Asian Australas. J. Biosci. Biotechnol. 2023, 8 (1), 8-16; https://doi.org/10.3329/aajbb.v8i1.65830

Asian-Australasian Journal of Bioscience and Biotechnology
ISSN 2414-1283 (Print) 2414-6293 (Online)
https://www.ebupress.com/journal/aajbb/

Article

Monitoring of pesticide residues in yard long bean collected from Mymensingh district of Bangladesh

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Received: 24 February 2023/Accepted: 27 April 2023/Published: 30 April 2023

Abstract: The farmers of our country mostly depend on chemical pesticides for crop protection due to the scarcity of competent pesticide alternatives and enough knowledge of safe pest management. As a result, there is a great possibility to remain residues in the harvested crops. Keeping this view, this study was conducted to analyze organophosphorus pesticide residues in yard long bean collected from five local markets of Mymensingh district of Bangladesh. The collected samples were carried to the Pesticide Analytical Laboratory, Entomology Division of Bangladesh Agricultural Research Institute (BARI), Gazipur on the same sampling day. The collected samples were analyzed using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD). Among the 30 samples of yard long bean, 3 samples (10% of the total samples) contained residue of quinalphos, of which 2 samples contained quinalphos residue above MRL. This study reflects the overall scenario of pesticide residue remain in the harvested yard long bean collected from local markets of Mymensingh city. Therefore, monitoring of pesticide residues in vegetables should be strengthened.

Keywords: yard long bean; organophosphorus pesticide residues; QuEChERS extraction; gas chromatography

1. Introduction

Vegetables and fruits are very important for nutritional, financial, and food security in any country. These are strongly associated with improvement in digestive health, good vision, and decreased risk of heart disease, stroke, chronic diseases like diabetes, and certain types of cancer. These constitute a major part of human diet in various parts of the world and play a major role in human nutrition, particularly as sources of vitamins (A, B1, B6, B9, C, E), minerals, dietary fiber and phytochemicals (Slavin and Lloyd, 2012). On the other hand, fruits and vegetables can also be potential sources of noxious toxic substances like pesticides if the pesticides are not applied following Good Agricultural Practices (GAP). Generally, pesticides are applied in agriculture to restrain pest invasion and proliferation, thereby improving agricultural yields. Such pesticides include a large number of chemical compounds that cover a wide range of different chemical and physical properties. However, these substances exhibit toxic properties, environmental persistence and bioaccumulation capability that are potentially harmful to the environment and human health (Musarurwa *et al*., 2019).

With the increasing use and reliance on the application of pesticides, growing public attention has been focused on the safety of agricultural products, because improper use of pesticides may cause enormous environmental and economic burdens as well as human health problems (Grovermann *et al*., 2013). Among the various edible agricultural products, fruits and vegetables are more likely to be contaminated by pesticide residues because they are applied with several times more pesticides than other crops owing to their high profit (Fan *et al*., 2015). Therefore, fruits and vegetables are usually included in the national pesticide residue monitoring programs in many countries (Bempah *et al*., 2016). Especially, insecticides, fungicides, and acaricides such as organophosphates, synthetic pyrethroids and neonicotenoids are more preponderantly presented in fruits and vegetables (Philippe *et al*., 2021).

The farmers of our country mostly depend on chemical pesticides for crop protection due to the scarcity of competent pesticide alternatives and enough knowledge of safe pest management. It is reported that due to incompetent labeling and deficient knowledge of farmers, chemical pesticides are extensively misused in Bangladesh. While the use of pesticides is of great benefits, their residues in vegetables may pose a detrimental effect on human, ecology and the environment (Islam *et al*., 2021). Excessive and haphazard use of chemical pesticides in the crop fields, enhance the production costs and increase certain environmental and social concern. This also causes the destruction of biological ecosystem and development of resistance in insect pests, pathogens and weeds. Besides, it is necessary to ensure safe food for the consumers. To ensure safe food for the consumers, it is mandatory to use pesticides following Good Agricultural Practice (GAP). Monitoring of pesticide residues is the essential tool to ensure GAP. In Bangladesh, pesticides are not used by the farmers following GAP, the farmers are using pesticides indiscriminately and the farmers are harvested the treated crops without taking into account of the pre-harvest interval (PHI). As a result, the residues of pesticides may remain in the harvested fruits and vegetables.

Now a days, the quick, easy, cheap, effective, rugged and safe (QuECHERS) extraction and cleanup method is widely used for extraction and clean up of pesticide residues in a wide variety of matrices (Prodhan *et al.,* 2018; 2016; 2016a; 2015; 2015a). This method is gaining popularity day by day compared to the other existing methods like liquid liquid extraction, super critical fluid extraction etc. (Prodhan *et al*., 2017). Gas Chromatography coupled with flame thermionic detector is widely used for the quantification of pesticide residues in vegetables, fruits (Alam *et al*., 2022; Rahman *et al.,* 2021; Prodhan *et al.,* 2021; Ahmed *et al.,* 2021; 2021a; 2019), fish and dry fish (Hoque *et al.,* 2021; Prodhan *et al.,* 2018a; 2010; 2009), sugercane (Kabir *et al.,* 2007), betel leaf and water (Prodhan *et al.,* 2021a; 2021b), as they are very much sensitive for the quantification of organophosphorus pesticides. Up until now, several research works have been conducted for the quantification of pesticide residues in vegetables in Bangladesh (Tasnim *et al*., 2022; 2022a; Parvin *et al.,* 2021; Hasan *et al.,* 2021; Habib *et al.,* 2021; Nahar *et al.,* 2020; Islam *et al.,* 2021; 2019; 2019a; Prodhan *et al.,* 2022; 2022a; 2018b; 2018c; Hossain *et al.,* 2014; Islam *et al.,* 2014; Kabir *et al.,* 2008; 2008a). However, more research work is needed to find out the actual scenario of pesticide residues remain in vegetables in a specific area of Bangladesh. Based on the above fact, this study was initiated to quantify 7 selected organophosphorus pesticide residues in yard long bean collected from different local markets of Mymensingh city (Shankipara Bazar, Notun Bazar, Sodesi Bazar, Mesua Bazar, Mintu College Bazar) of Bangladesh.

2. Materials and Methods

2.1. Ethical approval

This study did not require ethical approval.

2.2. Chemicals and reagents

The acephate, chlorpyrifos, diazinon, dimethoate, fenitrothion, malathion and quinalphos standard were obtained from Sigma-Aldrich Laborchemikalien (St Louis, MO, USA) via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. The necessary solvents like methanol, acetone, acetonitrile, sodium chloride, anhydrous magnesium sulphate and Primary Secondary Amine (PSA) were obtained from Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh.

2.3. Sample collection and sample preparation

A total of 30 yard long bean samples were collected for this study. The samples were collected from 5 local markets (Shankipara Bazar, Notun Bazar, Sodesi Bazar, Mesua Bazar and Mintu College Bazar) of

Mymensingh city. The sources of samples along with the sample code are presented in Table 1. Six samples were collected from each market. The amount of each sample was 1 Kg. Sample was collected in individual polyethylene bag to prevent the contamination. The collected samples were taken to the Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI) on the same sampling day. The whole unit of each sample cut into small pieces and mixed properly. Clean air tight polythene bags were used to store chopped sample in a refrigerator at -20°C until extraction and cleanup process.

2.4. Extraction and clean up

The QuEChERS extraction method 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 properly homogenized sample was weighted in a 50 mL polypropylene centrifuge tube. Then, 10 mL of acetonitrile (ACN) was added into the centrifuge tube. The centrifuge tube was closed properly and shaken vigorously for 30 s by vortex mixer. Then, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added into the centrifuge tube, and it was shaken by the vortex mixer for 1 minute. Afterwards, the extract was centrifuged for 5 min at 5000 rpm. An aliquot of 3 mL of the upper ACN layer was transferred into a 15 mL micro centrifuge tube containing 600 mg anhydrous $MgSO_4$ and 120 mg Primary Secondary Amine (PSA). Then, it was thoroughly mixed by vortex for 30 s and centrifuged for 5 minutes 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.

2.5. Instrumental analysis

A Gas Chromatograph (GC-2010 Shimadzu) coupled to Flame Thermionic Detector (FTD) was used for the quantification of selected organophosphorus pesticides (acephate, chlorpyrifos, diazinon, malathion, quinalphos, fenitrothion and dimethoate). The separation was done by Rtx-OPPesticide2 capillary column (30 m long, 0.32 mm i.d and 0.32 µm film thicknesses), helium was used as carrier and make up gas as well. The column flow rate was 1.5 mL/min. The temperature for the injector and the detector was set to 250 °C and 280 °C, respectively, and for the column oven, the temperature was programmed, which was started from 150 °C (1 min hold) and went up to 220 °C with an incremental rate of 10 °C (2 min hold). Spit mode was used for the injection of samples (1 µL) and the split ratio was 10.0. The total run time was 10 min. Identification was performed by comparing the retention time of the matrix matched calibration standard and the quantification was done using the calibration curve prepared with matrix matched calibration standard. A typical chromatogram of all the selected pesticides are presented in Figure 1.

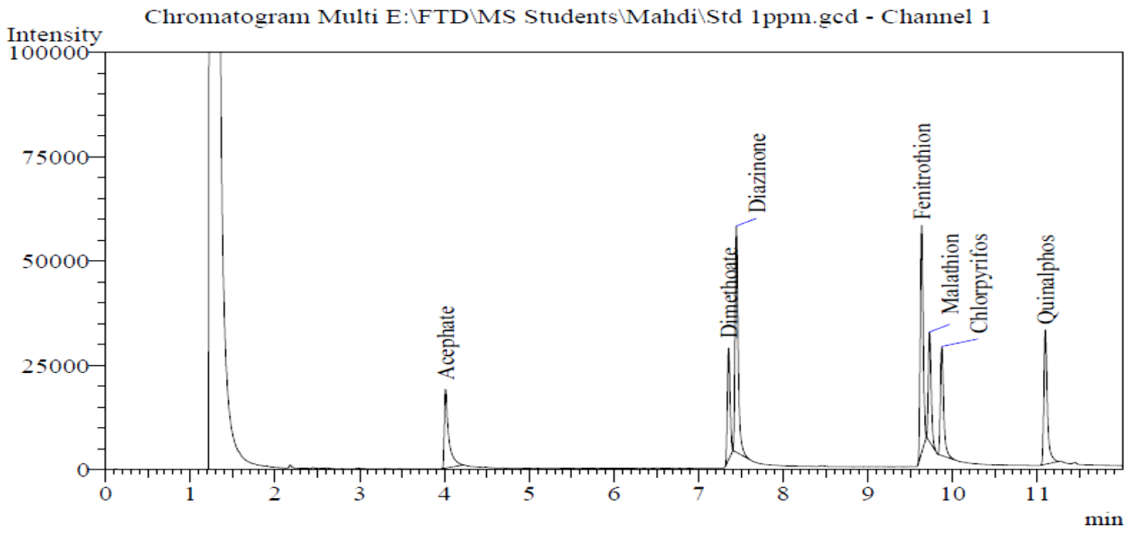

Figure 1. A typical Chromatogram of the selected organophosphorus pesticide standards.

Table 1. Sources and places of collection of yard long bean samples.

Area of collection	Sample ID	Source
Shankipara Bazar	YLB-Sb_1	Char nilakshmia
	YLB-Sb_2	Gafargaon
	YLB-Sb_3	Jamalpur
	YLB-Sb_4	Bobar char
	YLB–Sb_5	Paranganj
	YLB-Sb_6	Kalibari char
Notun Bazar	YLB-Nb_1	Bobar char
	YLB-Nb_2	Paranganj
	YLB-Nb_3	Kalibari char
	YLB-Nb_4	Jamalpur
	YLB-Nb_5	Bobar char
	YLB-Nb_6	Paranganj
Sodesi Bazar	YLB-Sdb_1	Char Gavindapur
	YLB-Sdb_2	Kalibari char
	YLB-Sdb_3	Char gobadia
	YLB-Sdb_4	Bobar char
	YLB-Sdb_5	Paranganj
	YLB-Sdb_6	Kalibari char
Mesua Bazar	YLB-Mb_1	Gafargaon
	YLB-Mb_2	Bobar char
	YLB-Mb_3	Nurondi
	YLB-Mb_4	Bobar char
	YLB-Mb_5	Paranganj
	YLB-Mb_6	Kalibari char
Mintu College Bazar	YLB-MCb_1	Bobar char
	YLB-MCb_2	Banganbari char
	YLB-MCb_3	Paranganj
	YLB-MCb_4	Jamalpur
	YLB-MCb_5	Bobar char
	YLB-MCb_6	Paranganj

3. Results and Discussion

The concentrated extracts of yard long bean samples collected from different markets of Mymensingh city were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. Figure 2-4 shows the chromatograms of the injected extracts of yard long bean sample containing detected pesticides. The level of pesticide residues found in the analyzed samples and their maximum residue levels are presented in Table 2.

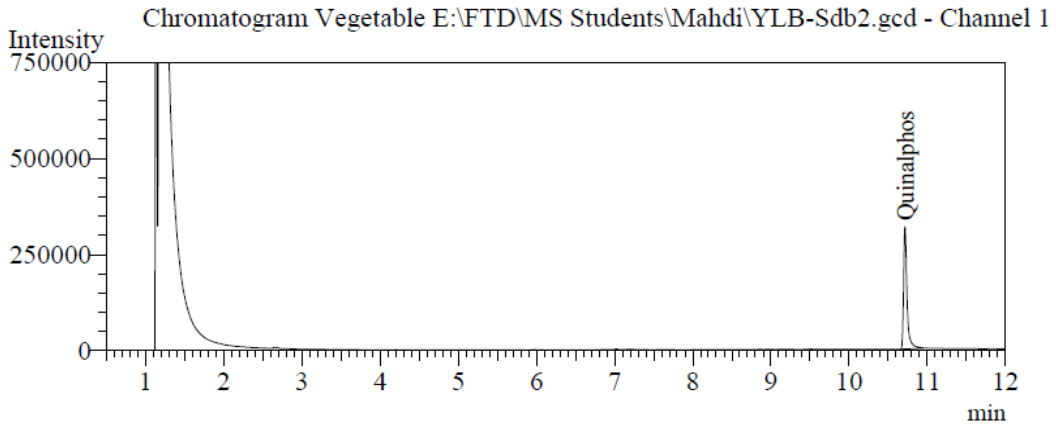

Figure 2. Chromatogram of quinalphos found in one of the yard long bean sample (YLB-Sdb$_2$).

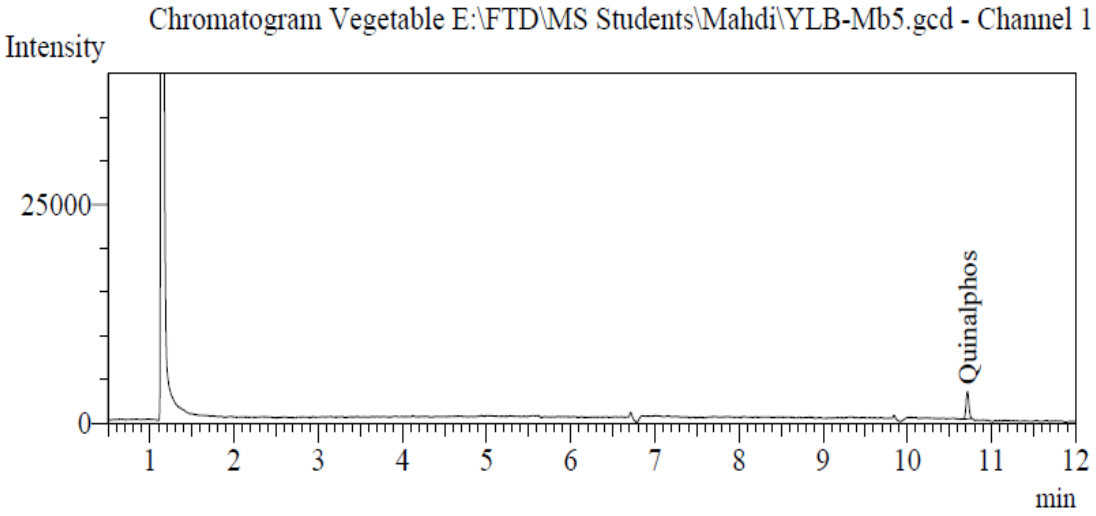

Figure 3. Chromatogram of quinalphos found in one of the yard long bean sample (YLB-Mb$_5$).

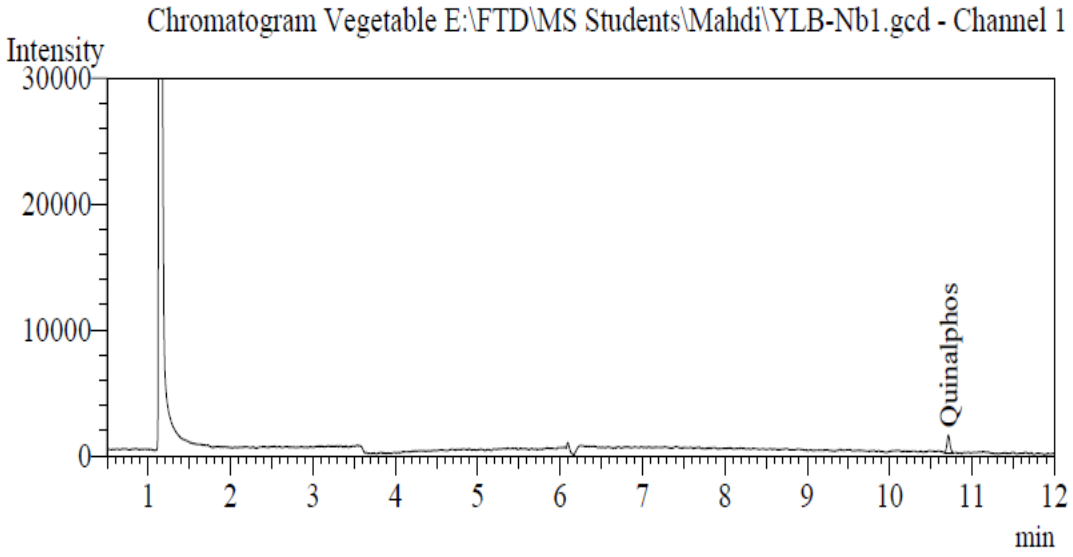

Figure 4. Chromatogram of quinalphos found in one of the yard long bean sample (YLB-Nb$_1$).

Table 2. The level of residues (mg/kg) of different pesticides found in the analyzed yard long bean samples.

Area of collection	Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)
Shankipara Bazar	YLB-Sb_1	ND	-	
	YLB-Sb_2	Quinalphos	0.562	0.01
	YLB-Sb_3	ND	-	
	YLB-Sb_4	ND	-	
	YLB-Sb_5	ND	-	
	YLB-Sb_6	ND	-	
Notun Bazar	YLB-Nb_1	Quinalphos	0.008	0.01
	YLB-Nb_2	ND	-	
	YLB-Nb_3	ND	-	
	YLB-Nb_4	ND	-	
	YLB-Nb_5	ND	-	
	YLB-Nb_6	ND	-	
Sodesi Bazar	YLB-Sdb_1	ND	-	
	YLB-Sdb_2	ND	-	
	YLB-Sdb_3	ND	-	
	YLB-Sdb_4	ND	-	
	YLB-Sdb_5	ND	-	
	YLB-Sdb_6	ND	-	
Mesua Bazar	YLB-Mb_1	ND	-	
	YLB-Mb_2	ND	-	
	YLB-Mb_3	ND	-	
	YLB-Mb_4	ND	-	
	YLB-Mb_5	Quinalphos	0.024	0.01
	YLB-Mb_6	ND	-	
Mintu College Bazar	YLB-MCb_1	ND	-	
	YLB-MCb_2	ND	-	
	YLB-MCb_3	ND	-	
	YLB-MCb_4	ND	-	
	YLB-MCb_5	ND	-	
	YLB-MCb_6	ND	-	

Thirty samples of yard long bean were collected from 5 different markets of Mymensingh Sadar (Shankipara Bazar, Notun Bazar, Sodesi Bazar, Mesua Bazar, Mintu College Bazar) and were analyzed to find out the presence of left over residues of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos). Out of Thirty samples, 3 samples (10% of the total no. of samples) contained pesticide residues and 27 samples (90% of the total number of samples) contained no detectable residues of the sought pesticides. The present results are supported by the findings of Parven *et al.* (2021). They have collected

70 samples of hyacinth bean and yard long bean from Bogura district of Bandgladesh, where they found 14% yard long bean samples were contaminated by pesticide residues. The results of this study also supported by the findings of Islam *et al*. (2019b), they have seen that among the 65 analyzed eggplant samples, 8 (12.3% of the total number of samples) had pesticide residues.

Among the six sample of yard long bean, one sample (YLB-Sb_2) from Sankipara Bazar contained pesticide residues of quinalphos (0.562 mg/kg) which was above MRL (0.01mg/kg). The other 5 samples contain no detectable pesticide residues.

In case of Notun Bazar, one sample (YLB-Nb_1) contained residues of quinalphos (0.008 mg/kg) which was below MRL (0.01mg/kg). The other 5 samples contain no detectable pesticide residues. The samples collected from Mintu College Bazar and Sodeshi Bazar contained no residues of the sought pesticides. However, one sample (YLB-Mb_5) collected from Mesua Bazar contained residues of quinalphos (0.024mg/kg) which was above MRL (0.01mg/kg).

4. Conclusions

In this study, 30 samples of yard long bean collected from different local markets (Shankipara Bazar, Notun Bazar, Sodesi Bazar, Mesua Bazar, Mintu College Bazar) of Mymensingh district of Bangladesh were analyzed for the quantification of seven organophosphorus pesticide residues. Among the 30 samples of yard long bean, 3 samples (10% of the total samples) contained residue of quinalphos, of which 2 samples contained quinalphos residue above MRL. The contaminated samples were collected from Shankipara Bazar, Notun Bazar and Mesua Bazar. This study will help the policy planners and the relevant stakeholders to take necessary action for the reduction of pesticide residue load in vegetables.

Acknowledgements

The authors are grateful to Md. Kamal Hossain in the Pesticide Analytical Laboratory, Pesticide Research & Environmental Toxicology Section, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh for his kind cooperation during the present study.

Data availability

All relevant data are within the manuscript.

Conflict of interest

None to declare.

Authors' contribution

Conceptualization: [Mohammad Dalower Hossain Prodhan]; Methodology: [Mohammad Dalower Hossain Prodhan], [Md. Mahadi Alam], [S M Mizanur Rahman]; Formal analysis and investigation: [Md. Mahadi Alam], [Mohammad Dalower Hossain Prodhan]; Writing - original draft preparation: [Md. Mahadi Alam], [Deeder Sultana], [Mohammad Dalower Hossain Prodhan]; Writing - review and editing: [Mohammad Dalower Hossain Prodhan], [S M Mizanur Rahman], [Deeder Sultana]; Supervision: [Mohammad Dalower Hossain Prodhan], [S M Mizanur Rahman]. All the authors have read and approved the final manuscript.

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