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Contamination and Health Risk Assessment of Pesticide Residues in Vegetables from Agricultural Fields of Gazipur District, Bangladesh

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Abstract: A study was conducted to investigate the contamination and health risk assessment of pesticide residues in vegetables from agricultural fields of Gazipur district, Bangladesh. Ten vegetable samples (VS) were analyzed for some selected organochlorines, organophosphorus, carbamates and pyrithrium pesticides using High Performance Liquid Chromatography (HPLC) with (photo diode array) PDA detector. The results showed that none of the vegetable samples were contaminated with organochlorine pesticides. Sample VS-5(lady's finger) was found contaminated with ethion residue at 1.80 mg/kg. VS-1 (Brinjal) and VS-5 (Lady's finger) were contaminated with fenitrothion residues at 0.166 mg/kg and 0.170 mg/kg, respectively. Health indices of acephate, cypermethrin and fenitrothion in brinjal were 0.363, 0.002 and 0.475 respectively and in lady's finger for ethion and fenitrothion were 10.350 and 0.490 respectively. The data indicated a significant effect for regular monitoring programs of pesticide residues in vegetables, to protect consumers' health.

Key words: HPLC · Pesticide residues · Vegetables · Health risk assessment · Estimated daily intake (EDI)

INTRODUCTION

Bangladesh as an agricultural country produces most likely rice, wheat, jute, potato, sugarcane, vegetables and tea [1]. In addition, every year pest attack causes a great damage of agricultural production in Bangladesh. About 62% of the population is involved in agriculture with total crop production of about 27.787 million metric ton [2]. As agricultural production increased every year to meet the growing demand of the people, uses of pesticides also being rose up to prevent deleterious pest attack. As the vegetable growers have been using the pesticides frequently to have the higher yield, the overdoses of pesticides make the residue problem, which might pollute food and be harmful for health [1]. The most alarming concern is that pesticide use is very indiscriminate in Bangladesh. It has been reported that some of the pesticides are used without maintaining any pre-harvest time. There are areas where pesticides are used in excessive quantities that make it difficult for monitoring and assessment of pesticide contamination. Therefore, the presence of these pesticide residues in food commodities has always been a matter of serious concern especially when these commodities are consumed fresh [3]. Pesticides have been associated with a wide spectrum of human health hazards, ranging from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm and endocrine disruption [4].

WHO [5] estimated an annual worldwide total of 3 million cases of acute and severe pesticide poisoning with some 220,000 deaths. The majority of these cases of poisoning and deaths occur in the developing countries,

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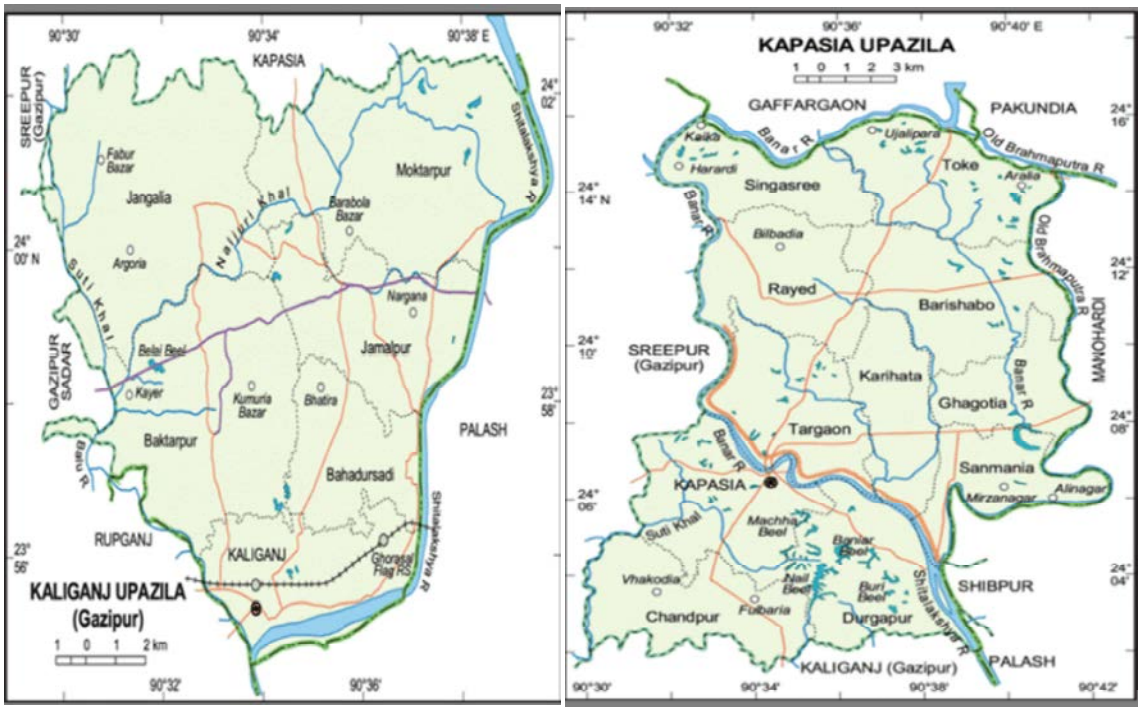
although far greater quantities of pesticides are used in the developed countries [6]. Several indices of residue levels can be used to predict pesticide residue intake. In particular, the effects of a regular intake of pesticide residues in food are hard to detect and quantify. An exposure or risk assessment is necessary in order to ascertain the effects due to regular intake of pesticide residues in food.

In our present study, we have selected some top agro based areas of Kapasia and Kaligonj upazila of Gazipur district under Dhaka division, from where a large part of vegetables are supplied to capital city. We selected 5 organophosphorus pesticides (fenitrothion, parathion, ethion, acephate, fenthion), 2 carbamate pesticides (carbaryl and carbofuran), 1 pyrethroid pesticide (cypermethrin), to determine the concentration and potential health risk assessment of pesticide, based on investigation of the use of pesticides in that areas. We also analyzed organochlorine pesticide (DDT and methoxychlor) if those were still used illegally by farmers. After collection of vegetable, they were analyzed by HPLC with PDA detector at laboratory of Agrochemicals and Environmental Research Division (AERD), Institute of food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE).

MATERIALS AND METHODS

Sample Collection and Preservation: Ten vegetable samples viz. brinjal, lady's finger, cauliflower and cabbage were collected from different agricultural fields that were exposed to various pesticides from two upazila (Kapasia and Kaligonj) of Gazipur district in Bangladesh (Fig. 1). The details of different vegetables sampled during the experiment are given in Table 1. Keeping in sterile polyethene bag samples were transported in ice carrier to the laboratory where they were marked, labelled and registered immediately before storing at 4°C until analysis within. Fifty grams (g) homogenized samples from mother vegetation were taken for analysis.

Chemicals: The solvents, such as acetone (BDH, England) and n-hexane (Merck, Germany) were of analytical grade, while acetonitrile (Scharlau, Barcelona, Spain) was of HPLC grade. Anhydrous sodium sulfate (Na_2SO_4) (BDH, England), florisil (Magnesium Silicate) (Sigma, USA) and dichloromethane (BDH, England) were also used. DD n-hexane was prepared for sample preparation in the laboratory.



(a) (b)

Fig. 1: Chowdhury *et al*.

Table 1: Plant samples collected from the experimental sites.

Edible part of vegetable	Common name	Botanical name	Family
Leaf	Cabbage	Brasssica oleracea var. capitata L.	Brassicaceae
Inflorescence	Cauliflower	Brassica oleracea var. Botrytis L.	Brassicaceae
Fruit	Lady's finger	*Abelmoschus esculentus* L.	Malvaceae
Fruit	Brinjal	*Solanum melongena* L.	Solanaceae

Extraction: A 50 g portion of homogenized laboratory sample was extracted with 100 ml solvent mixture (Hexane: Dichloromethane = 9: 1) in the presence of 20 g sodium hydrogen carbonate using electric blenders at about 20-25°C. Sixty grams sodium sulphate was added to remove remaining water. The slurry was well mixed. The sample solvent mixture was kept in a fume hood for about 15-30 minutes to let the solvent separate from the solid material. The separated solvent was transferred to round bottom flask and evaporated in a rotary evaporator (Buchi, Switzerland) at 45° under mild pressure and finally transferred to a vial and the evaporation was continued with gentle nitrogen stream to nearly dryness, based on the method described in the Deutsche Forschungsgemeinschaft [7]. The extract was dissolved in hexane to make a particular volume (5 ml).

Clean-up: Florisil column chromatography was used to clean up the extract. The florisil (Mesh size 60-100) was activated at 200°C for 6 hours and deactivated with 2% distilled water. The top 1.5 cm of the florisil column was packed with anhydrous sodium sulphate. Elution was done with a solvent mixture of double distilled hexane (65%) and dichloromethane (35%) at 5ml/min. The elute was concentrated in a rotary vacuum evaporator and transferred to a vial. Solvents were completely removed under mild fresh nitrogen flow. The Evaporated sample was dissolved in acetonitrile and made to a particular volume (5 ml) for analysis by HPLC.

HPLC Analysis: Following the sample cleanup, aliquots of the final volume were quantified using an HPLC (Shimadzu, Japan) LC-10 ADvp, equipped with an SPD-M 10 Avp attached to a photo-diode array detector (PDA) (Shimadzu SPD-M 10 Avp, 200-800 nm). The analytical column was a C_{18} reverse phase Alltech (250 × 4.6 mm, 5µm) that was maintained at 30 °C in a column oven. The mobile phase, a combination of 70% acetonitrile and 30% water, was filtered using a cellulose filter of 0.45µm before each use. The flow rate was 1.0 mL/min and all solvents used were of HPLC grade. Prior to HPLC analysis, the samples were passed through 0.45µm of nylon (Alltech Associates, IL, USA) syringe filters. The 20µL samples were manually injected each time. The identification of the suspected pesticide was performed, relative to the retention time of the pure analytical standard. Quantification was performed based on the method described elsewhere [8]. A typical chromatogram from the analysis is shown in Fig. 2.

Recovery: The mean percentage recoveries for the various pesticides were calculated using the following equation:

$$\text{Percentage recovery} = (C_E/C_M \times 100)$$

Where C_E is the experimental concentration determined from the calibration curve and C_M is the spiked concentration.

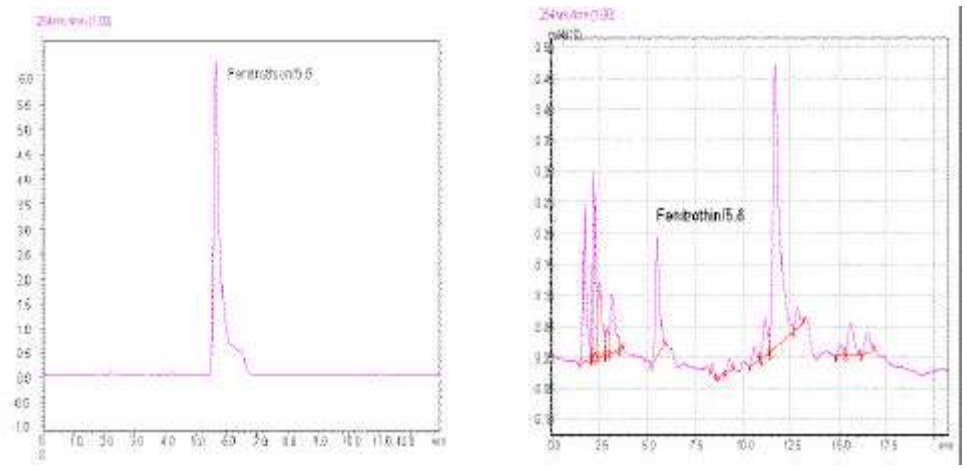
The calibration curves for all the pesticides in this study were prepared at four concentrations (1, 2, 5 and 10 mg/kg) and exhibited good linear correlation coefficients (0.982-0.998). The percentage recoveries of pesticides studied in respective vegetable matrices ranged between 75 and 105.4% for various spiking levels and indicated the suitability of the method for the analysis.

Quantification Procedures: Tentative identification of the suspected pesticides was carried out in relation to the retention time (RT) of the pure analytical standards. For this purpose, injection of equal volumes of differently concentrated standard solutions into HPLC prepared calibration curve for each pesticide. The retention featured ±0.05% difference is acceptable.

To determine the residual levels of pesticides, the following calculating equation was used:

$$R \sim © \frac{H_A \, V_{END} \, W_{ST}}{H_{ST} V_i \, G}$$

Here, R' = mg/Kg for vegetable samples; G = Sample weight (Kg); V_{END} = Terminal volume of the sample solution (ml); V_i = Portion of volume V_{END} injected into HPLC (µL) column; W_{ST}= Amount of standard pesticides injected with standard solvent (µg); H_A = Peak area obtained from V_i (mm^2); H_{ST}= Peak area obtained from W_{ST} (mm^2).



(a) (b)

Fig. 2: Chowdhury *et al.*

Health Risk Index (HRI): Health risk indices of the residues were computed using the obtained and food consumption. Health risk index was calculated using following equation described elsewhere [9].

$$\text{Health risk Index (HRI)} = \frac{\text{EDI}}{\text{ADI}}$$

Where, EDI is estimated daily intake and ADI is acceptable daily intake. An index more than 1 is considered as not safe for human health [10]. Estimated Daily Intake (EDI) was found by multiplying the residual pesticide concentration (mg/kg) by the food consumption rate (kg/day) and dividing by a body weight of 60 kg for adult population.

RESULTS AND DISCUSSION

Level of Pesticide Residues in Vegetables: The levels, concentration ranges and identity of pesticide residues found in the analyzed samples are outlined in Table 2. Pesticides Rule (PR), 1985 of Bangladesh strictly prohibits any kind of unauthorized use of pesticides. It does not work effectively to control hazardous pesticides because of lack of strong monitoring and proper evaluation facilities. In present study, neither methoxychlor nor DDT residues were detected in any of the vegetable samples. These may be the conversion of these parent pesticides to their metabolite. The presence of DDT metabolite is still under investigation.

The use of organophosphorus, carbamate and pyrethrium pesticides has greatly increased because of their less detrimental effects on the environment owing to their less persistency. Among the organophosphorus pesticides ethion, fenitrothion, parathion, acephate and fenthion were analyzed in vegetable samples. VS-5 (Lady's finger) was contaminated with 1.80 mg/kg ethionand 0.17 mg/kg fenithrothion residues.VS-1 (Brinjal) and VS-3 (Brinjal) were contaminated with fenitrothion (Figure 3) and acephate residues at a concentration of 0.166 mg/kg and 0.19 mg/kg, respectively. Ethion was recorded [11] in the range of 0.017 to 0.034 mg/kg in vegetable in Egypt. The concentration of fenitrothion was detected 0.054 mg/kg and 0.014 mg/kg in brinjal (eggplant) and lady's finger, respectively [12]. The concentration of acephate was 0.053 mg/kg in lady's finger also recorded [12]. It have been revealed that the concentration of acephate and fenitrothion were in the range o f nd (not detected) to 4.082 mg/kg and nd to 0.651 mg/kg, respectively in different vegetables from Xiamen city, China [13]. In this study, no fenithrothion, acephate residues were detected in cabbage. It have been reported that no fenithrothion and acephate residues were detected in cabbage and cauliflower, but acephate residues were found in the concentration of 0.035 mg/kg in cabbage [13]. The mean concentration of fenithrothion and acephate residues was recorded 0.012 mg/kg and 0.0025 mg/kg in cauliflower, respectively [12].

None of the vegetable samples were found contaminated with carbofuran and carbaryl residues due to their low persistency. These might be the conversion of these parent pesticides to their metabolite and hence they might remain below detection limit, though some previous have recorded carbamate residues in different vegetables in different region of the world [14-17]

Table 2: Pesticide residues (mg/kg) in vegetable samples of kaligonj and kapasiaUpazila of Gazipur district.

Sample ID	Location Union	Methoxychlor	DDT	Acephate	Fenthion	Fenitrothion	Parathion	Ethion	Carbaryl	Carbofuran	Cypermethrin
VS-1 (Brinjal)	Mokterpur	BDL	BDL	BDL	BDL	0.166	BDL	BDL	BDL	BDL	BDL
VS-2 (Brinjal)	Mokterpur	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
VS-3 (Brinjal)	Mokterpur	BDL	BDL	0.19	BDL	BDL	BDL	BDL	BDL	BDL	BDL
VS-4 (Lady's finger)	Mokterpur	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
VS-5 (Lady's finger)	Kapasia	BDL	BDL	BDL	BDL	0.17	BDL	1.8	BDL	BDL	BDL
VS-6 (Lady's finger)	Kapasia	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
VS-7 (Cauliflower)	Jamalpur	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
VS-8 (Cauliflower)	Jamalpur	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
VS-9 (Cabbage)	Durgapur	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
VS-10 (Cabbage)	Durgapur	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

VS = Vegetable Sample, BDL = Below Detection Limit, *Mean value of triplicates. Limit of detection (LOD): 0.01 mg/kg

Table 3: Maximum residue levels (MRLs) of identified pesticide in several vegetables.

	Maximum residue levels (MRLs) of identified pesticide in mg/kg			
	Brinjal		Lady's finger	
Pesticide	MRL[a]	MRL[b]	MRL[a]	MRL[b]
Acephate	0.02	NA	0.02	NA
Fenitrothion	0.01	NA	0.01	NA
Ethion	0.01	NA	0.01	NA
Cypermethrin	0.5	0.03	0.5	0.5

[a]MRLs refer to maximum residue limits issued by European Union Pesticides Database (2010).

[b]MRLs refer to maximum residue limits issued by Codex Alimentarius Commission (FAO/WHO, 2004).

NA refer to MRL not available for commodities analyzed

recorded that the mean concentration of carbaryl residue was 0.09 mg/kg in brinjal and 0.72 mg/kg in cabbage in Al-Qassim region of Saudi Arabia.

Pyrithrium is the chemical insecticide that contains Permethrin and cypermethrin that are commonly used in agricultural purposes. During field study we assumed that most of the farmers use cypermethrin and these gave the opportunity to study only with cypermethrinin vegetable samples. Only VS-2 (Brinjal) was contaminated with cypermethrin at a concentration of 0.02 mg/kg. Cypermethrin was found in brinjal in the range of nd to 0.012 mg/kg [18] which was similar compared to the present investigation. Cypermethrin concentrations also have recorded 0.012 mg/kg in brinjal (eggplant) in China [13]. In this study cypermethrin were not detected in cabbage, lady's finger and cauliflower, but concentration of cypermethrin were recorded in cabbage in the range of nd to 0.017 mg/kg in China [13].

When the present concentrations of pesticides were compared with the MRLs established by European Union Pesticides Database [19] and Codex Alimentarius Commission [20], then it was found that all pesticides detected was higher than the MRLs value in all vegetable samples except cypermethrin in brinjal (Table 3).

However, the persistent nature of the pesticides is of great concern due to their bio-accumulative nature and toxic biological effects on human and wildlife [21]. The wide use of pesticides in the world causes major health and environmental problems. A continuous monitoring of residual pesticides level in different food materials from different areas is obvious to understand the trend of contamination. A continuous monitoring is also essential from a government regulatory body to prevent indiscriminate use, misuse and overuse of pesticides. It would be valuable to learn more about the problems caused by exposure to pesticides with respect to safety, health and the environment.

Daily Intake and Risk Assessment Based on Pesticides: From a potential health perspective, it is important to compare exposure estimates to established toxicological criteria such as estimate daily intake (EDI). Results obtained were used to calculate EDI expressed as milligram pesticides per kilogram body weight per day (mg/kg b.w/day).Estimated Daily Intake (EDI) was found by multiplying the average residual pesticide concentration (mg/kg) by the food consumption rate (kg/day). Estimated the hazard indices were obtained by dividing the EDI (mg/kg/day) by their corresponding values of acceptable daily intakes (ADI) for agricultural and veterinary chemicals [22]. The estimated daily intake values of the residues and their corresponding hazard indices in the vegetable samples were given in Table 4. The data illustrated that the intakes were much lower than the ADIs and the exposure level to whole pesticide residues except ethion was below the level to produce health risk. The EDIs have been estimated between 2×10^{-5} and 0.597 mg/kg body weight/day, while the hazard indices (EDI/ADI) ranged from 7×10^{-8} to 0.20 in vegetables on Al-Qassim markets, Saudi Arabia [17]. Thus, lifetime consumption of these vegetables could not pose health risk for population as the indices for all the residues were less than one [10].

Table 4: Health risk assessment based on average daily intake of pesticide residues in vegetables

		Brinjal		Lady's finger	
	ADI* (mg/ kg/ day)	EADI (mg/kg/day)	Hazard index	EADI (mg/kg/day)	Hazard index
Acephate	0.003	0.00109	0.363	-	-
Cypermethrin	0.05	0.00012	0.0024	-	-
Ethion	0.001	-	-	0.01035	10.35
Fenitrothrion	0.002	0.00095	0.475	0.00098	0.49

*Source: Australia, 2005 [22]

In the present study, estimated dietary intakes of pesticides considered only exposures from vegetables and did not include other food products such as grains, dairy, fish and meats. On the other hand, processing factors (such as peeled, cooked and boiled before consumption) were ignored which might result in an overestimation of the actual exposure of pesticide residues. Moreover, the effect of pesticides on more vulnerable group such as children, disorder and pregnant women could all affect these calculations.

CONCLUSION

Among the detected compounds, fenitrothrion were found in both brinjal and lady's finger, but acephate and cypermethrin were found in only brinjal and ethion in only lady's finger. Routine monitoring programs for these pesticide residues in vegetables are needed to prevent, control and reduce the pollution and to minimize health risks. It should also emphasize for control at production, tighter regulation of spraying and also tighter regulation in the sale of pesticides as well as for education of farmers.

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