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Article

Analysis of pesticide residues in vegetables purchased from local markets of Mymensingh district of Bangladesh based on QuEChERS Extraction and Gas Chromatography

Md. Mahadi Alam[1*], Rakibul Hasan[1], S M Mizanur Rahman[1], Md. Abdur Razzak Choudhury[2] and Mohammad Dalower Hossain Prodhan[3]

[1]Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh
[2]Department of Entomology, Sylhet Agricultural University, Sylhet 3100, Bangladesh
[3]Pesticide Analytical Laboratory, Pesticide Research & Environmental Toxicology Section, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh

[*]Corresponding author: Md. Mahadi Alam, Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. Phone: +8801917853203; E-mail: mahadialam1104437@gmail.com

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Abstract: The study was conducted to analyze organophosphorus pesticide residues in two common vegetables (cauliflower and eggplant) 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). In total, 60 vegetable samples were analyzed in this study. Out of 30 analyzed samples of cauliflower, 5 samples (17% of the total number of samples) contained residues of dimethoate, chlorpyrifos and quinalphos, where 3 samples contained residues above the maximum residue limits (MRLs). Among the 30 analyzed samples of eggplant, 5 samples (17% of the total number of samples) contained residues of chlorpyrifos, quinalphos, and dimethoate, where only one sample contained residues above MRL. This study reflects the actual scenario of pesticide residues remain in cauliflower and eggplant collected from local markets of Mymensingh district, which will help the consumer to be aware of their health and safety. Thus, continuous monitoring of pesticide residues in vegetables should be strengthened.

Keywords: vegetables; organophosphorus pesticide residues; QuEChERS extraction; gas chromatography

1. Introduction

As an overpopulated country food shortage and malnutrition has become a general problem in Bangladesh. Not only in Bangladesh but also around the world the food demand is changing rapidly because of population growth, economic growth, rising income and rapid urbanization. Demand is changing away from traditional commodities towards high value food commodities like vegetables, fruits, spices, fish etc. In this regard, vegetable growing has become an important farming activity from the point of view of dietary fulfillment as well as economics returns (Aktar *et al.*, 2017).

The consumption of vegetables is increasing day by day. Vegetables are important components of the human diet since they provide essential nutrients that are required for most of the reactions occurring in the body. The eggplant and cauliflower are the two important vegetables grown commercially in Bangladesh. Eggplant is one of the most common and popular vegetable throughout the entire tropical and subtropical regions of the world. It is grown extensively in China, India, Bangladesh, Pakistan, Philippines, Japan, Indonesia, Turkey, Greece, Italy, France, USA, and Mediterranean and Balkan countries. The benefits of eggplant are well known. It helps to prevent colon cancer, reduces cholesterol level, helps in the type 2 diabetes management, is very rich in antioxidant, and also helps to control weight (Dome, 2013). Cauliflower is a member of the cruciferous family of vegetables. It contains vitamins, fiber, and minerals and helps to prevent several diseases (Ambroson *et al*., 2009).

It is undoubtedly true that a negative economic impact on the production of vegetables is occurred by the insect pests and diseases. Due to plant pests and diseases 20 to 40 percent crop yields are reduced globally, besides the world will need to produce 60 percent more food for the over increasing world population by 2050 (FAO, 2012). To ensure this demand control of insect pests and diseases plays a key role. Till to date for the control of insect pests and diseases pesticides plays a vital role. There is no doubt that due to the use of pesticides, production is increased day by day. However, pesticides create several adverse effects on human health and the environment. Extensive use of pesticides has resulted in contamination of 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. Therefore, it is an urgent need to monitor pesticide residues in food.

To monitor pesticide residues, an effective extraction and clean-up method is essential. Without the effective extraction method it is not possible to analyze pesticide residues accurately. 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.

Gas Chromatography coupled with flame thermionic detector is widely used for the quantification of pesticide residues in vegetables, fruits (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 (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.,* 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. Considering this view, this study was aimed to quantify seven selected organophosphorus pesticide residues in cauliflower and eggplant 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. Chemicals and reagents

The certified reference material (CRM) of acephate, chlorpyrifos, diazinon, dimethoate, fenitrothion, malathion and quinalphos were obtained from Sigma-Aldrich Laborchemikalien (St Louis, MO, USA) via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. Standards of all the 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.

2.2. Preparation of pesticide standard solution

Pesticide standard stock solutions of acephate, chlorpyrifos, diazinon, dimethoate, fenitrothion, malathion and quinalphos were prepared separately in acetonitrile at a concentration of 1000 mg/L and stored at -20°C until use. A mixed standard solution of 50 mg/L in acetonitrile containing all the aforementioned pesticides was prepared by adding the appropriate volume of each individual stock solution in a 50 ml volumetric flask and

made to volume by addition of acetonitrile. An intermediate mixed standard solution of 10 mg/L in acetonitrile 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 acetonitrile were prepared by transferring the appropriate amount from 10 mg/L intermediate mixed standard solution into ten separate 10-mL volumetric flasks. All the standard solutions were kept in a freezer at -20°C until use.

2.3. Sample collection and sample preparation

In total 60 samples (30 cauliflower and 30 eggplant) 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 in Bangladesh. Six samples of eggplant, and six samples of cauliflower were collected from each market. The amount of each sample was 1 Kg for all the selected vegetables. The samples were collected in clean transparent air tight polyethylene bag and each bag was properly labeled with sample number and sources. Sample was collected in individual polyethylene bag to avoid cross contamination. The collected samples were taken to the Pesticide Analytical Laboratory, Pesticide Research & Environmental Toxicology Section, 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 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 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.

3. Results and Discussion

3.1. Pesticide residues in cauliflower

The concentrated extracts of cauliflower samples collected from different markets of Mymensingh city were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. The level of pesticide residues found in the analyzed cauliflower samples and their maximum residue levels are presented in Table 1.

Table 1. The level of residues (mg/kg) of different pesticides found in the analyzed cauliflower samples.

Area of collection	Sample ID	Detected pesticide	Level of residues (mg/kg)	MRLs (mg/kg)
Shankipara Bazar	CF-Sb_1	ND	-	
	CF-Sb_2	ND	-	
	CF-Sb_3	ND	-	
	CF-Sb_4	ND	-	
	CF-Sb_5	Dimethoate	0.721	0.02
	CF-Sb_6	ND	-	
Notun Bazar	CF-Nb_1	ND	-	
	CF-Nb_2	ND	-	
	CF-Nb_3	Dimethoate Chlorpyrifos	0.092 0.045	0.02 0.05
	CF-Nb_4	ND	-	
	CF-Nb_5	ND	-	
	CF-Nb_6	ND	-	
Sodesi Bazar	CF-Sdb_1	ND	-	
	CF-Sdb_2	ND	-	
	CF-Sdb_3	ND	-	
	CF-Sdb_4	ND	-	
	CF-Sdb_5	ND	-	
	CF-Sdb_6	Chlorpyrifos	0.036	0.05
Mesua Bazar	CF-Mb_1	ND	-	
	CF-Mb_2	Quinalphos	0.025	0.01
	CF-Mb_3	ND	-	
	CF-Mb_4	ND	-	
	CF-Mb_5	ND	-	
	CF-Mb_6	ND	-	
Mintu College Bazar	CF-MCb_1	Quinalphos	0.009	0.01
	CF-MCb_2	ND	-	
	CF-MCb_3	ND	-	
	CF-MCb_4	ND	-	
	CF-MCb_5	ND	-	
	CF-MCb_6	ND	-	

Thirty samples of cauliflower 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 residue of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos). Out of 30 samples of cauliflower, 5 samples (17% of the total number of samples) contained pesticide residues and 25 samples (63% of the total number of samples) contained no detectable residues of the sought pesticides. The present results can be compared to Islam *et al.* (2014). They have collected 42 samples of brinjal, cauliflower and country bean from fields and markets of Narsingdi district, Bandgladesh, where they found 15 samples (above 68% of total samples) contained no residues of the sought pesticides. The findings of the present study are in a good agreement with the findings of Akter *et al.* (2017), they found that among the 50 analyzed samples, 11 (22% of the total number of the samples) contained residues of diazinon, dimethoate, quinalfos, and chlorpyrifos in eggplant collected from Mymensingh district of Bangladesh, of which, 2 had multiple pesticide residues and 5 contained residues above the EU-MRLs. The results of this study also supported by the findings of Hasan *et al.* (2017), they found that among the 50 analyzed samples, 10 samples (20%) contained residues of dimethoate and quinalphos in country bean collected from different markets of Dhaka district of Bangladesh.

In case of Sankipara Bazar, six cauliflower samples were collected, among them one samples (CF-Sb_5) contained dimethoate at a level of 0.721 mg/kg, which was above the EU-MRL (European Commission, 2015). The other 5 samples contain no detectable pesticide residues. From Notun Bazar, six samples were collected. Of which one sample (CF-Nb_3) contained multiple residues of dimethoate (0.092 mg/kg) and chlorpyrifos (0.045 mg/kg). But other five samples contained no detectable pesticide residues. The level of detected residue of dimethoate was above MRL (0.02 mg/kg) and the residue of chlorpyrifos was below MRL (0.05 mg/kg). In case of Sodeshi Bazar, among the six collected samples one sample (CF-Sdb_6) of cauliflower contained residue of

chlorpyrifos (0.036 mg/kg), which was below MRL (0.05 mg/kg). The other 5 samples contain no detectable pesticide residues.

One sample (CF-Mb_2) of cauliflower contained residue of quinalphos (0.025 mg/kg) among the six samples collected from Mesua Bazar, which was above MRL (0.01 mg/kg). The other 5 samples contain no detectable pesticide residues. While, out of six samples collected from Mintu College Bazar, one sample (CF-Mcb_1) contained residue of quinalphos (0.009 mg/kg) which was below the MRL (0.01 mg/kg).

3.2. Pesticide residues in eggplant

The concentrated extracts of eggplant samples collected from different markets of Mymensingh Sadar were analyzed by GC-2010 (Shimadzu) with Flame Thermionized Detector (FTD) with the pre-set parameters. The level of pesticide residues found in the analyzed eggplant samples and their maximum residue levels are presented in Table 2.

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

Area of collection	Sample ID	Detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)
Shankipara Bazar	EP-Sb_1	ND	-	
	EP-Sb_2	ND	-	
	EP-Sb_3	ND	-	
	EP-Sb_4	ND	-	
	EP-Sb_5	ND	-	
	EP-Sb_6	Chlorpyrifos	0.173	0.40
Notun Bazar	EP-Nb_1	ND	-	
	EP-Nb_2	ND	-	
	EP-Nb_3	ND	-	
	EP-Nb_4	Dimethoate	0.013	0.02
	EP-Nb_5	Dimethoate	0.018	0.02
	EP-Nb_6	ND	-	
Sodesi Bazar	EP-Sdb_1	ND	-	
	EP-Sdb_2	Quinalphos	0.042	0.01
	EP-Sdb_3	ND	-	
	EP-Sdb_4	ND	-	
	EP-Sdb_5	ND	-	
	EP-Sdb_6	ND	-	
Mesua Bazar	EP-Mb_1	Dimethoate	0.017	0.02
	EP-Mb_2	ND	-	
	EP-Mb_3	ND	-	
	EP-Mb_4	ND	-	
	EP-Mb_5	ND	-	
	EP-Mb_6	ND	-	
Mintu College Bazar	EP-MCb_1	ND	-	
	EP-MCb_2	ND	-	
	EP-MCb_3	ND	-	
	EP-MCb_4	Chlorpyrifos	0.108	0.40
	EP-MCb_5	ND	-	
	EP-MCb_6	ND	-	

Thirty samples of eggplant collected from 5 different markets of Mymensingh sadar (Shankipara Bazar, Notun Bazar, Sodesi Bazar, Mesua Bazar, Mintu College Bazar) were analyzed to find out the presence of left over residues of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos). Out of 30 samples, 5 samples (17% of the total no. of samples) contained pesticide residues and 25 samples (83% of the total number of samples) contained no detectable residues of the sought pesticides. The present results can be compared to Parven *et al.* (2021). They have collected 70 samples of country bean and yard long bean samples 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 found that among the 65 analyzed eggplant samples, 8 (12.3% of the total number of samples) were contaminated with pesticide residues.

In case of Sankipara Bazar, six samples of eggplant were collected and analyzed. Among them, only one sample (EP-Sb_6) contained residue of chlorpyrifos (0.173 mg/kg), which was below MRL (0.4 mg/kg). The other 5 samples contain no detectable pesticide residues. Among the six samples of eggplant collected from Notun Bazar, two samples (EP-Nb_4 and EP-N b_5) contained residue of dimethoate (0.013 mg/kg) and (0.018 mg/kg), respectively, which were below the MRL (0.02 mg/kg). The other 4 samples contain no detectable pesticide residues. In case of Sodeshi Bazar, six samples of eggplant were collected and analyzed. Of them, one sample (EP-Sdb_2) contained residues of quinalphos (0.042 mg/kg), which was above MRL (0.01 mg/kg). The other 5 samples contain no detectable pesticide residues. On the other hand, among the six sample of eggplant collected from Mesua Bazar, one sample contained residue of dimethoate (0.017 mg/kg), which was below MRL (0.02 mg/kg). The other 5 samples contain no detectable pesticide residues. In case of Mintu College Bazar, out of six samples, only one sample (EP-Mcb_4) contained residue of chlorpyrifos and the other five samples contained no detectable pesticide residues of the sought pesticides. The level of detected chlorpyrifos residue was 0.108 mg/kg, which was below MRL (0.4 mg/kg).

4. Conclusions

In total 60 vegetable samples containing 30 samples of cauliflower and 30 samples of eggplant 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 ensuring food safety. Among the 30 analyzed samples of cauliflower, 5 samples (17% of the total number of samples) were contaminated with chlorpyrifos, quinalphos and dimethoate, and 3 samples contained residues above the maximum residue limits (MRLs). On the other hand, out of 30 analyzed samples of eggplant, 5 samples (17% of the total number of samples) contained residues of chlorpyrifos, quinalphos, and dimethoate, where only 1 sample had residues above MRL. This study will help the policy planners and the relevant stakeholders to take necessary action for the reduction of pesticide residue load in vegetables.

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Data availability

The data used to support the findings of this study are included within the article.

Conflict of interest

None to declare.

Authors' contribution

Conceptualization: [Mohammad Dalower Hossain Prodhan]; Methodology: [Mohammad Dalower Hossain Prodhan], [Rakibul Hasan], [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], [Mohammad Dalower Hossain Prodhan], [Rakibul Hasan], [Md. Abdur Razzak Choudhury]; Writing - review and editing: [Mohammad Dalower Hossain Prodhan], [S M Mizanur Rahman], [Md. Abdur Razzak Choudhury]; Supervision: [Mohammad Dalower Hossain Prodhan], [S M Mizanur Rahman]. All authors have read and approved the final manuscript.

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