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Health risk assessment of selected pesticide residues in locally produced vegetables of Bangladesh

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Abstract

Consumption of pesticide contaminated vegetables pose a major threat to public health. High Performance Liquid Chromatography (HPLC) with a Photo Diode Array detector (PDA) was used to determine two organochlorines (methoxychlor and DDT), seven organophosphorus (acephate, chlorpyrifos, fenthion, fenitrothion, malathion, parathion, and ethion), two carbamate (carbaryl and carbofuran) and one pyrethroid (cypermethrin) pesticide residues in 15 samples of three common vegetables (Tomato, Lady's Finger and Brinjal). Acephate, chlorpyrifos, fenitrothion, malathion, parathion, ethion and carbaryl residues were detected in some of the tested vegetable samples. Except chlorpyrifos and cypermethrin, all of the pesticides were found at higher levels than the corresponding MRLs in all vegetable samples. Highest percentage of samples was found contaminated with chlorpyrifos and its level ranged from BDL (<0.01 µg/kg) to 1.03 mg/kg. The highest health index (5.7) was found for ethion in lady's finger, whereas health index for carbaryl in tomato, chlorpyrifos and carbofuran in brinjal were 1.09, 1.97 and l.17, respectively. The present study suggests the need for strict regulation and regular scrutinizing of pesticide residues in vegetables, to protect consumers' health.

Introduction

Agriculture is the main economic backbone of Bangladesh, which contributes about one third to the country's gross domestic product (GDP) and 80% of the people depend on agriculture for their livelihood (Chowdhury *et al*., 2011; Bhattacharjee *et al*., 2012). Like many other developing countries, pesticides are used extensively in Bangladesh to increase the crop yield per acre (Dasgupta *et al*., 2007; Chowdhury *et al*., 2012a). As agricultural production is being increased every year to meet the growing demand of the people, uses of pesticides also being rose up. In Bangladesh context, the vegetable growers have been using the pesticides frequently to have the higher yield. The widespread use of pesticides may contaminate the environment as well as foods, which may create health problem (Rahman, 2000; Parveen and Nakagoshi, 2001; Islam *et al*., 2009).

It has been reported that some of the pesticides are being used in vegetables where no pre-harvest time frame after application is maintained because of their high demand for farm produce and low perception of the toxic effects of pesticides in food, which resulted in the occurrence of residues in vegetables (Darko and Akoto, 2008). The most alarming concern is that pesticide use is very indiscriminate in Bangladesh. There are areas where pesticides are used in excessive quantities. 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 (Solecki *et al*., 2005; Chen *et al*., 2011; Osman *et al*., 2011). 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 (Ali and Tahir, 2000; Berrada *et al*., 2010; Chowdhury *et al*., 2012a).

WHO (2002) estimated about 849,000 people death globally from acute toxicity of the pesticide in 2001. Pesticide poisoning and deaths occurred in the developing countries are far greater quantities than that of the developed countries (Bhanti *et al*., 2004). The aim of this paper was to determine the concentration of the selected pesticide residues in vegetables of Savar upazila and to assess public health risk of pesticide residues in vegetables via daily intake of pesticides contaminated vegetables.

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Table 1. Mean recoveries of pesticides analyzed in vegetables

Pesticide	Clean up system	Concentration (µg)[a]		Recovery (%)
		Spiked	Measured	
Methoxychlor	Control[b]	0.00	0.00	0.00
	Florisil clean up	40.00	35.1	87.75
DDT	Control	0.00	0.00	0.00
	Florisil clean up	40.00	34.0	85.00
Acephate	Control	0.00	0.00	0.00
	Florisil clean up	40.00	37.5	93.75
Chlorpyrifos	Control	0.00	0.00	0.00
	Florisil clean up	40.00	38.55	96.38
Fenthion	Control	0.00	0.00	0.00
	Florisil clean up	40.00	34.93	87.33
Fenitrothion	Control	0.00	0.00	0.00
	Florisil clean up	40.00	36.77	91.93
Malathion	Control	0.00	0.00	0.00
	Florisil clean up	40.00	37.79	94.47
Parathion	Control	0.00	0.00	0.00
	Florisil clean up	40.00	35.50	88.76
Carbaryl	Control	0.00	0.00	0.00
	Florisil clean up	40.00	38.29	95.72
Carbofuran	Control	0.00	0.00	0.00
	Florisil clean up	40.00	37.06	92.66
Cypermethrin	Control	0.00	0.00	0.00
	Florisil clean up	40.00	36.33	90.82

[a] Mean value of triplicates
[b] Control is blank sample without spiking of any concentration of pesticides

Materials and Methods

Chemicals

Reference grade pesticide standards with purity from 98.5 to 99.5 % were purchased from GmbH (D-86199 Augsburg, Germany).

Sample collection and preservation

Fifteen samples of three different vegetables viz., brinjal (*Solanum melongena* L.) and tomato (*Lycopersicon esculentum* L.) and lady's finger (*Abelmoschus esculentus* L.) samples were collected randomly from Savar bazar – a top ranked agro-based market located in Savar city from which a large proportion of vegetables are supplied to the city dwellers. One kilogram of each sample was collected in a separate sterile polyethylene bag, sealed, labelled with unique sample identity, placed in an insulated ice box and transported to laboratory for analysis. Samples were kept at 4°C for maximum of 24 h before being analyse.

Sample processing

For Analysis, vegetables samples were chopped and grounded using an electric blender to obtain a homogenous composite. Fifty gram of blended samples were transferred into a 250 ml Erlenmeyer flask and treated with 150 ml of solvent mixture (135 ml DD-hexane and 15 ml dichloromethane) and allowed to mix well in multi-Shaker over 12 h at 150 rpm at about 20-25°C. The content was then stands for 10-15 minutes for settling down and layered. The hexane layer (upper layer) was transferred to another conical flask. Sixty grams sodium sulphate was added to remove remaining water and this separated solvent was then transferred to a round bottom flask and concentrated in a rotary evaporator at 45° under mild pressure, based on the method described by Thier and Zeumer (1987).

The samples were cleaned up according to the method of Fardous *et al*. (2007). The n-hexane extract was subjected to clean up using florisil column chromatography. The florisil (60–100 mesh) was activated at 200 ^{0}C for 6 h and was subsequently deactivated with 2% distilled water. The top 1.5 cm of the florisil column was packed with anhydrous sodium sulfate. Elution was performed with a solvent mixture of double distilled hexane (65%) and dichloromethane (35%) at 5 ml/min. The elute was concentrated using a rotary vacuum evaporator and

Table 2. Pesticide residues detected in vegetables samples collected from Savar Baazer

Sample ID	Concentration (mg/kg)[a]											
	Methoxychlor	DDT	Acephate	Chlorpyrifos	Fenthion	Fenitrothion	Malathion	Parathion	Ethion	Carbaryl	Carbofuran	Cypermethrin
VS-1 (Brinjal)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	**0.17**	BDL	BDL	BDL	**0.16**
VS-2 (Brinjal)	BDL	BDL	BDL	**1.03**	BDL	**0.23**	BDL	BDL	BDL	**0.82**	BDL	BDL
VS-3 (Brinjal)	BDL	BDL	BDL	BDL	BDL	BDL	**0.29**	BDL	BDL	BDL	**0.61**	BDL
VS-4 (Brinjal)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
VS-5 (Brinjal)	BDL	BDL	**0.21**	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
MRLs[b,c]	-	-	**0.02**	**0.5**	-	**0.01**	**0.02**	**0.05**	**0.01**	**0.05**	**0.02**	**0.5**
VS-6 (Tomato)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	**0.09**
VS-7 (Tomato)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	**1.6**	BDL	BDL
VS-8 (Tomato)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	**0.31**	BDL	BDL	BDL	BDL
VS-9 (Tomato)	BDL	BDL	BDL	**0.01**	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
VS-10 (Tomato)	BDL	BDL	BDL	BDL	BDL	BDL	**0.33**	BDL	BDL	BDL	BDL	BDL
MRLs[d]	-	-	**0.02**	**0.5**	-	**0.01**	**0.02**	**0.05**	**0.01**	**0.5**	**0.02**	**0.5**
VS-11 (Lady's finger)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
VS-12 (Lady's finger)	BDL	BDL	BDL	BDL	BDL	**0.19**	BDL	BDL	BDL	BDL	BDL	BDL
VS-13 (Lady's finger)	BDL	BDL	BDL	**0.14**	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
VS-14 (Lady's finger)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	**0.98**	BDL	BDL	BDL
VS-15 (Lady's finger)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
MRLs[e]	-	-	**0.02**	**0.5**	-	**0.01**	**0.02**	**0.05**	**0.01**	**0.05**	**0.02**	**0.5**

[a]Mean value of triplicates, BDL = Below Detection Limit, Limit of detection (LOD): 0.01 mg/kg

[b]MRLs refer to maximum residue limits.

[c]MRLs for brinjal issued by European Union Pesticides Database (2010).

[d]MRLs for tomato issued by European Union Pesticides Database (2010).

[e]MRLs for lady's finger issued by European Union Pesticides Database (2010).

was transferred to a vial. Solvents were completely removed under a gentle flow of fresh nitrogen. The evaporated sample was dissolved in acetonitrile, and a 1 ml volume was used for the HPLC analysis.

Pesticide analysis by HPLC

Aliquots of the final volume were quantified with a HPLC system (SHIMADZU LC- 10 Avp-Series Automated with LC Solution Software LabSolutions (LC solution Release 1.11SP1) that was equipped with a SPD-M 10 Avp outfitted with a photodiode array (PDA) detector. A C18 Reverse Phase Alltech analytical column (5 lm, 250 9 4.6 mm) was used and maintained at 30^0C in a column oven. The mobile phase, which was a combination of 70% acetonitrile and 30% water, was filtered using a cellulose filter (0.45 lm) prior to use and was allowed to run at 1.2 ml/min. Prior to the HPLC analysis, the samples were passed through 0.45 µm nylon syringe filters and were manually injected (20 µl) into the HPLC system each time. The identification of the suspected pesticide was performed by comparing peak retention times in samples to those of peaks in the pure analytical standards. Quantification was performed using the method described by Chowdhury *et al.* (2012b). A typical chromatogram from the analysis is shown in Figure 1.

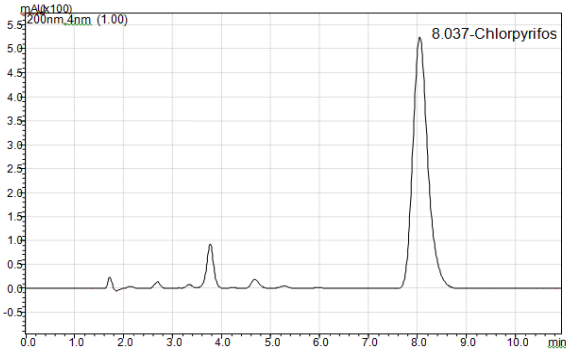

Figure 1. A typical Chromatogram showing chlorpyrifos standard peak (RT = 8.037 min.)

The percentage recoveries were calculated using the equation: Percentage of recovery = [CE/CM ×100], where CE is the experimental concentration determined from the calibration curve and CM is the spiked concentration. The mean percentage recoveries of all tested pesticides were more than 85% which was satisfactory. These values were quite satisfactory and meet the requirements of

Table 3. Health risk assessment of pesticide residues in studied vegetables

Pesticides		Brinjal			Lady's finger			Tomato		
	ADI* mg/kg/d	EDI mg/kg/d	HRI	Health Risk	EDI mg/kg/d	HRI	Health Risk	EDI mg/kg/d	HRI	Health Risk
Acephate	0.003	0.0012	0.4	No	-	-		-	-	
Chlorpyrifos	0.003	0.0059	1.97	Yes	0.0008	0.27	No	0.0001	0.023	No
Fenitrothrion	0.002	0.0013	0.65	No	0.0011	0.545	No	-	-	
Parathion	0.005	0.0009	0.196	No	-	-		0.0018	0.36	No
Ethion	0.001	-	-		0.0057	5.7	Yes	-	-	
Malathion	0.003	0.0017	0.57	No				0.0019	0.63	No
Carbaryl	0.008	0.0047	0.59	No	-	-		0.0092	1.15	Yes
Carbofuran	0.003	0.0035	1.17	Yes						
Cypermethrin	0.05	0.0009	0.018	No	-	-		0.0005	0.011	No

*Source: Australian Government (2005), ADI= Acceptable Daily Intake, HRI=Health Risk Index, EDI=Estimated daily intake

the European Commission (EC, 2000) (Table 1), indicating that the method can be considered accurate and precise when the accuracy of data is between 70 and 110 %.

Health risk index (HRI) analysis

Health risk estimations were done based on pesticide residues detected in vegetables in the present study. Health risk indices of the pesticide residues via dietary intake of vegetables were assessed according to the guidelines recommended by the USEPA, where the estimated daily intake (EDI) was compared with the acceptable daily intake (ADI) (Wang *et al*., 2011). Estimated Daily Intake (EDI) was found by multiplying the residual pesticide concentration (mg kg^{-1}) by the food consumption rate (kg day^{-1}) and dividing by a body weight of 60 kg for adult population. The average daily vegetable intakes for adults were considered to be 0.345 kg/person/day according to the previous report (Wang *et al*., 2005; Arora *et al.*, 2008). Health risk index (HRI) was calculated using the equation:

Health risk Index (HRI) = $\frac{EDI}{ADI}$(EFSA, 2007), 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 (Darko and Akoto, 2008).

Results and Discussion

Levels of pesticide residues in vegetable samples collected from Savar bazaar

The levels of pesticide residues found in the analyzed samples and their maximum residue limits are outlined in Table 2. The organophosphorus pesticides (acephate, chlorpyrifos, ethion, fenitrothion, parathion and malathion) were detected in the 10 samples out of 15 analyzed samples. In VS-2 Brinjal samples, 1.03 mg/kg of Chlorpyrifos (MRL 0.5) and 0.23 mg/kg Fenitrothion (MRL 0.01) were detected. On the other hand, 0.01 mg/kg and 0.14 mg/kg of Chlorpyrifos (MRL 0.5) were detected in VS-9 (Tomato) and VS-13 (Lady's finger), respectively. Malathion, parathion and ethion were found at the concentration of 0.33 mg/kg (MRL 0.02), 0.31 mg/kg (MRL 0.05) and 0.98 mg/kg (MRL 0.01) in VS-10 (Tomato), VS-8 (Tomato), and VS-14 (Lady's finger) sample, respectively. Fenitrothion, malathion and parathion were detected in two samples whereas, acephate and ethion was detected in only one sample. Any one of the tested samples was not found contaminated with fenthion, methoxychlor and DDT. The present results found much higher fenitrothion residues compared to the study of Sinha *et al*. (2012), they reported fenitrothion concentration 0.054 mg/kg and 0.014 mg/kg in brinjal (eggplant) and lady's finger, respectively. Concentrations of ethion, acephate, chlorpyrifos, malathion and parathion were also higher than those reported in other studies (Chen *et al.*, 2011; Farag *et al*., 2011; Osman *et al.,* 2011; Sinha *et al.*, 2012).

Among carbamate pesticides, carbaryl residues were detected 0.82 mg/kg (MRL 0.05) in VS-2 (Brinjal) and 1.6 mg/kg (MRL 0.5) in VS-7 (Tomato) sample, whereas only one sample was contaminated with carbofuran, though some previous studies recorded carbamate residues in different vegetables in different regions of the world (Chun and Kang, 2003; Dhas and Srivastava, 2010; Latif *et al*., 2011;

Osman *et al.*, 2011). Osman *et al*. (2011) recorded the mean concentration of carbaryl residue 0.10 mg/kg in tomato and 0.09 mg/kg in brinjal in Al-Qassim region of Saudi Arabia. In this study cypermethrin were not detected in lady's finger. Cypermethrin was recorded in two samples ranged from BDL to 0.16 mg/kg and the residue was higher than other studies reported (Chandra *et al.*, 2010; Chen *et al.*, 2011).

Pesticide residues of the present study were compared with the maximum residue level (MRL) (Table 2) established by European Union Pesticides Database (2010). It was found that all detected pesticides was at higher levels than their corresponding MRLs value in all vegetable samples except chlorpyrifos in tomato and lady's finger, and cypermethrin in tomato and brinjal, respectively. The presence of these pesticide residues in vegetables makes the safety of these vegetables more venerable to public health. Even though, in Bangladesh eggplant and ladys finger usually did not eat raw, the stir fry or boil of brinjal and ladys finger could reduce this problem. In case of tomato, consumers sometimes eat raw in different forms like salad and have health effect directly. However, the persistent nature of the pesticides is of great concern due to their bio-accumulative nature and toxic biological effects on human (Tanabe *et al*., 2000).

Daily intake and health risk assessment based on pesticide residues in vegetables

Estimated the health risk index (HRI) were obtained by dividing the EDI (mg kg^{-1} day^{-1}) by their corresponding values of acceptable daily intakes (ADI) for agricultural and veterinary chemicals (Australian Government, 2005). Table 3 expresses the estimated daily intake values of the residues and their corresponding health risk index in the vegetable samples. Health indices of acephate, chlorpyrifos, fenitrothion, malathion, parathion, carbaryl, carbofuran and cypermethrin in brinjal were 0.4, 1.97, 0.65, 0.57, 0.196, 0.59. 1.17 and 0.0184, respectively. The highest health indices were found for ethion (5.7) in lady's finger, carbaryl (1.09) in tomato, chlorpyrifos (1.97) and carbofuran (1.17) in brinjal. The main health risk may be posed by chlorpyrifos, ethion, carbaryl and carbofuran, while rest of the pesticide residues belonged to no risk class. 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 (Osman *et al.*, 2011). Thus, potential health risk to population may be posed by the lifetime consumption of these vegetables as the indices for chlorpyrifos, ethion, carbaryl and carbofuran residues were more than one.

Conclusion

The organophosphorus pesticides acephate, chlorpyrifos, ethion, fenitrothion, parathion and malathion were detected in the 10 samples out of 15 tested samples. Among them highest percentage of vegetable samples was found contaminated with chlorpyrifos. Most of the pesticides were found at higher levels than their corresponding MRLs values in all vegetable samples except chlorpyrifos in tomato and lady's finger, and cypermethrin in tomato and brinjal, respectively. The highest health indices were found for ethion (5.7) in lady's finger, carbaryl (1.09) in tomato, for chlorpyrifos (1.97) and carbofuran (1.17) in brinjal. Routine monitoring programs for these pesticide residues in vegetables are needed to prevent, control and reduce the pollution and to minimize health risks.

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