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Shrimp/prawn farming status in south-west region of Bangladesh in context of quality control and food safety issues

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Abstract. To determine hazardous antibiotics/chemicals and pesticides residues in Good Aquaculture Practice (GAP) and non-GAP farms and exploring the post-harvest management techniques of shrimp/ prawn for ensuring its food safety issues, a study was conducted from July 2016 to June 2019 in three upazilas *viz*. Bagerhat Sadar, Rampal and Kachua of Bagerhat district. A total of 550 shrimp/prawn farms were randomly surveyed considering GAP criteria. The farms were categorized as Category A- the farms which comply 90-100% GAP criteria; Category B- farms which comply 70-< 90% GAP criteria and Category C- farms which comply 50-<70% GAP criteria. From the study it was found that only 1.67±0.58 % farms were under Category-A, 37.67±6.81 % farms were under Category-B and 53±6.08 % farms were under Category-C. A total of 108 shrimp/prawn samples were collected in which 30% samples were from GAP farms and 70% samples were from non-GAP farms. Collected samples were analyzed for hazardous antibiotics/chemicals and pesticides residues (Heptachlor, Endrin, Dieldrin and DDT) using LC-MS and GC-MS machine. No hazardous Nitrofuran and Chloramphenicol metabolites were found from the collected samples and no residual concentrations of Heptachlor, Endrin, Dieldrin and DDT were found from GAP samples. In non-GAP samples, 14.8% samples contained some residues of Heptachlor, Endrin and Dieldrin in which 10.2% samples residues were lower than the Maximum Residual Limit (MRL) which is not harmful for human health. Only 4.6% samples had residues which were slightly higher than the MRL. Exploration of post-harvest management techniques of shrimp and prawn were done by observing the icing system quality and transportation system from farms to market/depot. It was found that 5.67±2.08% farmer used proper icing and transportation system as well, 16.33±3.21% farmers used moderate icing and transportation system and 78±2% farmers used poor icing and transportation system. It was also observed that pathogenic bacterial load was higher in poor icing and transportation system as well than the proper icing and transportation system. From the study it can be concluded that, if farmers follow at least 50% of the GAP criteria then the foods (shrimp/prawn) will be safer for human consumption.
Keywords: Good Aquaculture Practice, Antibiotics, Pesticides, Food safety issues

Introduction

Bangladesh is one of the pioneers of frozen sea food exporters' country and ship mainly frozen shrimps to mainstream market in the USA, EU, Japan, Saudi Arabia, the UAE and Gulf States (Ali *et al*. 2012, Khan *et al*. 2012). However, the exported shrimp is rejected many times by the importing countries because of high bacterial load, decomposition and due to the presence of filth, unexpected foreign materials as well as pathogenic microbes *(Escheritia coli, Salmonella, Vibrio cholerae* etc.). After 17 December, 1997 it was mandatory to prepare all the seafood products under the HACCP regulations (USFDA 1997). Good Manufacturing Practices (GMP) as well as Good Aquaculture Practices (GAP) and sanitation are the perquisite for the implementation of HACCP system. Now-a-days, imported countries have developed standard criteria and a full certification system for the shrimp production line from farm to table. In recent times, the GMP and sanitation procedures of many fish processing plants are excellent, hence their product performance (quality) earn a better place in the foreign markets. However,

sometimes remarkable contamination in shrimp is found due to improper management in culture stage. That's why GAP has become a mandatory task for implementation of HACCP system in a better way. GAP are a series of considerations, procedures and protocols designed to foster efficient and responsible aquaculture production and expansion and to help in ensuring the final product quality, safety and environmental sustainability (Schwarz *et al*. 2010).

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 rice. Some of these pesticides residues are gradually accumulating in shrimp/prawn body. In Bangladesh, import and production of all types of Persistent Organic Pollutants (POPs) like DDT and heptachlor is banned but at least five POPs pesticides including DDT are still in use under different name or label. Besides, some banned antibiotics such as Nitrofurans, Chloramphenicol, Malachite green, Leuco Malachite green etc. also accumulating in shrimp/prawn which are mostly approved for veterinary products and used in farms for disease control and shrimp/prawn health management. All of these products are highly carcinogenic and threatening for food safety. According to WHO estimation, 1.8 million deaths related to contaminated food or water occurs every year. 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. In this context, consciousness of our farmers is inadequate. So, it is very much essential to identify and quantify of these pesticides and antibiotics residues for assessment of risk on human health.

Materials and Methods

Expt. I: Survey the existing shrimp farms for exploration of its present farming status based on GAP (Good Aquaculture Practice)

This survey were conducted in the shrimp farming areas for 3-4 months and data were collected from 550 randomly selected shrimp farms considering the following GAP Criteria : (i) Good Practice Status in Site Selection for Shrimp, (ii) Good Practice Status in Farming, (iii) Good Practice Status in Water Use, (iv) Good Practice Status in Surrounding Environment and Pest Control of the Farm, (v) Good Practice Status in Feed Management, (vi) Good Practice Status in Disease Management, and (vii) Good Practice Status in Harvesting and Post Harvesting Management. The experimental design is shown in Table I.

Table I. Experimental design

Sampling Site	Survey type	Survey method	Surveyed farms	Target aspects
Bagerhat Sadar, Rampal, Kochua Upazila of Bagerhat district	Questionnaire Survey	Random Survey from shrimp/prawn farms and in few cases from market with PRA method	20 nos. /Union	Site Selection, Farming management, Water Use, Surrounding Environment and Pest Control of the Farm, Feed Management, Disease Management as well as Harvesting and Post Harvesting Management

Expt. II: Assessment of the hazardous antibiotics/chemicals and pesticides residues to determine shrimp/prawn quality

Table II. Experimental design

Sampling sites	Sampling farms	No. of sampling (monthly)	Sample species	Sample weight	Target analyses
Bagerhat Sadar, Rampal, Kochua Upazila of Bagerhat district	GAP farm	05 (Approx.) based on survey result	*M. rosenbergii* / *P. monodon*	500 g	**Antibiotics:** Nitrofuran Metabolites, Chloramphenical) **Pesticides:** Organochlorine pesticides such as DDT, Dieldrin, Heptachlor, Endrin
	non-GAP farm	05 (Approx.) based on survey result			

Sampling procedure: From the surveyed farms, 10% farms were selected as sampling sites. Multisampling was done whenever necessary. Shrimp and prawn samples were collected randomly from the farms when those attained 3+ months age. Then those were brought into Quality Control lab. of Shrimp Research Station (SRS) and preserved in refrigerator for further analysis.

Extraction, derivatization and clean-up procedures for antibiotic test using LC-MS/MS: Frozen whole shrimp and prawn were thawed, rinsed under running water, and the shell and head were removed. Muscle was chopped with knife on a clean board and collected in a beaker and kept in the fridge until analysis. About 2.0 g of chopped sample was taken into 50 ml PE-centrifugation tube. Then 4ml HPLC-Water and 3mL 1 M HCL was added. The mixture was homogenized using Ultra Terex for 2 minutes. Washing the rotor with HPLC water was done in order to make sure that the total volume do not to exceed 17 ml. Adding of 400μl of 2-nitrobenxaldehyde solution and 500 μl internal standards were insured. The PE-centrifugation tubes with the dedicated cap was kept in the dark at room temperature in the water bath keeping at constant 37^{0}C for 16 hours for derivatization. In order to adjust the pH adding of 2 ml di-potassium hydrogen phosphate solution and 2.5 mL 0.8 mol/L NaOH (aq.) was done. When necessary 0.125 mol/L hydrochloric acid or the 0.125 mol/L. NaOH (aq.) were used to adjust the pH 7. After that 8 ml ethyl acetate was added and shaked for 5 min and centrifuged for 5 min at 4000 rpm and the ethyl acetate phase (upper phase) was transferred to a 15 ml centrifugation tube. The sample was extracted again with 4 ml ethyl acetate, centrifuged and combined the ethyl acetate phases in the 15 ml PE-centrifugation tube. Concentration of the extract was done at 45^{0}C under the stream of dry nitrogen. Then the residue was mixed with 1.0 ml methanol: water (50% / 50%) and 1.0 ml n-hexane in 15 ml PE centrifugation tube, for centrifugation at 4000rpm at 5 min at 4^{o}C. The upper hexane phase was discarded by using disposable syringe and long needle syringe was used to draw the bottom (clear) part into the syringe. Then it was filtered by 0.25μm syringe filter to the vial. At last, the vial with sample was taken for injection by LCMS/MS API 3200.

Extraction and clean-up procedure for pesticides residue analysis using GC-MS/MS: The extraction was carried out according to the procedure described by QuEschERS Method and

necessary modification was also adopted for extraction, separation and clean-up sample. 10g of shrimp and prawn sample was taken in a Teflon tube. 20 ml ethyl-acetate/aceto-nitrile was added to it and hand shaken for 1 min. Then it had to shake with vortex mixture for 2 min and 1.5g NaCl and 6g anhydrous $MgSO_4$ was added to it and then hand shaken for 1 min. Again it had to shake with vortex mixture for 1/2 min. Then it was filtered with 20g $NaSO_4$ and 10 mL anhydrous ethyl-acetate. The mixture had to centrifuge at 5000 rpm for 5 minutes. Then 10 ml supernatant was taken in a round bottom flask. The supernatant was evaporated with rotary evaporator keeping temperature not more than 40^0C. 5 ml n-hexane was added in a round bottom flask. Then 2 ml n-hexane soln. was taken in a test tube.

For cleaning-up: 2ml H_2SO_4 was added with that 2 ml n-Hexane soln. Then it was vortex for 1 min. After vortex the mixture was centrifuged at 4000 rpm for 3 minutes. After that the supernatant was taken in a tube and was filtered with 0.45μg syringe filter in a vial. Then finally 2mL sample was taken in a vial for GC-ECD or GC-FID analysis.

Expt. III: Post-harvest management techniques of shrimp/prawn to ensure food safety issues

Table III: Experimental Design

Sampling Sites	Sampling farms	No. of sampling (monthly)		Sample Species	Sample weight	Analysis aspects
		Sampling from farms	Sampling from local market/ depot			
Bagerhat Sadar, Rampal, Kochua Upazila of Bagerhat district	GAP following farm	05 (Approx.) based on survey result	05	*M. rosenbergii* /*P. monodon*	500 g	Risk analysis through risk assessment, Risk management, Risk communication, Hazard identification, Hazard characterization, Exposure assessment, Risk characterization
	non-GAP farm	05 (Approx.) based on survey result	05			

Sampling and analysis procedure: Samples were collected randomly from the farms when those became attain 3+ months age. Same samples of pre-selected farms were collected from the market/depot after their transportation from farm to market. Then their icing quality as well as transportation quality was observed to ensure food safety. Food safety issues were ensured after risk analysis through risk assessment, risk management, risk communication, hazard identification, hazard characterization, exposure assessment, risk characterization process.

Issues concerning risk analysis: (i) Icing system has been observed from farm to depot/market, (ii) Marketing chain/channel observation, (iii) Transportation techniques was observed from farm to depot/market.

Statistical analysis: Statistical analysis was done using MS-EXCELL as well as SPSS software for the meaningful interpretation of the research outputs.

Results and Discussion

Expt. I: Survey of shrimp farms for exploration of its present farming status based on GAP

A comprehensive survey was conducted randomly among 550 farms of Bagerhat Sadar, Rampal, and Kochua Upazila of Bagerhat district covering 27 Unions. On the basis of surveyed data, the surveyed farms were categorized into 03 categories *viz.* Category-A, Category-B and Category-C where Category-A indicates the farms which comply 90-100% GAP criteria; Category-B indicates the farms which comply 70-< 90% GAP criteria and Category-C indicates the farms which comply 50-<70% GAP criteria. Then from the surveyed data, it was found that 1.67±0.58 percent farms were under Category-A, 37.67±6.81 percent farms were under Category-B and 53±6.08 percent farms were under Category-C. Rest of the farms (7.67±1.53 percent) did not follow the criteria of GAP (Table IV).

Table IV. Present status of shrimp farms at Bagerhat Sadar, Rampal, Kochua Upazila, Bagerhat

Category of Farms	Percentage of Farms (%) (Mean ± SD)
Category-A	1.67±0.58
Category-B	37.67±6.81
Category-C	53±6.08
Out of GAP Category	7.67±1.53

Expt. II: Assessment of the hazardous antibiotics/chemicals and pesticides residues for determination of shrimp/prawn quality of GAP and non -GAP farms

Assessment of antibiotic residues in shrimp/prawn farms: For analysis of banned antibiotic Nitrofuran and Chloramphenicol, the shrimp and prawn samples were analyzed by LC-MS following standard analysis protocol. No hazardous Nitrofuran and Chloramphenicol metabolites were found from shrimp and prawn samples collected from sampling farms (Table V).

Table V. Tested parameters of hazardous antibiotic residue in collected samples

Test Parameter	MRPL/RPA (ppb)	Reporting limit (ppb)	Result (ppb)	Method of test	Analytical tools
Nitrofuran 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	

Nitrofurans, particularly Furazolidone (FZD), Furaltadone (FTD), Nitrofurantoin (NFT) and Nitrofurazone (NFZ), belong to a class of synthetic broad spectrum antibiotics which contain a characteristic 5-nitrofuran ring. Nitrofaurans 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 Koten-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 Nitrofaurans, Oxytetracycline is generally considered a very stable substance in the environment, with half-life of 101 to 364 days (Rahman 2013).

Assessment of pesticide residues in shrimp and prawn farms: Shrimp and prawn samples were collected from sampling sites for pesticidal residue analysis. A total of 108 shrimp/prawn samples were collected from the sampling site in which 30% samples were from the GAP farms and 70% samples were from the non-GAP farms. The samples were analyzed for DDT, Dieldrin, Endrin and Heptachlore using GC-MS Machine following standard analysis protocol. Analyzed report from ECD detector of Gas Chromatography shows that no residual concentrations of Heptachlor, Endrin, Dieldrin and DDT were found from the GAP samples. In the non-GAP samples, 14.8% contained some residues of Heptachlor, Endrin, Dieldrin and DDT in which 10.2% samples residues were lower than the Maximum Residual Limit (MRL) which is not harmful for human health. Only 4.6% samples had residues which were slightly higher than the MRL (Table VI).

Table VI. Residual concentration of hazardous pesticides in collected sample

Sampling sites	Upazila	Heptachlor (ppm)	Dieldrin (ppm)	Endrin (ppm)	DDT (ppm)
Badhal	Kochua	00	0.022	00	00
Dhopakhali		00	00	00	00
Gopalpur		00	0.018	00	00
Moghia		00	0.012	00	00
Raripara		00	0.012	00	00
Gozalia		00	0.010	00	00
Kochua Sadar		00	0.025	00	00
Bagerhat Sadar	Bagerhat sadar	0.042	0.086	0.391	00
Karapara		00	0.00023	00	00
Jatrapur		0.00019	00	00	00
Gurumba	Rampal	0.00031	00	00	00
Rajanagar		0.00014	00	00	00
Rampal Sadar		0.00012	00	00	0.00022
Acceptable limit		0.01	0.02	0.01	0.05

Zaman *et al*. (2012) studied 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

consist some other harmful organic contaminants in tissues. Rahman (2013) studied on organochlorine pesticides in marine sediments along the coast of Alicante, Spain. The levels of organochlorines in sediments were low.

Expt. III: Post-harvest management of shrimp/prawn to ensure food safety issues

The experiment about the exploration of post-harvest management techniques of shrimp/prawn for ensuring its food safety issues was done by observing the icing system quality and transportation system quality from farms to market/depot. It was found that (5.67±2.08)% farmers used proper icing and transportation system, (16.33±3.21) % farmers used moderate icing and transportation system, and (78±2) % farmers used poor icing and transportation system (Table VII). It was also observed that pathogenic bacterial load was higher in poor icing and transportation system as well than the proper icing and transportation system.

Table VII. Observation of icing and transportation system quality from farms to market /depot

Icing System Quality	% of Farms	Duration (from farm to depot / market)	Risk category
Good (Maintaining proper ice ratio)	5.67±2.08	0.5 to 2 hrs.	Low
Moderate (Not maintaining proper ice ratio)	16.33±3.21		Medium
Poor (No icing)	78±2		High

Conclusions

In the present study, no residual concentrations of Heptachlor, Endrin, Dieldrin and DDT were found from GAP samples. In non-GAP samples, 14.8% samples comprising some residues of Heptachlor, Endrin, Dieldrin and DDT in which 10.2% samples residues were lower than the Maximum Residual Limit (MRL) which is not harmful for human health. Only 4.6% samples had residues which were slightly higher than the MRL. Though the presence of residues was 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. It can be concluded from the study that, if farmers follow at least 50% of the GAP criteria then the foods (shrimp/prawn) will be safer for human consumption.

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