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Extent of Insecticide Residue Load in Vegetables Grown under Conventional Farming in Bangladesh

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Abstract

The study was carried out to detect and quantify the left over residues of eight commonly used insecticides (fenvalerate, diazinon, quinalphos, fenitrothion, acephate, chlorpyriphos, cypermethrin and malathion) in brinjal, yard long bean, bitter gourd, snake gourd, pointed gourd, okra, tomato, hyacinth bean and cabbage samples collected from local market of eight different regions like Jessore, Comilla, Narsingdi, Tangail, Rangpur, Jamalpur, Gazipur and Dhaka during 2011-2012 seasons. Among the170 analyzed samples, 21.78% were contaminated with four insecticides (chlorpyriphos, quinalphos, acephate and cypermethrin) either single or multiple product residue, in which 18.26% samples had residue above MRL. The vegetables of Norsingdi, Jessore and Comilla had more insecticide residue in comparison to other locations. Most of the samples contain chlorpyriphos (13.53%) and quinalphos (8.4%) residue. Acephate, chlorpyriphos and quinaphos were also found as multiple product residues expressing 2.35% of the total samples which were above MRL and 19.41% sample contained single product residue with chlorpyriphos, quinaphos and cypermethrin where 15.88% were of above MRL. Cypermethrin residue was detected only in two samples (brinjal and yard long bean) which were below MRL.

Keywords: Insecticide, organophosphorus, residue, vegetables, MRL.

1. Introduction

Bangladesh has an irresistible agricultural economy. Production of agricultural produces is being increased every year to meet the growing demand of the people. Diversification of vegetable crops and increasing commercialization can support the development of the agriculture sector in several ways. But, in case of vegetables, data show the output growth in Bangladesh mainly through area expansion, without much improvement in yield. One potential drawback associated with a shift toward more intensive vegetable production is the common reliance of most vegetable producers on heavy application of pesticide (Hossain *et al.*, 2000). Among the vegetables grown in Bangladesh, brinjal, tomato, cabbage, bitter gourd, snake gourd, pointed gourd, okra, hyacinth bean and yard long bean are nutritious, valuable and very popular to consumers. It has been reported that in brinjal, bitter gourd and beans, the attack of insect pests are severe and farmers sprayed pesticides quite frequently even every day (Anonymous, 2001; Ahmed *et al.*, 2005). Extensive deliberate use of pesticides has resulted in contamination of vital supplies, air, water and food. The risk to humans may be short term as well as long term depending on the persistence of the pesticide and the exposure period. Pesticide residue in food makes deleterious effect on human health such as headaches and nausea to chronic impacts like cancer, reproductive harm and endocrine disruption (Berrada *et al.,* 2010). Now pesticide residue in food has become a consumer's safety issue and the consumers have the right to know how much pesticide get incorporated in the food they eat. However, very little references are available on the presence of pesticides in vegetables in Bangladesh (Khatoon *et al.,* 2004). Under such circumstances, the present study was undertaken to detect and quantify the amount of leftover residue of pesticide in different vegetable samples collected from local market of different regions of Bangladesh.

2. Materials and Methods

One hundred seventy vegetable samples viz., brinjal, cabbage, hyacinth bean, yard long bean, bitter gourd, snake gourd, pointed gourd, okra and tomato were collected from local market of eight different regions namely Comilla, Gazipur, Jessore, Norsingdi, Tangail, Dhaka, Jamalpur and Rangpur (Table 1). The weight of the sample of each vegetable was 1 kg. Samples were collected according to regulation made in the "Guidelines for the control of pesticide residues in foods" (Anonymous, 1996), which incorporate the EU directive (Anonymous, 1979) and Codex recommendations (Anonymous, 1993) regarding sampling. Collected samples were kept in "Chilled box" and carried to the laboratory within the quickest time and stored in refrigerator (-20^{0}C) until analysis.

The standard for diazinon, malathion, quinalphos, fenitrothion, chlorpyriphos, acephate, fenvalerate and cypermethrin were obtained from Sigma-Aldrich Laborchemikalien, Seelze, Germany via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. Standards of all the insecticides contained >99.6% purity. Chromatograms obtained from standard were used to compare residue of the vegetable samples.

2.1 Extraction, separation and clean up of vegetable samples

The collected vegetable samples (250g) were chopped by knife on white board and mixed well. A sub sample of 20g was taken into a wide mouth jar with the help of a spatula then 100 ml of hexane was added to it. Sodium sulphate (Na_2SO_4) was also added with sample until water was removed from the sample. The mixture was then macerated with high-speed homogenizer (Ultra-turrax, IKA T18 basic, Germany) for 2 minutes. The homogenized material was then poured into 250 ml conical flask and placed into the shaker (Refrigerated Shaker, Rexmed, Sweden) for 12 hrs continuous shaking. After shaking, the slurry was filtered through whatman filter paper no.40 and a buchner funnels with suction. The flask and filter cakes were rinsed with 25 ml of hexane each. The filtrate was then transferred into 250 ml round bottom flask and was dried to around 5-7 ml by evaporation using a rotary vacuum evaporator (Laborota-4001, Heidolph, Germany) at 35-40ºC. Then, the concentrate filtrate was collected in a centrifuge tube adjusted at 10 ml volume which was then centrifuged at 16500 rpm for 10 minutes with laboratory refrigerated centrifuges (Sigma-3K30, Germany). After centrifuge, supernatant was collected and cleaned up by Super Phase Extraction (SPE) cartridge. Then the final volume was kept in 10 ml volumetric flask. Before injection, this volume was again cleaned up by High Performance Liquid Chromatograph (HPLC) filter (0.2 PTFE) which was ready for injection in Gas Chromatograph.

2.2 Detection and quantification of insecticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) with Flame Thirmionic Detector (FTD) for detection of organophosphorus (diazinon, quinalphos, acephate, chlorpyriphos, fenitrothion and malathion) insecticide. For detection of pyrethroid (cypermethrin, fenvalerate) each sample extract was again analyzed with Electron Capture Detector (ECD). The capillary column used was AT-1, length 30m, ID 0.25mm and film thickness 0.25µm in case of both detectors. Helium was used as carrier and make up gas in FTD and in case of ECD, it was Nitrogen.

The instrument parameters for detecting organophosphorus and pyrethroid insecticides were as follows:

Organophosphorus Insecticides

[Injection Port SPL]
Injection Mode: Split
Temperature: 250^0C
Flow Control Mode: Linear Velocity
Split Ratio: 30.0

[Column Oven]
Initial Temperature: 150^0C
Column Oven Temperature Program:
Total Program Time: 10.00 min

Rate(C/min)	Temperature (^{0}C)	Hold Time (min)
-	150	1
10	220	2

[Detector Channel 1 FTD]
Temperature: 280^0C
Stop Time: 10 min
Current: 1.00 pA
Makeup Flow: 30 ml/min
H_2 Flow: 1.5 mL/min
Air Flow: 145 ml/min

Pyrethroid Insecticide
[Injection Port SPL]
Injection Mode: Split
Temperature: 280^0C
Flow Control Mode: Linear Velocity
Split Ratio: 10

[Column Oven]
Initial Temperature: 160^0C
Column Oven Temperature Program:
Total Program Time: 18.00 min

Rate(C/min)	Temperature (^{0}C)	Hold Time (min)
-	160	1
10	270	6

[Detector Channel 1 ECD]
Temperature : 300^0C
Stop Time: 18 min
Current: 1.00 pA
Makeup Flow: 30 ml/min

Prior to the injection of the sample extract, standard solutions of different concentrations of each pesticide were prepared and injected with selected instrument parameters. The samples were calibrated (retention time, peak area etc.) against three to four pointed calibration curve of standard solution of concerned pesticide. Each peak was characterized by its retention time. Sample results were expressed in $mgkg^{-1}$ automatically by the GC software which represented the concentration of the final volume injected. From this value, the actual amount of pesticide residue present in the sample was determined by using the following formula:

Residue in sample ($mgkg^{-1}$):

$$= \frac{\text{Conc. obtained in injected volume } (mgkg^{-1}) \text{ X Quantity of final volume (L)}}{\text{Amount of sample taken (kg)}}$$

Table 1. Basic information about vegetable samples collected from different locations of Bangladesh during 2011-12

Locations	English name	Common name	Edible part	Scientific name	Family
Jessore, Comilla,	Hyacinth bean	Sheem	Pod	*Lablab niger*	Leguminoseae
Narsingdi, Tangail,	Yard long bean	Borboti	Pod	*Vigna sesquipedalis*	Fabaceae
Rangpur, Jamalpur,	Cabbage	Bandhakopi	Head	*Brassica oleracea* var capitata	Cruciferae
Gazipur	Tomato	Tomato	Fruit	*Lycopersicon esculentum*	Solanaceae
and Dhaka	Brinjal	Begoon	Fruit	*Solanum melongena*	Solanaceae
	Okra	Dherosh	Fruit	*Abelmoschus esculentus*	Malvaceae
	Bitter gourd	Karala/Ucche	Fruit	*Momordica charantia*	Cucurbitaceae
	Snake gourd	Chichingga	Fruit	*Trichosanthes anguina*	Cucurbitaceae
	Pointed gourd	Potal	Fruit	*Trichosanthes dioica*	Cucurbitaceae

3. Results and Discussion

3.1 Determination of insecticide residue

The analytical results of the vegetable samples for the detection and quantification of ***insecticide*** residue have been summarized in Table 2. Figure 1 shows the chromatograms of standards of organophosphorus insecticides. Three chromatograms of quinalphos residue obtained from the yard long bean in Norsingdi, Comilla and Jessore regions are shown in Figure 2-4. In this way the results of other samples were also made by in-built GC-2010 software.

A total of 170 samples of nine vegetables viz., brinjal, hyacinth bean, cabbage, bitter gourd, tomato, pointed gourd, snake gourd, okra and yard long bean were collected from local markets of eight districts as Comilla, Jamalpur, Tangail, Gazipur, Dhaka, Rangpur, Jessore and Norsingdi. Analytical results showed that 21.76% samples were contaminated with four insecticides (chlorpyriphos, quinalphos, acephate and cypermethrin) irrespective of single or multiple product residues, in which 18.26% samples contained residue above MRL (European Commission, 2016; FAO/WHO, 2016). The detected residue of chlorpyriphos and quinalphos were found more as compared to acephate and cypermethrin. Acephate was detected as multiple products with chlorpyriphos in two samples of snake gourd from Norsingdi which were above MRL but sample of other locations did not have any acephate residue. Another two multiple product (chlorpyriphos, quinalphos) residues were found in brinjal and bitter gourd samples of Jessore location which were also above MRL (Table 2). The presence of multiple product residues indicated that farmers applied more than one pesticide on brinjal and bitter gourd in Jessore and snake gourd in Norsingdi location. Most of the commercial farmers of vegetables especially in Jessore region have been spraying “cocktail” (mixture of 3-5 pesticides) at every or every alternate day (Kabir *et al*., 1996; Rashid *et al.*, 2003). This whimsical and injudicious use of pesticides on vegetables might cause the multiple residues of pesticides even at or above MRL level.

Cypermethrin residue was found in two samples; one in brinjal from Jessore and another one in yard long bean from Rangpur location and the levels of residue were below MRL which might be due to higher rate of dissipation.

Table 2. Quantity of residue of different insecticides estimated from vegetable samples of local market collected from different regions of Bangladesh during 2011-2012

Vegetables	Samples analyzed (no.)	Contaminated samples (no.)		Detected insecticide and Level of residue (mgkg^{-1})		Frequency	* MRL (mgkg^{-1})
		Single	Multi				
Brinjal	30	3	1	Quinalphos:	0.326 0.069	2	0.01
				Chlorpyriphos:	0.420 0.445	2	0.05
				Cypermethrin:	0.026	1	0.03
Hyacinth bean	15	3	-	Quinalphos:	0.260	1	0.01
				Chlorpyriphos:	0.196 0.407	2	0.05
Cabbage	6	4	-	Quinalphos:	0.143 0.098	2	0.01
				Chlorpyriphos:	0.406 0.063	2	0.01
Bitter gourd	20	7	1	Quinalphos:	0.226 0.132 0.083 0.065	4	0.01
				Chlorpyriphos:	0.200 0.230 0.441 0.396 0.094	5	0.05
Tomato	30	3	-	Chlorpyriphos:	0.138 0.443 0.397	3	0.01
Pointed gourd	10	2	-	Chlorpyriphos:	0.302 0.267	2	0.05

Vegetables	Samples analyzed (no.)	Contaminated samples (no.)		Detected insecticide and Level of residue ($mgkg^{-1}$)	Frequency	* MRL ($mgkg^{-1}$)
		Single	Multi			
Snake gourd	23	2	2	Chlorpyriphos: 0.035	3	0.05
				0.080		
				0.120		
				Acephate: 0.066	2	0.01
				0.236		
				Quinalphos: 0.094	1	0.01
Okra	20	4		Quinalphos: 0.160	1	0.01
				Chlorpyriphos:0.090	3	0.50
				0.056		
				0.075		
Yard long bean	16	5	-	Chlorpyriphos: 0.368	1	0.05
				Quinalphos: 0.247	3	0.01
				0.205		
				0.096		
				Cypermethrin: 0.563	1	0.70

*MRL= Maximum Residue Limit (European Commission, 2016; FAO/WHO, 2016).

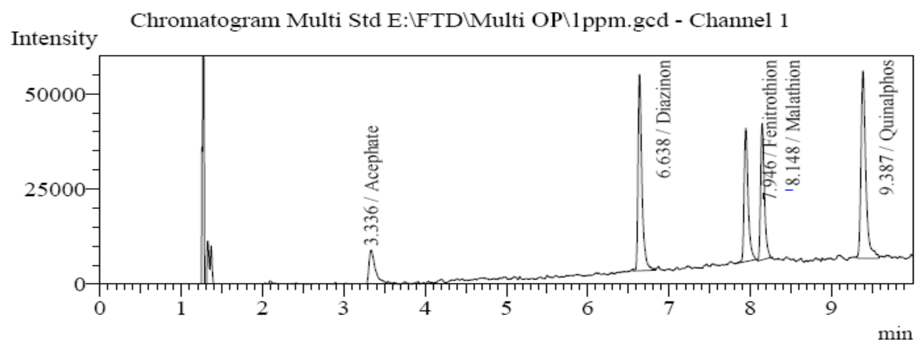

Figure 1. Chromatograms of standards of organophosphorus insecticides run by GC-FTD

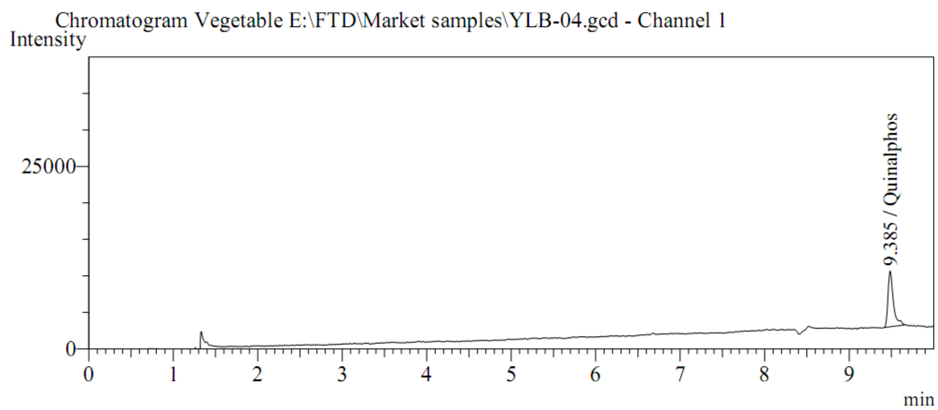

Figure 2. Chromatograms of quinalphos obtained from the extract of yard long bean (YLB-04) collected form Jessore region

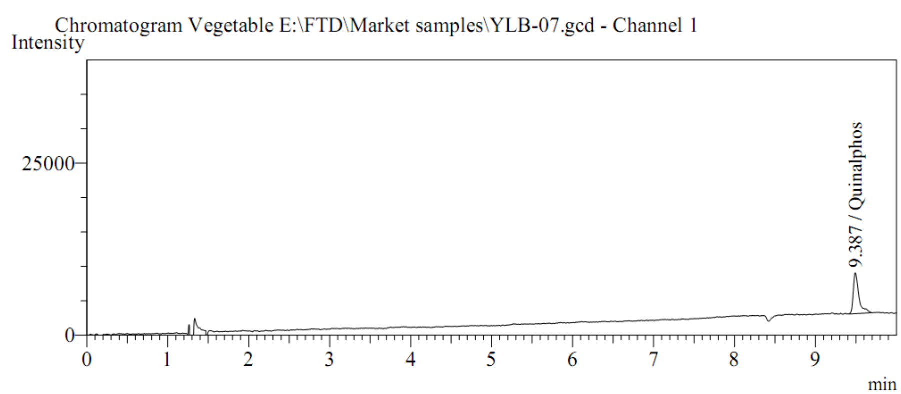

Figure 3. Chromatograms of quinalphos obtained from the extract of yard long bean (YLB-07) collected form Norsingdi

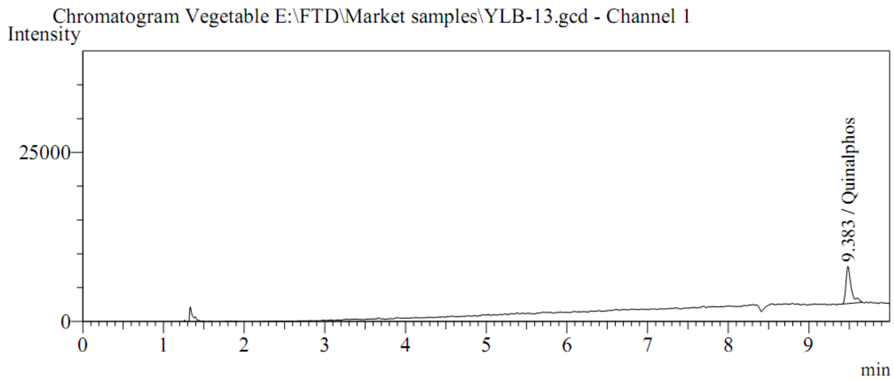

Figure 4. Chromatograms of quinalphos obtained from the extract of yard long bean (YLB-13) collected form Comilla

There were 33 contaminated samples of single product in which 19 samples were of chlorpyriphos (frequency 23), 12 samples were of quinalphos (frequency 14) and two samples of cypermethrin (frequency 2). Fifteen samples contained 0.063 to 0.445 $mgkg^{-1}$ chlorpyriphos residue as a single product which were above MRL and other four samples contained 0.035 to 0.090 mg/kg^{-1} residue in okra and snake gourd in Gazipur, Tangail, Dhaka and Mymensingh which were below MRL. One sample of tomato in Jessore and two samples of each pointed gourd and tomato in Norsingdi showed chlorpyriphos residues (0.138- 0.443 mg/kg^{-1}) which were above MRL (Table 2).

Twelve samples showed quinalphos residue as a single product in brinjal, hyacinth bean, cabbage, bitter gourd, snake gourd, okra and yard long bean in Norshindi, Comilla and Jessore region. All the detected residue levels of quinalphos were above MRL. Fenitrothion, fenvalerate, diazinon, and malathion residue was not detected at any of the analyzed samples (Table 2). In comparison to locations the vegetables of Norsingdi, Jessore and Comilla received more insecticide residue then other locations. Some workers of India have found contamination with organophosphorus and pyrethroid pesticides in farm gate vegetables (Deka *et al.,* 2005; Battu Joia 2006). Chen *et al.* (2011) have revealed that the concentration of acephate and fenitrothion were in the range of not detected (nd) to 4.082 $mgkg^{-1}$ and nd to 0.651 $mgkg^{-1}$, respectively in different vegetables from Xiamen city, China. El-Saeid and Selim (2013) detected residues of organophosphorus, organochlorine, pyrethroid and carbamate from market vegetables (viz., beans, egg plant, cauliflower, tomato, pepper, carrot, cucumber, squash, potato, onions and okra) which showed above the MRL in 15.89% of the total tested samples. Rahman *et al*. (2014) found 26% vegetables (viz., brinjal, hyachinth bean, cabbage, cauliflower and red amaranth) samples from five major vegetables growing regions (i.e., Bogra, Jessore, Norsingdi, Jamalpur and Rajshahi of Bangladesh) were contaminated with dimethoate, chlorpyriphos and quinalphos residues, of which 24% of the contaminated samples contained residue above MRL. The results of the present study were similar to the works of the above authors with little exception. However, contamination of single and multiple insecticide residues in vegetables over MRL is very harmful for human consumption. An effort should immediately be taken for quantification and documentation of insecticide residues in vegetables that helps decision maker, researchers and consumers to undertake activities for food safety.

4. Conclusions

The results of the present study clearly indicated that vegetables those consumed daily indeed are contaminated by a number of insecticides. However, insecticide residue in vegetables over MRL can cause serious health hazard both wild life and human. So, continuous monitoring of pesticide residue would be needed in a large scale. Pesticide dealers/retailers and vegetables growers should be given training on the safe use and handling of pesticides in order to protect crops from pest infestation and reduce health hazards of end users.

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