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Assessment of Some Organochlorine Pesticides Residues in Shrimp and Prawn of South-west Region of Bangladesh

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Abstract: ***An attempt was made to know the residual status of some organochlorine pesticides in shrimp (Peneus monodon) and prawn (Macrobrachium rosenbergii) by Electron Capture Detector (ECD) of Gas Chromatography during their culture with rice. A Total of six (06) sites viz. Bagerhat Sadar, Kochua, Fakirhat, Mollarhat of Bagerhat District and Dumuria, Fultola of Khulna District were selected for this study from where the shrimp/prawn samples were collected to assess some organochlorine pesticides named Heptachlor, DDT, Dieldrin and Endrin. The heptachlor residue concentration was 0.035~0.155 ppm; Endrin residue was 0.008~0.020 ppm; Dieldrin residue concentration was 0.003 ppm and DDT residue concentration was 0.005 ppm in Shrimp body. In Prawn sample, Heptachlor residue concentration was 0.586~0.806 ppm; Endrin concentration was 0.044~0.073 ppm. Dieldrin and DDT residue were not detected in Prawn body. From the assessment of these four pesticide residues concentration in Shrimp and Prawn body, it was noticeable that Heptachlor concentration was in alarming level according to acceptable limit of EU. Endrin, Dieldrin and DDT concentration were not in harmful level in accordance with EU acceptable level. So, proper initiatives should take to inhibit the uses of all classes of hazardous pesticides and chemicals for avoiding any health hazard situation to ensure food safety.***

Keywords: Shrimp and Prawn; Organochlorine Pesticides: DDT, Heptachlor, Dieldrin, Endrin; GC-ECD detector etc.

1. Introduction

Shrimp and frozen products is an important item in Bangladesh which contribute huge amount of foreign currency after the readymade garments. Total export of fish and frozen products in 2010-2011 was 96469 mt contributing 4604 crore Tk. Shrimp contributes 54891 mt of 3568 crore Tk. But now-a -day's export of shrimp facing difficulties due to malpractices in shrimp processing and culture. In south-west region of Bangladesh like Khulna, Bagerhat and Shatkhira district most of the farmers' culture shrimp and prawn with rice. As a result they have to use various groups of pesticides to control pest of their rice field which ultimate accumulate in sediment and from sediment it transmitted to shrimp and prawn body. Organochlorine (OC) pesticides are among the agrochemicals that have been used extensively for long periods. They have been used widely in agriculture, as well as, in mosquito, termite and tsetse fly control programs [1]. Residues and metabolites of many OC pesticides are very stable, with long half lives in the environment [2].

Studies have shown that DDT is still in its highest concentration in biota of some areas. It is a hydrophobic molecule which disrupts ionic channels like Na^+-K^+ pumps in nervous cell membrane leading to automatic stimulation of neurons and involuntary contraction of muscles [3]. The persistent nature of organochlorine residues in the environment may pose the problem of chronic toxicity to animals and humans via air, water and foods intake. Many of these OC pesticides and their metabolites have been implicated in a wide range of adverse human and environmental effects including reproduction and birth defects [4], immune system dysfunction, endocrine disruptions and cancer [5]. Fish are used extensively for environmental monitoring [6], because they uptake contaminants directly from water and diet. Generally the ability of fish to metabolize organochlorines is moderate; therefore, contaminant loading in fish is well reflective of the state of pollution in surrounding environments [1]. Some analyses in Bangladesh show alarming pollutants in fish like DDT and heptachlor [7]. In Bangladesh at present, all Persistent Organic Pollutants (POPs) like DDT and heptachlor import and production have been banned but at least five POPs pesticides including DDT are still in use under a different name or label [8]. However, there is no specific legislation for controlling the production and use of hazardous industrial chemicals [9]. Among the dirty dozen the chlorinated hydrocarbons (organochlorine compounds), were the first generation of pesticides called wonder drug introduced following the Second World War which comprises DDT, Dieldrin, heptachlor and others. In areas where it is used for malaria control, infants can be exposed via breast milk in levels that exceed the W. H.O's acceptable daily intake value for DDT [10]. Thus, the present study has to design for the assessment of residue level of hazardous chemicals in shrimp farming systems of Bangladesh.

2. Materials and Methods

2.1 Sample Collection

A Total of six (06) sites *viz.* Bagerhat Sadar, Kochua, Fakirhat, Mollarhat of Bagerhat District and Dumuria, Fultola of Khulna District were selected for this study from where the shrimp/prawn samples were collected. The experiment was conducted during the period of October/2014 to April/2015. The control shrimp samples (not used any insecticides) were collected from the internal pond complex of Shrimp Research Station, Bagerhat of Bangladesh Fisheries research Institute, Bangladesh.

2.2 Apparatus

Mincer fish chopper (Weisser No. 81 K), round bottomed flask (500 and 100 mL), volumetric flask (50 and 10 mL), Homogeniger IKAR T25 digital ULTRA-Turrax, Nitrogen evaporator(N-EVAPTM111), SPE Cartidge (C18-REC 300 mg/3 mL) Magnetic Starrier, Gas Chromatograph (GC-2010, Shimadzu).

2.3 Reagents

Methanol, n-Hexane, Ethyl acetate/Acetonitrile, Primary Secondary Amine (all were with high purity 99.99%, HPLC grade) and anhydrous sodium sulphate, anhydrous magnesium sulphate were purchased from Merk Company (Germany). DDT, Dieldrin, Heptachlor and Endrin reference standards were obtained from Sigma Alorich Chemicals (USA).

2.4 Extraction and cleanup procedure

The samples were extracted following QuEChERS method and few of its modification. At first Ten (10) g shrimp sample was taken in a Teflon Tube. Then 20 mL Ethyl acetate was added with that sample and the sample was shaken by hand for 1 min. Then the shaken sample was mixed properly with vortex mixture for 2 min. After the vortex, 1.5 g NaCl and 6 g anhydrous $MgSO_4$ was added with the mixture and again shaken by hand for 1 min. Then was shaken with vortex machine for 2 min. After vortex, the mixture was centrifuge at 5000 rpm for 5 min which created two layer *viz.* supernatant (upper aqueous layer) and denatant (lower non-aqueous layer). Then filtration the supernatant with 20g Na_2SO_4 and 10 mL anhydrous Ethyl acetate. Ten (10 mL) supernatant was taken in a round bottom flask. Then the filtered solution was evaporated with rotary evaporator at a temperature not more than 40^0C until it was completely dry. Then 5mL n-Hexane was added in that round bottom flask. After that 2mL solution of the previous round bottom flask was taken in a test tube for cleaning-up.

Cleaning-up procedure

Adding of 150 mg PSA (Primary Secondary Amine) and 750 mg anhydrous $MgSO_4$ in that previous test tube was done. Then the solution was mixed with vortex mixture for 1 min. After that the solution mixture was centrifuged at 4000 rpm for 5 min which created two layer *viz.* supernatant (upper aqueous layer) and denatant (lower non-aqueous layer). Then the supernatant was taken in a tube and filtered with a 0.45 µ syringe filter in a 1.5 mL vial. Finally, the vial was kept in injection tray for its real time analysis through GC-ECD or other detector.

2.5 Sample analyses

The target pesticides residues were analyzed by GC-2010, Shimadzu with an Electron Capture Detector (ECD), an auto injector (Shimadzu, AOC 20i) and GC solution software. The capillary column used was Rtx-5MS, length 30.0 m x ID 0.25 mm x film thickness 0.25µm. The GC was run under the following conditions: injector temperature: 200^0C; detector temperature 270^0C; oven temperature programme: 250^0C starting from 100^0C for 1 minute and continued at 5^0C/minute to 250^0C held for 3 minute; injected sample volume: 1µL; mode of injection: Splitless; The carrier gas was N_2 with a 172.0 kPa flow rate. Run time; 16 min. Standards' peak were identified by injecting high concentration of the standard (1 ppm, 0.5ppm and 0.25 ppm) and the retention time for DDT, Heptachlor, Endrin and Dieldrin was determined. Then calibration was done at 3 points (1, 0.50 and 0.25 ppm) by composite stock standard solution. GC system was calibrated using external standard technique. Individual standard stock solution (100 mg/L) was prepared by weighing appropriate amounts of active ingredients in a brown bottle with a Teflon-lined screw cap and dissolving the weighed standard in HPLC grade methanol. Stock standard solution was used to prepare primary dilution standards. An appropriate volume of each individual stock solution was taken in a volumetric flask and mixed the solutions to obtain stock standard solution.

2.6 Analytical Quality Control

Gas chromatograph equipped with ECD was checked for linearity. Instrumental limit of detection for GC-ECD was 1.0 µg/l for Organochlorine pesticides. An aliquot of shrimp samples were collected as blank and treated exactly as a sample including exposure to all glassware, equipment, solvents and reagents used with the sample matrix. No analytic peak was detected in laboratory reagent blank. An aliquot of fortified samples matrix were prepared for known quantities of the pesticides which were added in the laboratory in ppm range. This laboratory fortified matrix was analyzed. Extraction and clean up were done as mentioned and the recoveries from untreated control samples of shrimp fortified with the analyzed compounds at the level of 0.50 ppm were 96 to 100% for heptachlor, 98 to 100% for DDT, 80 to 100% for Dieldrin and 86 to 100% for Endrin. Prior to injection of the first sample solution, a standard solution was injected at least three times to check the operating conditions and the constancy of the detector signals. Further linearity of the ECD signal was checked by injecting serial dilutions of DDT, Heptachlor, Endrin and Dieldrin. A standard solution injected after at least every other sample solution so that any alterations of the gas chromatographic system recognized due to column contamination. Any insecticide detected from the tested samples was identified and quantified by the chromatogram of standards. Sample results were quantitated in ppm automatically by the GC-Solution software, which represented the concentration of the final volume injected and from the value, the actual amount of pesticides residues present in the sample was determined by using the following formula:

$$= \frac{\text{Concentration of obtained in injected volume (ppm)} \times \text{Quantity of final volume (L)}}{\text{Amount of sample taken (Kg)}}$$

3. Results

Prawn and Shrimp samples were collected from Bagerhat Sadar, Kochua, Fakirhat, Mollarhat of Bagerhat District and Dumuria, Fultola of Khulna District for analysis of DDT, Dieldrin, Heptachlor and Endrin residue. From the analyzed result, it is found that few pesticide residue are in alarming level of few sites. As shown in Table 1, different concentrations of DDT, Dieldrin, Heptachlor and Endrin residues were found.

Table 1: Concentration of pesticides residues in Shrimp and Prawn body of different sampling sites

Samples Name	Target Pesticides	Name of sampling location					Control	
		B'hat Sadar	Kochua Upazila	Fokirhat Upazila	Mollarhat Upazila	Dumuria Upazila	Fultola Upazila	SRS Pond Complex
Shrimp (Peneus monodon)	Heptachlor	0.131 ppm	0.155 ppm	0.035 ppm	0.119 ppm	0.041 ppm	0.058 ppm	ND
	Dieldrin	ND	ND	ND	ND	ND	0.003 ppm	ND
	Endrin	0.009 ppm	0.020 ppm	0.017 ppm	0.008 ppm	0.026 ppm	0.026 ppm	ND
	DDT	0.005 ppm	ND	ND	ND	ND	ND	ND
Prawn (Macrobrachium rosenbergii)	Heptachlor	0.806 ppm	0.586 ppm	ND	ND	ND	ND	ND
	Dieldrin	ND	ND	ND	ND	ND	ND	ND
	Endrin	0.073 ppm	0.044 ppm	ND	ND	ND	ND	ND
	DDT	ND	ND	ND	ND	ND	ND	ND

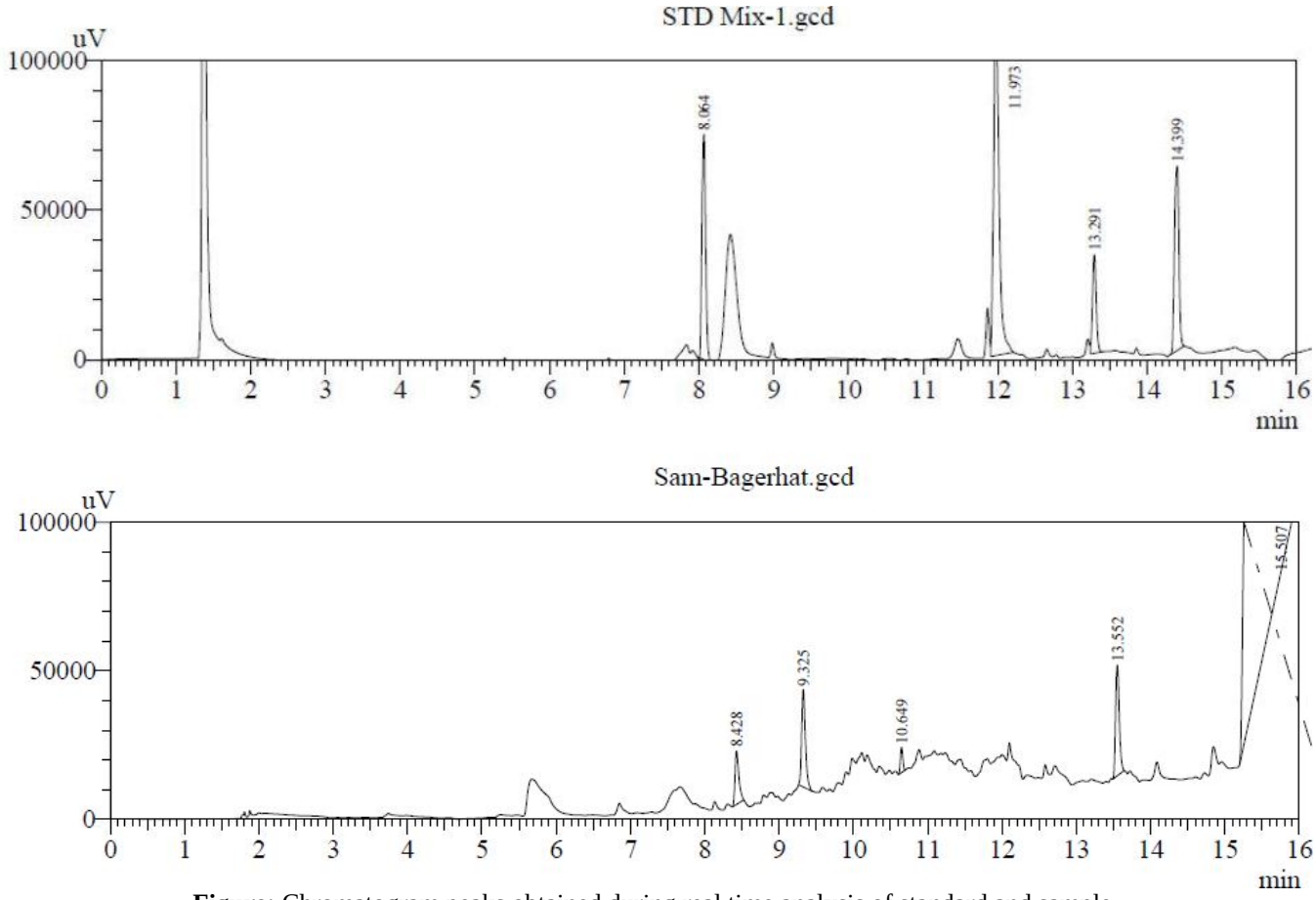

Figure: Chromatogram peaks obtained during real time analysis of standard and sample

4. Discussion

Until to date a few researchers have done research on DDT, Dieldrin, Heptachlor and Endrin residues assessment of shrimp and prawn in Bangladesh. To my best knowledge, this is the first time reported preliminary information on the residual concentration of DDT, Dieldrin, Heptachlor and Endrin in these selected sites *viz*. Bagerhat Sadar, Kochua, Fakirhat, Mollarhat of Bagerhat District and Dumuria, Fultola of Khulna District. Among the obtained results of pesticides residues few are showing alarming concentration level as these are above the acceptable limit of European Union (EU). According to the EU, acceptable limit for Heptachlor and Endrin in shrimp/prawn is 0.01 ppm. Acceptable limit for Dieldrin in shrimp/prawn is 0.02 ppm and DDT in shrimp/prawn is 0.05 ppm. Organochlorine compounds in general have linked to diabetes [11]. For mammals LD_{50} (acute oral) value for p, p'DDT ranging between 100 and 2500 mg/Kg have been reported by [12]. Almost all industrialized countries and many developing countries around the whole world have recognized the hazards of persistent pesticides banned. But still now some developing countries, POPs insecticides are willingly available in spite of official bans or severe restrictions. In those countries a government may lack the resources and infrastructure to implement and enforce the legislation fully.

People are selling these chemicals in isolated villages may have no idea that sale or use of the pesticides is restricted. The government of Bangladesh should take all the necessary steps to combat the situation. It can be implementation the legislation and improving the awareness of the related people through some program, public education campaigns and announcement of harmful rule of those insecticides and make difficulty the availability of those insecticides in market.

Due to our limitation of facilities, we could not study about other organochlorine and organophosphorus but we suspect that there may available other organochlorine pesticides contaminations shrimp and prawn sample. Further work on DDT, Dieldrin, Heptachlor and Endrin residues in blood serum of consumers of the study area is recommended.

5. Conclusion

The aim of this study was to provide baseline information on the concentration level of OCs in the shrimp and prawn sample collected from the selected sampling sites. Heptachlor residue was identified as the dominant OC in the sampling sites. In some cases the concentration level of Heptachlor cross the maximum acceptable limit recommended by EU. So, we should identify the probable source of this contaminant to reduce such contaminants.

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