rate, increased gill mucus secretions, flaring of the gill arches, head shaking, and listlessness before death.

The main acute haematological response of rainbow trout and common carp to the effects of pyrethroid was a significant change in the RBC, Hb, MCV, MCHC, lymphocyte, and segmented neutrophilic granulocyte counts. The reduction in RBC count and PCV value and the higher erythrocyte haemoglobin of fish can be attributed to haemodilution due to damage of organs and changes in the haematological parameters PCV, RBC, and Hb, which can be interpreted as a compensatory response to increase the O_2 carrying capacity of the blood to maintain gas transfer, also indicating a change of the water-blood barrier for gas exchange in gill lamellae. Haematological results indicated decrease in nonspecific immunity (Velisek *et al*. 2011). A decrease in PCV, Hb, Leuko and RBC has been reported in carp after poisoning with cypermethrin (Dorucu & Girgin, 2001), and a decrease in total leukocyte count and neutrophil granulocyte count was observed in carp following acute poisoning with permethrin (Sopinska & Guz 1998).

The change in blood GLU concentration after pyrethroid exposure demonstrated the response of exposed fish to metabolic stress. Cypermethrin caused an increase in plasma NH_3 levels, presumably due to an increase in amino acid catabolism and a failure of ammonia excretion mechanisms. Increased NH_3 concentration indicates organism inability to convert the toxic ammonia to less harmful substances (Velisek *et al*. 2011). An enhanced energy demand caused by short-term pyrethroid stress stimulates the activity of glutamate dehydrogenase (GDH) which induces glutamate fission into ammonia and α-ketoglutaric acid utilized in the TCA cycle. The enzymes used for the purpose are LDH, CK, and transaminases AST and ALT. A significant increase in the activity of the above mentioned plasma enzymes indicates stress-related tissue impairment. Increased activity of transaminases indicated amplified transamination processes (Velisek *et al*. 2011). An increase in transamination occurs with amino acid input into the TCA cycle to cope with the energy crisis during pyrethroid induced stress (Philip *et al*. 1995). The changes in LDH level indicated metabolic changes, i.e. glycogen catabolism and glucose shift to the formation of lactate in stressed fish, primarily in the muscle tissue (Velisek *et al*. 2011). Jee *et al*. (2005) found an increase in levels of serum glutamic-acid-oxylacetic-acid-transaminase, glutamic-acid-pyruvic-acid-transaminase, GLU, and ALP and a decrease in the concentration of plasma TP, ALB, cholesterol, and lysozyme in Korean rockfish (*Sebastes schlegeli*) exposed to cypermethrin. Balint *et al*. (1995) observed an increase of GLU in *Cyprinus carpio* after exposure to deltamethrin. Atamanalp *et al*. (2002) found changes in the concentration of Ca^{2+} and phosphorus in rainbow trout following cypermethrin exposure.

Teleangioectasia of secondary lamellae of the gills and degeneration of hepatocytes in periportal zones in rainbow trout were observed after cypermethrin exposure (Velisek *et al*. 2011). Teleangioectasia indicate acute respiratory distress. Sarkar *et al*. (2005) found significant changes such as hyperplasia, disintegration of hepatic mass, and focal coagulative necrosis in *Labeo rohita* exposed to cypermethrin. Edwards *et al*. (1986) reported acute toxicity symptoms of cypermethrin in rainbow trout including gill flailing, hyperactivity, loss of buoyancy, and inability to remain upright. Cengiz (2006) observed histopathological effects of deltamethrin on the gill (desquamation, necrosis, aneurysm in secondary lamellae, lifting of the lamellar epithelium, oedema, epithelial hyperplasia, and fusion of the secondary lamellae) of common carp after acute exposure in concentration of 0.029 and 0.041 mg/L.

Acute effects of pyrethroid pesticides in fish include damage of gills and behavioural changes. Because fish are highly lipophilic, pyrethroids are likely to be strongly absorbed by the gills, even from water containing low levels of pyrethroids. Degeneration of hepatocytes in periportal zones can imply the influence of toxic compounds in the digestive tract. The biochemical changes in liver profile may also be related to hepatocyte damage (Velisek *et al*. 2011). Significant changes such as hyperplasia, disintegration of hepatic mass, and focal coagulative necrosis were found in *Labeo rohita* exposed to cypermethrin (Jee *et al*. 2005). Velisek *et al*. (2009b) reported accelerated respiration and loss of movement coordination in rainbow trout and carp following acute poisoning with metribuzin. These characteristics have also been reported in *Oreochromis niloticus* and *Chrysichthyes auratus* (Hussein *et al*. 1996) and in *Carassius auratus* by Saglio & Trijasse (1998) following acute poisoning with atrazine. Movement imbalance in freshwater fish (*Labeo rohita, Mystus vittatus* and *Cirrhinus mrigala)* exposed to simazine and cyanazine has been reported by Dad & Tripathi (1980). Oropesa *et al*. (2009) reported respiratory distress such as rapid ventilation, increased rate of gill cover movements, and floating at the surface of water in common carp after exposure to simazine.

Biometric parameters of common carp exposed to simazine in concentrations of 2 and 4 µg/L showed increases in HSI relative to controls (Velisek *et al*. 2011). Biometric parameters are regarded as general indicators of fish health and the quality of the aquatic environment. The hepatosomatic index is a non-specific biomarker influenced by factors such sex, season, disease, and nutritional level (Velisek *et al*. 2011). Dewey (1986) reported reduction in body weight and length and decrease of condition in brook trout (*Salvelinus fontinalis*) exposed 306 days to atrazine at a concentration of 120 µg/L. Davies *et al*. (1994) observed growth rate reduction in the inanga (*Galaxias maculatus*) following exposure to low concentrations of atrazine.

Chronic exposure to terbutryn at 0.2 and 2 µg/L resulted in a significant increased in plasma GLU concentration, demonstrating the response of exposed fish to metabolic stress (Velisek *et al*. 2011). Mekkawy *et al*. (1996) observed increases in GLU levels in Nile tilapia (*Oreochromis niloticus*) and catfish (*Chrysichtheys auratus*) after atrazine exposure at 3 mg/L. Chronic exposure to simazine at 0.06, 2, and 4 µg/L resulted in a significant decrease in plasma ALP activity. The source of ALP includes synthesis in the intestinal epithelium, kidney, and liver and is often increased in response to a biliary obstruction. Velisek *et al*. (2008) also reported decreased ALP in rainbow trout after acute exposure to metribuzin. Chronic exposure to terbutryn at 0.2 and 2 µg/L resulted in significant increase in plasma AST and LDH activity. Chronic exposure to simazine at 2 and 4 µg/L resulted in a significant decrease in plasma ALT activity.

LDH is the terminal enzyme of anaerobic glycolysis and therefore of crucial importance in muscle physiology, particularly in conditions of chemical stress when high levels of energy may be required for a short period of time (Monteiro *et al*. 2007). The increase in LDH level indicated metabolic changes, i.e. glycogen catabolism and a glucose shift towards the formation of lactate, primarily in muscle. A significant change in the activity of plasma enzymes LDH and the transaminases ALT and AST indicates stress-based tissue impairment. Change in activity of transaminases indicates amplified transamination processes. An increase in transamination occurs with amino acid input into the TCA cycle to cope with the energy crisis during pesticide stress (Velisek *et al*.

2011). Chronic exposure to simazine at 4 μg/L resulted in significant decrease in plasma TP and ALB concentration (Velisek *et al*. 2011). In these circumstances, changes in serum protein concentration might arise from protein leakage from damaged tissue. Reduction of plasma protein and albumins with chronic exposure are related to the toxic effects of simazine on the immune system and/or the haemodilution effect, and may account for the pathological effects on caudal kidney (Velisek *et al*. 2011). Decrease of TP in atrazine exposed Nile tilapia and catfish was observed by Hussein *et al*. (1996) and Mekkawy *et al*. (1996). Davies *et al*. (1994) also observed a decrease in TP in rainbow trout after acute exposure to atrazine at a concentration of 50 μg/L. Velisek *et al*. (2009a) found a decrease in the activity of AST and an increase in GLU, NH3, LDH, CK, and CREA levels in common carp after subchronic exposure to simazine.

Simazine was associated with decreased leukocyte count relative to controls (Velisek *et al*. 2011). Leukocytes are involved in the regulation of immunological function and a protective response to stress in fish. The reduction in leukocyte count occurs through an alteration in lymphopoesis and/or altered release of lymphocytes from lymphoid tissues. Decrease in leukocyte count and the lymphopenia in carp exposed to terbutryn indicated a reduction in non-specific immunity (Velisek *et al*. 2011). Prolonged stress may have caused disruption of leukopoiesis, resulting in reduction in the total leukocyte count. Exposure to terbutryn was associated with the highest RBC value in fish exposed to the higher concentrations, when stress-induced RBC release from spleen to blood circulation was reported (Tort *et al*. 2002). Crain *et al*. (1997) showed that chloro-striazine herbicides have the ability to stimulate production of the enzyme aromatase, which converts androgens to oestrogens, and presumably could interfere with sex differentiation and development. Moore & Waring (1998) observed that an atrazine concentration of 3.6 μg/L altered plasma testosterone and, at 6.0 μg/L, affected KT in Atlantic salmon (*Salmo salar*). Triazine pesticides have a direct effect on kidney structure and function in freshwater fish (Velisek *et al*. 2008, 2009b). Caudal kidney of carp with chronic exposure to simazine showed destruction of the tubules (Velisek *et al*. 2011). Velisek *et al*. (2010) found cell shape changes and lipid inclusions in hepatocytes of common carp with subchronic terbutryn exposure in concentrations of 4, 20, and 40 μg/L. Similar alterations in liver were observed by Arufe *et al*. (2004), who exposed the larvae of gilthead sea bream (*Sparus aurata*) to terbutryn triasulfuron at a concentration of 2.5 mg/L for 72 h. Steatosis in liver of grey mullet (*Liza ramada*) (Biagianti-Risbourg & Bastide 1995) has been observed after atrazine exposure. Changes in metabolism of hepatic lipids and vacuolar degeneration of hepatocytes have been observed in various fish species exposed to herbicide such as clomazone (Crestani *et al.* 2007).

From the above literature, it is clearly indicated that chemicals and drugs play an important role in present aquaculture systems, whether they are intensive, semi-intensive, semi-closed or closed systems. Certain types of chemicals, if used inappropriately, can cause damage to aquatic animals and the environment. Improper use of pesticides /insecticides in rice field and their runoff from agriculture also affect water and sediment quality, alter microbial communities and biodiversity, kill non-target animals and plants, and affect the health of farm workers and consumers. Overuse of chemicals, especially antibiotics, not only increases production costs but also intensifies adverse consequences. The adverse impacts of chemical use in aquaculture may be outweighed by their advantages; however, there has been increasing concern about their uses, and they must be used with great caution. Impact of drugs on water, pond sediments, fish productivity and biodiversity should also be studied. Research should be focused not only on the effects of pesticides alone, but also on interactions of pesticides with other pollutants in environmental concentrations with long-term exposure, since the aquatic environment may be polluted by many substances, the effects of which can be potentiated with concurrent exposures. With these aims the present proposal has been made with the following objectives.

4. Objectives

i. To make check list of chemicals and drugs used in aquaculture in Bangladesh and identification of their sources

ii. To study the impact of drugs and chemicals on aquatic ecology

iii. To study the residual deposition of toxic chemicals and drugs in fish and shrimp

iv. To make awareness among the farmers/entrepreneurs regarding the toxic effect of chemicals/drugs in aquatic environment through training, workshop, publication etc

5. Research areas and Training programmes

Based on the demand and field requirements following research areas (Table 2) and training programmes (Table 3) were identified to fulfil the objectives.

Table 2: Identified Research Areas for the project of Impact Aquaculture Drugs and Chemicals on Aquatic Ecology and Productivity

Sl.	Research Areas
1	Assessment of degree of damage of microbial community due to use/over dose of chemicals and drugs in aquaculture
2	Survey on the available chemicals and drugs used in aquaculture and fish feed and make a checklist
3	Study on impact of aqua-drugs and chemicals on fish productivity and biodiversity
4	Determination of safety doses of aqua-drugs
5	Investigation on the sources of aquaculture chemicals and drugs
6	Efficacy of toxic aqua-drugs in respect of seasons
7	Study on the seasonal and regional variations of toxic drugs and chemicals in fish and shrimp
8	Investigation on the means through which the toxic drugs and chemicals gain access into shrimp/fish
9	Development of protocols for identification of unknown toxic component in water and fish
10	Identification of risk factors associated with diseases and development of health management strategies
11	Testing of local herbal drugs for shrimp/fish disease prevention and control
12	Evaluation of the present status, bio-chemical composition, shelf life, performance and effects of commercial fish and shrimp feeds and additives available in the country
13	Development of species-wise feed for the commercial fish and shrimp
14	Any other emerging issues (Station & Sub-Station, University)

Table 3 Training Programmmes for the project of Impact Aquaculture Drugs and Chemicals on Aquatic Ecology and Productivity

Sl. No.	Subject of Training	Trainees
1	Environment friendly aquaculture and quality fish production	DoF officers/ Entrepreneurs
2	Use of insecticides in pond preparation and their harmful effect	Fish farmers
3	Fish/shrimp disease diagnosis, prevention & control measures	Progressive Farmers
4	Safety doses of chemicals and drugs in aquaculture/rice field	Fish farmers/ Rice farmers
5	Microbial community in aquatic environment and their importance in aquaculture	Progressive Farmers
6	Soil -water characteristics for environment friendly aquaculture	Farmers/ Stakeholders
7	Proper fertilization and manuring for production of natural feed in pond	Fish/shrimp Farmers
8	Culture technique of live feed for shrimp	Shrimp farmers
9	Research Methodology, PPA 2008, Office management	BFRI officers/Staff
10	EU and USFDA guideline for safety aquaculture	Shrimp farmers
11	Impact of aqua-drugs on fish biodiversity	Fish farmers
12	Effect of toxic chemical on fish productivity	Fish farmers
13	Role of mass media to create awareness regarding the usage and effect of toxic chemicals	Journalists
14	Best management practices for maximizing fish production	Progressive Farmers
15	Improved technique for rice-cum-prawn culture	Progressive Farmers
16	Semi-intensive culture technique of fish without using drugs/chemicals	Progressive Farmers
17	Office management and financial rules	BFRI Staffs
18	Application and efficacy of herbal medicines in fish/shrimp disease control	BFRI scientists/ DoF Officials
19	Hazardous effect of insecticides in aquaculture	Fish farmers/Rice farmers

6. eneral Observations

6.1 Habitat degradation and aquatic life

Habitat is inversely related to aquatic life. Species diversity, density and production are directly related to the suitability of aquatic habitat. Degraded habitat usually harbour low number of fish species and produce low production. Each fish species has characteristic tolerances for habitat, water quality and other conditions. They have specific requirements for breeding, feeding, growth, recruitment and survival. Indeed, within each family, sets of species may be ranked for their tolerances. Some species are tolerant to the degradation of habitat while some are not. A single species may be highly tolerant of one form of pollution but intolerant to another.

In Bangladesh, *Tenualosa ilisha*, *Ailia coila*, *Clupisoma garua*, *Silonia silondia*, *Eutropiichthys vacha* and *Amblypharyngodon mola* are less tolerant to temperature changes while high tolerant species include *Anabas testudineus*, *Channa orientalis*, *Channa marulius*, *Channa punctatus*, *Channa striatus*, *Clarias batrachus*, and *Heteropneustes fossilis*. According to the limit of upper lethal temperature, the most tolerant species is goldfish (41°C) and the least tolerant species is grayling (24.1°C). Tolerance capacity of a species to pollution and environmental degradation depends on its genetic and physiological characters. Moreover, tolerance capacity varies with the nature and type of degradation (Varley 1967, Horoszewicz 1973). Tench, common carp, crucian carp, goldfish and 3-spined stickleback are highly tolerant of poor water quality whilst chub and barbel are less tolerant. Dace and grayling are classified as intolerant (Cowx *et al.* 1995). In terms of key water quality parameters, such as ammonia, dissolved oxygen, suspended solids, the major freshwater species of Bangladesh can be categorised according to their tolerance as follows (Fig. 3):

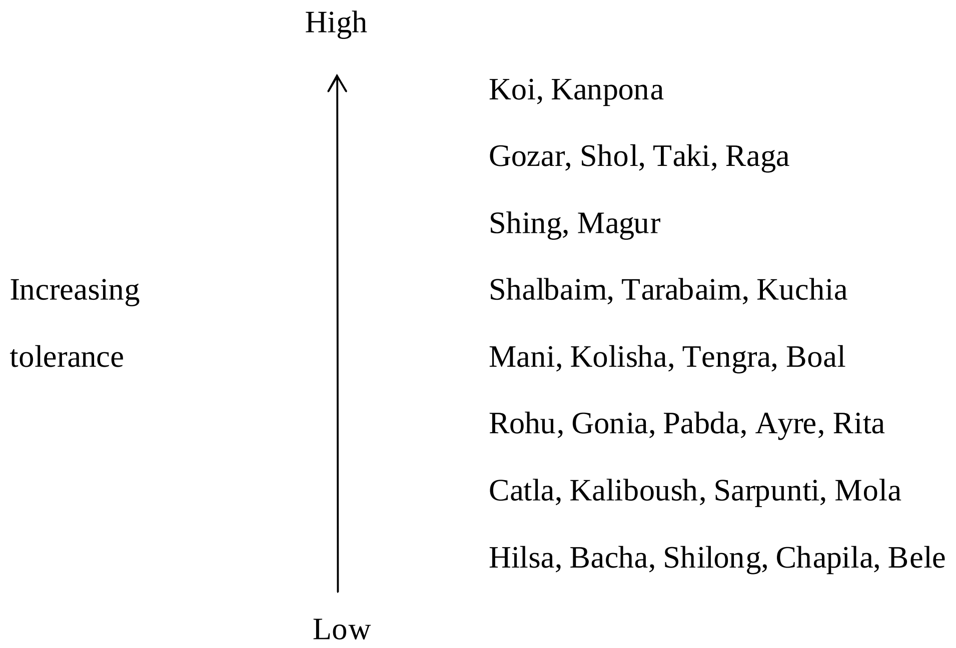

Fig. 3 Tolerance of fish to environmental degradation

6.2 Pollution due to agro-chemicals

Agro-chemicals are also responsible for pollution of river waters. Residues of many agro-chemicals (e.g., pesticides, inorganic fertilisers) washed down through surface run-off to the river systems, which also responsible for water pollution, and disappearance of many fish species and decreased fish production. Intensive use of agro-chemicals starts in the name of so called "Green Revolution" in early sixties when High Yielding Variety (HYV) of rice has introduced in former East Pakistan (Bangladesh). Agro-crop farmers were not trained before introducing such agro-chemicals. As a result farmers applied higher doses than prescribed; consequently, major carps, minor carps and many small fish species have disappeared from many beels and haors. Total fish production from beels and haors has declined severely. Rice production has increased in the country at the cost of disappearance of many fish species and loss of total fish production from beels and haors. In 1981-1982, a total of 5 578 t of different agro-chemicals were used in crop fields in Khulna division (Bhouyain 1983). About 30-40% of the input of fertilisers and agrochemicals in the form of insecticides are generally washed down the rivers during the rainy season (Haider 1988) causing water pollution. Fecundity of fish decreases up to 40% due to effect of pesticides and fertilisation and hatching rates decrease to 15% and 25%, respectively. Due to detrimental effects of agro-chemicals on aquatic biota, Government of Bangladesh banned different types of agro-chemical products (Table 4).

Table 4 List of banned agro-chemicals in Bangladesh

Sl. No.	Name of Pesticides	Registration Number
1	Aerovap 100EC	AP-41
2	Aerodriel 20EC	AP-42
3	Aeromal 57EC	AP-44
4	Actellic 2% dust	AP-99
5	Aerocypermethin	AP-137
6	Agridhan 3G	AP-154
7	Agrine 85WP	AP-187
8	Arifos 20EC	AP-299
9	Azodrin 40WSC	AP-336
10	Amcodrin 40SL	AP-340
11	Bizguard 2P	AP-09
12	Bidrin 24 WSC	AP-74
13	Bidrin 85 WSC	AP-80
14	Bkzne 14G	AP-135
15	Benicron 100WSC	AP-86
16	Cureterr 3G	AP-30
17	Cureterr 3G	AP-69
18	Chlordane 40WP	AP-40
19	Carbin 85 WP	AP-54
20	Carbaryl 85 WP	AP-147
21	Sekumetrin 10EC	AP-219
22	Cekuthoate 40 EC	AP-225
23	Cardan 5G	AP-234
24	Cythrin	AP-220
25	Chemo DDVP	AP-245
26	Corophos 40SL	AP-342
27	Diazinon 60EC	AP-23
28	Diazinon 14G	AP-236
29	Diazinon 14G	AP-08
30	Diazinon 90L	AP-20
31	Dankavapon 100	AP-13
32	Damfin 2P	AP-19
33	Damfin 950EC	AP-25
34	Diamal 57EC	AP-55
35	Dichlorvos 100	AP-57

Sl. No.	Name of Pesticides	Registration Number
36	Detia Gas EXT	AP-56
37	Dieldrin 20EC	AP-69
38	Dieldrin 50WP	AP-82
39	Dieldrin 40WP	AP-83
40	Draiwzon 60EC	AP-190
41	Devison Glyphosphate	AP-29
42	Dimecron 100SL	AP-22 & 276
43	DDVP 100WP	AP-03
44	DDVP 100EC	AP-151
45	Daman 100EC	AP-325
46	Darsban 20EC	PHP-5
47	Darsban 20EC	PHP-85
48	Delapon Na – 85	AP-66
49	Enthio 25EC	AP-64
50	Folithion USVC 98	AP-36
51	Fenitrothin 98	AP-53
52	Furadan 3G	AP-85
53	Fenkil 20EC	AP-169
54	Phoskil 40SL	AP-339
55	Gastoxin	AP-195
56	Heptachlor 40WP	AP-39
57	Kamex	AP-145
58	Kekthion 57EC	AP-178
59	Kadette 40WSC	AP-284
60	Luphos 40SL	AP-388
61	Methyl bron	AP-38
62	Methyl bromide 98	AP-58
63	Melathion 57EC	AP-68
64	Melathion 57EC	AP-78
65	Melathion 57EC	AP-230
66	Manex 2	AP-163
67	Melbromid 98	AP-185
68	Mebrom	AP-186
69	Morestan 25WP	AP-269
70	Manzate 200	AP-301
71	Megaphos 40SL	AP-175
72	Monophos 40WSC	AP-328
73	Monodrin 40WSC	AP-09
74	Monotaf 40WSC	AP-331
75	Macupex 65%	AP-65
76	Mortein King Mosquito Coil	PHP-54
77	Mortein King Mosquito Coil	PHP-101
78	Nogos 100EC	AP-26 & 274
79	Nuvacron 40SL	AP-98 & 275

Sl. No.	Name of Pesticides	Registration Number
80	Padan 10G	AP-52
81	Pillarcron 100SL	AP-22 & 276
82	Phosvit 100EC	AP-46
83	Polythion 50EC	AP-32
84	Paramound Cctype	AP-300
85	Phytox MZ80	AP-164
86	Poligor 40EC	AP-180
87	Quick phos	AP-102
88	Rentokill CCtype 75%	AP-221
89	Ridan 3G	AP-131
90	Rizinon 60EC	AP-239
91	Rexion 40EC	AP-11
92	Sunfuran 3G	AP-171
93	Torquue 550 G/L	AP-115
94	Tamaron 40SL	AP-188
95	2,4-DNA salb	AP-34
96	Tecto 2% Dust	AP-157
97	Uniflow TM Sulphar	AP-167
98	Vitacron 40SL	AP-341
99	Vapona	AP-79
100	Zithilon 57EC	AP-126
101	Zolone 35EC	AP-67
102	Zincphospide	AP-258

Source: Mazid & Haldar 2005

6.3 Pollution due to pest controlling chemicals

Currently, a total of 11 000 to 12 000 t of pesticides under 92 groups belonging to 380 types are used annually, mainly for rice, jute, tea, vegetable and sugarcane crops (Mazid & Haldar 2005) while in 1988, only 20 insecticides, 18 fungicides and two rodenticides were useed (ESCAP 1988, Showler 1989). Of the total, about 25% i.e., 2 000 t washed down to rivers, canals and beels through rainwater (Mazid & Haldar 2005). Although import and production of organo-chlorine pesticides like BHC, DDT, Disulfoton, Eldrin, Dieldrin, methoxychlor, lindane, ethyl and methyl parathion, phorate, telodrin and mercury compounds, except for sugarcane crop, have been banned due to their acute toxicity, environmental persistence, and bio-accumulation (Table 5).

Table 5 Different types of pesticides and their toxicity

Name	Trade name	Recommended dose (ha^{-1})	Mortality (%)after 96 h	Remarks
Karbaril	Sevin 85 SP	1.3 kg	-	Low toxic
Heptachlore	Heptachlore 40 P	4.5 kg	100	Highly toxic
Dieldrin	Dieldrin	4.5 kg	100	Highly toxic
Permethrine	Pounce 1.5 EC	3.4 kg	100	Highly toxic
Diazinon	Basudin 10 G	16.8 kg	100	Highly toxic
Dezomat	Basmid G	16.8 kg	100	Highly toxic
Carbophoran	Carbophoran 3G	16.8 kg	10	Relatively low toxic
Carbophoran	Furadan 5G	16.7 kg	10	Relatively low toxic
Carbosulphan	Marshal 6G	6.0 kg	-	Not toxic
Fenvelarate	Sumisidin 20 EC	250 ml	100	Highly toxic
Cypermethrine	Ripchord 10 EC	560 ml	100	Highly toxic
Cypermethrine	Simbush 10 EC	500 ml	100	Highly toxic
Deltametrhine	Decis 1.5 EC	500 ml	100	Highly toxic
Fenprapathrine	Danitol 10 EC	500 ml	100	Highly toxic
Phosphamidon	Dimacron 100 SCW	840 ml	18	Relatively low toxic
Dichlorvos	Nogos 100 EC	560 ml	5	Low toxic
Diazinon	Diazinon 60 EC	1.68 l	100	Highly toxic
Fenitrothion	Sumithion 50 EC	1.12 l	-	Low toxic
Malathion	Malathion 57 EC	1.12 l	-	Low toxic

Each year floodwater inundates about one-third of the total area of Bangladesh. The flood and rain waters carry a portion of the agro-chemical residues to the river, haor and beel systems for final discharge in the coastal regions. Most fish species cannot survive insecticides in concentrations higher than 1-10 ppb (parts per billion). In lethal doses, the run-off water kills fish and other aquatic organisms. A sub-lethal dose for fish may not bring immediate danger but in the long run, it will induce reproductive failure, decrease fecundity, early maturity, low hatching rate, low survival of spawn, total sterility, deform body and behavioural changes in fishes.

In sub-lethal doses, residues of pesticides accumulate in various parts of body of fish, prawn and other aquatic organisms. From fish and prawn, the residues go into the body of humans who consumed them causing various diseases. Uptake of organo-chlorines and mercury by three species of shrimp (*Penaeus indicus*, *Penaeus monodon* and *Metapenaeus monoceros*) and four species of finfish (*Tenualosa ilisha*, *Coila dussumerii*, *Johnius belangerii* and *Pampus chinensis*) from the Bay of Bengal and estuaries is presented in Table 6.

Table 6 Agro-chemical levels (ng g^{-1} dry weight of muscle) in marine and estuarine fishes and shrimps of Bangladesh
DDT= Di-chloro-di-phynyl-tri-chloro-ethene, DW= Dry weight, N= Number of samples

Species	N	% DW	PBC	DDT	Lindane	Heptachlor	Aldrin	Deildrin	Hg
P. indicus	10	245	134	4.24	29	127	9	15	252
P. monodon	10	505	16	58	5	25	2	0	68
M. monoceros	10	275	81	66	22	75	5	6	192
T. ilisha	10	395	202	307	56	394	11	0	80
C. dussumerii	10	18	113	138	31	190	24	0	17.8
J. belangerii	10	28	327	84	29	105	35	6	96
P. chinensis	10	22	100	68	23	36	11	6	201

6.4 Pollution due to chemical fertilisers

Intensive use of chemical fertilisers also starts in the name of "Green Revolution". Chemical fertilisers can increase agro-crop as well as aqua-crops, but indiscriminate use and large doses have negative impacts on aquatic as well as crop biota. About 1.6 million t of chemical fertilizers are used in Bangladesh. In aquatic environment, larger doses usually produce excessive phytoplankton and macrophytes and also have direct chemical effects. Beel and haor fisheries have declined severely due to indiscriminate and excessive use of chemical fertilisers. Residues of chemical fertilisers usually washed down to openwater systems with surface run-off and may cause eutrophication in rivers, beels and haors. In 1992-1993, a total of 2.32 million tons of inorganic fertilizers were used, which increased to 3.30 million tons in 2002-2003, an increment of 41.58%. Within that period increment of urea fertilizer was about 45%. Urea is very toxic to fish. Due to impacts of chemicals and drugs many fish species are going to be disappear from the waters of Bangladesh.

6.5 Threatened to disappeared fishes of Bangladesh

The IUCN made a list of threatened to disappeared fishes of Bangladesh. These species are considered as threatened fishes of Bangladesh. The National Categories of Threatened Animals are based on the Global Threatened Categories of IUCN. Bangladesh National Criteria were developed on the basis of qualitative data due to the lack of quantitative ones. The National Categories and Criteria are as follows:

6.5.1 Categories of status

Critically Endangered (CR): A taxon is Critically Endangered when it is facing an extremely high risk of extinction in the wild in Bangladesh in the immediate future (Table 7).

Endangered (EN): A taxon is Endangered when it is not Critically Endangered but is facing a very high risk of extinction in the wild in Bangladesh in the near future (Table 8).

Vulnerable (VU): A taxon is Vulnerable when it is not Critically Endangered or Endangered but is facing a high risk of extinction in the wild in Bangladesh in the medium-term future (Table 9).

Data Deficient (DD): A taxon is Data Deficient where there is inadequate information to make a direct, or indirect assessment of its risk of extinction in Bangladesh.

Not Threatened (NO): A taxon is Not Threatened when it is out of the four above-mentioned categories. i.e. which has no apparent threat of extinction in Bangladesh.

Table 7 Critically Endangered fish species in Bangladesh

Scientific name	English name	Local name
Bagarius bagarius	Gangetic goonch	Bagahair
Channa barca	Barca snakehead	Pipla shol, Tila shol
Clupeisoma garua	Garua bacha	Ghaura
Eutropiichthys vacha	Batcha bacha	Bacha
Labeo boga	Boga labeo	Bhangan, Bata
Labeo nandina	Nandi labeo	Nandina, Nandil
Labeo pangusia	Pangusia labeo	Ghora muikha, Longu
Pangasius pangasius	Pangas	Pangas
Puntius sarana	Olive barb	Sarpunti
Rita rita	Rita	Rita
Sisor rhabdophorus	Sisor catfish	Sisor
Tor tor	Tor mahseer	Mahashol

Table 8 Endangered fishes in Bangladesh

Scientific name	English name	Local name
Badis badis	Badis dwarf	Napit, Kio bandi
Barilius bendelisis	Hamilton's barila	Joia, Chedra, Koksa
Barilius bola	Indian trout	Bhol, Bol
Barilius vagra	Vagra baril	Koksa, Khoksa
Batasio tengana	Assamese batasio	Tengra
Bengala elonga	Bengal barb	Along, Sephatia
Botia Dario	Necktic Loach	Rani, Bou
Botia lohachata	Y-loach	Rani, Putul, Beti
Chaca chaca	Indian chaca	Chaga, Cheka
Channa marulius	Giant snakehaed	Gazar, Gajal
Chela laubuca	Indian glass barb	Laubuca, Kash Khaira
Crossocheilus latius	Gangetic latia	Kalabata
Dermogenys pussilus	Wrestling half beak	Ek thota
Labeo bata	Bata labeo	Bhangan, Bata
Labeo calbasu	Black labeo, Kalbasu	Kalibaus, Baus, Kalia
Labeo gonius	Laria labeo	Goni, Kurchi, Ghainna
Mastacembelus armatus	2-track spinyeel	Baim, Salbaim, Bam
Microphis deocata	Deocata pipefish	Kumirer khil
Mystus seenghala	Giant river catfish	Guizza, Guizza ayre
Notopterus chitala	Humped featherback	Chital
Ompok bimaculatus	Indian butter catfish	Kani pabda, Boali pabda
Ompok pabda	Pabdha catfish	Madhu pabda, Pabda
Ompok pabo	Pabo catfish	Pabda
Ptenops nobilis	Indian paradise fish	Neftani
Rasbora rasbora	Gangetic scissortail rasbora	Darkina, Leuzza darkina
Rohtee cotio	Cotio	Dhela, Dhipali, Keti
Scatophagus argus	Spotted scat	Bistara, Chitra
Silonia silondia	Silondia vacha	Shilong

Table 9 Vulnerable fishes in Bangladesh

Scientific name	English name	Local name
Ailia punctata	Jamuna ailia	Kajuli, Baspata
Anguilla bengalensis	Indian longfin eel	Bamosh, Bao baim, Telkoma
Chanda nama	Elongate glass perchlet	Chanda, Nama chanda
Chanda ranga	Indian glassy fish	Chanda, Lal chanda
Channa orientalis	Asiatic snakehead	Telo taki, Raga, Cheng
Cirrhinus reba	Reba carp	Raik, Tatkani, Bata, Laacho
Macrognathus aral	One-spine spinyeel	Tara baim
Monopterus cuchia	Gangetic mud eel	Kuchia
Mystus aor	Long-whiskered catfish	Ayre, Aor
Mystus cavasius	Gangetic mystus	Kabashi tengra, Golsha-tengra, Gulsha
Nandus nandus	Mud perch	Meni, Bheda
Notopterus notopterus	Grey featherback	Foli, Pholi
Plotosus canius	Canine catfish-eel	Gang magur
Puntius ticto	Ticto barb	2-spot barb; Tit punti

7. Investigation on Chemicals and Drugs Used in Aquaculture Practices in Bangladesh

7.1 Background and Justification

Fish catches from open waters in Bangladesh are declining day by day due to over exploitation and degradation of habitats. Therefore, aquaculture is being looked upon as a panacea for meeting the increasing demand. Bangladesh has 146,890 ha of ponds, 5,488 ha of oxbow lakes and 140,000 ha of brackishwater areas, which offers excellent opportunities for culture of various species of carps, tilapia, catfish and various species of prawn and shrimp. In order to fulfill the increasing demand, aquaculture in Bangladesh is being intensified. People are stocking their pond at a greater rate, which necessitates greater use of fertilizers and supplementary feeding. Feed manufacturers are using different feed additive for increased growth. Some organic/inorganic products as coming to farmers as fish/shrimp growth enhancer. High stoking density and use of artificial feed increases the risk of diseases outbreak in culture waters leading to mass mortalities and economic losses. EUS disease in fish seriously out broke in 1988. Shrimp aquaculture in Bangladesh seriously affected by SEMBV (Systematic Ectodermal Monodon Baculovirus) in 1994, which has become a major problem in recent years and led to great losses to shrimp producers.

In aquaculture, chemicals are used mainly in the treatment and prophylaxis of disease problems, which constitute the largest single cause of economic losses. However, the increasing use of chemicals in aquaculture has lead to widespread public concern. The concerns related to human health due to chemical use in aquaculture are repeatedly found in the published literature. They include allergic reactions in the previously sensitized persons triggered by chemical residues, and the potential impact on human health resulting from the emergence of drug-resistance bacteria caused by used of sub-therapeutic levels of antibiotic and by antibiotic residues persisting in the sediments of aquaculture environments. Generally, small-scale farmers in Bangladesh use rice bran and oil cakes as supplementary feed in carp culture, while fish meal is used additionally in prawn and catfish culture. The chemicals they use are different forms of limes, such as, calcium oxide (CaO), slake lime {$Ca(OH)_2$}, agricultural lime ($CaCO_3$) and dolomite lime {$CaMg(CO_3)_2$} and fertilizers, such as, urea, TSP and MP.

No significant work has been done on the impact of use of chemical and feed additives in aquaculture in Bangladesh, which may be a health hazards to human. Little work has been conducted on the use of chemicals and feed additives in aquaculture in their impact on fish/shrimp body. Work has been done on the use of pesticide in agriculture on the fishery resource. With the intensification of aquaculture, different farm are trying to introduce chemotherapeutics, growth enhancers, feed additives and hormones in order to increase the growth and combat disease. BFRI tested some of that products, which indicated that those products are not so effective as demanded. Information on the accumulation of chemicals used in aquaculture is scarcely available.

Fish/shrimp culture in Bangladesh is sifting gradually towards commercial practice where stocking densities is increasing and formulated feed 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 to combat disease and increase growth. Different business firm are advocating for their chemicals and feed additive products as remedy to diseases and as growth enhancers. Unfortunately, at present there is no complete information on the use of chemicals and feed additives in aquaculture in Bangladesh. This study will provide a complete list of chemicals and feed additives used in aquaculture in Bangladesh. There is no sufficient regulation on the use of chemicals and feed additives in Bangladesh. Findings of this study will also help to formulate registration, licensing, regulation and guideline for the use of chemicals and feed additives in aquaculture.

In aquaculture, as in all other food production systems, one of the inputs required for enhance production is chemicals. In the most simple, extensive systems, this may be limited to fertilizers, while in more complex semi-intensive and intensive systems a wide range of natural and synthetic compounds may be used. It is safe to say that, as in aquaculture, chemicals are an essential “ingredient” to production increase, one which has been used in various forms for centuries.

Chemicals have many uses in aquaculture, the types of chemicals used depending of the nature of the culture system and the species being cultured. They are essential components in:

- pond preparation,

- soil and water management,
- enhancement of natural aquatic productivity,
- transportation of fry and fingerlings,
- feed formulation,
- manipulation and enhancement of reproduction,
- growth promotion,
- health management, and
- processing and value enhancement of the final product.

The benefits of chemical usage are many. Chemicals increase production efficiency and reduce the waste of other resources. It assists in increasing hatchery production and feeding efficiency, and improves survival of fry and fingerlings to marketable size. It is also used to reduce transport stress and to control pathogens, among many other applications. Many aquaculture chemicals are, by their very nature, biocidal, and achieve their intended purpose by killing or slowing the population growth of aquatic organisms. Chemicals used in this manner include disinfectants, algicides, herbicides, pesticides, and antibacterials. There are several important concerns with regard to the use of chemicals in aquaculture. These include:

- Human health concerns related to the use of feed additives, therapeutants, hormones, disinfectants and vaccines.
- Product quality concerns related to such issues as the occurrence of chemical residues in aquaculture products, their use in the enhancement of product quality and in the preparation of value-added products, the need for consumer protection from hazardous usage, and issues surrounding consumer acceptance of the use of chemicals in the production of fish and shellfish destined for human consumption.
- Environmental concerns, such as the effects of aquaculture chemicals on water and sediment quality (nutrient enrichment and loading with organic matter), natural aquatic communities (toxicity, disturbance of community structure and resultant impacts on biodiversity), and effects on microorganisms (alteration of microbial communities and the generation of drug resistant strains of bacteria).

Human health and environmental concerns regarding the use of chemicals in aquaculture are reflected in the FAO Code of Conduct for Responsible Fisheries (FAO 1995). Many aquaculture chemicals degrade rapidly in aquatic systems. For example, formalin, a widely used parasiticide and fungicide, has a half-life in water of 36 hours (Katz 1989). Furazolidone, an antibacterial, has a half-life in sediments of less than one day (Samuelsen *et al.* 1991). Other chemicals may persist for many months, retaining their biocidal properties. Very little is known about the environmental fate of many aquaculture drugs with available data (Graslund *et al.* 2003).

With the intensification of aquaculture, different farms are trying to introduce chemotherapeutics, feed additives and growth enhancers in order to increase the 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). The general lack of knowledge concerning the effects and fates of chemicals and their residues in cultured organisms and within the aquaculture system itself. Similarly, information is lacking on the impacts of chemicals and drugs on non-cultured organisms, sediments and the water column.

The present study investigates the sources of chemical and biological products used in aquaculture in Bangladesh, and case study results of some such products. Findings of this study will help to formulate registration, licensing, regulation and guideline for the use of chemicals and biological products in aquaculture industry of the country.

7.2 Literature Review

A survey conducted under the ADB/NACA Regional Study included 96 extensive carp farms and 522 semi-intensive carp farms of Bangladesh (ADB/NACA 1996 & 1998). The most commonly used chemical for pond preparation in carp culture was lime (used on 7% of extensive farms and 80% of semi-intensive farms). Rotenone was used on 1% of extensive farms and 11% of semi-intensive farms as a piscicide to remove "weed fish" prior to stocking of culture fish. Various forms of organic and inorganic fertilizers (1-70%) were used for pond preparation. Use of Phostoxin (a compound used as a piscicide) was also reported by only 6% of semi-intensive carp farmers. The only chemical used during grow-out operations was lime, which was used on 9% of semi-intensive farms, and organic/inorganic fertilizer, used on 5% of the extensive farms and all (100%) of the semi-intensive farms. The main disease problem in freshwater carp culture in Bangladesh is epizootic ulcerative syndrome (EUS). Fish diseases affected 31% of extensive farms and 24% of semi-intensive carp farms. Most farmers made no attempt to control disease outbreaks. Where farmers did use chemicals, the most common treatments were salt, lime (between 28 and 33% of farms), potassium permanganate (15% of semi-intensive farms) and occasionally dipterex. Antimicrobials (e.g., Oxytetracycline and Oxolinic acid) were used on less than 5% of farms affected by disease.

Formalin has a very old history as an aquatic chemotherapeutant. It is active against a wide range of organisms, including fungi, bacteria and ectoparasites (Herwig 1979). However, its action is slow. At a concentration of 5,000 ppm, 6-12 h is required to kill bacteria and 2-4 d to kill spores. At 80,000 ppm (8% solution), it requires 18 h to kill spores (Harvey 1975). It is also ineffective against internal infections. Formalin has been approved by the US FDA for use in treatment of food fish. However, formalin also causes oxygen depletion (Plumb 1992) and this excess can be deleterious in the long run. Even if effective, the action of formalin would be too slow to be of any significant use.

Copper sulphate is a broad-based disinfecting agent used in some shrimp and fish farms. It is effective against a wide range of organisms including blue-green algae, bacteria, fungi, protozoans, digeneans, leeches and monogeneans (Herwig 1979). Overdosing can easily kill the animals being treated, and thus caution is called for in its use.

Organophosphate pesticides are used in both freshwater fish ponds and marine shrimp hatcheries to control infections by crustaceans, and monogeneans and ciliates, respectively. The main organophosphates used are Malathion and Dipterex. Both are effective against crustacean parasites (especially *Lernaea* and *Argulus*), and protozoans (*Ichthyophthirius*, *Trichodina*), and are used as broad-spectrum anthelminthics to control monogeneans. In many freshwater fish farms, these organophosphates are also used to control aquatic insects that prey upon fish fry, such as dragonfly larvae (Shariff *et al.* 1996).

The ability to control the sex of fish populations would be advantageous to producers of economically important species, and is especially suited for prolific species such as tilapias and common carp. Androgens, 17 α methyltestosterone (MT) induced masculinization when fed to tilapia (Shelton *et al*. 1981). Culture of mixed sex tilapia may result in limited growth, 30-40% of the harvested fish being under marketable size. However, the use of sex steroids has sometimes yielded inconsistent results (Hunter & Donaldson 1983), presumably due to differences in duration, dose, temperature, timing of treatment, availability of natural food and species studied.

7.3 Objectives

Study on sources of existing chemical usage would be useful to properly assess the extent of the problems of use of chemicals and drugs in aquaculture practices. However, the specific objectives are:

- to list down the chemicals used in aquaculture practices in Bangladesh.
- to assess the sources of drugs and chemicals in farm aquaculture and fish hatchery production systems.
- to build awareness on safe and effective use of chemicals in aquaculture. This information could be in the form of technical guidelines, extension materials for farmers.
- to formulate guidelines for environmentally sound aquaculture practices which can help prevent the indiscriminate use of chemicals.

4.4 Methodology

7.4.1 Survey on the use of chemicals and feed additives in aquaculture

A survey was conducted to find out the present status of the use of chemicals and feed additives in fish/shrimp farming in Bangladesh. A questionnaire was prepared and tested to collect information (Annexure 2). Representative fish/shrimp farmers, extension workers, feed manufacturers and busniess agencies were interviewed. Data on product classification (pesticide, veterinary drug, medicated feedstuff, feed additive, poison), manufacturer's name, product name (official, scientific), active ingredient, common name and content, applicable mandatory rule, agencies involved in the approval process, chemical and properties, efficacy, toxicity for assessment of human health hazards, residues and possible environmental impacts were collected and analyzed.

7.4.2 Study the effects of chemicals and feed additives on fish/shrimp, soil and water

Laboratory trial: Fish/shrimp was exposed to different chemicals and feed additives commercially used in Bangladesh. Samples were collected at certain intervals and analyzed for chemical residues.

7.5 Results and Discussion

Chemicals use in aquaculture

Chemicals have many uses in aquaculture, the types of chemicals used depending of the nature of the culture system and the species being cultured. They are essential components in pond and tank construction, soil and water management, enhancement of natural aquatic productivity, transportation of live organisms, feed formulation, manipulation and enhancement of reproduction, growth promotion, health management, and processing and value enhancement of the final product.

The benefits of chemical usage are many. Chemicals increase production efficiency and reduce the waste of other resources. It assists in increasing hatchery production and feeding efficiency, and improves survival of fry and fingerlings to marketable size. It is also used to reduce transport stress and to control pathogens, among many other applications.

7.5.1 Environmental and human health issues

7.5.2 Persistence in aquatic environments

Many aquaculture chemicals degrade rapidly in aquatic systems. For example, formalin, a widely used parasiticide and fungicide, has a half-life in water of 36 hours (Katz 1989). Furazolidone, an antibacterial, has a half-life in sediments of less than one day (Samuelsen *et al.* 1991). Other chemicals may persist for many months, retaining their biocidal properties. Metal-based compounds, such as the organotin molluscicides and copper-based algaecides are likely to be quite persistent in aquatic sediments, although precise data are lacking. Some antibacterials, notably oxtetracycline, oxolinic acid and flumequine, can be found in sediments at least 6 months following treatment (Weston 1996). The persistence of chemical residues is highly dependent on the matrix and ambient environmental conditions. Very little is known about the environmental fate of many aquaculture drugs with available data being derived argely from temperate latitudes.

7.5.3 Residues in non-cultured organisms

Use of pesticides, antibacterials and other therapeutants in aquaculture has the potential to result in chemical residues appearing in wild fauna of the local environment. For example, uningested medicated feeds or faeces containing drug residues provide routes by which local fauna may ingest and incorporate medicants. Filter-feeding molluscs in down-current areas are particularly vulnerable to "secondary medication" from contaminated particulates. Such inadvertent chemical exposures and subsequent human consumption of aquaculural products theoretically can present hazards to human health although risks are probably extremely low in most aquaculture situations.

7.5.4 Toxicity to non-target species

Toxicological effects on non-target species may be associated with the use of chemical bath treatments, pesticides, disinfectants or leaching of toxicants from antifouling chemicals employed in aquaculture. Among the pesticides that may have toxicological effects on the surrounding invertebrate fauna are the organophosphate ectoparasiticides, such as those employed in fish culture. The use of carbaryl pesticides to eliminate burrowing shrimp from oyster beds in the north-western United States results in the unintended mortality of Dungeness crab, a commercially exploited species (WDF/WDOE 1985).

7.5.5 Stimulation of resistance

Since the first true antibacterial agents were introduced in the 1930s, users have been coping with the emergence of drug resistance among target organisms. As each new drug was developed, major successes in therapy were achieved but, within a few years, the first cases of drug resistant strains began to appear. In intensive aquaculture antibacterial agents are used universally to treat bacterial disease and there is widespread prophylatic use. The most common routes of application are oral or by immersion. In both cases, significant quantities of antibacterial may reach the environment and lead to the selection of resistance. Considering the possible health hazards, US FDA and EU approved few chemicals and drugs for use in aquaculture (Table 10).

Table 10 List of chemicals approved for aquaculture

Product	Use
THERAPEUTANTS	
Acetic acid	Parasiticide
Formalin	Paraiticide and fungicide
Romet 30 (sulfadimethoxine and orthomeprim)	Bactericide
Salt	Osmoregulatory enhancer
Sulfamerizne	Bactericide
Oxytetracycline (Terramycin)	Bactericide
ANESTHETICS	
Carbonic acid	Anesthetic
MS 222 (tricaine methane-sulfonate)	Anesthetic and sedative
Sodium bicarbonate	Anesthetic
DISINFECTANTS	
Calcium hypochlorite	Disinfectant, algicide, and bactericide
WATER TREATMENT	
Fluorescein sodium	Dye
Lime (calcium hydroxide, oxide, or carbonate)	Pond sterilant
Potassium permanganate	Oxidizer and detoxifier
Rhodamine B and WT	Dye
Copper sulfate	Algicide and herbicide
Copper, elemental	Algicide and herbicide
2, 4-D	Herbicide
Diquat dibromide	Algicide and herbicide
Endothall	Algicide and herbicide
Simazine	Algicide and herbicide
Clean-Flo (aluminum sulfate, Calcium Sulfate, and boric acid)	Algicide and herbicide
Glyphosate	Herbicide
Potassium ricinoleate	Algicide
Xylene	Herbicide

7.5.6 Use of chemicals in aquaculture

A survey conducted at field level showed that use of chemicals in aquaculture was not well established. Farmer uses different chemicals rather than most commonly used lime in a limited scale. Although large numbers of chemicals are now introduced by different agrochemical industries/agencies, their wide scale use could not be quantified. Use of chemicals in shrimp culture was more than carp culture and a variety of chemicals found in the markets believed to be use in fish and shrimp culture (Table 11).

Table 11 Available chemicals to be used in fish and shrimp culture

Sl No.	Product name	Producer	Marketing by	Ingredient	Purpose
1	ABACUS	Biostadt Europe Ltd., India	Syngenta, Bangladesh	*Lactobacillus sporogenes, L. acidophilis, Bacillu subtilis, B. licheniformis, Saccharomyces cerevisiae,* sea weed extract	Improves efficacy of digestive system, better absorption of nutrients, improves the digestion of complex pertinacious materials, higher feed consumption rate with better FCR, faster growth rate and weight gain, enhances the body's ability to resist stress and the attacks of pathogens, increases yields
2	Ablaze	Biostadt Europe Ltd., India	Syngenta, Bangladesh	Doxycycline, Collistine sulphate, minerals	Effectively prevents and cures a broad range of viral and bacterial diseases, helps in reducing trace in fishes, build resistance against disease
3	ACIMIX Super-B		ACI Animal Health, Dhaka	Vitamins, trace elements, amino acid	Rapid growth, increase FCR, increase disease resistance
4	AGOX	BROOKSIDE ARA L.C., USA	QADSIDE BANLADESH LTD.	BHA, Ethoxion, EDTA, phosphoric acid, citric acid, mono/diglycerides, calcium silicate, silicon dioxide	Anti-oxidant & chelating agent, increase appetite and disease resistance
5	Aldrin				Predator kill
6	Alphamax	Biolab, Chittagong	Biolab, Chittagong	Calcium, Aluminium, Magnesium, trace elements, N.P.K	Reduce acidity and sulfide gas, maintain oxygen balance, remove lesion, reduce antenna rot
7	Alphavit C+E Premix		Biolab, Chittagong	Vitamin C, vitamin E, Herbal Q.S	Increase FCR, reduce stress, increase growth

Sl No.	Product name	Producer	Marketing by	Ingredient	Purpose
8	Alphavit Fish & Prawn	Biolab, Chittagong	Biolab, Chittagong	Vitamin B1, B2, B6, B12, niacin, folic acid, Calpantho, lysine, methionine, iron, coper, manganese, zinc, boron, sodium selinite, cobalt chloride	Increase plankton, source of vitamin, minerals and amino acid, increase disease resistance
9	Alphavit Fish & Prawn WS	Biolab, Chittagong	Biolab, Chittagong	Vitamin B1, B2, B6, B12, K, niacin, folic acid, pantothenic acid, choline, lysine, methionine, tryptophan,	Tail and gill rot, dropsy control
10	AMNOVIT	Hoechst Roussel Vet Ltd., India	-	Vitamin B2, B6, B12, niacin, folic acid, Calpantho, lysine, methionine, iron, coper, manganese, zinc, boron,	Non-antibiotic growth promoter
11	Anafish	-	-	Vitamins, minerals, antibiotic	Growth, sickness prevention, anti-tress
12	Aquakleen	Square	Square	Tetradacile trimethyl ammonium bromide, BKC, Aminonitrogen	Water cleaner
13	Aqua Grow	USA	Eion Animal Health Products, Dhaka	DHA enriched micro diet supplement	Larval growth, disease resistance
14	Artemia	USA	Eion Animal Health Products, Dhaka	Artemia	Larval feed
15	Ayumin	Dabur India Ltd,	Dabur Ayurvet Ltd., India	Copper, cobalt, magnetium, iron, zinc, iodine, methionine, lysine, calcium, phosphorus	Rapid growth, increase FCR, increase disease resistance
16	BANJO	Biostadt Europe Ltd., India	Syngenta, Bangladesh	Tetraacetyl ethylene diamine, sodium perborate, absorbants, de-odorizers	Releases required amount of oxygen, relieves shrimp from stress, checks the growth of anerobic bacteria, protozoa and secondary pathogens
17	BIO-ADE	USA	Eion Animal Health Products, Dhaka	Extract of Euca cidigera	Decrease ammonia and maintain healthy environment

Sl No.	Product name	Producer	Marketing by	Ingredient	Purpose
18	Biolive Powder	-	Biolab, Chittagong	Kalomegh, Azoan, Radhoni, Naxvum, sodium selenite, cobalt chloride, herbal & sincona	Reduce ulcerative disease, enteric hemorrhage, hepatic septicemia, tail rot, gill rot
19	Biophos 1x	-	Biolab, Chittagong	Ferum phos, trace elements, herbal	Reduce ulcerative disease, enteric hemorrhage, hepatic septicemia, tail rot, gill rot
20	Biophyl	Avitec Lab., France	-	Minerals, vitamins, amino acids	Growth, sickness prevention, anti-tress
21	Cencalcium-plus	Senavisa Lab. Spain	Impex Marketing Ltd.	Minerals, vitamins, trace elements, amino acids	Better formation of scales and bones
22	CHLORSTE CLIN	Huazhong Chia Co. Ltd., China	Novertis (Bangladesh) Ltd.	Chlorotetracycline 15%	Prevent and cure tail and gill roty, dropsy, EUS
23	Cevit-Aqua	Square	Square	Ascorbic acid	Increase health and daily growth
24	Copper sulphate				Algal control enhancer
25	Dolo-lime	-	Ideal Trading Ltd., Dhaka	Dolomite (Mg & Ca enriched lime)	Reduce acidity of soil and water, release calcium and magnesium, incease plankton, reduce turbidity
26	Dropper	-	-	Chloro Alkyl Phenyl Urea	Inhibit growth of blue green algae and toxic plankton, reduce algae and toxic plankton, control plankton growth rate and water quality
27	Fish Curepus	M/S M.R. Enterprise, Dhaka	M/S M.R. Enterprise, Dhaka		Increase nutrient and oxygen, decrease disease, cure ulcerative disease
28	Fish Premix	USA	Eion Animal Health Products, Dhaka	Vitamins, minerals	Increase health and daily growth
29	FLASH	Biostadt Europe Ltd., India	Syngenta, Bangladesh	Synthetic Gonadotropin releasing hormone analogue	High fertilization and hatching rate, single dose administration, no stress to the brood stock
30	FLO-BOND	BROOKSID E ARA L.C., USA	QADSIDE BANLADE SH LTD.	Hydrated sodium calcium alumino-silicate	Micotoxin binder in feed
31	Gastrap	Square	Square	Lactic acid bacillus, cellouse, Lipase, Aminonitrogen	Bioside /disinfectant
32	Hanter	-	Ion Animal Health	Rotenon 9%	Weed fish control
33	M:H-10	Agrosystem s, Italy	Genetica, Bangladesh	Protein, vitamins, minerals, UGF	Rapid growth, increase disease resistance, increase plankton
34	Malachite green		Aquatic, Dhaka		Disease control

Sl No.	Product name	Producer	Marketing by	Ingredient	Purpose
35	Malathion			Malathion	Insecticide
36	Methylene blue		Aquatic, Dhaka		Pond treatment
37	NOVASIL PLUS	USA	Eion Animal Health Products, Dhaka	Toxin binder	Prevent alpha-toxin in feed
38	Oxistat	Agril Ltd. UK	-	Butylated hydroxytoluine, butylated hydroxyanisole, ethoxyquin, sodium citrate	Anti-oxidant for feed
39	Oxycare pH-7	Beijing Lily Agrochemistr, Chaina	National Agricare, Bangladesh	-	Growth enhance, disease treatment
40	Oxyflow				Increase oxygen
41	Oxylife	Square	Square	Oxygen precursors, Probiotics	Increase oxygen
42	Oxymex	-	Ion Animal Health	CaO_2	
43	Oxytetracycline			Oxytetracycline	Antibiotic
44	Panvit-Aqua	Square	Square	Multivitamin	Growth enhancer
45	Pegabind	USA	Eion Animal Health Products, Dhaka	Pellet feed binder	Feed binder
46	Phostoxin				Weed fish control
47	Potassium permanganate			Potassium permanganate	Oxygen generation
48	Prokura Efinol-L	USA	Eion Animal Health Products, Dhaka		Anti-tress, reduce mortality rate
49	QL-fishmeal	Malaysia	Eion Animal Health Products, Dhaka	Meal from marine fishes	Feed ingredient, 55% protein
50	Rotenone		Aquatic, Dhaka		Weed fish control
51	Sex-F		Aquatic, Dhaka		All female fish
52	Sex-M		Aquatic, Dhaka		All male fish
53	SPA	Biostadt Europe Ltd., India	Syngenta, Bangladesh	Protein, cholesterol, calcium, vitamin-D3, careotenoid	Binds all powder form medicinal, nutrient and probiotic supplements to the feed, water stable and increase the bioavailability of the feed supplements, improves pigmentation in shrimps

Sl No.	Product name	Producer	Marketing by	Ingredient	Purpose
54	Sumithion				Insecticide
55	Super Grow	China	Eion Animal Health Products, Dhaka	Zn premix.	Rapid growth, enhance molting
56	Tea Seed Powder	China	Eion Animal Health Products, Dhaka	Natural saponin and crude protein	Weed fish control, snail control
57	Tetracycline				Antibiotic
58	Timsen	United Promotions Inc, USA	Eion Animal Health Products Ltd., Dhaka	En-alkylbenzyl dimithyl chloride	Bioside /disinfectant Prevention of bacterial, viral, protozoan, algal and fungal infection, reduce hardness
59	VitaFish-V	-	Agro Products, Dhaka	Vitamin B1, B2, B6, manganese sulphate	Rapid growth, increase appetite, disease resistance, helps in protein, lipid and carbohydrate metabolism
60	Vitam-C	-	AGRIMAN, Dhaka	Vitamins, trace minerals, amino acids mix.	Vitamin, trace mineral, amino acid mix. for carp
61	Vitam-P	-	AGRIMAN, Dhaka	Vitamins, trace minerals, amino acids mix.	Vitamin, trace mineral, amino acid mix. for pangus
62	Vitam-S	-	AGRIMAN, Dhaka	Vitamins, trace minerals, amino acids mix.	Vitamin, trace mineral, amino acid mix. for shrimp
63	Zeofresh	Square	Square	SiO_2, Al_2O_3, Fe_2O_3, CaO, MgO, Na_2O, K_2O, TiO_2, LOI	Bioside /disinfectant
64	Zeolite	Zeoan Forein Trade Co., Chaina	National Agricare, Bangladesh	Calcium, magnesium, potassium, phosphorus, silicon, aluminum, ferrous, sodium	Remove toxic gases, alternative to lime, produce plankton

7.5.7 Chemicals used in fish culture

7.5.8 Pond preparation

The most commonly used chemical for pond preparation in carp culture was lime (used on 7% of extensive farms and 80% of semi-intensive farms). Rotenone was used on 1% of extensive farms and 11% of semi-intensive farms as a piscicide to remove "weed fish" prior to stocking of culture fish. Various forms of organic and inorganic fertilizers (1-70%) were used for pond preparation. Use of Phostoxin (a compound used as a piscicide) was also reported by only 6% of semi-intensive carp farmers.

7.5.9 Grow-out operations

The only chemicals used during grow-out operations were lime, which was used on 9% of semi-intensive farms, and organic/inorganic fertilizer, used on 5% of the extensive farms and all (100%) of the semi-intensive farms.

7.5.10 Fish disease control

The main disease problem in freshwater carp culture in Bangladesh is epizootic ulcerative syndrome (EUS). Fish diseases affected 31% of extensive farms and 24% of semi-intensive carp farms. Most farmers made no attempt to control disease outbreaks. Where farmers did use chemicals, the most common treatments were salt, lime (between 28 and 33% of farms), potassium permanganate (15% of semi-intensive farms) and occasionally dipterex. Antimicrobials (e.g., oxytetracycline and oxolinic acid) were used on less than 5% of farms affected by disease.

7.5.11 Chemicals used by feed manufacturers

There was no report on use of antibiotics in fish/shrimp feeds by fish feed industries in Bangladesh, although some use anti-oxidant or anti-mold in feed as preservatives. The reported anti-oxidant and anti-mold are:

Anti-oxidant: Butylated hydroxytoluene, butylated hydroxyanisole, ethoxyquin, sodium citrate etc.

Anti-mold: Polymethyl carbamide, manoic starch blend, hydrated calcium sulphate etc.

7.5.12 Marketing of chemicals

In Bangladesh, there is no specific legislation regarding the use of therapeutic drugs and chemicals in aquaculture. Most drugs are similar to those used in human medicine, while chemicals used in aquaculture are the same as those used for agricultural purposes. Recently, some products are marketing directly for aquaculture. There is no registration system of importation of chemicals for use in aquaculture. As consequence of the expansion of aquaculture, chemical usage has become increasingly a part of management. Various types of commercial products are produced to meet the demand. There is an increasing level of suppliers. Some suppliers import the product and some produce their product locally. Efficiency of most of the products generally does not tested before marketing. A few leading companies contact DoF or MoFL for testing their products before importing and/or marketing. They are ultimately referred to the BFRI for testing their products. Some time they directly contact BFRI for testing. On the other hand most of the suppliers of the country are indiscriminately marketing their products without prior testing. For the effective use of chemicals in aquaculture, a national policy should be developed, where marketing of chemicals should clearly be indicated. The chemical manufacturers, suppliers and traders should play key role, they should:

- Undertake proper labeling of drugs/chemicals (ingredients, methods of use, handling, risks) and undertake responsibilities for substantiating claims on products.
- Work closely with the farmers and researchers in the development of effective chemicals for use in aquaculture. The need for pharmaceutical companies to fund research on aquaculture chemicals is necessary.
- Consider ways to establish industry codes of practice or certification for the marketing of aquaculture chemicals. The certification might be in close consultation with scientists and appropriate government agencies. Self-policing of manufacturing and marketing practices, in accordance with agreed-upon standards and practices is desirable.
- Importer should test the appropriateness of their chemicals in local condition before marketing.
- Assist farmers by providing correct information on product usages and substantiating claims, mainly through labeling of products.

7.6 Recommendations

7.6.1 Recommendations for theAaquaculture Industry

- Chemotherapeutants should not be the first option when combating disease but used only as a last resort environmental conditions, nutrition and hygiene have been optimized.

- Prophylactic treatment should be avoided since the selective pressure for development of antibacterial resistance poses a threat to the long-term efficacy of a drug.
- When multiple chemical alternatives are available, aquaculturists should select drugs not only on the basis of efficacy data but also available information regarding environmental persistence, potential effects on non-target organisms, propensity to stimulate microbial resistance and rate of residue elimination.
- Aquaculturists should utilize antibacterials having as narrow a spectrum of activity as possible but without loss of efficacy, so as to minimize selective pressure for resistance in other micro-organisms.
- In order to document cost-effectiveness and guide future treatment, aquaculturists should maintain records of chemical use including agents used, amounts, reasons for use, methods of application, dates of use, amount/number and size of stock treated, success/failure of treatments and times of harvest of treated stock.
- Aquaculturists should not discharge to natural water bodies any effluent containing chemical residues at concentrations likely to cause adverse biological effects and should first reduce concentrations, preferably by residue removal or increased residence time, and/or by dilution with other effluent waste streams within the farm.
- Farms in close physical proximity should collaborate in minimizing the risk of contaminating of their water supplies and those of neighboring facilities with chemical residues and drug resistant bacteria.

7.6.2 Recommendations for the Farmers

It is the impression from the survey results and other discussions with farmers that farmers are mainly searching for solutions to the day-to-day problems they face, particularly when disease outbreaks are encountered. It is the responsibility of government and scientific community to ensure that farmers are properly supported with and technical assistance which provides appropriate advice on farm management and on environmentally safe ways of using approved chemicals. The importance of basic preventative health care for cultured fish and shrimp cannot be overemphasized as one key way to minimize need for using disease control chemicals.

7.6.3 Recommendations for the Drug and Chemical Industry

- Producers of chemicals used in aquaculture should support the development of efficacy, fate environmental effects data specific to the species and the geographical region(s) of chemical use.
- Aquaculture chemicals should be provided to the aquaculturist with labeling and/or data sheets in the principal local language(s). Information should be provided on active ingredients, intended use, route of treatment, environmental and health hazards, species and life stage to me treated, storage conditions, expiration dates and disposal requirements. Aquaculturists should be encouraged to purchase only chemicals with complete labeling and to follow all instructions regarding their use.
- Chemical producers and marketers should be responsible for proper labeling of products, substantiation of claims and precautionary measures for reducing risks from improper use of the chemicals.

7.6.4 Recommendations for Government Agencies and Institutions

Government agencies have a responsibility for fair regulations on chemical use and provision of appropriate information to farmers. Researchers also have a responsibility for provision of information on chemical use and impacts and for development of farming systems and practices which promote environmentally sound aquaculture production.

8. Investigation and Identification of Emerging Fish Diseases and Development of Their Control Strategies

8.1 Background and Justification

Disease is one of the most important problems of fish production both in culture system and wild condition of Bangladesh (Rahman & Chowdhury 1996). Fishes have been suffering from many diseases such as Epizootic Ulcerative Syndrome (EUS), tail and fin rot, fungal, parasitic and bacterial infections (Chowdhury *et al.* 1999). In most cases hemorrhages, septicemia, lesions, gill damage are the common symptoms of the diseased fish (Chowdhury 1993 & 1998). Thus it is essential to investigate the cause of the occurrence of disease by using suitable techniques. Clinical investigation provides information on the nature of diseases in fish.

Edwardsiellosis and bacterial haemorrhagic septicaemia disease was identified as problem and associated in cultured *Pangasiadon hypopthalmus. Streptococcus agalactiae* is a major problem in tilapia culture, especially in the cage culture. The problem is still unresolved. *Flavobacterium columnaris* is a global problem in the freshwater environment. It is known to cause up to 90% mortality in fry of Thai koi (*Anabas testudineus*) and Tilapia (*Oreochromis niloticus*). Now-a-days, diseases in Shing (*Heteropnaustes fossilis*), Thai koi (*A. testudineus*) and Tilapia (*O. niloticus)* are threat to the fish growers. Pathogens, excessive high stocking density, higher input and inadequate husbandry practices accelerate diseases in the fish farm. Taking all these factors in consideration the research program has been undertaken.

The importance of prevention and control of disease risks as a measure to reduce production losses in commercial, semi-commercial and small-scale aquaculture systems has thus increased attention. Over the past three decades, aquaculture has expanded, intensified and diversified, such that modern-day aquaculture practices often involve significant domestic and international movement of live aquatic animals and animal products. This has led to the movement and spread of associated pathogens, and such introductions of pathogens have not only caused losses and mortalities in commercial systems, but also affected small-scale, rural aquaculture and fisheries operations (Arthur *et al*. 2002).

However, indiscriminate and unplanted use of feed and fertilizer, with subsequent effects on water quality in pond ecosystems correspondingly increases stress on fish and accelerates susceptibility to pathogens. The effects of disease in improved culture systems are significant; however, proper systemic information on disease outbreaks is not yet available. So, the present study is undertaken to determine the relation between risk factors and disease occurrences and to develop control strategies against emerging diseases. Finally, it is urgently needed to improve bio-security of fish farms through responsible use of eco-friendly chemotherapeutics.

8.2 Literature Review

By using epidemiological approaches it is possible to identify pond/farm's level risk factors for disease outbreaks. Intervention strategies can then be developed to minimize or eliminate such risk factors and reduce the risk of disease outbreaks. Epidemiological approaches for identifying pond level risk factors for fish disease outbreaks are becoming more popular in the development of aquatic animal disease prevention and management strategies (Corsin *et al*. 2001 & 2002a, MPEDA/NACA 2003).

Common fish diseases in Bangladesh are epizootic ulcerative syndrome (EUS), tail and fin rot, gill rot, columnaris disease, dropsy, various types of fungal diseases, protozoan and other parasitic diseases and nutritional deficiency diseases. Among them, major diseases are suspected to be occurred by pathogens. In most cases, hemorrhage, different kinds of lesions and gill damages are the common symptoms of the affected fish (Chowdhury 1997). Chowdhury (1997) reported that *Aeromonas* spp., *Pseudomonas* spp, and *Flexibacter columnaris* were initially suspected to have their involvement in the outbreak of diseases like different kinds of lessions on the body surface, tail and fin rot and gill damage in fishes of Bangladesh. Kashem (1998) identified *F. columnaris* as the causative agent of columnaris disease of *Anabas testudineus*.

Sebastiao *et al*. (2010) reported that *Flavobacterium columnaris*, the etiologic agent of columnarie disease, has a broad geographical distribution and accounts for a large number of mortalities in fish species. *Flavobacterium columnaris* 16S rDNA gene from bacteria isolated from Nile tilapia *(Oreochromis niloticus*). The bacteria were characterized biochemically and by PCR-RFLP. The use of PCR-RFLP for identification of the bacteria was shown to be a more efficient and rapid tool than current biochemical techniques, which are time consuming and often inconclusive. It is reported that epizootic ulcerative syndrome (EUS) affected *Cirrhinus mrigala*, *Labeo rohita*, *Puntius sarana* and *Heteropneustes fossilis* were treated with different drugs *viz*., $KMnO_4$, NaCl and herbal drug (antiseptic & water clean) where 10% of *H. fossilis* were recovered from the disease (BFRI, Annual Report 1999-2000).

8.3 Objectives

i) Find out risk factors associated with emerging fish diseases on farm level.

ii) Identification of causative agent(s) for emerging fish diseases outbreak with special reference to Shing (*Heteropneustes fossilis*).

iii) Histological changes in different organs of diseased fish.

iv) Development of control strategies to minimize fish mortality using better management practices and eco-friendly chemicals.

8.4 Results

8.4.1 Risk factors on farm level

An interview-based questionnaire survey was carried out in 25 disease affected and 25 disease free farms to study the risk factors associated with outbreaks of emerging fish diseases in five Upazillas of Mymensingh district. Both internal and external factors are associated with disease outbreak. There were 16 relative sources of risk identified from the disease farms. Data showed that the higher relative sources of risk for disease outbreak of higher stocking density, water quality deteriorate, pond connected to other water body, incomplete pond preparation and disease in the previous season in culture ponds were found 92%, 88%, 84%, 72% and 72%, respectively in the disease farm (Table 12). The use of lime and salt regularly in *H. fossilis* farm comparatively found to be low disease outbreak than the others. It was also observed that birds carried specific pathogens to the disease free farms and disease outbreak nearest all fish farms.

Table 12 Different risk sources cause fish diseases on farm level

Risk sources	Disease affected farm (N=25)	Disease free farm (N=25)
POND CONNECTION		
High embankment and fencing in pond	8%	68%
Pond connected to other water body (rice field/other pond/ditch)	84%	0%
PRE-STOCKING POND PREPARATION		
Pond drying	28%	80%
Removal of bottom mud	12%	84%
Liming of pond	36%	100%
Incomplete pond preparation	72%	4%
STOCKING		
Low quality fingerlings stock	68%	4%
Higher stocking density	92%	4%
POST-STOCKING MANAGEMENT		
Liming and salting in pond	32%	92%
Use preventive measure for disease control	20%	76%
Black water color (high organic debris)	64%	12%
Deteriorate water quality (Temperature, pH, DO & ammonia)	88%	8%
HYGIENE		
Dried/disinfected of farm nets and equipments	16%	96%
Parasites observed on fish	64%	8%
Disease outbreak by birds	68%	0%
SEASONALLY		
Disease in the previous season	72%	8%

8.4.2 Identification of causative agent(s) for fish diseases outbreak and treatment trials

Emerging diseases causing severe mortality were observed in *H. fossilis,* Pangas (*Pangasianodon hypophthalmus*) and Thai koi (*Anabas testudineus*) in different Upazillas under Mymensingh district. Sudden mass mortality of fry of *P. hypophthalmus* and *H. fossilis* were noticed in several farms of the district. Pond size varied from 15 to 210 decimal having a depth range of 1.5 to 2.5 m. In December 2012, mass mortality was observed in Catla (*Catla catla*) at Mahamaya Lake in Mirsorai Upazilla under Chittagong district. Diseased fish were collected and carried to the laboratory for detail study.