Growth Rate	0.23^{a} ± 0.01	0.13^{b} ± 0.01	0.12^{b} ± 0.01	0.12^{b} ± 0.01	*
Net production (kg/ha	1955.64^{a} ± 24.28	1501.14^{b} ± 47.66	1455.12^{b} ± 61.94	1500.5^{b} ± 30.11	*
Gross production (kg/ha	835.38^{a} ± 10.16	648.58^{b} ± 19.85	629.34^{b} ± 25.72	649.57^{b} ± 12.65	*

NS = Means are not significantly different (*P> 0.05*)
* Mean values with different superscript letters in the same row indicate significant difference at 5% significance level.

Gross production kg/ha

The mean (±SD) values of Gross production were recorded after using of drugs 835.38 ± 10.16kg/ha, 648.58 ± 19.85kg/ha, 629.34 ± 25.72 kg/ha, and 649.57 ± 12.65kg/ha in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments, respectively.

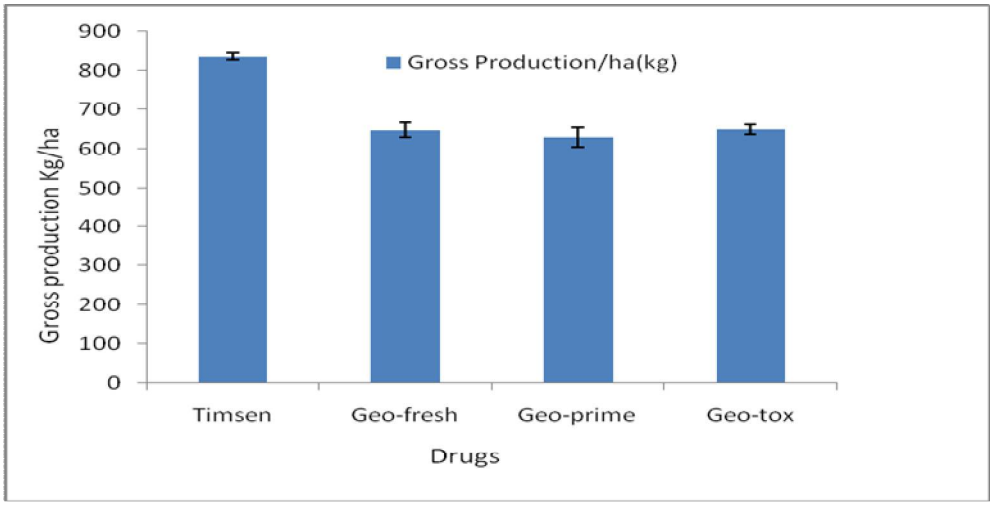


Fig4.9: Impact of different water quality treatment drugs on Gross fish production.

Net fish production (kg/ha)

The mean (±SD) values of Net fish production were recorded after using of drugs 1955.6 4 ± 24.28 kg/ha, 1501.14 ± 47.66kg/ha, 1455.12 ± 61.94 kg/ha, 1500.50 ± 30.11 in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments, respectively.

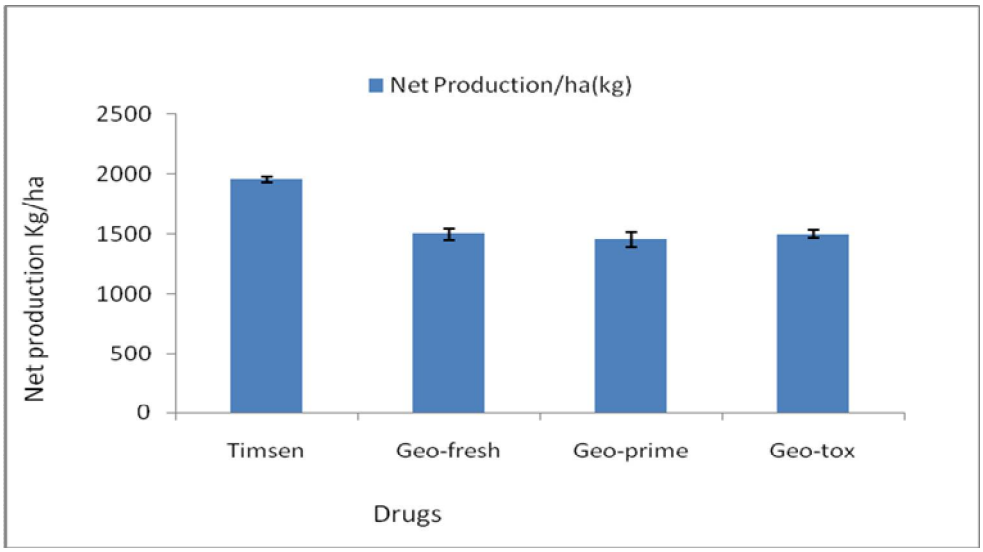


Fig4.10: Impact of different water quality treatment drugs on Net fish production.

Survival (%)

The mean (±SD) values of survival of % fish survival were recorded after using of drug 85.00 ± 1.73, 77.00 ± 2.00, 75.67 ± 1.53, 79.00 ± 1.00 in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments, respectively.

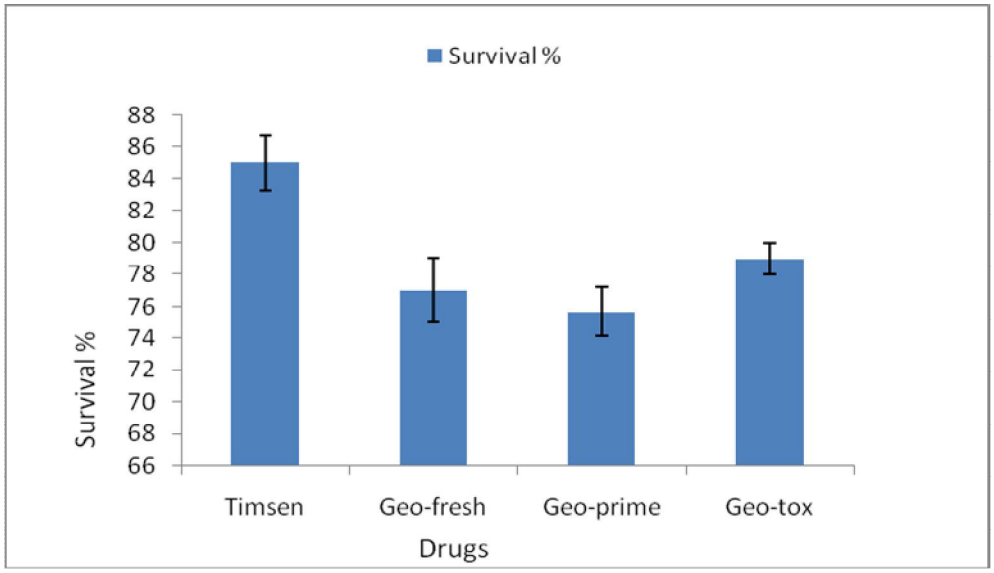


Fig4.11: Impact of different water quality treatment drugs on %fish survival.

Histopathological study of different organs of Tilapia (*O. niloticus)*

To identify effects of drugs and chemicals on different organ of fish liver, gill, muscle & kidney were observed by histological study. Histological study did not show any change of the following organ, so drugs had no negative impact on different organ of fish.

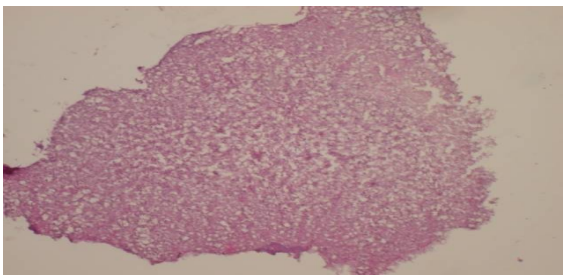
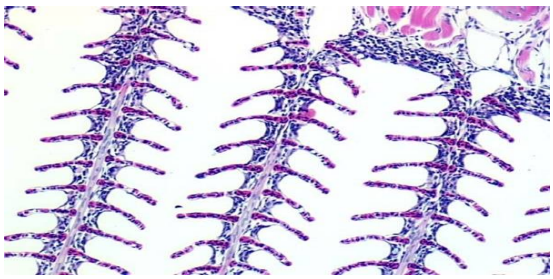
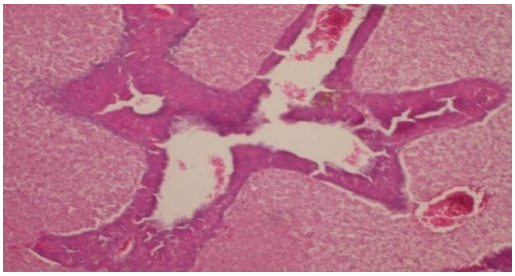


Plate no 4.1 Liver



Plates no 4.2 Gill



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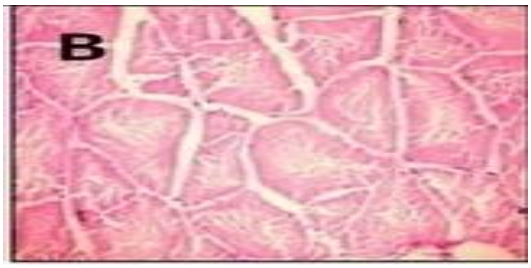


Plate no 4.4 Muscle

The research was conducted for a period of 120 days from August to November 2012 in 12 ponds, each with area 40 m^2. The research was done for the determination on efficacy of selected common drugs used in Bangladesh. For enhancing production of the Gift Tilapia,

Timsen, Geofersh, Geoprime, Geotox, were selected to maintain water quality of the ponds. The experiment was designed into four treatment groups of T_1 -(Timsen), T_2- (Geofersh) T_3 – (Geoprime) and T_4- (Geotox), each having 100 fingerlings, equally divided into three replications. Male GIFT tilapia *(O. niloticus)*, (ABW: 6.25 ± 0.27 gm) fingerlings were used as test animals. Feeding of the fingerling was done twice daily in the morning & evening with commercial nursery feed (Saudi-Bangla Fish Feed Limited) at the rate of 10% of the body weight at first week. For second week daily ration was adjusted at the rate of 5% of the body weight. Data on water quality parameters, survivality, and growth were recorded before and after using of drugs. Essential water quality parameters were recorded weekly and growth, survivality were recorded after ten days interval. Histological studies were done 1 month's interval.

It was observed that after using drugs in all treatments the value of pH (8.12±0.58), alkalinity (119.40±9.67), nitrate (0.28±0.04) and phosphate (1.00±0.19), dissolved oxygen (4.56 ± 0.29), were maintained. Ammonia became reduced due to use of drugs. During observation of physical water quality parameters transparency (31.27±3.06) became increased and temperature (28.25±3.38) became lower than control water body. Gift Tilapia production also provided higher in Timsen treated pond than other treated ponds. Histopathological study did not show any negative changes on the organ of fishes.

Among all the drugs, Timsen was the best in all aspects (improve fish health and water quality). Other three drugs (Geo-tox, Geo-fresh, and Geo-prime) resulted more or less same result.

Conclusion

The research was conducted for a period of 90days from September- November, 2010 in 12 ponds, each with area 40 m^2. The research was done for the determination on efficacy of selected common drugs used in Bangladesh. For enhancing production of the Gift Tilapia, Timsen, Geofersh, Geoprime, Geotox, were selected to maintain water quality of the ponds. The experiment was designed into four treatment groups of T_1 -(Timsen), T_2- (Geofersh) T_3 – (Geoprime) and T_4- (Geotox), each having 100 fingerlings, equally divided into three replications. Male GIFT tilapia *(O. niloticus)*, (ABW: 6.25 ± 0.27 gm) fingerlings were used as test animals. Feeding of the fingerling was done twice daily in the morning & evening with commercial nursery feed (Saudi-Bangla Fish Feed Limited) at the rate of 10% of the body weight at first week. For second week daily ration was adjusted at the rate of 5% of the body weight. Data on water quality parameters, survivability, and growth were recorded before and after using of drugs. Essential water quality parameters were recorded weekly and growth,

survivability were recorded after ten days interval. Histological studies were done 1 month's interval.

It was observed that after using drugs in all treatments the value of pH (8.12±0.58), alkalinity (119.40±9.67), nitrate (0.28±0.04) and phosphate (1.00±0.19), dissolved oxygen (4.56 ± 0.29), were maintained. Ammonia became reduced due to use of drugs. During observation of physical water quality parameters transparency (31.27±3.06) became increased and temperature (28.25±3.38) became lower than control water body. Gift Tilapia production also provided higher in Timsen treated pond than other treated ponds. Histopathological study did not show any negative changes on the organ of fishes.

Among all the drugs, Timsen was the best in all aspects (improve fish health and water quality). Other three drugs (Geo-tox, Geo-fresh, and Geo-prime) resulted more or less same result.

CHAPTER V

Determination on efficacy of selected Oxygen Releaser drugs used in Aquaculture in Bangladesh

Description of study Area:

Three experiments were conducted, one in Laboratory condition, one in on station experimental pond of the Freshwater station and another one is farmers pond of Trishal , Muktagacha and Fulpur upazilla.

Experimental Design: 1.

Impact of Oxygen releaser in controlled condition

This experiment was conducted in 15 plastic buckets. The size of buckets was 30 liter. Five selective drugs were tested and each had three replications. Before using drug, initial concentration of DO was recorded. After that oxygold, oxymore, oxymax, oxyflow and oxylife were used in different doses. Data were recorded at 30min., 60 min., 120 min., 180 min., and 240 min.interval for each drug simultaneously. It was observed that concentration of dissolved oxygen was initially increased and later gradually decreased in successive hours.

Fig: 5.1 Determination oxygen concentration and analysis of different water quality parameters.

Table: 5.1 Impact of Oxygen releaser in control condition

Name of drugs	Initial level of O2(ppm)	After30min.levelsofO2(ppm)	After 60min.levelsofO2(ppm)	After120min.levelsofO2 (ppm)	After180 min.level O2(ppm)	After240 min.level O2(ppm)
Oxygold	1.83± 0.05	3.17± 0.15	4.77± 0.55	3.77± 0.55	3.77± 0.55	2.97± 0.45
Oxymore	1.83± 0.05	2.63± 0.65	4.53± 0.45	3.63± 0.45	3.63± 0.45	2.43± 0.35
Oxymax	1.83± 0.05	2.88± 0.34	4.58± 0.64	3.88± 0.34	3.28± 0.34	2.85± 0.34
oxylife	1.83± 0.05	3.23± 0.85	4.70± 0.85	3.73± 0.75	3.73± 0.75	2.34 0.75
Oxyflow	1.83± 0.05	3.23± 0.55	4.62± 0.73	3.63± 0.73	3.63± 0.73	2.79± 0.73

Expt: 2. Impact of Oxygen releaser in pond condition

Materials and methods

To determine the effect of suitable dose of oxygen releasing drug an experiment was conducted in the earthen ponds of Bangladesh Fisheries Research Institute (BFRI), Mymensingh from the period of August -October 2011. Twelve ponds having an area of 40.00 m^2 (1decimal=40.48 m^2) each were prepared through sun drying and liming the bottom soil with agricultural lime @ 250 kg/ha and mustard oil cake @ 500 kg/ha. The ponds were filled in with underground water up to a depth of 1 m and inorganic fertilizers of TSP and urea were applied @ 35 kg/h with 3:1 ratio. After 5 days of fertilization, Tilapia, *O. niloticus* fry (ABW: 3.33 gm, ABL: 5.53±0.83 cm), were stocked in the ponds with same stocking patterns 100 fish/pond. The experiment was designed with three different doses of drugs (T-1 Recommended dose, T-2 1.5 times lower than recommended dose, T-3 1.5 times lower than recommended dose)). Each treatment had three replications and those were assigned into a completely randomized design. Five selective drugs were tested and each had three replications. Before using drug we recorded data and after using drugs simultaneously (30minutes, 60 minutes, 120minutes, 180minutes, 240minutes) also recorded data. It was observed that concentration of dissolved oxygen was at first increased and gradually decreased in all treatments. Results are shown in Table 2-8

Table: 5. 2 Mean values (±SD) of different water quality parameters of ponds under three treatments (Before drug use)

water quality para.meters	T-1 (RD)	T-2 (1.5 times LD)	T-3 (1.5 times HD)	T-4 (control)
Temp. (°C)	30.23 ± 2.15	30.38 ± 2.58	30.43 ± 2.15	30.53 ± 2.18
DO(mg/l)	4.65 ±0.91	4.35 ±0.78	4.78 ±0.88	4.3 5 ±0.94
PH	6.5-7.14	6.27-7.18	6.22-7.62	6.28-8.11
Total Alka.(mg/l)	116.35 ±6.25	115.65 ±8.78	114.00 ±12.38	124.75 ± 12.34
Ammonia (mg/l)	0.65 ±0.09	0.52 ±0.08	0.41 ±0.08	0.85 ± 0.05

Table: 5. 3 Mean values (±SD) of different water quality parameters of ponds under three treatments (After drug use)

water quality parameter.	T-1 (RD)	T-2 (1.5 times LD)	T-3 (1.5 times HD)	T-4 (control)
Temp. (°C)	32.26 ± 3.15	32.28 ± 2.58	32.43 ± 2.13	32.53 ± 2.15
DO(mg/l)	6.85 ±0.91	5.65 ±0.78	8.88 ±0.98	4.75 ±0.94
P^H	7.5-8.11	7.37-7.69	7.82-8.62	7.98-8.11
Total Alkalinity(mg/l)	196.85 ±16.25	165.65 ±8.78	214.00 ±12.38	174.75 ± 12.34
Ammonia (mg/l)	0.22 ±0.19	0.22 ±0.04	0.24 ±0.03	0.28 ± 0.15

Table 5.4 : Showed the oxygen concentration of different drugs in laboratory condition. All the selective drugs enhanced the oxygen concentration in water.

Name of drugs	Initial level of O_2 (ppm)	After 30min. levelsof O_2 (ppm)	After 60 min. levels of O_2 (ppm)	After120min. levels of O_2 (ppm)	After180min. level O_2 (ppm)	After 240min. level O_2 (ppm)
Oxygold	1.83± 0.05	3.17± 0.15	4.77± 0.55	3.77± 0.55	3.77± 0.55	2.97± 0.45
Oxymore	1.83± 0.05	2.63± 0.65	4.53± 0.45	3.63± 0.45	3.63± 0.45	2.43± 0.35
Oxymax	1.83± 0.05	2.88± 0.34	4.58± 0.64	3.88± 0.34	3.28± 0.34	2.85± 0.34
oxylife	1.83± 0.05	3.23± 0.85	4.70± 0.85	3.73± 0.75	3.73± 0.75	2.34 0.75
Oxyflow	1.83± 0.05	3.23± 0.55	4.62± 0.73	3.63± 0.73	3.63± 0.73	2.79± 0.73

The results of the experiments were shown in Table 2 and 3.

Table: 5.6 Mean values (±SD) of different water quality parameters of ponds under four treatments (Before using drug)

Water quality para.	T-1 (RD)	T-2 (1.5 times LD)	T-3 (1.5 times HD)	T-4 (control)
Temp. (°C)	30.23 ± 2.15	30.38 ± 2.58	30.43 ± 2.15	30.53 ± 2.18
DO(mg/l)	4.65 ±0.91	4.35 ±0.78	4.78 ±0.88	4.3 5 ±0.94
PH	6.5-7.14	6.27-7.18	6.22-7.62	6.28-8.11
Total Alka.(mg/l)	116.35 ±6.25	115.65 ±8.78	114.00 ±12.38	124.75 ± 12.34
Ammonia (mg/l)	0.65 ±0.09	0.52 ±0.08	0.41 ±0.08	0.85 ± 0.05

Table: 5.7 Mean values (±SD) of different water quality parameters of ponds under four treatments (After using drug)

Water quality para.	T-1 (RD)	T-2 (1.5 times LD)	T-3 (1.5 times HD)	T-4 (control)
Temp. (°C)	32.26 ± 3.15	32.28 ± 2.58	32.43 ± 2.13	32.53 ± 2.15
DO(mg/l)	6.85 ±0.91	5.65 ±0.78	8.88 ±0.98	4.75 ±0.94
PH	7.5-8.11	7.37-7.69	7.82-8.62	7.98-8.11
Total Alka.(mg/l)	196.85 ±16.25	165.65 ±8.78	214.00 ±12.38	174.75 ± 12.34
Ammonia (mg/l)	0.22 ±0.19	0.22 ±0.04	0.24 ±0.03	0.28 ± 0.15

RD = Recommended dose, LD= Lower dose, HD= Higher dose

Plankton determination

During the trial period, total phytoplankton population was higher in T-3 than that of T-1 &T-2. The mean abundance of total phytoplankton was 10.67±2.06, 7.15±1.4 and 20.28±2.06×10^3 cells/l in T-1, T-2 and T-3 respectively (Table 9). In T-3, 50.22% higher phytoplankton population was produced in T-1 where pond was treated by higher (1.5 times) dose. While, The mean abundance of total zooplankton population was found to be 2.64±0.22×10^3 ,1.71±0.31x10^3 and 5.22±2.16×10^3unit/l T-1, T-2 and T-3 respectively. The zooplankton value of T-3 was higher than that of T-1(recommended dose).

Table 5.8: Mean (±SD) abundance of plankton (x10^3cells/l) in ponds under three treatments

Plankton group	T-1(Recommended dose)	T-2 (1.5 times lower dose)	T-3(1.5 times higher dose)
Phytoplankton	$10.67\pm2.06\times10^3$	$7.15\pm1.4\times10^3$	$20.28\pm2.06\times10^3$
Zooplankton	$2.64\pm0.22\times10^3$	$1.71\pm0.31\times10^3$	$5.22\pm2.16\times10^3$

Production of phytoplankton and zooplankton was higher in the T-3, where 1.5 higher dose (Oxygen releaser drug) used than that of recommended dose-treated pond.

Expt: 3. Impact of Oxygen releaser drugs (Oxymax/Oxymore/Oxylife) in farmers pond

In Mymensingh regions farmers culture mostly Thai pangus, Thai Koi, Tilapia ,Magur and Shing. Production of Pangus and koi in Tarakanda and Muktagacha upazillas were almost double in the chemical treated ponds compared with non treated ponds.

Site	Pond size (decimal)	Species culture	Stocking densities/decimal	Before drug use(O_2 mg/l)	Recommend dose(O_2 mg/l)
Trishal	42	Pangas Silver Rui Catla Carpio	238	4.64 ± 0.67	7.36 ± 0.36
Muktagacha	38	Shing pangus silver	450	5.64 ± 0.67	8.24 ± 0.67
Tarakanda	40	Tilapia Shing Magur	860	3.84 ± 0.27	6.94 ± 0.68

Analysis

Fish production between culture systems using chemicals and without chemicals were compared. For this purpose, three upazillas like Trishal, Muktagacha and Tarakanda were selected. For this purpose, three possible culture systems like carp polyculture, Thai pangas, Shing ,Magur and tilapia were chosen and total fish production and water quality were determined from ponds using chemical and without chemicals. Production of fish was slightly higher in the treated pond (Oxygen releaser drug) than that of non-treated pond.

Conclusion

From the findings it could be suggested that 1.5 times higher dose than recommended dose of each drug have been optimized for increasing dissolved oxygen in critical event. However elaborate studies are necessary to observe the effects of oxygen releaser drugs and others with more bio-chemical parameters before making comments.

It was observed that concentration of dissolved oxygen was initially increased and later gradually decreased in successive hours.

Histological observation of different organ of fish show no remarkable changes after using drugs.

CHAPTER V1

Determination on efficacy of selected Antibiotics used in Aquaculture in Bangladesh

Effects of Antibiotics on Fish fry health and Water quality of Cistern

Experimental Procedure and design : The feeding trail with antibiotic mixing feed were carried out a static indoor rearing system of Bangladesh Fisheries Research Institute (BFRI), Mymensingh consisting a series of rectangular cistern (2500L each) for 8 weeks. The same aged uniform size of each fish fry were randomly distributed into groups of 100fish (averaging 1.5gm in weight) per cistern .Three selective antibiotics were tested and each had three replications. The fish were individually weighed at the starting of the experiment by weekly. A weekly fish weight (fish sampling) was done to adjust the daily feed ration for the following week. . Before using antibiotics all Water quality parameter such as temperature, pH, dissolve oxygen and ammonia were recorded though weekly sampling. At the beginning of the experiment, 10 fish were randomly sacrificed for histopathological analysis.

Experimental Design

After 5 days of fertilization, Thai koi *Anabus testudeneaus.* fry (1.53 ± 0.14 gm) were stocked in the cisten with same stocking patterns 100 fish/cistern. Three antibiotics [Renamycin (Reneta), Oxysentin (Novartis), Aquamycin (ACI)] were applied with pelleted Koi nursery feed (Saudi-Bangla Fish Feed Limited). Three replication (feeding feed with antibiotic) were used for each antibiotic, these cistern were marked as T_1, T_2, T_3, (T_1, for Renamycin, T_2, for Oxysentin, and T_3 for Aquamycin) and two cistern (feeding feed without antibiotic) were used for control these cistern marked as T_4

Fig 6.1: Application of antibiotics mixed feed in the cistern

Table 6.2: Mean (±SD) of water quality parameters of cistern under three treatments

Parameters	Renamycine		Oxysentin		Aquamycine	
	BT	AT	BT	AT	BT	AT
DO (mg/l)	3.28- 3.52	4.68-6.24	4.12-5.98	4.5-6.10	3.99-5.20	5.1-6.5
pH	6.50-6.82	7.48-7.98	6.58-7.90	7.20-7.45	6.0-7.2	7.12-7.5
Total alkalinity (mg/l	80- 95	125-140	85-98	98-111	84-96	111-120
Ammonia (mg/l)	0.32-0.58	0.05-0.08	0.09-0.13	0.03-0.06	0.09-0.11	0.03-0.06
Phosphate (mg/l)	0.22-.32	0.28-0.65	0.22-.32	0.28-0.65	0.22-.32	0.28-0.65
Nitrate (mg/l)	0.10-0.24	0.18-0.30	0.10-0.24	0.28-0.55	0.10-0.24	0.18- 0.83

- **BT= Before Treatment / AT= After Treatment**

Table: 6.3 Growth and survival (±SD) of (*A. testudineus*) under different

antibiotics treated commercial feed for 60 days in cistern

Growth Parameters	Renamycine	Oxysentin	Aquamycine	Control
Av.Initial wt. (gm)	1.50 ± 0.14	1.55 ± 0.15	1.55 ± 0.15	1.53 ± 0.11
Av.Final wt. (gm)	8.78 ± 0.35^{a}	8.50 ± 3.31^{b}	8.15 ± 0.27^{c}	6.65 ± 0.29
Av. live weight gain (%)	485.73 ± 7.02^{a}	448.28 ± 8.60^{ab}	434.72 ± 0.28^{c}	334.50 ± 0.24^{c}
Survival rate (%)	92 ± 3.06^{a}	90.00 ± 3.03^{a}	90.23 ± 2.15^{a}	70.15 ± 2.12^{b}
Specific growth rate (%)	2.96 ± 0.55^{a}	$2.84 \pm 0.54^{a\,b}$	2.83 ± 0.55^{b}	2.45 ± 0.08^{c}

Effects of Antibiotics on Fish fry health and Water quality of Pond

The follow up the antibiotic trail and experiment was designed with three treatments with three replicates of each. Twelve (12) Nursery ponds (each pond 40 m^2) were used for nursing of fry. Ponds were prepared through sun drying and liming the bottom soil with agricultural lime @ 250 kg/ha and mustard oil cake @ 500 kg/ha. The ponds were filled up with underground water up to a depth of 1 m and inorganic fertilizers of TSP and urea were applied @ 35 kg/h with 3:1 ratio. After 5 days of fertilization, Tilapia, *O. niloticus* fry (ABW: 3.33 gm, ABL: 5.53±0.83 cm), were stocked in the ponds with same stocking patterns 100 fish/pond.

Experimental Design

Three antibiotics [Renamycin (Reneta), Oxysentin (Novartis), Aquamycin (ACI)] were applied with pelleted Tilapia nursery feed (Saudi-Bangla Fish Feed Limited). Three replication (feeding feed with antibiotic) were used for each antibiotic, these ponds were marked as T_1, T_2, T_3, (T_1, for Renamycin, T_2, for Oxysentin, and T_3 for Aquamycin) and three ponds (feeding feed without antibiotic) were used for control these ponds are marked as T_4. All of the above ponds were prepared by drying, liming, and fertilization before stocking fish fry (Stocking density 400/m^2). Water quality parameters were measured and recorded before applying feed. Antibiotic mixing feed applied twice a day for 25 days (50% body weight) from 26th day to 45 days (25% body weight) and from 46th day to 60 days (10% body weight) and 61th day to 90 days(5%body weight). Growth, survivability, Water quality and plankton monitoring were done after 15 days interval. Histopathological study also done before and after the end of experiment.

Fig 6.4: Histopathological study in the laboratory

Result and Discussion The water quality parameters of antibiotic treated ponds were recorded using commercial test kits(HANNA Test kit,) water temperature (oC), pH, dissolved oxygen (mg/l) total alkalinity were measured every 7 days interval for talapia and koi fishes separately. Water quality parameters of antibiotic treated ponds have been presented in the Table. 6.1&6.2

The influence of various antibiotics and drugs were tested in experimental ponds. Water quality parameters found to be more or less similar in antibiotic treated ponds when compared to those in the control pond. Microbial load decreased significantly in antibiotic treated ponds as compared to control. This is an indication that the antibiotics administered with the fish feed had significant effect to reduce the total load of bacteria in the pond. Histopathological changes of different organs like muscle, gill ,kidney and liver of non-treated ponds were observed. In that cases necrosis, vacuum, hemorrhage, pyknosis and fungal granuloma observed.

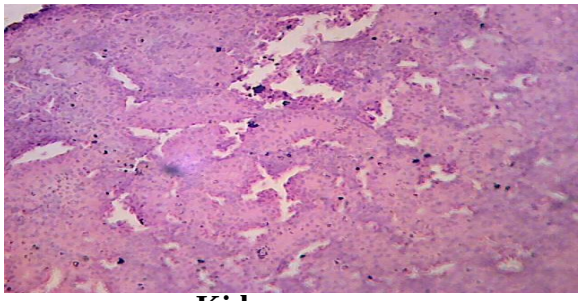
Table : 6.5 Mean (±SD) abundance of plankton (x10^3cells/l) in ponds under three treatments

Aquatic Organisms	Renamycine		Oxysentin		Aquamycine	
	BT	**AT**	**BT**	**AT**	**BT**	**AT**
Chironomidae	37.65± 11.11	22 ± 13.1	44.07± 9.98	30.12 ±10.33	23.1.98±9.06	24 ± 3.98
Oligochaeta	25.93± 3.23	10.12 ± 3.12	16.05± 3.04	11.53 ± 3.15	22.35± 24.69	11.,36 ± 2.65
Mollusca	9.51± 2.14	8.23 ± 2.23	6.46 ± 2.56	5.23 ± 2.17	8.10± 1.13	7.11 ± 2.12
Unidentified	34.57± 7.11	36.16 ± 7.40	39.51± 8.06	30.13 ± 7.99	35.19± 9.85	28.33 ± 6.23
Bacillariophyceae	13.54 ± 3.01	10.21 ± 2.11	12.04 ± 4.74	5.14 ± 1.76	11.37 ± 4.86	8.0 ± 2.1
Chlorophyceae	13.79 ± 4.48	8 ± 2.8	19.92 ± 8.24	10.32 ± 2.87	17.95 ± 3.61	12.09 ± 3.65`
Cyanophyceae	10.38 ± 3.67	6 ± 2.1	7.79 ± 1.88	3.97 ± .2.13	10 ± 5.76	6.12 ± 2.56
Euglenophyceae	1.63 ± 0.96	1.0 ± 0.12	2.29 ± 1.18	2.1 ± 1.0	1.5 ± 1.11	1 ± 0.23
Crustacea(Nauplius)	10.79 ± 2.66	6.12 ± 1.34	11.92 ± 3.18	6.12 ± 1.34	14.58 ± 5.33	11 ± 1.2
Bacillariophyceae	8.1± 1.38	3.33 ± 1.0	6.67± 1.89	4.23 ± 1.9	4.1± 1.74	3.21 ± 0.9
Chlorophyceae	22.27± 2.95	15 ± 1.77	2.43 ± 1.68	2 ± 0.2	4.4 ± 1.54	2.0 ± 0.25
Euglenophyceae	12.17 ± 6.72	8.1 ± 2.13	9.97 ± 2.49	5.1 ± 1.37	7.5 ± 3.89	6 ± 1.5
Rotifera	3.73 ± 1.62	2.1 ± 0.2	2.5 ± 1.46	1.44 ± 0.2	2.43 ± 1.49	2.2 ± 0.5
Cladocera	3.9 ± 1.7	2.0 ± 1.0	2.43 ± 1.43	1.55 ± 0.1	2.2 ± 1.01	1.4 ± 0.44

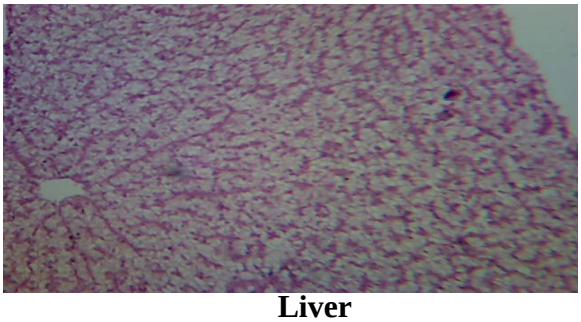
Table 6.6: Mean (±SD) value of water quality parameters under three treatments

Parameters	Renamycine		Oxysentin		Aquamycine	
	BT	AT	BT	AT	BT	AT
DO (mg/l)	3.28- 3.52	4.68-6.24	4.12-5.98	4.5-6.10	3.99-5.20	5.1-6.5
pH	6.50-6.82	7.48-7.98	6.58-7.90	7.20-7.45	6.0-7.2	7.12-7.5
Total alkalinity (mg/l	80- 95	125-140	85-98	98-111	84-96	111-120
Ammonia (mg/l)	0.32-0.58	0.05-0.08	0.09-0.13	0.03-0.06	0.09-0.11	0.03-0.06

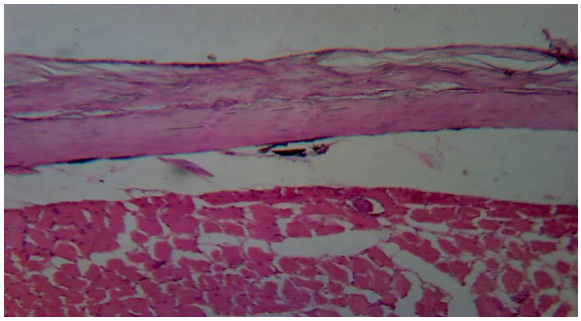
Histopathological study of different organs of Koi, *Anabas testudineus*



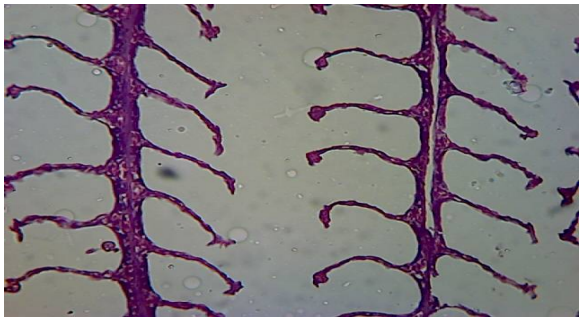
Kidney



Liver



Muscle



Gill

Table: 6.7 Growth and survival (±SD) of (*A. testudineus*) under different **antibiotics treated pond with commercial feed**

Parameters	Renamycine	Oxysentin	Aquamycine	Control
Initial Body weight(gm)	6.26±0.27	6.26±0.28	6.26±0.23	6.26±0.29
Final weight Body (gm)	98.30±0. 45[a]	84.45±0. 35[b]	83.10±0.45[b]	73.18 ±0.48 [b]
Weight gain (gm)	92.04±0.37[a]	78.55±0.25 [b]	77.04±0.38[b]	67.53±0.39 [c]
Weight gain (%)	1470.28±0.33	1256.80±0.30	232.67±0.32	1168.10±0.38
Specific Growth Rate (SGR) (% day)	2.29±0.35	2.17±0.34	2.16 ±0.36	2.26±0.38
Survival rate(%)	89.25±5.41	88.72±6.09	76.20±4.77	70.34±5.71

Statistical analyses were done to find-out the correlation and impact of different antibiotics on growth and production following the standard statistical package.

CHAPTER V11

DISCUSSION

Few studies have been conducted with the status of aqua-drugs and chemicals in Bangladesh but no such study was carried out with impact of aqua-drugs and chemicals on health and production of fish. The purpose of this work was to provide current status and impact of aqua-drugs and chemicals in aquaculture activities in Bangladesh. Aquaculture activities in Bangladesh region influenced by numerous aqua-drugs and chemicals. As a result different types of aqua-drugs and chemicals were frequently used in this sector.

The present study provided the impact of drugs & chemicals on water quality parameters, fish health & production. In the present study, it was found that most of the farmers used aqua-drugs and chemicals for pond preparation, health management and disease treatment. However, fish disease treatment was the major area where most of the aqua-drugs and chemicals were used. There are many purposes of the use of aqua-drugs and chemicals in aquaculture such as pond preparation, water treatment, health management, enhancement of natural aquatic productivity and growth promotion (Subasinghe *et al.*, 1996). This research was also done maintaining water quality, disease free, and high fish production by using water quality treatment drugs.

Alam (2011) found that commonly used chemicals in aquaculture were lime, salt, urea, triple Super phosphate (TSP) , potassium permanganate, vitamins, antibiotics (mainly oxytetracycline and chlorotetracycline), rotenone, phostoxin, sumithion, melathion and some hormones. The author observed that for treatment of water quality Timsen, Geo prime, Geo fresh, Geotox is commonly used in Bangladesh.

Geotox, Geolite gold, Mega-geo plus and Miracol lime mila were widely used by farmers in study area. Most of the farmers used Geolite gold whose active ingredients were SiO_2, Al_2O_3, Fe_2O_3, TiO_2 and MgO. Faruk *et al.* (2008) mentioned that Geotox, JV Zeolite, Timsen, Green Zeolite, Pontox plus, Zeolite, Zeo care, Mega Zeo, Bis Zeolite, Bio-Tuff, Well Zeolite and Aqua zet were found in Mymensingh region. The impact of water quality treatment drugs on physico–chemical parameters and fish production were discussed below.

Water quality parameters

The present investigation showed that fish farmers of different categories used various types of chemicals. Commonly used chemicals found in the present study were lime, salt, oxygen

suppliers, disinfectants, growth promoters, antibiotics, pond preparation, fish poisons, insect killers and microbe's killer. Few previous studies also revealed the similar reports about the use of aqua-drugs and chemicals used in aquaculture of Bangladesh (Brown and Brooks, 2002; Phillips, 1996). There are problems associated with the use of chemicals. With the expansion of aquaculture

in Bangladesh, there has been increasing trend in using chemicals in aquatic animal health management. Commonly used chemicals in Bangladesh aquaculture are lime, rotenone, various forms of inorganic and organic fertilizers, phostoxin, salt, dipterex, antimicrobials, potassium permanganate, copper sulphate, formalin, sumithion, melathion etc. (Phillips, 1996; Hasan and Ahmed, 2002; Brown and Brooks, 2002; DoF, 2002 and Faruk *et al.,* 2005). These chemicals and drugs had impact on water quality parameters. The present study determined efficacy of water quality treatment drugs and their impact on water quality parameters.

Physical parameters

This study resulted after using of water quality treatment drugs in pond water transparency became increased. Before using of Timsen and after using Timsen transparency was 28.40±2.16cm and 31.27±3.06cm respectively. Before and after using of Geofresh, Geoprime, and Geotox water transparency was 27.73±1.75cm, 27.47±2.39cm, 28.67±2.23cm and 29.53±1.88 cm, 29.20±2.76cm, 30.07±2.52cm respectively. Among Timsen, Geoprime, Geofresh Geotox, Timsen was suitable to increase water transparency. Other three drugs provided more or less same result in case of transparency. Water transparency did not provided that water was more productive way turbid water was not suitable for aquaculture. Water quality treatment drugs increased water transparency which was suitable to aquaculture (Author observation).

After using drugs water temperature became reduced. In case of temperature before and after using of Timsen temperature was 28.41±3.36℃ and 28.25±3.38 ℃ respectively. Before and after using of Geofresh, Geoprime, and Geotox water temperature was 28.48 ± 2.77°C, 27.78 ± 2.09°C, 28.29 ± 2.98°C and 28.38 ± 2.73°C 27.71 ± 2.07°C, 28.27 ± 2.96°C respectively. This also provided suitable for fish culture, Swann (2009) described the suitable ranges of water quality parameters for aquaculture water temperature suitable for warm water species would be 24 to 32°C this study agreed to the present study. The author observed that Timsen & other three drugs maintained water temperature is 28.41 ± 3.36 is suitable to fish culture.

Chemical parameters

The present study showed after using of water quality treatment drugs pH, DO, phosphate, Nitrate, became increased, before and after using of Timsen pH was 6.93±0.25 and 8.12±0.58 respectively. Before and after using of Geofresh, Geoprime, and Geotox water pH was 6.62 ± 0.34, 6.49 ± 0.19, 6.42 ± 0.17 and 7.71 ± 0.31, 7.64 ± 0.14, 7.56 ± 0.18 respectively. Before and after using of Timsen DO was 3.72 ± 0.33mg/l and 4.56 ± 0.29 respectively. Before and after using of Timsen DO was 6.93 ± 0.25 and 8.12 ± 0.58 respectively. Before and after using of Geofresh, Geoprime, and Geotox water DO was 3.44 ± 0.28, 3.30 ± 0.16, 3.33 ± 0.26 and 4.14 ± 0.29, 4.07 ± 0.17, 4.06 ± 0.26 respectively. Before and after using of Timsen Total Alkalinity was 95.00 ± 8.84 and 119.40 ± 9.67 respectively. Before and after using of Geofresh, Geoprime, and Geotox water Total Alkalinity was 94.07±6.56, 93.33±8.01, 92.47±6.32 and 115.73±11.13 , 115.33± 6.97 , 110.33±9.19 respectively.

Than control water body, which was similar to Swann (2009), he described the suitable ranges of water quality parameters for aquaculture water temperature suitable for warm water species would be 24 to 32°C, dissolved oxygen content of water would be 5 mg/l, P^H would be 6.5 to 9.0, alkalinity would be at least 20 mg/l for recirculation system, nitrite-nitrogen would be 0.03 to 0.06 mg/l and nitrate-nitrogen would be 0.0 to 3.0 mg/l. It was found that ammonia, nitrite, alkalinity, dissolved oxygen, hardness and P^H ranged from 0.7 to 4.0 mg/l, 0 to 0.2 mg/l, 115 to 180 mg/l, 3.0 to 4.0, 100 mg/l and 7.3 to 8.2 during the study period after used of drug. This study agreed to the present research.

Timsen kept ammonia (0.01±0.01) level close to 0.1. Boyd (1998) reported that desired concentration of ammonia in an aquaculture pond should be <0.1 mg/l. He observed that ammonia was toxic to culture animals in the gaseous form and can cause gill irritation and respiratory problems.

Hossain (2002) stated that a huge cumulative amount of supplementary feed utilization (57.1 to 134.4 tons/ha/18 months) and high level of both plankton and fish biomass lowered the dissolved oxygen level to 0.7 mg/l but caused no fish mortality and formation of black soil at pond bottom was also minimum. This experiment agreed to Hossian (2002) because using of drugs oxygen level in case of Timsen (4.56±0.29) which was suitable for fish culture.

The author observed that using water quality treatment drugs P^H became increased that was similar to Chinabut *et al.* (1992), he stated that quick lime and slaked lime both have a very high pH and in addition to increased alkalinity, can have a sterilizing effect against disease.

The present experiment agreed to Hoq *et al.* (1996), he measured dissolved oxygen from 4.00 to 5.90 mg/L in chemical treated polyculture of Thai pangus with 7.50 months culture period, which was almost similar with the values of present experiment.

Authors observed that these drugs was acted as water treatment due to no disease was introduced & drugs containing active ingredients like aluminium sulfate, lime , ctc. GESAMP (1997) found that for soil and water treatments, alum (aluminium sulfate) at the rate of 10-20 mg/L, gypsum at concentrations of 250-1000 mg/L, lime at dose of 100-8000 Kg/ha, geolite at a dose of 100-500 Kg/ha. Antibacterial agent amoxicillin, nitrofuran, macrolides active against gram-positive bacteria. Used of sulphonamides to control diseases such as furunculosis, enteric red mouth disease and vibriosis.

The author observed after using of water quality treatment drugs DO, pH, Phosphate, and nitrate became increase and temperature became reduced. Which agreed to Hossain *et al.* (1999), he studied the growth and survival of the Indian major carps and found a negative correlation between temperature and dissolved oxygen, pH and carbon dioxide, dissolved oxygen and free carbon dioxide. In the present experiment.

The author was also agreed to DoF (1996), because drugs kept water suitable to aquaculture. In this research using of water quality treatment drugs showed DO level (4.56 ± 0.29) and pH level (8.12 ± 0.58) & DOF reported that dissolved oxygen and P^H of a suitable water body for fish culture would be 5.0 to 8.0 mg/l and 6.5 to 8.5 mg/l respectively.

Davis *et al.* (2009) described that feeds used for fish growth had some negative impact on water quality because feeds were also source of pollutant, which ultimately caused water quality deterioration and disease outbreak. This agreed to author due to use of drugs water remained optimum in case of quality.

Tamuli and Shanbhogue (1996) reported the efficacy of some commonly available chemicals in the treatment of anchor worm (Lernaea maelraensis) infection in India. The authors observed that efficacy of some commonly available chemicals in the treatment of water quality in Banladesh, among all Timsen was best in case of water quality.

Fish production

To determine efficacy of water quality treatment drugs fish production was considered as indicator of determination of water quality treatment drugs. Four drugs were treated in different

fish culture pond to determine efficacy of water quality treatment drugs. Among all water quality treatment drugs Timsen provided best result in fish production. Timsen showed (1955.64 ± 24.28 kg/ha/60 days) as net production which was higher than other drugs.

The present study provided higher fish production in drugs treated ponds where as Khaled and Mamun (1996) achieved production of *tilapia* as 339.39 Kg/ha, 600.00 Kg/ha and 624.24 Kg/ha over a period of three months in three earthen ponds by using two prepared and one commercial diets. Hussain *et al*. 2000 found tilapia production 125-140 kg/m^2 in cage system.

Nahid, S.A.A *et al*. reported tilapia production 13832kg/ha/ya. Production of Thai pangus in the control pond at BAU pond was higher (7328.16 Kg/acre) than in the treated ponds (6400.08 Kg/acre) which disagrees to this experiment. Whereas, production of Thai koi in the treated pond at BAU pond was higher (1471.92 Kg/acre) than that of control ponds (1296.00 Kg/acre) which agrees to the present study.

In the present study survival rate showed 85.00±1.73 which agreed to the present investigation at BAU ponds, survival rate of Thai pangus was recorded 85.67% and 81.67% in treated and control ponds, respectively. Sayeed *et al.* (2008) also found the survival rates of Thai pangus was 94 to 97% in nine earthen chemicals treated ponds with a period of 11 months which is more or less similar to this experiment. Islam (2009) studied survival rates (%) were 70.4, 80.75, and 72.25% for native koi, Thai koi and other two hybrids respectively.
Khan, *et al* 2011 reported that using of water treatment drugs mostly Geoprime was suitable to fish production that's disagrees to the present investigation because this experiment show Timsen was best. Faruk *et al.* (2008) mentioned that Geotox, JV Zeolite, Green Zeolite, Pontox plus, Zeolite, Zeo care, Mega Zeo, Bis Zeolite, Timsen, Bio-Tuff, Well Zeolite and Aqua zet were found in Mymensingh region but Faruk *et al.* (2008) did not mentioned among which was best this research showed Timsen was best for fish culture among Geo fresh, Geoprime,Geotox.

Histopathology

Histopathological study was done to know negative impact of water quality treatment drugs on different organ of fishes. In this study water quality treatment dugs did not show any remarkable changes to the different organ (liver, kidney, gill, muscle). Clinically the fishes of treated and control ponds did not show any remarkable changes which is similar to the present research, but another.

(Samsuzzaman.*et.al* 2011) disagreed to the author study, he reported histopathologically in the control treatments at both the BAU ponds and farmer's level ponds, skin, muscle, liver, kidney and gill of fish had almost normal structure the present experiment did not show any remarkable change to the different organ of fish, However, in the chemical treated ones, the above mentioned investigated organs of fishes had remarkable pathological changes like necrosis, hemorrhage, vacuum, pyknosis, necrosis, hypertrophy and partial loss of some parts. It was observed that loss of epidermis, necrosis, vacuum, haemorrhage and pyknosis were found in the skin and muscle layer of aqua-drugs and chemical treated fishes which may be occurred other chemicals and drugs except water quality treatment drugs.

Some important pathological changes such as haemorrhage, necrotic hepatocytes, pyknotic cells and vacuum were recorded in the liver of chemical treated fishes. Anderson *et al.* (2005) reviewed that malachite green is readily absorbed by fish tissue and is metabolically reduced to leucomalachite green (LMG) which is lipohilic and can be stored in edible fish tissues for extended periods of time.

Ahmed *et al.* (2009) also found similar result for freshwater eel in winter season which was occurred due to antibiotic treatment. This disagreed to the present study.

Ahmed *et al.* (2007) found that necrosis, pyknosis, inflammation, hypertrophy, hyperplasia, missing of gill lamellae in the months of December and January in *Anabas testudineus*. The author agreed to Ahmed *et al.* (2007) the author did not found any remarkable changes in the organ of fishes by using water quality treatment drugs and culture period was april to June.

Haemopoeitic necrosis, hemorrhages, vacuolation in haemopoeitic cell were common pathological changes in kidney of chemical treated fishes. Ahmed *et al*. (2009) also observed necrosis, vacuums, hemorrhage and blood cells in kidney tubule of *Anabas testudineus* during the month of November. Water quality treatment drugs using in aquaculture did not show any remarkable changes in the different organ of fishes.

In case of gills of aqua-drugs and chemical treated fish exhibited pathological changes which include hypertrophy, haemorrhage, missing of secondary gill lamellae and necrosis Ahmed *et al*. (2009).In this study gill had no remarkable change due to treated water quality treatment drugs.

Liver had highly necrotic hepatocytes, pyknotic and inflammatory cell during the months of December and January (Roy *et al*. 2006). Hossain *et al*. (2009) reported that severe necrosis of hepatocytes, pyknosis, vacuoles, fat droplets and hemorrhage were observed in small indigenous species during December and January. The author agreed to that research because the present

research duration is April to june. Ahmed *et al.* (2007) found that necrosis, pyknosis, inflammation, hypertrophy, hyperplasia, missing of gill lamellae in the months of December and January in *Anabas testudineus*. The author agreed to Ahmed *et al.* (2007) the author did not fiund any remarkable changes in the organ of fishes by using water quality treatment drugs during the culture of April to June.

CHAPTER VIII
SUMMARY AND CONCLUSION

Recommendations

Due to large demand of fish protein, it is the appropriate this to extend the production of fish. Now a day's farmers are interested to produce more fish in the water body, they culture fish with high stocking densities for this reason they use aqua drugs to protect fish from any disease. Among all the drug, Timsen was the best in all aspects (improve fish health and water quality). Other three drugs (Geo-tox, Geo-fresh, and Geo-prime) resulted more or less same result.

Among all the common drugs, Timsen is the best in all aspects (improve fish health and water quality). Other three drugs (Geo-tox, Geo-fresh, and Geo-prime) results more or less same result. Histopathological study do not show any negative changes on the organ of GIFT tilapia.These drugs may be used in aquaculture when ammonia becomes increased in pond, moreover it will also use to increase energy of water for fish culture.

Among all the drug, Timsen was the best in all aspects (improve fish health and water quality).

Other three drugs (Geo-tox, Geo-fresh, and Geo-prime) resulted more or less same result.

Among all the antibiotic Renamycine was the best in all aspects (improve fish health), other two antibiotic (Oxysentin and Aquamycine) are more or less same result However more elaborate studies are necessary to observe the effects of drugs and others with more bio-chemical parameters before making comments.

CHAPTER IX

Training:

Within the project period four training course has been completed in 2011-12 and 2012-13. Each training consisted 3-day long program on "Impact of Aqua drugs on fish culture practices in Bangladesh" One hundred twenty farmers (including women and unemployed youths) participated in the training course. During the training course, the trainee farmers were provided with the hands-on knowledge on improved fish culture and protocol of appropriate drug management techniques. Most of the trainees felt encouraged on the improved fish culture technique and expressed their interest of adopting it in their own farms. The trainees requested to the organizing authority to arrange more training with upgraded knowledge and technology. Details of training conpleted under the project have been furnished in Table 12.

Table 12. **Details of training conducted during the project period**

Details	**Year 2011-12**	**Year 2012—13**
Topic(s)	Impact of Aquadrugs on Fish culture	Impact of Aquadrugs on Fish culture
No. of trainings	02	02
No. of trainees	60	60
Duration	06 days (02 to 04 May 25 to-27 May)	06 days (11 to 1 3 May 18 to 20 June)

Field days:

Under the project one field day was implemented in 2012- 13. Fish farmers including women and unemployed youths, and at least twenty office staff, GO & NGO representative, have been participated on the occasion. After the inaugural session the participants were provided with the hands-on knowledge on improved fish culture and and protocol of appropriate drug management techniques. Most of the trainees felt encouraged on the drug management technique both in ponds and in fish hatchery. The participants were divided into groups to identify the problems and prospects of this sector in a focus group discussion (FGD) session. After group discussion open discussion was done on the problems identified from the group discussion. The participants have shown their keen interest about the ongoing Aqua drug research activities Details of field days have been shown below.

Venue **:** Farmer’s field (Haluaghat ,Mymensingh)

No. of field day **:** 01

No. of participants : 50

Duration **:** From 09.00 am to 05.00 pm

Fig; Course cordinator discuss with the trainee

Fig- Teacher teaches in training class

REFERENCES

Ahmed, G.U. and E.S.P. Tan. 1992. The responses to tetracycline treatment of the epidermis of injured catfish (*Clarias macrocephalus*) raised under intensive culture conditions. *Aquaculture*, 105: 101-106.

Ahmed, G.U. and M.A. Hoque.1999. Mycotic involvement in epizootic ulcerative syndrome of freshwater fishes of Bangladesh: A histopathological study. *Asian Fish. Sci.*, 12: 381-390.

Ahmed, G.U., M. Dhar, M.N.A Khan and J.S. Choi. 2007. Investigation of disease of Thai koi, *Anabas testudineus* (Bloch) from farming conditions in winter. *J. Life Sci.*, 17 (10): 1309-1314.

Ahmed, M. and M.A. Rab. 1995. Factors affecting outbreaks of epizootic ulcerative syndrome in farmed and wild fish in Bangladesh. *J. Fish. Dis.*, 18: 263-271.

Ahmed. G.U., M.M. Hossain and M.M. Hassan. 2009a. Seasonal variation of disease and pathology of a perch, *Nandus nandus* (Hamilton) from Oxbow Lake fisheries of Bangladesh. *Eco-friendly Agril. J.*, 2 (8): 761-767.

Akhteruzzaman, M. 1999. A study on the production of Thai Koi (*Anabas testudineus)* under semi-intensive culture system. *Bangladesh J. Zool.*, 25: 39-43.

Alam. 2011 The efficacy and performances of three most commonly used Eon Animal Health Products.

Alderman, D.J. 1992. Malachite green and alternatives as therapeutic agents. *Europ. Aquacult. Soc. Spec. Pub*., 16: 235-44.

Anderson, D.I. and B.R. Levin. 1999. The biological cost of antibiotic resistance. *Curr. Opin. Microbiol.*, 2: 489-493.

Anderson, W.C., S.B. Turnipseed and J.E. Roybal. 2005. Quantitative and confirmatory analyses of malachite green and leucomalachite green residues in fish and shrimp. *USFDA Lab. Inform. Bull*. LIB No. 4363. 21 (11): 12-15.

Davis. 2009 impact of feeds on fish growth, water quality & disease outbreak of fish health.

Brown, D. and A. Brooks. 2002. A survey of disease impact and awareness in pond aquaculture in Bangladesh, the Fisheries and Training Extension Project- Phase 11. In: Arther, J.R., M.J. Phillips, R.P. Subasinghe, M.B. Reantaso and I.H. MacRae (eds.). Primary Aquatic

Animal Health Care in Rural, Small Scale and Aquaculture Development. *FAO Fish. Tech. Pap.*, No. 406. pp. 85-93.

Bart, A., M.M. Haque and M.A. Wahab. 2004.Technologicalconstrains and future of tilapia culture in Bangladesh.Abstract of a workshop held in BRAC in, Dhaka, Bangladesh.

Boyd. (1998) reported that desired concentration of ammonia in an aquaculture pond should be <0.1 mg/l.

Good water quality in fish ponds is essential for survival and adequate growth Burford, 1997

Chinabut, S. and J. H. Lilley. 1992. The distribution of EUS lesions on snakehead fish, *Channa striatus* (Bloch). *AAHRI Newsletter*, 1 (2): 1-2.

DoF (Department of Fisheries). 2011. Jatiyo Matshya Soptaho. Ministry of Fisheries and Livestock, The Government of Peoples republic of Bangladesh, Dhaka. pp. 56.

Faruk, M.A.R., N. Sultana and M.B. Kabir. 2005. Use of chemicals in aquaculture in Mymensingh district, Bangladesh. *Bangladesh J. Fish.*, 29(1-2): 1-10.

Faruk, M.A.R., M.J. Alam, M.M.R. Sarker and M.B. Kabir. 2004. Status of fish disease and health management practices in rural freshwater aquaculture of Bangladesh. *Pakistan J. Biol. Sci.*, **7** (12): 2092-2098.

Faruk, M.A.R., M.M. Ali and Z.P. Patwary. 2008. Evaluation of the status of use of chemicals and antibiotics in freshwater aquaculture activities with special emphasis to fish health management. *J. Bangladesh Agril. Univ.*, **6** (2): 381-390.

Floyd, R.F. 1993. The Veterinary Approach to Game Fish. Pergamon Press. pp. 395-408.

GESAMP, (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). 1997. Towards safe and effective use of chemicals in coastal aquaculture. *Rep. Stud.* (IMO/ FAO/ UNESCO/ IOC// WMO/ WHO/ IAEA/ UN/ UNEP). pp. 40.

Hoq, M.E., M.M. Islam and M.M. Hossain. 1996. Polyculture of freshwater prawn (*Macrobrachium rosenbergii*) with Chinese and Indian carps in farmer's pond. *J. Aquacult.*, 57: 135-141.

Hussain, M.G. 2009. A future for the tilapia in Bangladesh. AQUA Culture AsiaPacific Magazine. pp 38-40.

Islam. 2009 comperative survival rates (%)of native koi, & Thai koi and other two hybrids respectively.

Khan, M.R., M.M. Rahman, M. Shamsuddin, M.R. Islam and M. Rahman. 2011. Present status of aqua-drugs and chemicals in Mymensingh district. *J. Bangladesh Soc. Agric. Sci. Technol.*, **8** (1&2): 169-174.

Mazid, M.A. 2001. Fish diseases and prevention. Bangladesh Fisheries Research Institute (BFRI). Mymensingh. pp. 36.

Philips, M. 1996. The use of chemicals in carp and shrimp aquaculture in Bangladesh, Cambodia, Lao PDR, Nepal, Pakistan, Sri Lanka and Vietnam. In: J.R. Arthur, C.R. Lavilla-Pitogo, R.P. Subasinghe (eds.). Use in aquaculture in Asia. Southeast Asian Fisheries Development Centre, Aquaculture Department Tigbauan, Iloilo, Philippines. pp. 75-85.

Prasad, Y., S.A. Mastan, T.A. Qureshi and C.H. Samuel. 1996. Effect of different antibiotic on EUS affected fish *Channa striatus* (Bloch). In: The Fourth Indian Fisheries Forum. Asian Fisheries Society, Indian Branch. pp. 191.

Rahman, M.M. and M.B.R. Chowdhury. 1999. Incidence of ulcer disease in African catfish (*Clarias gariepinus*) and trial for its chemotherapy. *Bangladesh J. Fish. Res.***,** 3: 193-200.

Subasinghe, R.P., U. Barg and A. Tacon. 1996. Chemicals in Asian aquaculture: need, usage, issues and challenges. In: Arthur, J.R., C.R. Lavilla-Pitogo and R.P. Subasinghe (eds.). Use of Chemicals in Aquaculture in Asia. Southeast Asian Fisheries Development Center, Aquaculture Department Tigbauan, Iloilo, Philippines. pp. 1-6

Sayeed, M.A.B., G.S. Hossain, S.K. Mistry, and K.A. Huq. 2008. Growth performance of Thai Pangus (*Pangasius hypophthalmus*) in polyculture system using different supplementary feeds. *Rajshahi Univ. J. Zool.*, 27: 59-62.

Tamuli, K.K. and S.L. Shanbhogue. 1996. Efficacy of some commonly available chemicals in the treatment of anchor worm (*Lernaea bhadraensis*) infection. *Environ. Ecol.*, 14 (2): 259-267.