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Article

Analysis of organophosphorus pesticide residues in selected vegetables purchased from Narsingdi district of Bangladesh using QuEChERS Extraction

Nuzat Tasnim[1*], Md. Nasrul Millat[2], Sadia Sultana[3], S M Mizanur Rahman[1] and Mohammad Dalower Hossain Prodhan[4]

[1]Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh
[2]Upazila Agriculture Officer, Department of Agricultural Extension, Fakirhat, Bagerhat, Bangladesh
[3]Upazila Agriculture Officer, Department of Agricultural Extension, Bagerhat Sadar, Bagerhat, Bangladesh
[4]Pesticide Analytical Laboratory, Pesticide Research & Environmental Toxicology Section, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh

[*]Corresponding author: Nuzat Tasnim, Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. Phone: +8801756040490; E-mail: nuzat@mblbd.com

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Abstract: Pesticide residues in different crops, including food grains and vegetables are burning issues in Bangladesh. Therefore, the present study was conducted to analyze organophosphorus pesticide residues in three popular vegetables (cauliflower, hyacinth bean and yard long bean) collected from five local markets of Narsingdi district of Bangladesh. The collected vegetable samples were extracted and cleaned up using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and analyzed by Gas Chromatography (GC) equipped with Flame Thermionic Detector (FTD). A total of 45 vegetable samples were analyzed. Among the 15 analyzed samples of hyacinth bean, three samples (20 % of the total samples) contained pesticide residues above the EU-MRL and the remaining samples did not contain any detectable residues of the selected organophosphorus pesticides. In case of cauliflower, out of 15 analyzed samples, two samples (13% of the total number of samples) contained pesticide residues above the EU-MRL. In case of yard long bean, of the 15 analyzed samples, four samples (27% of the total number of samples) contained pesticide residues above the EU-MRLs. This study reveals the present status of pesticide residue contamination in the selected vegetables.

Keywords: vegetables; QuEChERS extraction; organophosphorus pesticide residues; flame thermionic detector; gas chromatography

1. Introduction

Vegetables are considered essential for well-balanced diets since they supply vitamins, minerals, dietary fiber, and phytochemicals. They make up a major portion of the diet of humans in many parts of the world and play a significant role in human nutrition, especially as sources of phytonutrients: vitamins (A, B1, B6, B9, C, E), minerals, dietary fiber and phytochemicals (Dias *et al*., 2011). Not only vegetables are valuable in maintaining alkaline reserve of the body but also improve gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some forms of cancer. Having so much importance a world vegetable survey showed that 402 vegetable crops are cultivated worldwide, representing 69 families and 230 genera (Kays *et al*., 2011).

The yard-long bean has an adequate quantity of lysine and is rich in calcium, vitamins A and C, and lysine (Aykroiyd *et al.,* 1974). In Bangladesh, the pod borer *Euchrysops cnejus* F. (Lepidoptera: Lycaenidae) is regarded as one of the worst yard pests (Ali, 2006). Cauliflower has significant amounts of the vitamins B6, C, K, B6, folate, and pantothenic acid. Like other vegetables, cauliflower is vulnerable to insect infestation. Diamond back moth, tobacco caterpillar, aphids, and painted bug are the main pests that attack cauliflower. Hyacinth beans are currently cultivated often throughout the year and are a wonderful source of fiber and vitamins. According to Alam *et al.*,(1969) hyacinth bean is attacked by nine distinct insect species and one type of mite. The remaining species are considered minor pests, and four of these species are considered significant pests. However, many insect pests commonly target the leguminous plants. In 1975, an FAO panel sitting in Bangkok designated the bean pod borer (*Maruca testulalis* G.) as a legume pod borer (Reddy 1975). A significant insect pest of leguminous plants is *M. testulalis* G., according to Bakar et al. (1980).

In Bangladesh, the farmers are still mostly depends on different organophosphorus insecticides like malathion, diazinon, acephate, dimethoate, quinalphos, chlorpyrifos, fenitrothion etc. for controlling these vegetable insect pests. Pre-harvest intervals, or PHIs, are the amount of time between the last pesticide treatment and harvest that must pass for each pesticide. When the fruits and vegetables are picked after the PHI, foods will be safe for ingestion. Farmers do not adhere to the relevant PHI because of a lack of education and awareness. Multiple pesticide residues in fruits and vegetables can be detected using a variety of techniques. But a very little work has been done for these vegetables collected from local markets of Narsingdi district. A reliable and efficient approach is needed to monitor pesticide residues. Based on a flawed methodology, it is impossible to estimate pesticide residues. In the recent years, it have seen that a significant increase in the application of the quick, easy, cheap, effective, rugged, and safe (QuECHERS) extraction approach for the extraction and cleanup of pesticide residues in a wide range of matrices (Prodhan *et al*., 2018; 2016; 2016a; 2015; 2015a).

In order to assess pesticide residues, a variety of analytical methods (LC-MS/MS, GC-MS/MS, GC, and HPLC) have been used. Among these, gas chromatography is mostly used for the analysis of pesticide residues. In Bangladesh, a limited analytical method was developed using LC-MS/MS (Prodhan *et al*., 2022), but the majority of pesticide residue measurements are made using Gas Chromatography in conjunction with a flame thermionic detector in fruits and vegetables (Rahman *et al*., 2021; Prodhan *et al*., 2021; Ahmed *et al*., 2021; 2019), fish and dry fish (Prodhan *et al*., 2018a; 2010; 2009; Hoque *et al*., 2021), sugarcane (Kabir *et al*., 2007), betel leaf and water (Prodhan *et al*., 2021a; 2021b), because they are very much sensitive for the analysis of organophosphorus pesticides. Several studies have been done for the quantification of pesticide residues in vegetables in Bangladesh (Tasnim *et al*., 2022; Hossain *et al*., 2014; Alam *et al*., 2022; Parvin *et al*., 2021; Hasan *et al*., 2021; Islam *et al*., 2014; Kabir *et al*., 2008; 2008a; Habib *et al*., 2021; Nahar *et al*., 2020; Islam *et al*., 2021; 2019; 2019a; Prodhan *et al*., 2018b; 2018c). However, additional study is needed to know the actual situation of pesticide residues remaining in vegetables in a specific region of Bangladesh. In Bangladesh, the vegetable production is increasing day by day. Among different vegetable producing area, in Narsingdi, the farmers are producing a significant amount of different popular vegetables. With this view, the present study was started to measure the levels of seven selected organophosphorus pesticide residues in yard long bean, hyacinth bean, and cauliflower that were collected from various local markets in Narsingdi district, Bangladesh.

2. Materials and Methods

2.1. Chemicals and reagents

The selected pesticides (acephate, chlorpyrifos, diazinon, dimethoate, fenitrothion, malathion, and quinalphos) were bought as pure (>99.0%) analytical standards from Sigma-Aldrich Laborchemikalien (St. Louis, MO, USA) through Asa Scientific Pvt. Ltd. in Dhaka, Bangladesh. Asa Scientific Pvt. Ltd. in Dhaka, Bangladesh provided the methanol, HPLC grade acetonitrile, sodium chloride, anhydrous magnesium sulfate ($MgSO_4$), and primary secondary amine (PSA).

2.2. Preparation of pesticide standard solution

The separately produced 1000 mg/L primary stock solutions of diazinon, dimethoate, chlorpyrifos, acephate, malathion, fenitrothion, and quinalphos were stored at -20°C until use. A mixed standard solution of 50 mg/L in acetonitrile comprising all of the aforementioned pesticides was prepared by adding the required amount of each individual stock solution into a 50 ml volumetric flask and bringing the mixture to volume with acetonitrile. The 50 mg/L mixed standard solution was reduced to a 10 mg/L intermediate mixed standard solution in acetonitrile. Then, working standard solutions of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 mg/L were prepared by dividing the necessary amount from a 10 mg/L intermediate mixed standard solution into ten separate 10-mL volumetric flasks.

2.3. Sample collection and sample preparation

There were 45 samples altogether, 15 of each of the following: yard long bean, cauliflower, and hyacinth bean. The samples were collected from five markets of Narsingdi, namely Shibpur Bazar, C&B Bazar, Belabo Bazar, Marjal Bazar and Velanagar Bazar. Three samples of yard long beans, cauliflower, and hyacinth beans were collected from each market. A 1 kg sample of each of the selected vegetables was bought. To collect the samples, each clear, airtight polyethylene bag was thoroughly labeled with the sample number and the sources. On the same sampling day, the obtained samples were delivered to the Pesticide Analytical Laboratory, Pesticide Research & Environmental Toxicology Section, Entomology Division, Bangladesh Agricultural Research Institute (BARI). Each sample's entire unit was divided into smaller parts and thoroughly mixed. Chopped samples were kept in sterile, airtight polythene bags in a freezer at -20°C until extraction and cleanup.

2.4. Extraction and clean up

In order to sample extraction and cleanup the QuEChERS extraction and clean-up procedure was followed which was modified by Prodhan *et al*. (2015). The sample pieces were thoroughly ground in the fruit blender. A typical 10 g completely homogenized samples that had been weighted into a 50 mL polypropylene centrifuge tube. The centrifuge tube was then filled with 10 mL of acetonitrile (ACN). After being properly sealed, the vortex mixer vigorously shook the centrifuge tube for 30 seconds. After that, 1 g of NaCl and 4 g of anhydrous MgSO4 were added to the centrifuge tube, and the vortex mixer was used to stir the mixture for a minute. The extract was then centrifuged for 5 minutes at 5000 rpm. A 15 mL micro centrifuge tube containing 600 mg of anhydrous $MgSO_4$ and 120 mg of primary secondary amine was filled with an aliquot of 3 mL of the upper ACN layer (PSA). It was fully mixed using a vortex for 30 seconds, and it was then centrifuged at 4000 rpm for 5 minutes. Then, a clean GC vial was used to transfer a 1 mL supernatant that had been centrifuged and filtered through a 0.2 m PTFE filter.

2.5. Instrumental analysis

The acephate, malathion, chlorpyrifos, diazinon, dimethoate, fenitrothion, and quinalphos were among the organophosphorus pesticides chosen for analysis. A gas chromatograph (GC-2010 Shimadzu) linked to a flame thermoelectric detector (FTD) was used. Along with the Rtx-OPPesticide2 capillary column, which had a 30 m length, 0.32 mm i.d., and 0.32 m film thickness, helium was used as a carrier and make-up gas. The flow through the column was 1.5 mL/min. The temperature of the column oven was intended to begin at 150°C for one minute and increase to 220°C at an incremental rate of 10°C (2 min hold). Temperature for the injector and detector were set at 250°C and 280°C, respectively. The split ratio used for the sample injection was 10.0. The total run time was 10 min. The 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 hyacinth bean

The level of pesticide residues found in the analyzed hyacinth bean samples and their maximum residue levels are presented in Table 1.

In order to investigate residual pesticide contamination from seven different pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos), fifteen samples of hyacinth beans were obtained from five different marketplaces in Narsingdi city. Twelve of the 15 samples had no detectable pesticide residues, while three samples (20% of the total samples) had residues. Compared to Parven et al. (2021) the current findings are comparable. They gathered 70 samples of yard long and hyacinth beans from the Bogura district of Bangladesh, where they found that 14% of the yard long bean samples had pesticide residues. The findings of Islam et al. (2019b), who discovered that eight of the 65 examined eggplant samples or 12.3% of the total samples were contaminated with pesticide residues, confirm the findings of this investigation.

One of the 15 samples of country bean taken from the Narsingdi district, one smple (NMBe 06) had dimethoate with a residual level of 0.053 mg/kg, which was higher than the MRL. Quinalphos was present in another sample (NMBe 10) from Marjal Bazar with a residue level of 0.365 mg/kg, which was similarly above MRL. Quinalphos was also present in one sample (NMBe 14) from Velanagar Bazar with a residue level of 0.0454 mg/kg, which was also above MRL.

Table 1. The level of residues (mg/kg) of different pesticides found in the analyzed hyacinth bean samples collected from Narsingdi, Bangladesh.

Area of collection	Sample ID	Detected pesticide	Level of residues (mg/kg)	MRLs (mg/kg)
Shibpur Bazar	NMBe 01	ND		
	NMBe 02	ND		
	NMBe 03	ND		
C&B Bazar	NMBe 04	ND		
	NMBe 05	ND		
	NMBe 06	Dimethoate	0.053	0.02
Belabo Bazar	NMBe 07	ND		
	NMBe08	ND		
	NMBe 09	ND		
Marjal Bazar	NMBe 10	Quinalphos	0.365	0.05
	NMBe 11	ND		
	NMBe 12	ND		
Velanagar Bazar	NMBe 13	ND		
	NMBe 14	Quinalphos	0.454	0.05
	NMBe 15	ND		

3.2. Pesticide residues in cauliflower

The level of pesticide residues found in the analyzed cauliflower 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 cauliflower samples collected from Narsingdi, Bangladesh.

Area of collection	Sample ID	Detected pesticide	Level of residues (mg/kg)	MRLs (mg/kg)
	NMCF 01	ND		
Shibpur Bazar	NMCF 02	ND		
	NMCF 03	ND		
	NMCF 04	Dimethoate	1.266	0.02
C&B Bazar	NMCF 05	ND		
	NMCF 06	ND		
	NMCF 07	ND		
Belabo Bazar	NMCF 08	ND		
	NMCF 09	ND		
	NMCF 10	ND		
Marjal Bazar	NMCF 11	ND		
	NMCF 12	Chlorpyrifos	0.120	0.05
	NMCF 13	ND		
Velanagar Bazar	NMCF 14	ND		
	NMCF 15	ND		

Fifteen samples of cauliflower were obtained from five different markets of Narsingdi city and evaluated for the presence of seven pesticide residues. Out of 15 samples, two samples (13% of total samples) had pesticide residues, whereas the remaining 13 samples did not contain any pesticide residues. The current study's findings are consistent with those of Akter et al. (2017), who discovered that 11 (22% of the 50 analyzed samples) contained residues of diazinon, dimethoate, quinalfos, and chlorpyrifos in eggplant collected from the Mymensingh district of Bangladesh, two of which had multiple pesticide residues and five contained residues above the EU-MRLs. The outcome of this study were further corroborated by the findings of Hasan et al. (2017), who discovered that of the 50 tested samples, 10 (20%) had residues of dimethoate and quinalphos in hyacinth bean obtained from different marketplaces in Dhaka, Bangladesh.

Among the 15 cauliflower samples taken in the Narsingdi district, one sample (NMCF 04) from CNB bazar showed dimethoate residue (0.1.266 mg/kg), which was above the MRL. Another sample (NMCF 12) from Marjal Bazar contained chlorpyrifos residue (0.120 mg/kg), which was also above MRL.

3.3. Pesticide residues in yard long bean

The level of pesticide residues found in the analyzed yard long bean samples and their maximum residue levels are presented in Table 3.

Table 3. The level of residues (mg/kg) of different pesticides found in the analyzed yard long bean samples collected from Narsingdi, Bangladesh.

Area of collection	Sample ID	Detected pesticide	Level of residues (mg/kg)	MRLs (mg/kg)
Shibpur Bazar	NMLB 01	ND		
	NMLB 02	Dimethoate	0.098	0.02
	NMLB 03	ND		
C&B Bazar	NMLB 04	ND		
	NMLB 05	ND		
	NMLB 06	ND		
Belabo Bazar	NMLB 07	Chlorpyrifos	0.134	0.01
	NMLB08	ND		
	NMLB 09	ND		
Marjal Bazar	NMLB 10	ND		
	NMLB 11	Dimethoate	0.053	0.02
		Chlorpyrifos	0.119	0.01
	NMLB 12	ND		
Velanagar Bazar	NMLB 13	ND		
	NMLB 14	ND		
	NMLB 15	Chlorpyrifos	0.086	0.01

Fifteen samples of yard long bean were collected from five different markets of Narsingdi district and analyzed for the presence of seven commonly used pesticide residues. Four samples (approximately 27% of total samples) contained pesticide residues, while 11 samples did not contain any pesticide residues. The current findings can be compared to those of Parven et al (2021). They gathered 70 samples of hyacinth bean and yard long bean from the Bogura area of Bangladesh, where they discovered that pesticide residues contaminated 14% of the yard long bean samples. The findings of this study are corroborated by the findings of Islam et al. (2019b), who discovered that eight (12.3% of the total number of samples) of the 65 tested eggplant samples were contaminated with pesticide residues.

Among 15 samples of yard long bean collected from Narsingdi region, one sample collected from Shibpur Bazar (NMLB 02) contained dimethoate (0.098 mg/kg) residue which was above MRL. Another sample (NMLB 07) from Belabo bazar contained chlorpyrifos residue (0.134 mg/kg), which was also above MRL. Sample collected from Marjal Bazar (NMLB 11) contained dimethoate (0.053 mg/kg) and chlorpyrifos (0.119 mg/kg), both of them were also above MRL. Another sample from velanagar bazar (NMLB 15) contained chlorpyrifos (0.086 mg/kg), which was above MRL.

4. Conclusions

In the case of 15 analyzed samples of hyacinth bean, three samples (20% of total samples) contained pesticide residues above the EU-MRLs and 12 samples contained no detectable residues of the sought pesticides; in the case of 15 analyzed samples of cauliflower, two samples (13% of total samples) had pesticide residues above the EU-MRLs and 13 samples did not contain any residues of the selected pesticides; and in the case of 15 analyzed samples of yard long bean, four samples (27% of total samples) had pesticide residues above the EU-MRLs and 11 samples did not contain any residues of the selected pesticides. The findings of the current study may play a vital role for the relevant stakeholders to take necessary initiatives to reduce the level of pesticide residue 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], [Nuzat Tasnim], [Sadia Sultana], [S M Mizanur Rahman]; Formal analysis and investigation: [Nuzat Tasnim], [Mohammad Dalower Hossain Prodhan]; Writing - original draft preparation: [Nuzat Tasnim], [Mohammad Dalower Hossain Prodhan], [Sadia Sultana], [Md. Nasrul Millat]; Writing - review and editing: [Mohammad Dalower Hossain Prodhan], [S M Mizanur Rahman], [Md. Nasrul Millat]; Supervision: [S M Mizanur Rahman], [Mohammad Dalower Hossain Prodhan]. All authors have read and approved the manuscript.

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