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DETERMINATION OF PESTICIDE RESIDUES AND HEALTH RISK ASSESSMENT IN CUCUMBER AND EGGPLANT SOLD IN NORTHERN PART OF BANGLADESH

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ABSTRACT

The presence of pesticide residues in vegetables and their potential effects on health is one of the prime concerns in Bangladesh. This current research work is designed to assess the health risk related with the consumption of cucumber and eggplant. A modified QuEChERS extraction technique along with Gas Chromatography-Flame Thermionic Detector (GC-FTD) was used to determine some major organophosphorus insecticides specifically fenitrothion, acephate, dimethoate, quinalphos, chlorpyrifos, diazinon, and malathion, widely available in the local market. Pesticide residues were detected in nine (four cucumbers, five egg plants) samples which wereabout 11% of the total analyzed samples. Mostly the Diazinon were present at a level above the European Union(EU). However, comparing the Estimated Daily Intake (EDI) values with the Acceptable Daily Intake (ADI) indicated that the contaminated samples could be considered as safe for consumption. Continuous monitoring and development of an effective traceability system throughout the supply chain and control measures are suggested to protect consumers 'health.

KEY WORDS : Pesticide residues, Cucumber, Eggplant, Health risk assessment

INTRODUCTION

Eggplant and cucumber are exceptionally popular vegetables in Bangladesh. People here mostly consume cucumber as a raw or in salad while eggplant after cooking. Both vegetables constitute a good source of nutrition. For example, eggplant is a good source of vitamins, minerals and other bioactive compounds. Furthermore, eggplant is considered a healthiest vegetable because of its low caloric content (Plazas *et al*., 2014; Docimo *et al*., 2016). On the contrary, cucumber contains high levels of lignans, flavonoids vitamins (B, C, K) cucurbitacins and antioxidants (Mukherjee, 2013).

In the daily diet, consumption of different vegetables is associated with improvement of vision and gastrointestinal health, reducing the risk of stroke, heart attack, diabetes, and some kinds of cancer (Dias, 2012; Sarwar, 2012). It hasbeen reported that every year around 2.7 million people died because of poor diets with low vegetable intake (Dias and Ryder, 2011). In Bangladesh, it has been reported that above the half of the Bangladeshi are suffering in different from of malnutrition due to unbalanced diet (ICDDRB, 2020). Therefore, the government of Bangladesh emphasizes on the nutrition problem in SDG 2 (sustainable development goal) program (BBS, 2020).

The vegetable production in Bangladesh is in increasing trend and ranked 15th largest vegetable producer in the world and 3rd in South Asia (FAO, 2018). Being an agriculture based economy, it is a challenge for Bangladesh to keep the upraising production trend and minimize the pre and post-harvest production loss. The insect and pest infection cause 30-40% crop production losses even after insecticide application while it could be 100% production loss if control measure is not taken in the field (Sarwar *et al.*, 2013). Thus, one potential drawback associated with increasing vegetable production in Bangladesh is that most of the vegetable producers primarily rely on the intensive application of pesticides to control insects and pests (Hossain *et al.,* 2000). Interestingly, according to the FAO (2017), the pesticide application in Bangladesh is about 7-15 fold lower when compared to several countries like China, Japan, Malaysia and South Korea. Although the intensity of pesticide use could be varied in different part of a Bangladesh, nevertheless, there is still a significant need to improve the comprehensive harvesting procedure to eliminate of those pesticide residues.

Even though cucumber and eggplant are enormously popular and one of the most consumed vegetables in Bangladesh but, yet, there is very limited or no literature on health risk assessment associated with the consumption of these two vegetables. Therefore, the aim of this current research was to estimate the residues of major organophosphate pesticide in cucumber and eggplant collected from different location of Dinazpurdistrictin Bangladesh and to evaluate their long-term health risk.

Experimental

Study area

Five major markets of Dinazpurdistrict, namely Ambari, Bahadur, Fulbari, Laxmitola, and Parbotipur Notun Bazar (Figure 1) were selected. Some basic information of the two selected vegetables are presented in Table 1.

Collection and preparation of samples

Eighty samples (40 cucumber and 40 eggplant) were collected as such usually purchased by the consumers. Airtight zipper bags were used for sample collection. The temperature control cooler

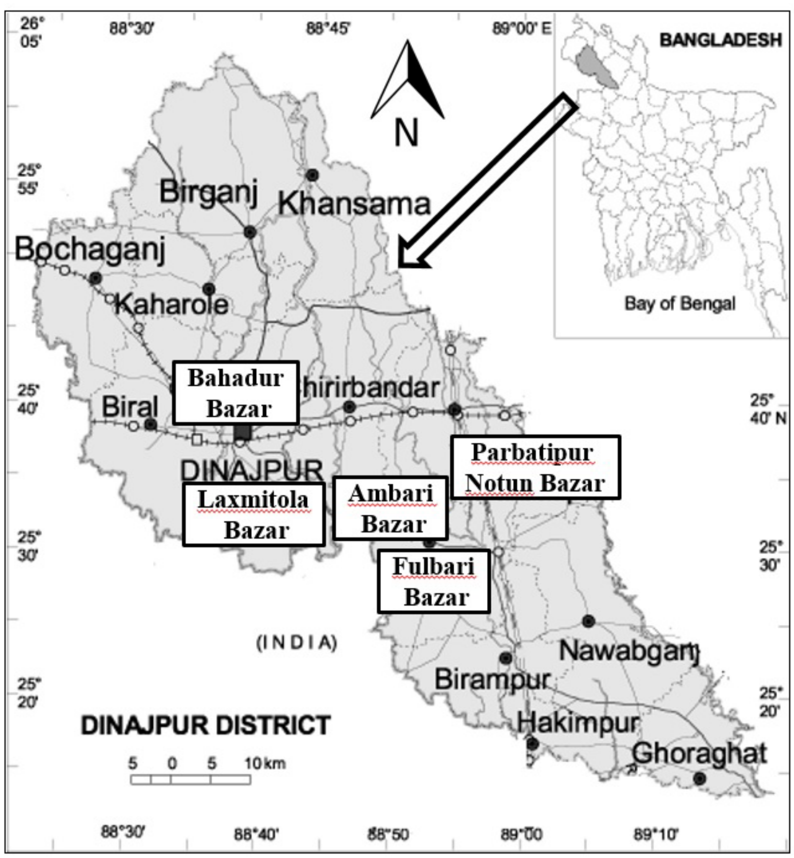

Fig. 1. Map showing different sampling areas of Dinazpur (Bangladesh)

Table 1. Vegetable samples used for the present research

Common Name	Scientific Name	Family	Edible part	Area (*TA)	Production (**TMT)
Cucumber	*Cucumissativus*	Cucurbitaceae	Fruit	24	65
Eggplant	*Solanummelongena* L.	Solanaceae	Fruit	126	516

* TA = thousand acres **TMT = thousand matric tones; Source: BBS, Agricultural Year Book 2018

boxwas used during sample collection and transportation. Day after sample collection, samples were transported to the pesticide residue analysis laboratory, Entomology Division, BARI, Gazipur. Samples werecut into a small pieces and mixed properly for homogeneity. Samples were stored in refrigerator at -20 °C until use.

Materials

The standard of all organophosphate pesticides (chlorpyrifos, diazinon, quinalphos, fenitrothion, acephate, malathion, and dimethoate) were purchased from Sigma-Aldrich (USA). The purity percentage of all standard compounds were > 99.6. The research gradeacetonitrile, acetone, methanol, Primary Secondary Amine (PSA), NaCl, anhydrous $MgSO_4$, and were obtained locally from Bangladesh Scientific Pvt. ltd.

Preparation of samples

The QuEChERSmodified method was followedfor the cleanup and extraction of the samples (Prodhan *et al.*, 2015). In brief, exactly 10g of ground sample was taken in 50 mL falcon tube containing 10 mL of acetonitrile,then the tube was shaken vigorously and homogenized properly with a vortex mixer for 30s. 4g of anhydrous $MgSO_4$ and 1g of NaCl were added into the mixture and was vortexed for one minute, and then, centrifuged (Laboratory Centrifuges, Sigma-3K30, Germany) for 5 min at 5000 rpm. From the centrifuged solution, 3 ml supernatant was placed in a 15 ml micro-centrifuge tube having 120 mg of PSA and 600 mg anhydrous $MgSO_4$. After that, the mixture was vortexed for 30s and centrifuged for 5 minutes at 4000 rpm. A 1 mL supernatant was filtered (0.2 µm, PTFE filter), and transferred into a vial for GC analysis.

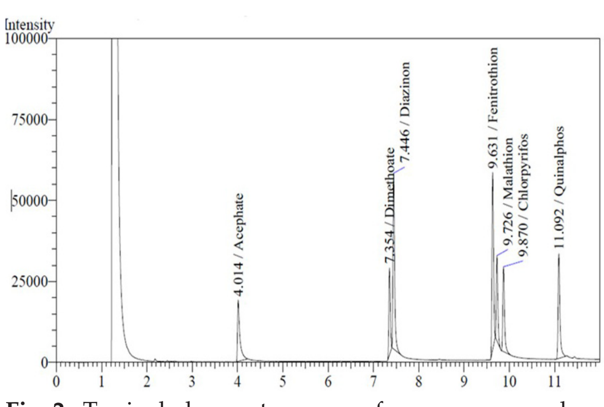

Fig. 2. Typical chromatograms of seven organophosphorus insecticide standards run by GC-FTD

Analysis of pesticide residue using GC-FTD

The analysis was carried out using Shimadzu GC-2010 equipped with Flame Thermionic Detector (FTD) for identification and quantification of acephate, chlorpyrifos, diazinon, quinalphos, dimethoate, fenitrothion and, malathion pesticide residues. The separation and quantification were conducted using AT-1 capillarycolumn, 30m × 0.25 mm (id) × 0.25 µm (film thickness). The inlet temperature was 250 °C with split mode (30:0). The FTD detector temperature was 280 °C. The H_2 flow, make flow, and air flow rate were 1.5, 30, and 145 ml/min, respectively. The column oven temperature started at 150 °C with a 1 min hold time and then ramped at 10 °C/min to 220 °C with a 4 min hold time. The total analysis time was 12 min. The identification of targeted pesticides was ensure by comparing the retention times of those pesticides' authentic standards.

Quantification of pesticide residues

The GC was calibrated using a seven- pointed calibration curve each ranged from 0.1 to 5.0 mg/l before injecting the sample extract. For example, methanolic standard solutions, of each pesticide group, of different concentrations (0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 mg/l) were prepared and injected with the same GC parameters as described above. In matrix-matched standards, the linearity of the calibration curves was excellent as the coeûcients of determination of targeted analytes was (R^2) ≥ 0.99. The detection limit (LOD) was 0.003 mg/kg while the quantification limit (LOQ) was 0.010 mg/kg. A typical chromatograph of seven organophosphorous insecticides presented in Figure 2.

Human health risk assessment

The EDI (mg/kg/day) for the specified pesticides in this study was evaluated separately by the following equation adopted byFAO/WHO (1997):

$$EDI = \frac{C \times FER}{BW} \quad \text{(equation 1)}$$

Where the EDI stands for the estimated daily intake of every pesticide, while C refers to the average residual concentration of each pesticide, and FER denotes the eating rate of food and BW represents the human body weight.

Since the FER of Bangladeshi people was not available, the FER values of the Indian National Sample Survey Office (NSSO, 2014) was used in this study, taking in considerationthat Bangladeshi and Indian individuals have almost the same cultural and food intake pattern. Children's and adults and body weight were considered as 10 kg and 60 kg, respectively (USEPA, 1996; USEPA 1989). In Bangladesh, the mean daily consumption of vegetable was considered 0.166 kg/person (ICN2, 2014).

To assess the long-term health effect following the consumption of those vegetables, the health hazard index (HHI) was calculated based on FAO/WHO (2017), as shown in equation 2. In case the calculated HHI is more than 1, then adverse health effects are possible to human health (Darko and Akoto, 2008).

$$HHI = \frac{EDI}{ADI} \quad \text{(equation 2)}$$

Where, HHI= Health Hazard Index, EDI = Estimated Daily Intake, and ADI = Acceptable Daily Intake.

RESULTS AND DISCUSSION

Pesticide residue in vegetable samples

The collected samples of cucumber and eggplant were analyzed for the determination of seven major organophosphorous insecticides available in the local market of the study area. As shown in Table 2, among the 40 cucumber samples, only 4 cucumber samples (10% of the total number of cucumber samples) were contaminated with pesticides. Furthermore, this study indicated that out of the 4 contaminated cucumber samples, 2 samples contained residue above the EU-MRL (European Commission, 2015). These samples contain Qunalphos, Acephate and Dimethoate residues above the EU level, whereas the other two samples contained Chlorpyripos and Fenitrothion below the EU level. Similarly, out of the 40 eggplant samples, 5 samples (13% of the analyzed samples) had revealed a contamination with pesticide residues of the investigated pesticides. All of the contaminated eggplant samples contained Diazinon above the EU-MRL (European Commission 2015). Frequency of contaminated samples among the five markets ranked as Bahadur Bazar>Fulbari Bazar>Parbatipur Notun Bazar>Ambari and Laxmitola Bazar.

The outcomes of this study is compared with a similar study conducted by Islam *et al.* (2019) where 50 samples of cabbage collected from Dhaka city were analyzed for the determination of four organophosphorous insecticides. The results showed about one-tenth (12%) samples the MRLs value were above the value set by EC. Similarly, Prodhan *et al.* (2016) conducted a study on cabbage samples in Thessaloniki, Greece, and have found that 31% of the total number of samples are pesticides contaminated. The reason could be in the study of Prodhan *et al.* they investigate for both insecticide and as well as fungicides in higher number of samples (132 samples in total). Whereas, Mohammed and Boateng (2017); Rahman (2010); and Islam *et al.* (2014) reported contamination within 45-64%. Location, time, frequency, number of total samples and number of total pesticides analyzed could be the probable reason for the variation. Furthermore, increasing number of market inspection and penalties seems to have a positive impact to reduce the number of contaminated samples.

Health risk assessment

Using the concentration of pesticide residues, the health risk assessment of the contaminated vegetable samples was also assessed foreach samples. The potential health risk related to contaminated vegetable samples presented in Table 3. Considering the specific food intake rate, the calculated EDI value was for cucumber was 0.01913 kg/person/day while for eggplant it was 0.01193 kg/person/day(NSSO, 2014). It is important to notice that all of the HHI values of the contaminated samples weresafe for consumption. However, cucumber sample no. C-15 collected from Bahadur Bazar was very close to HHI for children, thus considered as "alarming".Here, it is also important to state that all the vegetable samples we analyzed after collection werenot washed during sample preparation. Whereas, in Bangladesh, it is a common practice to wash and/or peel vegetablesthoroughly before consumption and/or cooking. Therefore, the residual effects of pesticide will reduce because of washing, peeling and cooking practices. As a consequence, the HHI will also reduce. There are reports indicating that these household pre-consumption activities can reduce up to 90% of residue levels (Alen *et al.,* 2017; Panhwar *et al.,* 2015). As a result, it is essential for policymakers to understand not to abolish these contaminated vegetables where we have high poverty rate. These samples may not suitable or illegal for international trade because of high MRL value, however, it can be

Table 2. The level of residues (mg/kg) of different pesticides found in the vegetable samples. The levels are compared with MRLs set by EC.

Area of collection	Cucumber Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)	Eggplant Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)
Ambari Bazar	C -01	BDL	—-		E -01	BLD	-	
	C -02	BDL	-		E -02	BDL	-	
	C -03	BDL	-		E -03	BDL	-	
	C -04	BDL	-		E -04	BDL	-	
	C -05	BDL	-		E -05	BDL	-	
	C -06	BDL	-		E -06	BDL	-	
	C-07	BDL	-		E -07	Diazinon	0.242	0.01
	C-08	BDL	-		E -08	BDL	-	
Bahadur Bazar	C -09	Chlorpyrifos	0.008	0.01	E -09	BDL	-	
	C -10	BDL	-		E -10	BDL	-	
	C -11	BDL	-		E -11	BDL	-	
	C -12	BDL	-		E -12	BDL	-	
	C -13	BDL	-		E -13	Diazinon	0.215	0.01
	C -14	BDL	-		E -14	BDL	-	
.	C -15	Quinalphos	0.241	0.01	E -15	BDL	-	
	C -16	BDL	-		E -16	BDL	-	
Fulbari Bazar	C -17	BDL	-		E -17	BDL	-	
	C -18	BDL	-		E -18	BDL	-	
	C -19	BDL	-		E -19	BDL	-	
	C -20	BDL	-		E -20	BDL	-	
	C -21	Acephate	**0.156**	0.01	E -21	BDL	-	
		Dimethoate	**0.168**	0.01				
	C -22	BDL	-		E -22	BDL	-	
	C -23	BDL	-		E -23	Diazinon	0.314	0.01
	C -24	BDL	-		E -24	BDL	-	
Laxmitola Bazar	C -25	BDL	-		E -25	BDL	-	
	C -26	BDL	-		E -26	BDL	-	
	C -27	BDL	-		E -27	BDL		
	C -28	BDL	-		E -28	BDL	-	
	C -29	BDL	-		E -29	BDL	-	
	C -30	BDL	-		E -30	BDL	-	
	C -31	BDL	-		E -31	BDL	-	
	C -32	BDL	-		E -32	Diazinon	0.442	0.01
ParbatipurNotun Bazar	C -33	BDL	-		E -33	BDL	-	
	C -34	Fenitrothion	0.009	0.01	E -34	BDL	-	
	C -35	BDL	-		E -35	BDL	-	
	C -36	BDL	-		E -36	BDL	-	
	C -37	BDL	-		E -37	BDL	-	
	C -38	BDL	-		E -38	BDL	-	
	C -39	BDL	-		E -39	Diazinon	0.298	0.01
	C -40	BDL	-		E -40	BDL	-	

*According to the EU Pesticide Database (European Commission 2005);
BDL= below detection limit

Table 3. Health hazard index only contaminated vegetable samples. The EDI calculated by considering the specific food intake rate 0.01913 kg/person/day of cucumber and 0.01193 kg/person/day of eggplant as mentioned in the National Sample Survey Office, household consumption of various goods and services in India (NSSO, 2014).

Sample ID	Area of collection	Name of pesticide	ADI mg/kg/d	BWkg	EDImg/kg/d	HHI	HR remark
C-09	Bahadur Bazar	Chlorpyrifos	—0.01	Adult	3.0×10^{-6}	0.0003	No
				Children	1.5×10^{-6}	0.0015	No
C-15	Bahadur Bazar	Quinalphos	—0.0005	Adult	7.7 v 10^{-5}	0.1537	No
				Children	4.6×10^{-4}	0.9222	Alarming
C-21	Fulbari Bazar	Acephate	0.03	Adult	5.0×10^{-5}	0.0017	No
				Children	3.0×10^{-4}	0.0099	No
	Fulbari Bazar	Dimethoate	0.02	Adult	5.4×10^{-5}	0.0027	No
				Children	3.2×10^{-4}	0.0161	No
C-34	Parbatipur Bazar	Fenitrothion	—0.006	Adult	3.0×10^{-6}	0.0005	No
				Children	2.0×10^{-5}	0.0029	No
E-07	Ambari Bazar	Diazinon	—0.003	Adult	5.0×10^{-5}	0.0160	No
				Children	2.9×10^{-4}	0.0963	No
E-13	Bahadur Bazar	Diazinon	—0.003	Adult	4.0×10^{-5}	0.0143	No
				Children	2.6×10^{-4}	0.0855	No
E-23	FulbariBazar	Diazinon	—0.003	Adult	6.2×10^{-5}	0.0208	No
				Children	3.7×10^{-4}	0.1249	No
E-32	LaxmitolaBazar	Diazinon	—0.003	Adult	8.8×10^{-5}	0.0293	No
				Children	5.3×10^{-4}	0.1758	No
E-39	Parbatipur Bazar	Diazinon	—0.003	Adult	0.6×10^{-5}	0.0020	No
				Children	3.6×10^{-4}	0.0119	No

Table 4. Health hazard index for only contaminatedvegetable samples. The EDI calculated by considering the average vegetable consumption 0.166 kg/person/day as mentioned in ICN2-Country Nutrition Paper, Bangladesh (ICN2, 2014) and assuming the total consumption of different other vegetable contains the same amount of pesticide.

Sample ID	Area of collection	Name of pesticide	ADImg/kg/d	BWkg	EDImg/kg/d	HHI	HR remark
C-09	Bahadur Bazar	Chlorpyrifos	—0.01	Adult	2.2´ 10^{-5}	0.0022	No
				Children	1.3´ 10^{-4}	0.0133	No
C-15	Bahadur Bazar	Quinalphos	—0.0005	Adult	6.7´ 10^{-4}	1.3335	**Yes**
				Children	4.0´ 10^{-3}	8.0012	**Yes**
C-21	Fulbari Bazar	Acephate	0.03	Adult	4.3´ 10^{-4}	0.0144	No
				Children	2.6´ 10^{-3}	0.0863	No
	Fulbari Bazar	Dimethoate	0.02	Adult	4.7´ 10^{-4}	0.0232	No
				Children	2.8´ 10^{-3}	0.1394	No
C-34	Parbatipur Bazar	Fenitrothion	—0.006	Adult	2.5´ 10^{-5}	0.0042	No
				Children	1.5´ 10^{-4}	0.0249	No
E-07	Ambari Bazar	Diazinon	—0.003	Adult	6.7´ 10^{-4}	0.2232	No
				Children	4.0´ 10^{-3}	1.3391	**Yes**
E-13	Bahadur Bazar	Diazinon	—0.003	Adult	5.9´ 10^{-4}	0.1983	No
				Children	3.6´ 10^{-4}	1.1897	**Yes**
E-23	FulbariBazar	Diazinon	—0.003	Adult	8.7´ 10^{-4}	0.2896	No
				Children	5.2´ 10^{-3}	1.7375	**Yes**
E-32	LaxmitolaBazar	Diazinon	—0.003	Adult	1.2´ 10^{-3}	0.4076	No
				Children	7.3´ 10^{-3}	2.4457	**Yes**
E-39	Parbatipur Bazar	Diazinon	—0.003	Adult	8.2´ 10^{-4}	0.0275	No
				Children	5.0´ 10^{-3}	0.1649	No

used for local consumption.

The EDI value was determined (Table 4) based on the average daily consumption of vegetable (0.166 kg/person/day) as declared in International Conference on Nutrition (ICN2, 2014). To predict a worst case scenario, HHIwas only determined in contaminated samples supposing that the total daily consumption of vegetables per person includes the same quantity of pesticide residue. Even in this worst scenario,six samples can be considered risky for health especially for children. It is clear that not all samples but only a certain percentage of samples are contaminated, therefore it is important to establish a traceability system throughout the supply chain to acquire the information of thosespecific sample for required preventive action.

CONCLUSION

In the modern world due to the high population pressure, agricultural inputs like pesticides are essential to increase crop production. Nevertheless, to safe guard the public health, residual effects of pesticides on food needs to be monitored continuously. During health risk assessment calculation and internal consumption, it is recommended to use ADI values instead of MRLs. Lastly, preventive measures need to be adopted to ensure the safe food.

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