Food Research 4 (6) : 2281 - 2288 (December 2020)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Health risk assessment of pesticide residues in vegetables collected from northern part of Bangladesh

[1]Nahar, K.M., [1]Khan, M.S.I., [1]Habib, M.,[2]Hossain, S.M., [3]Prodhan, M.D.H. and [1,*]Islam, M.A.

[1]*Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh*
[2]*Food and Agricultural Organization, Dhaka-1205, Bangladesh*
[3]*Pesticide Research and Environmental Toxicology Section, Division of Entomology, Bangladesh Agricultural Research Institute, Gagipur- 1701, Bangladesh*

Article history:
Received: 29 June 2020
Received in revised form: 23 July 2020
Accepted: 16 August 2020
Available Online: 18 October 2020

Keywords:
Pesticide residues,
Vegetables,
GC-FTD,
QuEChERS,
Health risk assessment

DOI:
https://doi.org/10.26656/fr.2017.4(6).309

Abstract

The use of synthetic pesticides for agricultural production in developing countries like Bangladesh is unavoidable. Therefore, there is also a concern for the residual effects in public health. The present study aimed to assess the health hazards associated with the residual effect of pesticides in two common vegetables (cauliflower and tomato) collected from five different markets of a northern city of Bangladesh. A total 80 samples (i.e. 40 of each vegetable) were collected for the analysis of seven major organophosphorus insecticides namely acephate, chlorpyrifos, diazinon, dimethoate, fenitrothion, malathion and quinalphos used in that region. Modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction technique and GC-FTD (Gas Chromatography coupled with Flame Thermionic Detector) used for the analysis of the samples. Results indicate that 11 (6 cauliflower, 5 tomato) analyzed samples contained residues which are about 14% of the total number of samples. Most of the samples contaminated diazinon at a level above EU-MRLs. However, health risk assessment based on ADI, the contaminated samples were safe. Continuous monitoring together with a sample traceability system is suggested to protect consumers' health from the cumulative effects of other contaminated dietary products.

1. Introduction

Vegetables are herbaceous plants that are cultivated for an edible part or any part of a plant which is consumable. The amount of vegetables needed for a person depends on age, sex and levels of physical activity. Vegetables are poorer in calories than a lot of other foods, but they do contain some amount of calories which is essential for human health. Their consumptions can also play a key job in neutralizing the acids created during digestion of greasy, proteins foods, and offer important roughages that assist in forwarding of food in the intestine (Sarwar, 2012).They are important sources of many nutrients, including potassium, folic acid, vitamin A, vitamin C and others (Mukherjee *et al.*, 2013; Afari-Sefa *et al.*, 2016). In the daily diet, vegetables have been closely associated with the improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some other forms of cancer (Dias, 2012).Consumption of vegetables is also valuable in maintaining the alkaline reserve of the human body. According to Dias and Ryder (2011) the unbalanced diets with low vegetable intake estimated to cause some 2.7 million deaths worldwide each year. Whereas, it is estimated that more than half of the population in Bangladesh are suffering from malnutrition (ICDDR,B, 2020). Bangladesh government, therefore, emphasis the malnutrition issue in the SDG 2 (sustainable development goal) which is mention as "end hunger, achieve food security and improved nutrition and promote sustainable agriculture" (BBS, 2020). Hence, it is necessary to maintain improved vegetable production for increasing nutritional status of the community. But the yield per unit area of vegetables could be affected significantly due to the insect infestation. Pests cause 30-40% losses in general and even 100% losses in case of menace if no control measure is applied (Rahman, 2006; Sapkota and Dahal, 2010; Sarwar *et al.*, 2013). To overcome these situations farmers are using pesticides as

*Corresponding author.
Email: *arif.sau.agch@gmail.com/arif.agch@sau.edu.bd*

it is the most convenient and economical way to control the insect pests and diseases and to increase the production. Control of insect pests and diseases, moulds and weeds over 1000 compounds are applied to agricultural crops (Ortelli *et al.,* 2004). There is no doubt that the use of pesticide is essential to increase production. To ensure food supply for over increasing world population the world will need to produce 60% more food by 2050 (FAO, 2012). Pesticides play a key role to fulfill this demand. Although the vegetable production is increasing day by day in Bangladesh, one potential drawback associated with a shift toward more intensive vegetable production is the reliance of most vegetable producers on the heavy application of pesticide (Hossain *et al.,* 2000). In Bangladesh, only 0.87 kg/ha pesticide is used in cropland which is in average 10-15 times lower compared to many developed countries like Japan, South Korea and the Netherlands (FAO, 2017).However, there is still huge uncertainty about the pre and postharvest management of those pesticides' residues and safety of agricultural products. Considering all the above facts, the aim of the present study was to determine pesticide residues in cauliflower and tomato collected from different areas of a northern city of Bangladesh and to assess their chronic health risk for the consumers.

2. Materials and methods

2.1 Study area

The study area included major five markets of Rangpur City, namely Paura Bazar, Tetultola Bazar, Lalbag Bazar, Termilan Bazar and Station Bazar (Figure 1). The scientific name, family, edible part, cultivation area and production information are presented in Table 1.

2.2 Sample collection and preparation

A total of 80 samples (40 cauliflower and 40 tomato) were collected for this study. A total of 8 samples of cauliflower and eight samples of tomato were collected from each market. The amount of each sample was 1 kg. The samples were collected in a clean transparent airtight polyethylene bag to avoid cross-contamination and kept in cool temperature box. Just after 1 day of collection all samples were carried to the Pesticide Analytical Laboratory, Division of Entomology, Bangladesh Agricultural Research Institute (BARI), Gazipur. This is an accredited laboratory for pesticide residue analysis in Bangladesh. Each sample was cut into small pieces and mixed properly. Airtight clean polythene bags were used to store chopped sample in refrigerator at -20°C until extraction and cleanup process started.

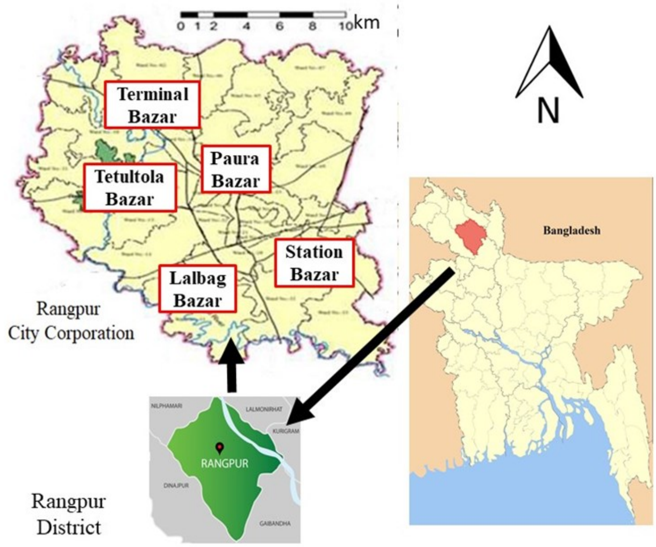

Figure 1. Map showing different sampling areas of Rangpur (Bangladesh)

2.3 Chemicals and reagents

The standard of chlorpyrifos, acephate, diazinon, dimethoate, quinalphos, malathion and fenitrothion were obtained from Sigma-Aldrich (St Louis, MO, USA) via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. Standards of all pesticides contained >99.6% purity. Methanol, acetone, gradient grade acetonitrile, sodium chloride (NaCl), anhydrous magnesium sulphate ($MgSO_4$) and Primary Secondary Amine (PSA) were purchased from Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh.

2.4 Preparation of pesticide standard solution

Pesticide standard stock solutions of chlorpyrifos, acephate, diazinon, dimethoate, quinalphos, malathion and fenitrothion were prepared separately in acetone. The concentration was 1000 mg/L. They were stored -20°C until use. A mixed standard solution containing all the aforementioned pesticides in 50 mg/L acetone was prepared in a 50 mL volumetric flask and made to the volume with acetone. An intermediate mixed standard solution of 10 mg/L in acetone was prepared from the mixed standard solution of 50 mg/L. Then working standard solutions of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0 and 5.0 mg/L in methanol was prepared by transferring the

Table 1. Vegetable samples used for the present research

Common Name	Scientific Name	Family	Edible part	Area (*TA)	Production (**TMT)
Tomato	*Solanum lycopersicum* L.	Solanaceae	Fruit	70	385
Cauliflower	*Brassica oleracea*	Brassicaceae	Head (florets)	48	274

* TA = thousand acres **TMT = thousand matric tones. Source: BBS (2018)

proper amount from 10 mg/L intermediate mixed standard solution into ten separate 10 mL volumetric flasks. All of the standard solutions were kept in a freezer at -20°C until use.

2.5 Preparation of calibration curve

Prior to the injection of the sample extract, standard solutions of different concentrations of each pesticide group were prepared and injected with suitable instrument parameters. The samples were calibrated (retention time, peak area etc.) against five-pointed calibration curve of the standard solution of concerned pesticide. Each peak was characterized by its retention time (Figure 2). The results were expressed in mg/kg automatically by the GC software.

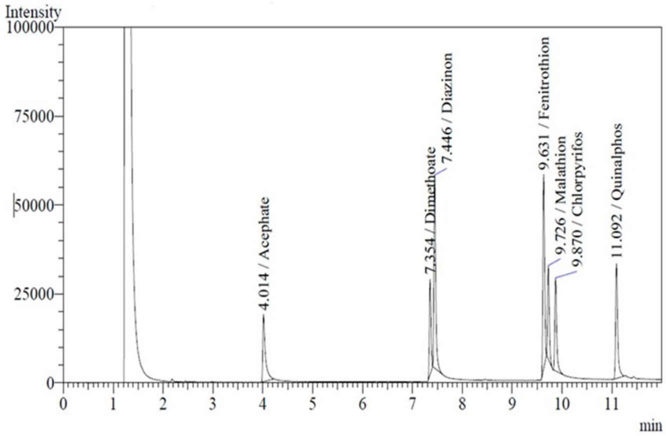

Figure 2. Typical chromatograms of seven organophosphorus insecticide standards run by GC-FTD

2.6 Extraction and cleanup

In this study, the QuEChERS extraction technique was used for the extraction and clean-up of samples which was modified by Prodhan *et al.* (2015). The chopped samples were grounded thoroughly with the fruit blender. A representative 10 g portion of the thoroughly homogenized sample was weighted in a 50 mL polypropylene centrifuge tube. Then 10 mL of acetonitrile (MeCN) was added into the centrifuge tube. The centrifuge tube was closed properly and shaken vigorously for 30 s by the use of a vortex mixer. Then, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added into the centrifuge tube, and it was shaken immediately by the vortex mixer for 1 min to prevent the formation of magnesium sulfate aggregates. Afterwards, the extract was centrifuged for 5 mins at 5000 rpm. An aliquot of 3 mL of the MeCN layer was transferred into a 15 mL microcentrifuge tube containing 600 mg anhydrous MgSO4 and 120 mg Primary Secondary Amine (PSA). Then it was thoroughly mixed by vortex for 30 s and centrifuged for 5 mins at 4000 rpm. (Laboratory Centrifuges, Sigma-3K30, Germany). After centrifuge, a 1 mL supernatant was filtered by a 0.2 µm PTFE filter, and then it was taken in a clean GC vial for injection.

2.7 Detection and quantification of pesticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) for the detection of chlorpyrifos, acephate, diazinon, dimethoate, quinalphos, malathion and fenitrothion. The capillary column was AT-1 length was 30m, ID was 0.25 mm and film thickness was 0.25µm. Helium was used as the carrier and make up gas for FTD. The identification of suspected pesticide was performed by peak retention times in samples to those of peaks in the pure analytical standards. The instrument conditions are described in Table 2. The level of detection (LOD) and level of quantification (LOQ) were 0.003 and 0.01 mg/kg, respectively.

2.8 Quality assurance

During this study, recovery tests were conducted on cauliflower and tomato samples. The collected samples were cut into small pieces and stored at -20°C until homogenization. Homogenization was done by fruit blender. A 10g homogenized sample was spiked prior to the determination procedure by the addition of a mixed pesticide standard working solution to reach the final fortification levels of 0.10 mg/kg. After the addition of each concentration in the matrix, the mixture was equilibrated by shaking and the samples were allowed to settle for 30 minutes prior to extraction in order to ensure the sufficient contact of the analytes with the whole matrix. Then the samples were prepared according to the method which was described earlier. Precision in case of Repeatability (RSD_r) was determined at the same fortification levels of 0.10 mg/kg on the same day. The average recoveries of the selected pesticides in cauliflower ranged from 80-104% with RSD ≤11, while in the case of tomato it was 83-101% with RSD ≤8.

2.9 Human health risk assessment

Table 2. The instrument parameters for GC-FTD

Instruments	Conditions
Injection port SPL	Injection mode: split; temperature:250°C; flow control mode: linear velocity; split ratio: 30:0
Detector channel 1 FTD	Temperature: 280°C; current: 1.00 Pa; H2 flow: 1.5 mL/min; stop time: 10 mins; make up flow: 30 mL/min; air flow: 145 mL/min
Column oven Temperature	It was started from 150°C (hold for 1 min) and went up to 220°C (hold for 2 min) with an incremental rate of 10 °C.

eISSN: 2550-2166

Estimated daily intake (EDI, mg/kg/day) was determined for each pesticide using the following equation proposed by FAO/WHO (1997).

$$\mathrm{EDI} = \frac{\mathrm{C} \times \mathrm{FER}}{\mathrm{BW}}$$

Where EDI is the estimated daily intake of each pesticide, C is the mean residual concentration of that pesticide, FER is the food eating rate and BW is the human body weight.

As we were not able to find suitable Bangladesh originated data, the food eating rate of was taken from the National Sample Survey Office, Household consumption of various goods and services in India (NSSO, 2014). The body weights of adults and children were assumed as 60 kg and 10 kg, respectively (USEPA, 1989; USEPA, 1996). An EDI value was also calculated by considering average vegetable consumption of 0.166 kg/person/day in Bangladesh (FAO/WHO, 2014).

The health hazard index (HHI) was calculated to predict the long-term effects on health due to consumption of pesticide residues via those vegetables. It is expressed as a ratio of estimated daily intake (EDI) to acceptable daily intake (ADI) as shown in the following equation adapted from CODEX Alimentarius International Food Standard (2017). If the calculated value of HHI is greater than 1 then it is indicating an unacceptable risk to human health (Darko and Akoto, 2008).

$$\mathrm{HHI} = \frac{\mathrm{EDI}}{\mathrm{ADI}}$$

3. Results and discussion

Dietary intake of pesticide residues has been linked with a wide range of chronic health hazards (Mir, 2018). So, it is most important matter to know the amount of pesticide residues contained in vegetables and to evaluate properly their health risk assessment.

3.1 Pesticide residue in vegetable samples

Cauliflower and tomato samples collected from different markets were analyzed for the detection of seven organophosphorus insecticides. Out of 40 samples of cauliflower 6 samples (15% of the total number of samples) contained pesticide residues and 34 samples (85% of the total number of samples) contained no detectable residues of the sought pesticides (Table 3). Among those 6 contaminated samples, 3 samples contained diazinon and quinalphos residues and 3 samples contained only diazinon residue. Most of the samples were above the EU-MRL, two markets namely Station Bazar, and Terminal Bazar contains most of the contaminated samples. Similarly, out of 40 samples of tomato, 5 samples (13% of the total number of samples) contained pesticide residues and 35 samples (87% of the total number of samples) contained no detectable residues of the sought pesticides. Four out of 5 samples were above the EU-MRL. Five different organophosphorus insecticides detected in five different markets.

The present results can be compared to Islam *et al.* (2019) where 50 cabbage samples were collected from different markets of Dhaka city for the analysis of four organophosphorus insecticides. Twelve percent samples were contaminated with MRLs above set by EC. Prodhan *et al.* (2016), however, found higher contamination (31% of the total no. of samples) in cabbage samples collected from different market places in Thessaloniki, Greece. The reason could be they look for insecticide (i.e. chlorpyrifos, cypermethrin and deltamethrin) and as well as fungicides (i.e. fluopicolide and propamocarb hydrochloride) in 132 samples. There as, Islam *et al*. (2014) and Mohammed and Boateng (2017) reported up to 64 and 45% of contaminated samples, respectively.

3.2 Health risk assessment

The health risk assessment was made by using the concentration of pesticide residues in the contaminated samples. The predicted health risk associated with the contaminated vegetable samples collected from the study area presented in Table 4. The EDI calculated by considering the specific food intake rate 0.011 kg/person/day of cauliflower and 0.027 kg/person/day of tomato as mentioned in the National Sample Survey Office, household consumption of various goods and services, India (NSSO, 2014). All of the samples were safe for consumption. Moreover, it should be considered that these samples were analyzed after collection without washing. Whereas, it is a common practice in our country to wash vegetable after buying from the markets. Most of them also peeled and cooked before consumption. These washing, peeling and cooking practices will definitely reduce the amount of pesticide residues. There are available reports that this simple household treatment can reduce 30-90% of residue levels (Panhwar *et al.*, 2014; Jankowska *et al.*, 2016; Alen *et al.*, 2017; Prodhan *et al.*, 2018). Therefore, it is important to consider for the policymakers not to destroy these contaminated vegetable products. These samples are not allowed for international trade due to higher MRL value but no problem for national consumption.

Table 5 shows the health risk assessment where EDI value calculated considering the average daily vegetable consumption (i.e. 0.166 kg/person/day) as mentioned in

Table 3. The level of residues (mg/kg) of different pesticides found in the vegetable samples.

Area of collection	Cauliflower Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)	Tomato Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)
Paura Bazar	C -1	BDL	---		T -1	Diazinon	0.119	0.01*
	C -2	BDL	-		T -2	BDL	-	
	C -3	BDL	-		T -3	BDL	-	
	C -4	BDL	-		T -4	BDL	-	
	C -5	BDL	-		T -5	BDL	-	
	C -6	Diazinon	0.247	0.01*	T -6	BDL	-	
	C-7	BDL	-		T -7	BDL	-	
	C-8	BDL	-		T -8	BDL	-	
Tetultola Bazar	C -9	BDL	-		T -9	BDL	-	
	C -10	BDL	-		T -10	BDL	-	
	C -11	BDL	-		T -11	BDL	-	
	C -12	BDL	-		T -12	Chloropyrifos	0.096	0.01*
	C -13	BDL	-		T -13	BDL	-	
	C -14	BDL	-		T -14	BDL	-	
	C -15	BDL	-		T -15	BDL	-	
	C -16	BDL	-		T -16	BDL	-	
Lalbag Bazar	C -17	Diazinon Quinalphos	0.146 0.102	0.01*	T -17	BDL	-	
	C -18	BDL	-		T -18	BDL	-	
	C -19	BDL	-		T -19	Quinalphos	0.095	0.01*
	C -20	BDL	-		T -20	BDL	-	
	C -21	BDL	-		T -21	BDL	-	
	C -22	BDL	-		T -22	BDL	-	
	C -23	BDL	-		T -23	BDL	-	
	C -24	BDL	-		T -24	BDL	-	
Station Bazar	C -25	Diazinon Quinalphos	0.162 0.031	0.01*	T -25	BDL	-	
	C -26	BDL	-		T -26	BDL	-	
	C -27	BDL	-		T -27	BDL		
	C -28	BDL	-		T -28	Dimethoate	0.202	0.01*
	C -29	Diazinon Quinalphos	0.093 0.009	0.01*	T -29	BDL	-	
	C -30	BDL	-		T -30	BDL	-	
	C -31	BDL	-		T -31	BDL	-	
	C -32	BDL	-		T -32	BDL	-	
Terminal Bazar	C -33	BDL	-		T -33	BDL	-	
	C -34	BDL	-		T -34	BDL	-	
	C -35	Diazinon	0.281	0.01*	T -35	BDL	-	
	C -36	BDL	-		T -36	BDL	-	
	C -37	BDL	-		T -37	Acephate	0.273	0.01*
	C -38	BDL	-		T -38	BDL	-	
	C -39	BDL	-		T -39	BDL	-	
	C -40	Diazinon	0.364	0.01*	T -40	BDL	-	

*According to the EU Pesticide Database. BDL= below detection limit

International Conference on Nutrition, Country Nutrition Paper-Bangladesh (FAO, 2014). Health hazard index was calculated for only contaminated samples assuming that total consumption of different vegetables per person per day contains the same amount of pesticide residues. In this extreme situation, 6 samples can be considered risky for health and another 6 samples as alarming for health risk especially for children with low body weight. As this HHI was prepared by only contaminated sample, not the average value of 8 samples collected from the same market, therefore it is import to have a traceability system to get the information of that particular sample for necessary action.

4. Conclusion

Pesticides are essential inputs in agriculture to increase crop production. The health risk associated with

eISSN: 2550-2166

Table 4. Health hazard index (HHI) only contaminated vegetable samples. The EDI calculated by considering the specific food intake rate 0.011 kg/person/day of cauliflower and 0.027 kg/person/day of tomato 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	BW kg	EDI mg/kg/d	HHI	HR remark
C-6	Paura Bazar	Diazinon	0.003	Adult	4.5×10^{-5}	0.0149	No
				Children	2.7×10^{-4}	0.0895	No
C-17	Lalbag Bazar	Diazinon	0.003	Adult	2.6×10^{-5}	0.0088	No
				Children	1.6×10^{-4}	0.0529	No
	Lalbag Bazar	Quinalphos	0.0005	Adult	1.8×10^{-5}	0.0369	No
				Children	1.1×10^{-4}	0.2217	No
C-25	Station Bazar	Diazinon	0.003	Adult	2.9×10^{-5}	0.0098	No
				Children	1.8×10^{-4}	0.0587	No
	Station Bazar	Quinalphos	0.0005	Adult	0.6×10^{-5}	0.0112	No
				Children	3.4×10^{-5}	0.0674	No
C-29	Station Bazar	Diazinon	0.003	Adult	1.7×10^{-5}	0.0056	No
				Children	1.0×10^{-4}	0.0337	No
	Station Bazar	Quinalphos	0.0005	Adult	0.2×10^{-5}	0.0033	No
				Children	1.0×10^{-5}	0.0196	No
C-35	Terminal Bazar	Diazinon	0.003	Adult	0.5×10^{-4}	0.017	No
				Children	3.1×10^{-4}	0.1018	No
C-40	Terminal Bazar	Diazinon	0.003	Adult	0.7×10^{-4}	0.022	No
				Children	4.0×10^{-4}	0.1318	No
T-1	Paura Bazar	Diazinon	0.003	Adult	0.5×10^{-4}	0.0178	No
				Children	3.2×10^{-4}	0.1066	No
T-12	Tetultola Bazar	Chloropyrifos	0.01	Adult	0.4×10^{-4}	0.0043	No
				Children	2.6×10^{-4}	0.0258	No
T-19	Lalbag Bazar	Quinalphos	0.0005	Adult	4.3×10^{-5}	0.0851	No
				Children	2.6×10^{-4}	0.5105	No
T-28	Station Bazar	Dimethoate	0.002	Adult	0.9×10^{-4}	0.0452	No
				Children	5.4×10^{-4}	0.2714	No
T-37	Terminal Bazar	Acephate	0.03	Adult	1.2×10^{-4}	0.0041	No
				Children	7.3×10^{-4}	0.0244	No

their residues on food needs to be continuously monitored and evaluated. About 14% of the samples were found to be contaminated with pesticide residues and their levels were mostly above EU recommendation MRLs. Based on the ADI, they were within the safe limit for consumption. Therefore, it is important to use ADI values rather than MRLs when calculating health risk assessment and internal consumption. However, based on HHI, all the samples were within the safe limit for consumption in the case of an adult, while several samples are alarming for health risk especially for children due to low body weight. In addition, traceability system can significantly help to improve the safety of the products.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to Md. Kamal Hossain, Momtaz Nahar and Prosenjit in the Pesticide Analytical Laboratory (an accredited pesticide analytical laboratory in Bangladesh) of Bangladesh Agricultural Research Institute for their cooperation during sample preparation and GC analysis. The authors are also grateful to Muhammad Muktader Rashid Bhuiyan for his help in SciFinder searching regarding the relevant research work.

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Table 5. Health hazard index (HHI) for only contaminated vegetable samples. The EDI calculated by considering the average vegetable consumption 0.166 kg/person/day as mentioned in ICN2-Country Nutrition Paper, Bangladesh (FAO, 2014) and assuming the total consumption of different other vegetable contains the same amount of pesticide.

Sample ID	Area of collection	Name of pesticide	ADI mg/kg/d	BW kg	EDI mg/kg/d	HHI	HR remark
C-6	Paura Bazar	Diazinon	0.003	Adult	6.8×10^{-4}	0.2278	No
				Children	4.1×10^{-3}	1.3667	Yes
C-17	Lalbag Bazar	Diazinon	0.003	Adult	4.0×10^{-4}	0.1346	No
				Children	2.4×10^{-3}	0.8079	Alarming
	Lalbag Bazar	Quinalphos	0.0005	Adult	2.8×10^{-4}	0.5644	Alarming
				Children	1.1×10^{-4}	0.2217	No
C-25	Station Bazar	Diazinon	0.003	Adult	4.5×10^{-4}	0.1494	No
				Children	2.7×10^{-3}	0.8964	Alarming
	Station Bazar	Quinalphos	0.0005	Adult	8.6×10^{-5}	0.1715	No
				Children	5.2×10^{-4}	1.0292	Yes
C-29	Station Bazar	Diazinon	0.003	Adult	2.6×10^{-4}	0.0858	No
				Children	1.5×10^{-3}	0.5146	Alarming
	Station Bazar	Quinalphos	0.0005	Adult	2.5×10^{-5}	0.0498	No
				Children	1.5×10^{-5}	0.2988	No
C-35	Terminal Bazar	Diazinon	0.003	Adult	7.8×10^{-4}	0.2591	No
				Children	4.7×10^{-3}	1.5549	Yes
C-40	Terminal Bazar	Diazinon	0.003	Adult	1.0×10^{-3}	0.3357	No
				Children	6.0×10^{-3}	2.0141	Yes
T-1	Paura Bazar	Diazinon	0.003	Adult	3.3×10^{-4}	0.1097	No
				Children	2.0×10^{-3}	0.6585	Alarming
T-12	Tetultola Bazar	Chloropyrifos	0.01	Adult	2.7×10^{-4}	0.0266	No
				Children	1.6×10^{-3}	0.1594	No
T-19	Lalbag Bazar	Quinalphos	0.0005	Adult	2.6×10^{-4}	0.5257	Alarming
				Children	1.6×10^{-3}	3.154	Yes
T-28	Station Bazar	Dimethoate	0.002	Adult	5.6×10^{-4}	0.2794	No
				Children	3.4×10^{-3}	1.6766	Yes
T-37	Terminal Bazar	Acephate	0.03	Adult	7.6×10^{-4}	0.0252	No
				Children	4.5×10^{-3}	0.1511	No

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