8.4.3 *Heteropneustes fossilis* disease

8.4.4 Clinical signs and symptoms of *H. fossilis* disease

Grayish white spot and slight lesion on skin (Fig. 4), reduce mucus, sometimes eroded lesion in caudal region (Fig. 5), hemorrhage with bacterial and fungal infection, whirling with up and down movement in water and high mortality in short period were observed.

8.4.5 Identification of bacteria and parasites

Aeromonas hydrophila and Pseudomonas spp. were isolated from the diseased *H. fossilis* using by API 20E bacterial diagnostic kit. Parasite *Trichodina* spp. was also found in diseased *H. fossilis*.

Fig. 4 Grayish white spot on skin

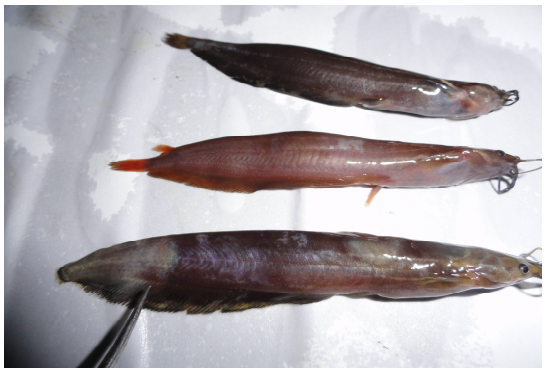

Fig. 5 Eroded lesion in caudal region

8.4.6 Treatment trials in aquarium, mini pond and farmer's pond

Diseased *H. fossilis* were treated with chlorotetracycline @ 1g + vit.C 1 tab./kg feed for 7 days with water exchange. Again, single dose of salt 200 g/dec and lime 200 g/dec were applied in the aquarium/pond. Recovery rate was found 50% in aquarium, 45% in experimental mini pond and 35% in farmer's pond.

8.4.7 Pangasianodon hypophthalmus disease

8.4.8 Clinical signs and symptoms observed

External, haemorrhage or inflammation occurs in the skin under the jaw (Fig. 6), on the operculum and belly, infection spreads to the skull and skin, thus creating the hole in the head (Fig. 7), sometimes spinning rapidly in circles and usually followed by death.

Fig. 6 Infection under jaw & the eye region

Fig. 7 Infection in skull

8.4.9 Identification of bacteria and parasites

Edwardsiella ictaluri was the causative agent that isolated from diseased *P. hypophthalmus* using by API 20E diagnostic kit. Parasites such as *Dactylogyrus* spp., *Gyrodactylus* spp. and *Trichodina* spp. were also found in the diseased fish.

8.4.10 Treatment trials in aquarium, experimental mini pond and farmer's pond

Diseased *P. hypophthalmus* were treated with oxytetracycline at the rate of 1g + vit.C 1 tab./kg feed for 7 days with water exchange. Again, single dose of potassium permanganate was applied at the rate of 10 g/dec in the aquarium/pond. Recovery rate was found 50% in aquarium, 46% in experimental mini pond and 38 % in farmer's pond.

8.4.11 *Anabas testudineus* disease

8.4.12 Clinical symptoms observed

Scale protrusion, deep ulcerative dermal lesion and tail rot were observed in diseased *A. testudineus*.

8.4.13 Identification of bacteria and parasites

Flavobacterium spp. was isolated from tail and fin rot diseased *A. testudineus*. Parasites such as *Dactylogyrus* spp. and *Trichodina* spp. were also found in the diseased fish.

8.4.14 Treatment trials in aquarium, experimental mini ponds and farmer's pond

Diseased *A. testudineus* were treated with oxytetracyclene at the rate of 1 g/kg + vit.C 1 tab./kg feed for 7 days with water exchange. Again, single dose of potassium permanganate was applied at the rate of 10 g/dec in the aquarium/pond. Recovery rate was found 48% in aquarium, 40% in experimental mini ponds and 30% in farmer's ponds.

8.4.15 Epidemic eye disease of *Catla catla*

8.4.16 Clinical signs and symptoms observed

Eyes of the diseased *C. catla* fish were the primary organs affected by the disease (Fig. 8).

Fig. 8 Infected eye in *C. catla* at primary stage

Fig. 9 Hollow eye-cup punctured walls in *C. catla*

In early stage of the disease, a reddish color develops in the cornea due to vascularisation, no haemorrage occurs, subsequent, the whole of the cornea turns milky white and becomes opaque (Fig. 9), finally gills fades and float on the surface of the water.

8.4.17 Identification of bacteria

Cultures prepared from the infected eyes and optic nerves showed that the etiological agent of the disease was a bacterium, which had been identified as *Aeromonas* sp.

8.4.18 Histological changes in different organs of diseased fish

Histopathological changes of different organs like muscle (Fig. 10), gill, kidney and liver (Fig. 11) of diseased *H. fossilis*, *P. hypophthalmus*, *C. catla* and *A. testudineus* were observed. Necrosis, vacuum, hemorrhage, pyknosis and fungal granuloma were observed in different organs of diseased fish.

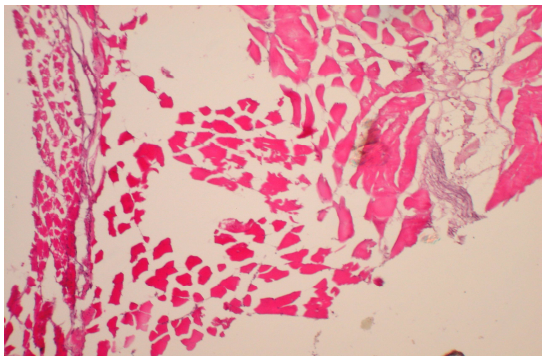

Fig. 10 Infected muscle of *H. fossilis*

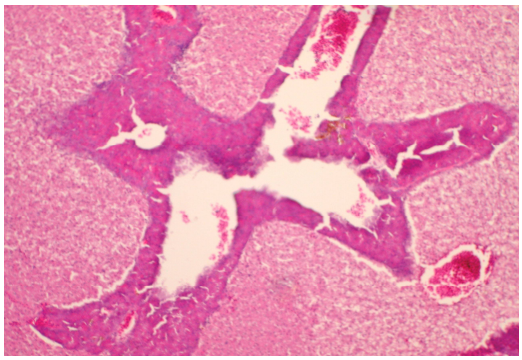

Fig. 11 Infected liver of *P. hypophthalmus*

8.4.19 Argulosis in fish

The parasite, *Argulus* sp., known as "fish lice" and sucking blood, is flat and more looks like a plate (Fig. 12). Hurt fish body with the help of cytolytic enzymes, in addition to the skin, ticks are also frequently found in fish gills under the cap. Almost all freshwater fish species are susceptible to this parasite infection. In high-intensity attacks, adult fish can suffer death from lack of blood.

Clinical Symptoms

Visually it looks like a flea parasites that attach to the body of the fish. accompanied by bleeding around the bite. Skin irritation, loss of balance, swimming in a zig-zag, jump into the water and rubbed his body on hard objects around him.

Diagnosis

Visually looks a parasite that attach to the fish body.

Control

Argulosis can be controlled by draining of the pond, followed by calcification. It can also be controlled by soaking in different chemicals and drugs. Soaking can be done by:

i. Dylox solution at doses of 0.25 ppm for 24 hours or more in the pool.

ii. Solution of Ammonium chloride (NH4CI) at a dose of 1.0 -1.5% for 15 minutes, or table salt at doses of 1.25% for 15 minutes.

iii. Dichlorvos solution 0.2 mg / L for 24 hours or more, every week for 4 consecutive weeks.

iv. Kitchen Salt 500 - 1000 ppm for 24 hours or more, repeated every week for 4 times giving

v. Potassium permanganate (PK) 2-5 mg / L for 24 hours or more.

Fig. 12 Adult *Argulus* sp.

9. Determination on Efficacy of Selective Chemicals and Drugs Used in Aquaculture in Bangladesh

9.1 Executive Summary

Three different experiments were conducted in the Bangladesh Fisheries Research Institute at different durations. 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 and enterpreneurs. From the findings it could be suggested that 1.5 times higher dose than recommended dose of each drug ,water treatment compound (Timsen, Geoprime, 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 experiment was conducted to determination the efficacy of common selected water treatment drugs for three months. Experiment was designed into four treatments **(**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 (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 the drugs tested, Timsen was found best in all aspects of improving fish health and water quality. Other three drugs (Geotox, Geo-fresh and Geo-prime) showed similar results in improving water quality in ponds.

The second experiment was conducted, in two ways one in laboratory condition and another one in experimental ponds of the Freshwater Station. First one was 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 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, *O. niloticus* fry (ABW: 3.33 g, 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 experiment, feeding trails with antibiotic mixing feed was carried out 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 100 fish (ABW 2.18±0.83 g) per cistern. Three selective antibiotics were tested and each had three replications. The fish were individually weighed at the starting of the experiment and biweekly fish sampling was also 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.

After using antibiotics 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 found suitable for fish health and culture. Ammonia became reduced due to application of drugs. Fish production was also 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 found best in all aspects of improving fish health and water quality. Other two drugs (Oxycentine and Aquamycine) showed similar results in treating fish diseases. However more elaborate studies are necessary to determine the efficacy of chemical, drugs and others with more bio-chemical parameters.

9.2 Background and 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 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.

9.3 Literature Review

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).

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 products such as 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 parameters was recorded after each hour for three hours.

A range of chemicals including antibiotics are applied 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

are oxytetracycline, sulphadiazine, chlorotetracycline, sulphamethoxazole, amoxicillin and co-trimoxazole (Faruk *et al*. 2008). According to Swann (2009) suitable ranges of water quality parameters for warm water species would be 24 to 32°C, dissolved oxygen content 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 feed used for fish growth had some negative impact on water quality because feed 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 used for controlling fish disease was reviewed by Chinabut & 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. They found that the hybrids resembled the native koi in skin color with high growth performances like Thai koi. Ahmed & Rab (1995) suggested that application of lime to ponds during the culture period decreased the severity of EUS outbreaks.

Tamuli & 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 & 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.

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 and geolite at a dose of 100-500 kg/ha are suitable for treating soil and water of ponds (GESAMP 1997). Antibacterial agents such as amoxicillin, nitrofuran and macrolides are active against gram-positive bacteria. Sulphonamides are used to control diseases such as furunculosis, enteric red mouth disease and vibriosis. Rahman & 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 & Brooks (2002) reviewed that 52% farmers in Bangladesh use potassium per-manganate, while 40% use lime, 11% use salt as a disease treatment. A few farmers use 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 and polyrnyxin have a significant bacteriostatic effect on *Pseudomonas* sp.

Oxytetracycline (OTC) is one of the most frequently used antibiotics in aquaculture, although negative side-effects were reported in some cases (Tafalla (1999). 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 are producing and marketing aqua-drugs and chemicals in Mymensingh region. Aqua-drugs 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. Liver had highly necrotic hepatocytes, pyknotic and inflammatory cell during the months of December and January (Roy *et al*. 2006). Skin, muscle, liver, kidney and gill of fishes had remarkable pathological changes like necrosis, hemorrhage, vacuum, pyknosis, necrosis, hypertrophy and partial loss of some parts due to chemical treatment Samsuzzaman *et.*a*l* (2011). 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 are lime, rotenone, various forms of inorganic and organic fertilizers, phostoxin, salt, dipterex, antimicrobials, potassium permanganate, copper sulphate, formalin, sumithion and melathion (Phillips 1996, Hasan & Ahmed 2002, Brown & 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 and adverse effect on species biodiversity, which ultimately affect the cultured species, human and environment. Several of these aspects have been well documented (Anderson & Levin 1999, Tendencia & 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 & 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).

9.4 Methodology

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:

a. Water treatment compounds
b. Oxygen releaser
c. Antibiotics

Three drugs/chemicals in each category were selected and tested in the laboratory and pond condition. The selected drugs/chemicals are as follows (Table 13):

Table 13 Selected chemicals and drugs for test

Category	Name of Drugs/Chemicals
Water treatment compounds	Timsen, Geotox, Geoprime and 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 to achieve the objectives of the project.

9.4.1 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.

9.5 Results

9.5.1 Water quality monitoring

Throughout the experimental period, the water quality parameters were recorded weekly. Water quality measurement and sample collection were done 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 drugs.

9.5.2 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).

9.5.3 Collection and Selection of fishes for research

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

9.5.4 Feeding

Feeding of fingerling was maintained twice daily in the morning and 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.

9.5.5 Description of selected drugs

Different types of water treatment compounds (Table 14), oxygen releaser (Table 15) and antibiotics (Table 16) are applied to maintain water quality parameters at optimum level.

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

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

Antibiotics **:** Aquamycin, Captox and Oxysentin

Table 14 Water treatment compounds and doses prescribed by Manufacturer

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 (Sio_2, Al_2O_3, $Fe2O_3$, CaO, MgO, LoI, K_2O) (Square Pharmaceutical Ltd.)	24 kg/acre (Water depth: 3-6ft)	10 kg/acre (Water depth: 3-6ft), every 4 weeks interval

Table 15 Oxygen releaser drugs and doses prescribed by Manufacturer

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

Table 16 Antibiotics and doses prescribed by Manufacturer

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

9.5.6 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 (^{o}C), 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.

9.5.7 Fish Sampling and harvesting

Sampling was done after 10 days interval by using a seine net to observe the growth of fish and to adjust the feeding rate. Small and inadequate sample of 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 g). The sampled fish were handled very carefully as the species are very susceptible to handling stress. Fishes were completely harvested after completing 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 a pump. During harvesting all fishes of each pond were collected and weighed individually to assess the survival rate and production.

9.5.8 Analysis of Plankton

9.5.9 Collection and preservation of plankton samples

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

9.5.10 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.

9.5.11 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)

9.5.12 Histopathological examination

For histopathological study, monthly sample was collected from various organs of fish 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) were used in the processor maintaining at various time schedules as mention below (Table 17).

Table 17 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 40^0C. 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 (37^0C) 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 18.

Table 18 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.

9.5.13 Description of study area (Water treatment compounds)

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

9.5.14 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.

9.5.15 Pond 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 pond. The ponds were filled with underground water up to a depth of 1m.

9.5.16 Water quality parameters

Water quality parameters represented productivity of the pond. Productive pond provides higher fish production. Less productive pond is not suitable for aquaculture. For ideal fish culture pH should be ranged 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 0.01mg/L. Water quality parameters of three treatments have been presented in the following Table 19.

Table 19 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 (mgL^{-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 (mgL^{-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 (mgL^{-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 (mgL^{-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 (mgL^{-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 (mgL^{-1}), nitrate (mgL^{-1}), ammonia (mgL^{-1}), DO (mgL^{-1}) and phosphate (mgL^{-1}) were significantly changed in all the treated ponds. After applying drugs all the above mentioned parameters were as optimum for aquaculture. Only temperature had no change due to use of drugs in drugs treated ponds.

9.5.17 Physical Parameters

9.5.18 Temperature

Before using drugs the mean values of water temperature were 28.41 ± 3.36 ^oC, 28.48 ± 2.77^oC 27.78 ± 2.09 ^oC and 28.29 ± 2.98^oC, respectively. After using drugs the mean

values of water temperature were recorded as 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 treated ponds, respectively.

9.5.19 Transparency

Before using drugs the mean (± SD) values of transparency were 28.40 ± 2.16 cm, 27.73±1.75 cm, 27.47 ± 2.39 cm and 28.67 ± 2.23 cm, respectively while, after using drugs it was 31.27 ± 3.06 cm, 29.53 ± 1.88 cm, 29.20 ± 2.76 cm, and 30.07 ± 2.52 cm in Timsen, Geo-fresh, Geo-prime and Geo-tox treated ponds, respectively.

9.5.20 Chemical Parameters

9.5.21 pH (Hydrogen ion concentration)

Before using drugs the mean (± SD) values of pH were 6.93 ± 0.25, 6.62 ± 0.34, 6.49 ± 0.19 and 6.42 ± 0.17, respectively. After using drugs the mean (± SD) values of pH were recorded as 8.12 ± 0.58, 7.71 ± 0.31, 7.64 ± 0.14 and 7.56 ± 0.18 in Timsen, Geo-fresh, Geo-prime and Geo-tox treated ponds, respectively.

9.5.22 Total Alkalinity

Before using drugs the mean (± SD) values of total alkalinity were 95.00 ± 8.84 mg l^{-1}, 94.07 ± 6.56 mgL^{-1}, 93.33 ± 8.01 mgL^{-1} and 110.33 ± 9.19 mgL^{-1}, respectively. After using drugs the mean (± SD) values of total alkalinity were recorded as 119.40 ± 9.67 mgL^{-} 115.73 ± 11.13 mgL^{-1}, 115.33 ± 6.97 mgL^{-1} and 110.33 ± 9.19mgL^{-1} in Timsen, Geo-fresh, Geo-prime and Geo-tox treated ponds, respectively.

9.5.23 Dissolved oxygen

Before using drugs the mean values of DO were 3.72 ± 0.33 mgL^{-1}, 3.44 ± 0.28 mgL^{-1}, 3.30 ± 0.16 mgL^{-1}, and 3.33 ± 0.26 mgL^{-1}, respectively. After using drugs the mean values of DO were 4.56 ± 0.29 mgL^{-1}, 4.14 ± 0.29 mgL^{-1}, 4.07 ± 0.17 mgL^{-1}, and 4.06 ± 0.26 mgL^{-1} in Timsen, Geo-fresh, Geo-prime and Geo-tox treated ponds, respectively.

Table 20 Production and survival of Tilapia in 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 (g)	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.

9.5.24 Gross production

After using drugs the mean (±SD) values of gross production were recorded as 835.38 ± 10.16 kg/ha, 648.58 ± 19.85 kg/ha, 629.34 ± 25.72 kg/ha and 649.57 ± 12.65kg/ha in Timsen, Geo-fresh, Geo-prime and Geo-tox treatments, respectively (Table 20, Fig. 13).