

Competitive Research Grant (CRG)

Sub-Project Completion Report

on

Residual Assessment of Hazardous Pesticides and Antibiotics in Shrimp/Prawn Farming Systems of South-West Bangladesh with its Traceability Identification and Risk Quantification on Human Health

Project Duration

May 2017 to September 2018

Bangladesh Fisheries Research Institute,
Shrimp Research Station, Bagerhat

Submitted to

Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215



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Citation

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Project Implementation Unit

National Agricultural Technology Program-Phase II Project (NATP-2)

Bangladesh Agricultural Research Council (BARC)

New Airport Road, Farmgate, Dhaka – 1215

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National Agricultural Technology Program-Phase II Project (NATP-2)

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Acronyms

BARC =Bangladesh Agricultural Research Council
BFRI = Bangladesh Fisheries Research Institute
Cm= Centimeter
Co-PI= Co-Principal Investigator
CRG = Competitive Research Grant
DFO= District Fisheries Officer
DoF = Department of Fisheries
EU =European Union
FGD = Focus Group Discussion
FIQC=Fish Inspection and Quality Control
GC = Gas Chromatography
GC-MS= Gas Chromatography Mass Spectrophotometer
Gm =Gram
GO = Government Organization
GPS=Global Positioning System
HPLC = High Performance Liquid Chromatography
LCMS = Liquid Chromatography Mass Spectrometer
Mm= millimeter
MS=Mass Spectrometer
N/A= Not Applicable
NATP= National Agricultural Technology Program
ND= Not Detected
NGO = Non-Government Organization
PI = Principal Investigator
PIU=Project Implementation Unit
POPs = Persistent Organic Pollutants
PP=Project Proposal
Ppm= Parts per Million
PSA=Primary Secondary Amine
SD = Standard Deviation
SGR= Specific Growth Rate
SPSS= Statistical Package For Social Sciences
SRS= Shrimp Research Station
SUFO= Senior Upazila Fisheries Officer
TK= Taka
UFO = Upazilla Fisheries Officer
WHO = World Health Organization

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Executive Summary

The study was carried out to identify the pesticides, antibiotics and aqua drugs used in the south-western shrimp/prawn farms of Bangladesh and quantify their residue level and their impact on human health. Samples were collected from three selected districts of the region such as Bagerhat, Satkhira and Khulna during July 2017 to September 2018. A pretested check list was prepared and a comprehensive baseline survey was performed through Focus Group Discussion (FGD) and Questionnaire approach to identify the available chemicals and antibiotics.

In addition to that, available pesticides being used in rice cum shrimp/prawn farm were also enlisted. A total of 492 respondent were interviewed, out of which 200 respondent were from different markets of seven Upazilas in Shatkhira District, 106 from nine Upazilas in Bagerhat and 186 from nine Upazilas in Khulna districts. Survey revealed that most commonly used aqua-drugs and chemicals such as Gastrap, Geotox, Zeolite and Aquaclean were being used for gher/farm preparation and water quality management. Bactitab, Cotrim Vet, Renamycin, Oxytetracyclin, Renamox, Sulfatrim were identified to be used for the treatment of shrimp especially against bacterial disease. On the other hand, most available pesticides were identified such as WINTIN 3% WG, Valor 40 WG, Nitro 50 EC, Dare 550 EC, Sydeor 550 EC, HARVEST 50 SP, Sumithion 50 EC, Deovit 90 WP, Malathion, SULFAVIT 80 WG, Dimethion were mostly being used to control the insects, fungal rot of rice leaves as well as growth enhancer of rice.

To determine the residual concentration of hazardous pesticides and antibiotics in shrimp/prawn body and its probable source, samples were collected from existing shrimp/prawn farms *i.e.* Khulna, Shatkhira and Bagerhat district and analyzed simultaneously. Integrated farming shrimp/prawns with rice/vegetable sites were given special priority for selection. Shrimp/prawn samples were collected from different farms *i.e.*, rice cum shrimp/prawn farms, vegetable cum shrimp/prawn farms, extensive shrimp/prawn farms, improved extensive shrimp/prawn farms and semi-intensive shrimp farms on a monthly basis to cover maximum number of farm locations. Water samples were collected from the farming systems into plastic bottles, which was pre-conditioned with 5% nitric acid and rinsed with distilled deionized water. Samples were taken into quality control lab of SRS, BFRI, Bagerhat for analysis of pesticides and antibiotics (Nitrofurantoin and Chloramphenicol) residues. Pesticides were analyzed by Gas Chromatography (GC-2010, Shimadzu) through real time analysis and antibiotics were analyzed by Liquid Chromatography Mass Spectrometer (LCMS: API-3200, ABSciex) through real time analysis. A total of 50 shrimp/ prawn samples, 50 water and 50 soil samples were collected from the sampling sites. Then samples were analyzed by LC-MS Machine using standard analysis protocol. No hazardous Nitrofurantoin metabolites and Chloramphenicol were detected in shrimp and prawn samples collected. A total of 50 shrimp/ prawn samples, 50 water and 50 soil samples were collected from the sampling sites. Then samples were analyzed for DDT, Dieldrin, Endrin and Heptachlore by GC-MS Machine using standard analysis protocol. One prawn sample of Bagerhat district was found having pesticides residues (DDT 0.00022ppm and Heptachlor 0.00012 ppm) which were much lower than the acceptable limit (DDT 0.05 ppm and Heptachlor 0.01ppm) and possessed no risk for human health.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. **Title of the CRG sub-project:** Residual Assessment of Hazardous Pesticides and Antibiotics in Shrimp/Prawn Farming Systems of South-West Bangladesh with its Traceability Identification and Risk Quantification on Human Health
2. **Implementing organization:** Bangladesh Fisheries Research Institute (BFRI)
3. **Name and full address with phone, cell and E-mail of PI/Co-PI (s):**
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4. **Sub-project budget (Tk):**
 - 4.1 Total: 4500000/-
 - 4.2 Revised (if any): Not Applicable
5. **Duration of the sub-project:** July 2017-September 2018
 - 5.1 Start date (based on LoA signed): 11 July' 2017
 - 5.2 End date: 30 September' 2018
6. **Justification of undertaking the sub-project:**

Shrimp as an export commodity of Bangladesh has been playing an important role in the country since mid 80's. In case of sectoral export, shrimp contribute more than 5% of the national earning. In south-west region of Bangladesh, shrimp/prawn is cultured mostly with rice and farmers use indiscriminately different groups of pesticides to control pest in the rice field. In Bangladesh, as in many other developing countries, chemicals and drugs are widely used for the treatment of bacterial diseases of fish and shrimp in commercial aquaculture. They are also used to improve water quality, killing unwanted fish and to enhance production. Some of these pesticides residues are gradually accumulating in shrimp/prawn body and ultimately affecting the consumers. In Bangladesh, import and production of all types of Persistent Organic Pollutants (POPs) like DDT and heptachlor are banned but at least five POPs pesticides including DDT are still in use under different name or label. Pesticide and antibiotic residue have received special attention in the recent years

because of ensuring food safety and public health concern. Presence of residues in animal food products arise socio-economic challenges as well as affecting international trading of shrimp/prawn and their products. Public health significance of such residues included development of drug resistance, carcinogenicity, mutagenicity, depression of bone marrow etc. According to EU regulation for shrimp/prawn as well as other frozen food exportation, appearance of any type of pesticides and chemical residues is fully prohibited. As major shrimp importing countries of the world European union and United States urges upon to full establishment of HACCP and US-FDA regulations in all frozen exportable commodities including shrimp that emerges another challenges for the Bangladeshi producers and exporters. As a follow up action, Bangladesh govt. has already initiated several technical means and brought all shrimp farmers under an association of production and export, established regional collaboration in addition to upgrading of processing industries and attempt for establishing traceability chain from larval production to culture production to processing to export level. However in spite of all efforts, farmers and traders still are not well aware about the bad effect of the all the malpractices for a quick return of bulk of money. Shortage of regulations and weak monitoring by the appropriate authority are the two most important draw backs of the sector. Therefore to increase consciousness among the producers and to develop awareness among the farmers and traders it is very much essential to know about the present use of harmful antibiotics and pesticides, their nature and degree of impact on human health. Under these considerations, the present study had been designed to identify and quantify the pesticides and antibiotics residues for the assessment of risk on human health.

7. Sub-project goal:

- To minimize pesticide and hazardous chemical adulterants in shrimp/prawn for reducing human health risk.
- To build-up awareness on safe usages of pesticides and antibiotics.

8. Sub-project objective (s):

- To scrutinize the available pesticides, antibiotics and other aqua-chemicals from existing shrimp/prawn farms of Khulna, Bagerhat and Shatkhira region through baseline survey
- To assess the hazardous pesticides, antibiotics and aqua-chemicals residues in shrimp/prawn and identification of its probable sources
- To assess the impacts of pesticides and antibiotics/chemicals residues on human health through risk quantification

9. Implementing location (s): Shrimp Research Station, Bagerhat

10. Methodology :

10.1: Scrutinizing available pesticides, antibiotics and other aqua-chemicals through baseline survey

Conduction of baseline survey:

At recent time a number of chemicals and antibiotics are available in the local market and with fish farmers as well. These are being used indiscriminately for disease treatment as well as other health management of shrimp/prawn. A pretested check list was prepared and a comprehensive baseline survey was conducted through FGD and Questionnaires approach to identify those available chemicals and antibiotics which may harm human health. A total of 25 FGD were done in 25 Upazilas with a group of 10 members in each group. Secondary information were collected from DFO, SUFO, representatives of drug companies etc. Thus a list of available pesticides, antibiotics and other aqua-chemicals had been prepared.

A pre-tested check list had been prepared and a comprehensive baseline survey was carried out through Focus Group Discussion (FGD) and Questionnaire approach to identify the available chemicals and antibiotics which might be harmful for human health. Besides, available pesticides had also been scrutinized which were used in rice cum shrimp/prawn farm. A total of 200 (Two hundred) questionnaire data were collected from different markets of 07 (Seven) Upazilas in Shatkhira District, where 153 brands of pesticides and 33 brands of aqua drugs were identified. A total of 106 (One hundred and six) questionnaire data were collected from the different markets of 09 (Nine) Upazilas in Bagerhat District, where 92 brands of different pesticides and 27 brands of different aqua drugs were found. 186 (One hundred and eighty six) questionnaire data were collected from different markets of 09 (Nine) Upazilas in Khulna district, where 109 brands of different pesticides and 24 brands of different aqua drugs were identified.

10.2: Determination of residual concentration in shrimp/prawn

Selection of sampling sites: Samples were collected from existing shrimp/prawn farming systems of south-west region of Bangladesh viz., Khulna, Shatkhira and Bagerhat district, as shrimp/prawn were cultured with rice/vegetable in an integrated way in some areas, those sites were given preference in case of site selection (Table 1).

Table 1: Sampling sites and number of sample collected from the sites

District	Upazila	No. of collected sample
Khulna	Batiaghata	2
	Dakope	2
	Digholia	2
	Dumuria	2
	Fultola	2
	Koyra	2
	Paikgacha	2
	Rupsha	2
	Terokhada	2
Shatkhira	Assasuni	2
	Debhata	2
	Kalaroa	2
	Kaliganj	2
	ShatkhiraSadar	2
	Shamnagar	2
	Tala	2
Bagerhat	Bagerhat	2
	Chitalmari	2
	Fakirhat	2
	Kachua	2
	Mollahat	2
	Mongla	2
	Morrelganj	2
	Rampal	2
	Sarankhola	2

Collection of Shrimp/Prawn sample:

Shrimp/prawn samples were collected monthly from different farms i.e., rice cum shrimp/prawn farms, vegetable cum shrimp/prawn farms, extensive shrimp/prawn farms, improve extensive shrimp/prawn farms and semi-intensive shrimp farms to cover maximum farming areas (Table 2). Samples were bought from the farmers and immediately kept with ice.

Table 2. Type of farm and samples

Upazila	Farm Type	Sample Type	Shrimp/Prawn size (g) at sampling time
Batiaghata	Vegetable cum shrimp/prawn	Shrimp, soil and water	40±5
Dakope	Vegetable/rice cum shrimp/prawn	Shrimp, soil and water	40±5
Digholia	Vegetable cum shrimp/prawn	Prawn, soil and water	60±10
Dumuria	Vegetable cum shrimp/prawn	Prawn, soil and water	60±10
Fultola	Vegetable cum shrimp/prawn	Prawn, soil and water	60±10
Koyra	Extensive	Shrimp, soil and water	40±5
Paikgacha	Improved Extensive	Shrimp, soil and water	40±5
Rupsha	Improved Extensive	Prawn, soil and water	60±10
Terokhada	Vegetable cum shrimp/prawn	Prawn, soil and water	60±10
Assasuni	Improved Extensive	Shrimp, soil and water	40±5
Debhata	Extensive	Shrimp, soil and water	40±5
Kalaroa	Extensive	Shrimp, soil and water	40±5
Kaliganj	Improved Extensive	Shrimp, soil and water	40±5
ShatkhiraSadar	Extensive	Shrimp, soil and water	40±5
Shamnagar	Semi-intensive	Shrimp, soil and water	40±5
Tala	Extensive	Shrimp, soil and water	40±5
BagerhatSadar	Vegetable /rice cum shrimp/prawn	Prawn, soil and water	60±10
Chitalmari	rice cum shrimp/prawn	Prawn, soil and water	60±10
Fakirhat	rice cum shrimp/prawn	Prawn, soil and water	60±10
Kachua	Vegetable /rice cum shrimp/prawn	Prawn, soil and water	60±10
Mollahat	rice cum shrimp/prawn	Prawn, soil and water	60±10
Mongla	Extensive	Shrimp, soil and water	40±5
Morrelganj	Vegetable /rice cum shrimp/prawn	Prawn, soil and water	60±10
Rampal	Extensive	Prawn, soil and water	60±10
Sarankhola	Extensive	Shrimp, soil and water	40±5

Collection of water and sediment sample:

Water samples were collected from the farming systems using plastic bottles that pre-conditioned with 5% nitric acid and rinsed with distilled deionized water. Prior to sampling, the pre-cleaned sample bottles were immersed about 10 cm below the water surface. For sediment, samples were collected in pyrogen-free zip-lock bags from the upper 5 cm layer of the same water area. Immediately after collection, sediment samples were dried at room temperature and sieved through a 2-mm nylon sieve to remove debris.

The sediments then grounded with a pestle and mortar and passed through a 200-mesh nylon sieve and preserved at -20°C for subsequent analysis. The water and sediment samples were collected from each sampling sites both before and after stocking of shrimp/prawn to identify the source of residues properly.

Sample analysis:

Samples collected from south-west region of Bangladesh *viz.*, Khulna, Shatkhira and Bagerhat district were taken into quality control lab of SRS for analysis of pesticides and antibiotics residues. Pesticides were analyzed by Gas Chromatography (GC: GC-2010, Shimadzu) and antibiotics were analyzed by Liquid Chromatography Mass Spectrometer (LCMS: API-3200, ABSciex). Twelve randomly selected samples were sent to Entomology Laboratory, Bangladesh Agricultural Research Institute (BARI) for analysis of pesticides. Also twelve shrimp/ prawn samples were sent to the laboratory of Fish Inspection and Quality Control (FIQC), Dhaka to test the presence of Nitro-furan Metabolites and Chloramphenicol.

Source identification:

If any antibiotic or pesticide residue was found in the collected prawn/shrimp samples then water, sample and feed sample associated with those farms were analyzed to identify the source of that antibiotic or pesticide residue.

Table 3. Experimental design

Sampling Area	Bagerhat	Shatkhira	Khulna
Samples	Shrimp/Prawn, Water, Sediment	Shrimp/Prawn, Water, Sediment	Shrimp/Prawn, Water, Sediment
Sample Weight	500 gm.	500 gm.	500 gm.
No of sampling	03 samples from each sampling site	03 samples from each sampling site	03 samples from each sampling site
Name of sample pesticide	Antibiotics such as Nitrofurans, Chloramphenicol and Organochlorine pesticides such as DDT, Endrin, Dieldrin, Heptachlor	Antibiotics such as Nitrofurans, Chloramphenicol and Organochlorine pesticides such as DDT, Endrin, Dieldrin, Heptachlor	Antibiotics such as Nitrofurans, Chloramphenicol and Organochlorine pesticides such as DDT, Endrin, Dieldrin, Heptachlor
Sampling interval	30 days	30 days	30 days

10.3: Impact assessment of the pesticides and antibiotics residues on human health through its risk analysis

Risk Assessment of the pesticides and antibiotics: Risk assessment was conducted based on local and country-wide consumption rates for key species. Data was analyzed individually for each of the collected shrimp/prawn species. An associated cumulative risk assessment was carried out for each contaminant (pesticides and antibiotics).

Statistical analysis: Data obtained from the experiments was analyzed and the result was expressed as mean \pm S.D. Statistical analysis had been performed using SPSS 20 software. ANOVA with post hoc test analyses based on Turkey was used to compare differences between samples. P-value of ≤ 0.05 was considered for the state of statistical significance.

11. Results and discussion:

11.1: Scrutinizing available pesticides, antibiotics and other aqua-chemicals

Conduction of baseline survey:

From the surveyed data, a total of 354 brands of pesticides and 84 brands of aqua drugs were found (Table 4).

Table 4. Types of pesticides and aqua drugs

District	No. of pesticides brands	No. of aqua drugs brands
Bagerhat	92	27
Khulna	109	24
Shatkhira	153	33
Total	354	84

Most available chemicals/aqua drugs and pesticides:

In the present study, most available aqua-drugs and chemicals were Gastrap, Geotox, Zeolite, Aquaclean. These were mostly used for gher/farm preparation and water quality management. Most available antibiotics were Bactitab, Cotrim Vet, Renamycin, Oxytetracyclin, Renamox, Sulfatrim which are used for disease treatment of shrimp especially for bacterial disease. Most available pesticides were Wintin, Valor, Nitro, Dare, Sydeor, Sumithion, Deovit, Malathion, Sulfavit, Dimethion, Diazinon etc. which were mostly used to control the insects, fungal rotten of rice leaves as well as growth enhancer of rice (Table 5-8). Similar findings obtained by Mazid (2002) that Diazinon, Sumithion, Malathion, Phostoxin, Endrin, Di-eldrin and DDT are widely used in pond aquaculture as insecticides and for killing predators/unwanted fish during pond preparation of fingerling stocking. It was also reported that annually 11,000 tons of 242 types of pesticides/insecticides are used in rice field in Bangladesh. The most commonly used pesticides/insecticides are Heptachlore, Diazinon, Ripchord, Sumisidin, Furadan, Basudin, Eldrin and Dieldrin. According to Fan *et al.* (2007), the major commercially used symmetrical triazines are simazine, atrazine, propazine, cyanazine, ametryn, prometryn, prometon, and terbutryn. Terbutryn is moderately toxic to fish (Meister 1992). Kidd & James (1991) reported the mean lethal toxicity of terbutryn (96 h LC50) 4 mg/L for common carp (*Cyprinus carpio*) and 3 mg/L for rainbow trout (*Onchorynchus mykiss*). As herbicides, the triazines may be used alone or in combination with other herbicide active ingredients to increase the weed control spectrum (Solomon *et al.* 1996). According to Velisek *et al.* (2011), eco-toxicological risks associated with pesticides depend on several factors such as type, concentration and frequency of application. Pyrethroids and triazines affect fish

physiology. Major negative impact of pyrethroids is their high toxicity to fish combined with their use for control of some parasitic diseases in fish. Triazine residues accumulate in fish tissue. Clinical symptoms of acute toxicity of pyrethroids in rainbow trout and common carp juveniles includes increased respiration, loss of coordination, and fish lying on their flank and moving in this orientation. Subsequent short excitation stages with convulsions, jumping above the water surface, and moving in circles alternated with resting. Necropsy performed after the acute toxicity test revealed increased watery mucus on body surfaces. The body cavity contained excess fluid and showed congestion of visceral vessels.

Table 5. Most available chemicals/aqua drugs identified available to traders and farmers in Khulna, Satkhira and Bagerhat Districts

Category	Aqua Drugs/ Chemicals	Active Ingredients	Purpose of Use
Gher preparation and water quality Management	Gastrap	Lactic acid, <i>Bacillus subtilis</i> , amilase, lipase	to remove gas
	Geotox	SiO ₂ , Al ₂ O ₃ , Fe ₂ O ₃ , CaO, MgO, Na ₂ O	to improve soil and water quality
	Zeolite	SiO ₂ , Al ₂ O ₃ , Fe ₂ O ₃ , CaO, MgO, Na ₂ O	-do-
	Aquaclean	Tetradesile trimethyle, amonium bromide, aminonitrogen	used as disinfectant to purify water
Disease Treatment	Bactitab	Oxytetracycline 20%	to inhibit pathogenic bacteria
	Cotrim Vet	Sulphamethoxazole+ Trimethoprim	-do-
	Renamycin	Oxytetracycline	to increase resistance capacity
	Oxytetracyclin	Oxytetracycline	-do-
	Renamox	Amoxacillintrihydrate	-do-
	Sulfatrim	Sulphadiazine and trimethoprim	to inhibit pathogenic bacteria

Table 6. Most available pesticides, fungicides and weedicides identified from the Traders and farmers in Bagerhat District

Category	Aqua Drugs/ Chemicals	Active Ingredients	Purpose of Use
Pesticides	Virtako 40 WG	Thiamethxam+Chlorentranilipron	To control insects of rice, tomato, bean, brinjals and other vegetables.
	Volume Flexi 300 SC	Thiamethxam+Chlorentranilipron	
	Ektara 25 WG	Thiamethxam	
	Vermitek 1.5 EC	Ebamectin	
	Karate 2.5 EC	Lamdacyhalthrins	
	Admire 20 SL	Emidachloropid	
	Dirsban 20 EC	Chlorpyriphos	
	Caught10 EC	Cypermethrine	
	Nitro 505 EC	Chlorpyriphos+ Cypermethrine	
	Grinchok	Deltamethrine	

	Lanirat	Bramadiolan	
Fungicides	Emister Top	Ezoxistrobin+Difenokonazol	To control the fungal disease of rice, chili & tomato.
	Bavistin DF	Carbendazim	
	Dithen M 45	Mencozeb	
	Ridomil Gold MZ 68 WP	Mencozeb+Metalixin	
	Tilt 250 EC	Propikonazol	
	Nativo 75 WP	Tebukonazol+Triflopristubin	
Antibiotic	Crasin AG 10 SP	Streptomycin+Tetracyclin	To control leaf back wilt, leaf back dye and leaf rot of rice
Weedicides	Lesar 10 WP	PirazosulfuranEthyal	To control the weeds of rice and vegetables.
	Rifit 500 EC	Pritilachlor	
	Ronstar	Oxadiazon	
	Gramoxol 20 SL	Parakoat dichloride	
	2, 4-D	2, 4, D-Amine	
	Neon 70 WG	Metribuzin	
	Afinity 50.75 WP	Karfentazon Ethyl+ Isoproteioron	
Organostimulant	Flora	Nitrobenzin	To stimulate the organic matters
Others	Grozin	Zinc+Sulfar	To supplement nutrients and minerals
	Soluber	Boron 20%	

Table 7. Most available pesticides, fungicides and weedicides identified from the traders and farmers in Khulna District

Category	Aqua Drugs/ Chemicals	Active Ingredients	Purpose of Use
Pesticides	Ripel 10 EC	Cypermethrine	To control insects of rice, tomato, bean, brinjals and other vegetables.
	Suntek 1.8 EC	Ebamectin	
	Emakto 5 WG	EbamectinBenzoit	
	Solar 55 EC	Ebamectin	
	Brikar 5 G	Carbofuran	
	Microthial Disperse 80 WG	Sulfar	
	Saifanon	Malathione	
	Clean Master	Acitachlor+Bensulfuran Methyl	
	Fotic 70 WG	Emidachlopid	
	Cibadin 10 G	Diazinon	
	Super Lamda	Synthetic Pyrethroid	
	Ravzum 14.5 SC	Indoxacarb	

	Tasla 10 WP	LamdaCihalothrin	
	Eunitoplus	Chloropyrifos+Cypermethrine	
	Hadak	Emidachloprid+Thiram	
Fungicides	Extra Care 300 EC	Propikonazol+Difenokonazol	To control the fungal disease of rice, chili & tomato.
Others	Magavit	Magnesium 9.5%+Sulfur 12.5%	To supplement nutrients and minerals
	China Monozinc	Monohydrate Zinc Sulfate 32%	
	Hay Zinc +	Zinc 36%+Sulfur 17%	
	Hay Mag +	Magnesium 9.5%+Sulfur 12.5%	
	Sandoz Mono	Zinc Sulfate (Mono 36%)	

Table 8. Most available pesticides, fungicides and weedicides identified from the traders and farmers in Satkhira District

Category	Aqua Drugs/ Chemicals	Active Ingredients	Purpose of Use
Pesticides	Damdama 440 EC	Profenofos+Cypermethrine	To control insects of rice, tomato, bean, brinjals and other vegetables.
	Lamah 25 WP	Isoprocarb 20%+Buprofezin 5%	
	Parthrin 10 EC	Cypermethrine	
	Caught 10 EC	Cypermethrine	
	Erad 55 EC	Chloropyrifos 50% + Cypermethrine 5%	
	Benthium 40 WDG	Emamectin Benzoate 20% + Thiamethoxam 20%	
	AXIS 10 EC	Alpha Cypermethrine	
	Aloron	ChlorofenoxyAcitic Acid	
	Morfos	Chloropyrifos 50% + Cypermethrine 5%	
	Gurphos 55 EC	Chloropyrifos + Cypermethrine	
	G-Five 55 EC	Chloropyrifos 50% + Cypermethrine 5%	
	Newthrin	Cypermethrine	
	Erenaphos 55 EC	Chloropyrifos + Cypermethrine	
	Nirjhor Super 40 WG	Emamectin Benzoate 20% + Thiamethoxam 20%	
	Corthrin 3 WG	Emamectin + Betacypermethrine	
	KASIR 48 EC	Chloropyrifos	
	SHEFA 10 EC	Cypermethrine	
JUBAS 2.5 EC	Chloropyrifos + Cypermethrine		

	LEADER	Cypermethrine	
	CYCLE	Chloropyrifos 50% + Cypermethrine 5%	
	PULSAR 3WG	Emamectin + Betacypermethrine	
	Tido+	Chloropyrifos	
	Modart	Chloropyrifos	
Fungicides	Shinshin 35 SC	Ezcristrobin 20% + Tebuconazol 15%	To control the fungal disease of rice, chili & tomato.
	Shabab 5 EC	Hexaconazol	
	Crizol	Hexaconazol	
	Megazol	Hexaconazol	
	TILT	Propiconazol	
	SCORE	Difenoconazol	
Weedicide	Rifit	Proteinachlor	
Others	Magavit	Magnesium 9.5%+Sulfur 12.5%	To supplement nutrients and minerals
	China Monozinc	Monohydrate Zinc Sulfate 32%	
	Hay Zinc +	Zinc 36%+Sulfur 17%	
	Hay Mag +	Magnesium 9.5%+Sulfur 12.5%	
	Sandoz Mono	Zinc Sulfate (Mono 36%)	

11.2 Determination of the residual concentration of hazardous pesticides and antibiotics

In case of water and soil no hazardous pesticides residues were found. In case of shrimp and prawn samples, only one prawn sample from Rampal of Bagerhat district, pesticides residues (DDT 0.00022ppm and Heptachlor 0.00012 ppm) were detected. For more confirmation randomly selected 12 shrimp/prawn samples out of 50 samples were sent to the Pesticide Analytical Laboratory, Entomology Division, BARI to test the presence of pesticide residues, but they did not find any residues. Zaman *et al.* (2012) studied on the level of organochlorine pesticide residues (OCPs), viz. α -, β -, γ -BHC, heptachlor, aldrin, heptachlor epoxide isomer B, dieldrin, 4,4'-DDE, endrin, 2,4-DDD, 4,4'-DDT, 4,4'-DDD, 2,4'-DDT in fish samples from Matlab floodplain areas. The result showed that none of the studied fishes contained organochlorine pesticide residues but contains some other harmful organic contaminants in tissues. Parts *et al.* (1992) studied on organochlorine pesticides in marine sediments along the coast of Alicante, Spain. The levels of organochlorines in sediments were low.

Table 9: Residual concentration of hazardous pesticides

District	Upazila	sample	DDT	Heptachlore	Endrin	Dieldrin
Khulna	Batiaghata	Shrimp	ND	ND	ND	ND
	Dakope	Shrimp	ND	ND	ND	ND
	Digholia	Prawn	ND	ND	ND	ND
	Dumuria	Prawn	ND	ND	ND	ND
	Fultola	Prawn	ND	ND	ND	ND

	Koyra	Shrimp	ND	ND	ND	ND
	Paikgacha	Shrimp	ND	ND	ND	ND
	Rupsha	Prawn	ND	ND	ND	ND
	Terokhada	Prawn	ND	ND	ND	ND
Shatkhira	Assasuni	Shrimp	ND	ND	ND	ND
	Debhata	Shrimp	ND	ND	ND	ND
	Kalaroa	Shrimp	ND	ND	ND	ND
	Kaliganj	Shrimp	ND	ND	ND	ND
	ShatkhiraSadar	Shrimp	ND	ND	ND	ND
	Shamnagar	Shrimp	ND	ND	ND	ND
	Tala	Shrimp	ND	ND	ND	ND
Bagerhat	Bagerhat	Prawn	ND	ND	ND	ND
	Chitalmari	Prawn	ND	ND	ND	ND
	Fakirhat	Prawn	ND	ND	ND	ND
	Kachua	Prawn	ND	ND	ND	ND
	Mollahat	Prawn	ND	ND	ND	ND
	Mongla	Shrimp	ND	ND	ND	ND
	Morrelganj	Prawn	ND	ND	ND	ND
	Sarankhola	Shrimp	ND	ND	ND	ND
	Rampal	Shrimp	ND	ND	ND	ND
Prawn		0.00022	0.00012	ND	ND	
Acceptable Limit			0.05	0.01	0.01	0.02

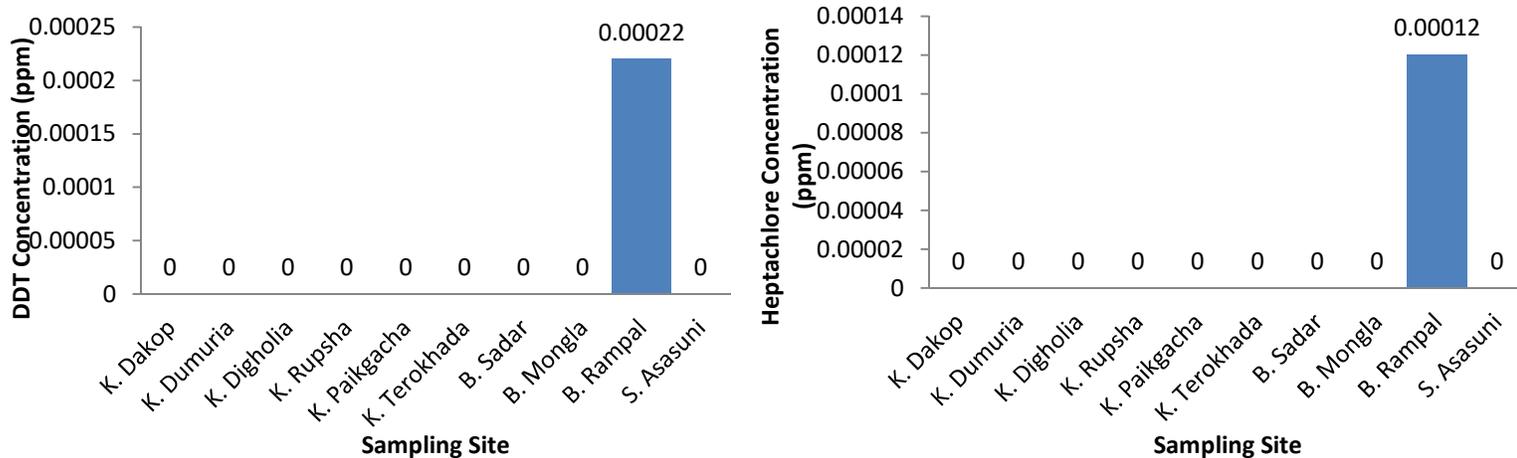


Figure 1 : Hazardous pesticides residue in Prawn/ Shrimp samples of Khulna, Shatkhiraand Bagerhat district

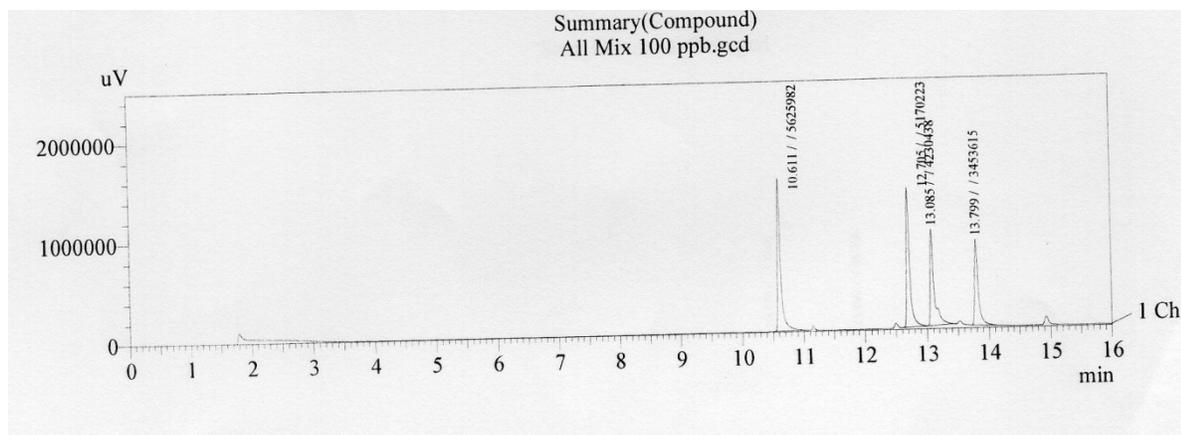


Figure 2: Chromatogram peaks obtained during real time analysis of standard

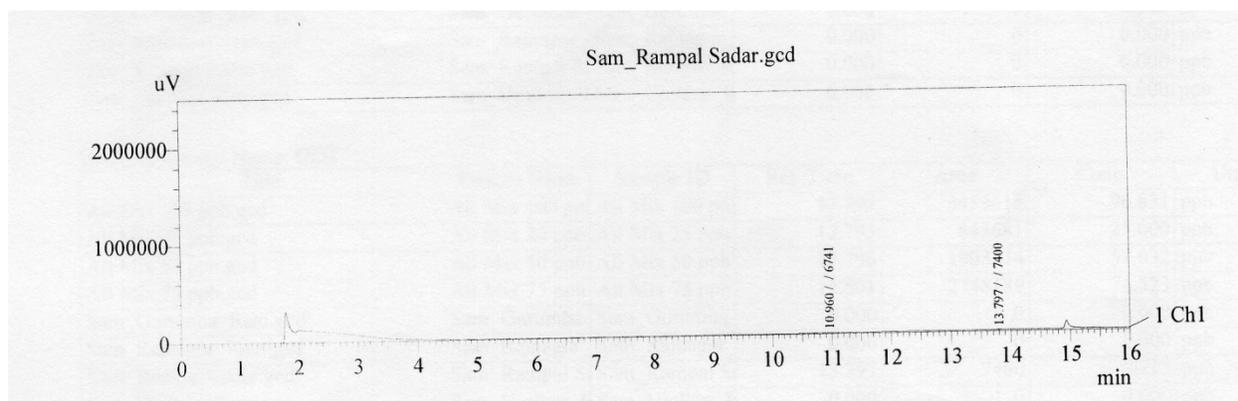


Figure 3: Chromatogram peaks obtained during real time analysis of sample

Assessment of antibiotic residues in shrimp/prawn farms:

For analysis of banned antibiotic Nitrofurantoin and Chloramphenicol, collected Shrimp and Prawn samples were analyzed by LC-MS Machine using standard analysis protocol. No hazardous Nitrofurantoin and Chloramphenicol metabolites were found from shrimp and prawn samples collected from sampling farms. For more confirmation randomly selected 12 shrimp/prawn samples out of 50 samples were sent to the FIQC, Dhaka to test the presence of Nitrofurantoin and Chloramphenicol residues but none of these were found.

Table 10: Tested parameters of Hazardous antibiotic residue in collected samples

Test Parameter	MRPL/RPA (ppb)	Reporting Limit & (ppb)	Result (ppb)	Method of Test	Analytic Tools
Nitrofurans Metabolites					
AMOZ	1.0	0.32	Not Detected	FLD_CD TMSOP-03 V.4	Analysis was carried out by LC MS/MS
AOZ	1.0	0.26	Not Detected		
AHD	1.0	0.19	Not Detected		
SEM	1.0	0.34	Not Detected		
Chloramphenicol	0.3	0.08	Not Detected	FLD_CD TMSOP-01 V.2	

Antibiotics are the group of chemicals that have the capacity to kill or inhibit the growth of microorganisms. Like heavy metals they are persistence in nature. Antibiotics (e.g. Nitrofurans and Oxytetracycline) are widely used in fish farms to control bacterial infectious diseases and to limit fish mortality. In fish farming, the widespread use of antibiotics (e.g. Nitrofurans and Oxytetracycline, Tetracycline) for treating bacterial diseases has been associated with the development of antibiotic resistance in *Aeromonashydrophila*, *A. salmonicida*, *Edwardsiellatarda*, *E. ictturali*, *Vibrio anguillarum*, *V. salmonicida*, *Pasteurellapiscida*, and *Yersinia ruckeri* (Hernandez 2005). Antibiotics are also used in pelleted feed stuffs. These substances are released to the aquatic environment from urine, feces and also uneaten feed (Hektoen *et al.* 1995).

Nitrofurans, particularly Furazolidone (FZD), Furaladone (FTD), Nitrofurantoin (NFT) and Nitrofurazone (NFZ), belong to a class of synthetic broad spectrum antibiotics which contain a characteristic 5-nitrofurans ring. Nitrofurans were commonly used in aquaculture as feed additive (Draisci *et al.* 1997). In 1995, EU banned the use of antibiotic due to concerns about the carcinogenicity of the drug residues and their potential harmful effects on human health (Van Kotten-Vermeulen 1993). Since 1993, the use of Nitrofurans in agriculture, livestock and aquaculture has also been prohibited in many countries like Australia, USA, Philippines, Thailand and Brazil because of a possible increased cancer-risk through long-term consumption (Khong *et al.* 2004). Like Nitrofurans, Oxytetracycline is generally considered a very stable substance in the environment, with half-life of 101 to 364 days (Zuccato *et al.* 2001). Oxytetracycline is used to control infectious diseases especially in carp polyculture. Use of antibiotics, in aquaculture is one of the major concerns because there is evidence that their use may cause bacterial resistance. Moreover, over indulgence use of antibiotic can cause serious damage of ecosystem and human being. It is estimated that over 75% of the majority of antibiotics disseminated to water at fish rearing facilities spread to the external environment (Halling-Sorenson *et al.* 1998). These drugs can accumulate in ecosystem and enter into the food chain.

11.3: Impact assessment of the pesticides and antibiotics residues on human health

In the present study, no hazardous Nitrofurantoin metabolites and Chloramphenicol was found in the samples. One prawn sample of Bagerhat district contained pesticides residues (DDT 0.00022ppm and Heptachlor 0.00012 ppm) which were much lower than the acceptable limit of DDT 0.05 ppm and Heptachlor 0.01ppm and have no risk for human health.

12. Research highlight/findings :

- A total of 354 brands of pesticides and 84 brands of aqua drugs were found.
- No hazardous pesticide residues were found in collected water and soil samples.
- No hazardous Nitrofurantoin metabolites and Chloramphenicol was found in the samples.
- One prawn sample of Bagerhat district contained pesticides residues (DDT 0.00022ppm and Heptachlor 0.00012 ppm) which were much lower than the acceptable limit.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk.)	Phy (#)	Fin (Tk.)	
(a) Office equipment					
Steel Almira	1	20000	100%	20000	
Laser Printer	1	20000	100%	20000	
Laptop computer	1	60000	100%	60000	
Scanner	1	10000	100%	10000	
Computer Table	1	10000	100%	10000	
(b) Lab &field equipment	a) Lab/Field chemicals/ Reagents		a) Lab/Field chemicals/ Reagents		
Acetonitrile LC -MS Grade	1	35,000	100%	35,000	
Acetonitrile HPLC -Grade	2	20000	100%	20000	
Methanol LC -MS Grade	2	36000	100%	36000	
Methanol Lab Grade	1	10000	100%	10000	
Dichloromethane Lab Grade	2	20000	100%	20000	
Ethyl Acetate - Lab Grade	3	15000	100%	15000	
Primary Secondary Amine (PSA)	5	450000	100%	450000	
Acetone	1	10,000	100%	10,000	
Sodium Sulfate	10	10,000	100%	10,000	
Magnesium Sulfate	10	10,000	100%	10,000	

Sodium Chloride	10	10,000	100%	10,000	
DDT--- 4,4'-DDT (Cl ₆ H ₄) ₂ CHCl ₃ , Analytical Standard	2	40000	100%	40000	
Heptachlor (C ₁₀ H ₅ Cl ₇) Analytical Standard	2	90000	100%	90000	
Endrin (C ₁₂ H ₈ Cl ₆ O) Analytical Standard	2	50000	100%	50000	
Dieldrin (C ₁₂ H ₈ Cl ₆ O) Analytical Standard	2	40000	100%	40000	
Chloramphenicol	2	32,000	100%	32,000	
DL-threo Chloramphenicol. D5	1	90000	100%	90000	
AMAZ	1	100000	100%	100000	
AOZ	1	110000	100%	110000	
AHD	1	20000	100%	20000	
SEM -HCl	1	20000	100%	20000	
AMAZ-D5	1	60000	100%	60000	
	b) Lab/Field Equipments /apparatus		b) Lab/Field Equipments/a pparatus		
Centrifuge tube (50 ml)	15	45000	100%	45000	
Centrifuge tube (15 ml)	10	25000	100%	25000	
Tube rack	5	5000	100%	5000	
Single use disposable syringe	2000	20000	100%	20000	
Ruffing pump Oil	3	30000	100%	30000	
Glass Vial 1.5 ml with septa and caps	5	125000	100%	125000	
Micropipette 5 ml	1	50000	100%	50000	
GC-MS auto sampler syringe	1	40000	100%	40000	
Water Purification Cartridge	4	100000	100%	100000	
PTFE Syringe filter 0.45 μ	5	90000	100%	90000	
Acid resistant hand gloves	2 pair	1000	100%	1000	
Operational Hand Gloves	5	3500	100%	3500	
Sampling zipper bag	10	8000	100%	8000	
GPS machine	1	40000	100%	40000	
(c) Other capital items					
Helium Gas Cylinder		200000	100%	200000	
Oxygen Gas Cylinder		50000	100%	50000	

2. Establishment/renovation facilities: N/A

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
					Not Applicable

3. Training/study tour/ seminar/workshop/conference organized:

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training	08	02	10	03 days	Pesticide Residue Analysis in Shrimp using QuEchERS Extraction and Gas Chromatography
(b) Workshop	N/A	N/A	N/A	N/A	

C. Financial and physical progress Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	191900	176345	189642	-13297	100%	
B. Field research/lab expenses and supplies	3018100	2989014	3007663	-18649	100%	
C. Operating expenses	410000	389122	383117	6005	100%	
D. Vehicle hire and fuel, oil & maintenance	200000	211243	195950	15293	100%	
E. Training/workshop/seminar etc.	150000	149280	97000	52280	65%	
F. Publications and printing	110000	101084	9875	91209	10%	This unspent fund is for PCR which will be printed by PIU
G. Miscellaneous	50000	48007	49700	-45693	100%	
H. Capital expenses	370000	365404	369923	-4519	100%	
Total	4500000	4429499	4302870	246945		

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output(i.e. product obtained, visible, measurable)	Outcome(short term effect of the research)
To scrutinize the available pesticides, antibiotics and other aqua-chemicals from existing shrimp /prawn farms of Khulna, Bagerhat and Shatkhira region through baseline survey	1. Conduction of baseline survey	A pretested check list had been prepared to identify the available chemicals and antibiotics which may harmful for human health.	1.To minimize pesticide and hazardous chemicals adulterant in shrimp/prawn by alleviating its dependency for reducing human health risk
To assess the hazardous pesticides, antibiotics and aqua-chemicals residues in shrimp / prawn and identification of its probable sources	1. Sample collection from the sampling sites 2. Pesticides and antibiotics (Nitrofurantoin, Chloramphenicol) residues in shrimp /prawn farms was assessed and confirmed	Sources of hazardous antibiotic metabolites and pesticides was identified	2.To build-up awareness on safe usages of pesticides and antibiotics
To assess the impacts of pesticides and antibiotics / chemicals residues on human health through risk quantification	1. Impacts of pesticides and antibiotics/chemicals residues on human health was measured	1. Impacts of pesticides and antibiotics/chemicals residues on human health can't be measured 2. Proper policy guideline can be developed in order to produce safe shrimp/prawn and ensure smooth export of shrimp, employment generation and poverty alleviation.	

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.	01	-	-
Journal publication	01	-	-
Information development	-	-	-
Other publications, if any	-	-	-

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

Not Applicable

ii. Generation of new knowledge that help in developing more technology in future

Not Applicable

iii. Technology transferred that help increased agricultural productivity and farmers' income

Not Applicable

iv. Policy Support

The present knowledge could be used for improving the fish/shrimp drug use policies/regulations of the country.

G. Information regarding Desk and Field Monitoring

ii) Monitoring - Monitoring Workshop

Monitoring Workshop organized by	Date	No. of visit	Output
PIU-BARC, NATP-2	21 December 2017	01	Suggested for improvement
PIU-BARC, NATP-2	15 May 2018	01	Expressed satisfaction with the output

i) Field Monitoring (time& No. of visit, Team visit and output):

Monitoring Team	Time & No. of visit	No. of visit	Output
Technical Division/unit, BARC	16/02/2018	01	Update monitoring report with enough observation of the team
PIU-BARC, NATP-2	09/04/2018	01	Same as above
Internal Monitoring	22/06/2018	01	Same as above
Others(BTV), Mati O Manus (In Bangla)	07/02/2018	01	Increased consciousness of consumer

H. Lesson Learned/Challenges (if any)

Fisheries sector plays an important role in the economy of the country in terms of nutrition, income generation, employment and foreign exchange earnings. Shrimp farming is one of the most important economic activities in the south and south-west parts of the country from the last three decades. A variety of chemicals and drugs are used in aquaculture for different purposes viz. sediment and water management, enhancement of natural aquatic productivity, transport of live organisms, feed formulation, manipulation and enhancement of reproduction, growth promotion, health management, processing and adding value to the final product. The reliance on these chemicals and drugs is increasing with the intensification of production, and their environmental toxicity has to be questioned. In the present study, the presence of residues found much lower than the acceptable limit but everyone should be aware about the indiscriminate use of pesticides, chemicals and aqua drugs because it may be harmful for the safe food production in the near future and more study is needed for the quantification of residues in shrimp/prawn in both captured and cultured fishery.

I. Challenges (if any)

Signature of the Principal Investigator
Date
Seal

Counter signature of the Head of the organization/authorized representative
Date
Seal

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