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Article

Multiple pesticide residue determination in major vegetables purchased from Gazipur district of Bangladesh

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Abstract: The present study was initiated to detect and quantify pesticide residues in three selected major vegetables (hyacinth bean, cauliflower and yard long bean) collected from 5 local markets of Gazipur district of Bangladesh. The vegetable samples were prepared by Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and analyzed by Gas Chromatography (GC) equipped with Flame Thermionic Detector (FTD). In this study, 45 vegetable samples were analyzed. Out of 15 samples of hyacinth bean, 2 samples (13% of the total samples) contained pesticide residues above MRL and the remaining samples did not contain any detectable pesticide residues. In the instance of cauliflower, 2 samples (13% of the total number of samples) contained pesticide residues above MRL, while the remaining samples had no detectable pesticide residues. Pesticide residues with levels exceeding MRL were found and measured in 3 samples (20% of the total number of samples) from 15 yard long bean samples. From remnant samples, no pesticide residue was detected. This study depicts the overall scenario of pesticide contamination in vegetables specially in hyacinth bean, cauliflower and yard long bean available in the local markets of Gazipur city, Bangladesh. As a result, it is important to intensify routine pesticide residue monitoring in commercially cultivated vegetables.

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

1. Introduction

Everybody wants to eat safe food. A large-scale food production of various agricultural products that are free of all types of contaminants, such as pesticide residues, heavy metals, and mycotoxins, is thus necessary to feed the rapidly growing global population. On the other side, Bangladesh is now experiencing a widespread issue with the scarcity of safe food. The world's food demand is changing quickly due to population increase, economic expansion, growing income levels, and fast urbanization. In this sense, growing vegetables has developed into a crucial farming activity that provides both dietary benefits and financial rewards (Aktar *et al.*, 2017). The way we eat is evolving every day. Because they offer vital nutrients, vegetables are a crucial part of the human diet. Hyacinth beans, yard long beans, and cauliflower are some of the most well-known rabi season crops among

main vegetables. Despite being a potential crop, the crop's low yield and poor quality have been a result of widespread insect pest infestations. Due to the harsh invasion of numerous insect pests, farmers in our nation experience a large crop loss each year.

Hyacinth beans are frequently grown from the rainy to the Rabi seasons and are a good source of important vitamins. Country bean is attacked by nine different insect species and one kind of mite, according to Alam et al. (1969). Four of these species are regarded as serious pests, and the remaining species are minor pests. But typically, a variety of insect pests attack the leguminous vegetables. The bean pod borer (*Maruca testulalis* G.), which was recognized as a legume pod borer in 1975 at FAO panel meeting in Bangkok (Reddy 1975). *M. testulalis* G. is a major insect pest of leguminous plants, according to Dina *et al.* (1979) and Bakar *et al.* (1980). Yard-long bean is rich in different vitamins like A and C, and calcium (Aykroiyd *et al.*, 1974) with a sufficient amount of lysine (Ferry *et al*., 1981). The pod borer *Euchrysops cnejus* F. (Lepidoptera: Lycaenidae) is considered a major pests of yard long been in Bangladesh (Ali, 2006). The nutrients folate, pantothenic acid, vitamin B6, vitamin C, and vitamin K are all abundant in cauliflower. Cauliflower, like other vegetables, is prone to pest infestation. Cauliflower is mostly affected by diamond backmoth, leaf webber, stem borer, tobacco caterpillar, aphids, and painted bug.

Presently the farmers use various pesticides such as dimethoate, quinalphos, chlorpyrifos, diazinon, acephate, fenitrothion etc. for controlling these vegetable pests. Each pesticide has a waiting period or pre-harvest interval (PHI), which is defined as the number of days needed to lapse, between the final pesticide application and harvest. Food products will be safe for consumption when the fruits and vegetables will be harvested following the PHI . Due to lack of education, the growers do not follow the PHI. A number of methods are used to determine multiple pesticide residues in fruits and vegetables. But a very little work has been done for these vegetables collected from local markets of Gazipur district. In order to monitor pesticide residues, an effective and accurate method is required. It is not possible to quantify pesticide residues based on a faulty method. 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).

Recently, a number of chromatographic techniques have been employed to quantify pesticide residues. Among these, the determination of pesticide residues is frequently done using gas chromatography. In Bangladesh, the measurement of pesticide residues is done using gas chromatography in conjunction with a flame thermionic detector in fruits and vegetables (Ahmed *et al.,* 2021; 2019; Rahman *et al.,* 2021; Prodhan *et al.,* 2021;), fish and dry fish (Prodhan *et al.,* 2018a; 2010; 2009; Hoque *et al.,* 2021;), sugercane (Kabir *et al.,* 2007), betel leaf and water (Prodhan *et al.,* 2021a; 2021b), because they are quite sensitive for the analysis of organophosphorus pesticides. Numerous studies have been done up to this point for the measurement of pesticide residues in vegetables in Bangladesh (Habib *et al.*, 2021; Nahar *et al.*, 2020; Islam *et al.*, 2021; 2019; 2019a; Prodhan *et al.*, 2018b; 2018c; 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). To know more about the actual situation of pesticide residues remaining in vegetables in a particular region of Bangladesh, more research is required. With this in mind, the current study was started to find and measure the levels of seven organophosphorus pesticide residues in yard long beans, hyacinth bean, and cauliflower that were gathered from various local markets in Gazipur city, Bangladesh (Joydebpur Bazar, Harinal Bazar, BIDC Bazar, Pubail Bazar, and Luxmipura Bazar).

2. Materials and Methods

2.1. Chemicals and reagents

The pure (>99.0%) analytical standard of acephate, chlorpyrifos, diazinon, dimethoate, fenitrothion, malathion and quinalphos were purchased from Sigma-Aldrich Laborchemikalien (St Louis, MO, USA) via Asa Scientific Pvt. ltd. Dhaka, Bangladesh. The methanol, HPLC grade acetonitrile, sodium chloride, anhydrous magnesium sulfate ($MgSO_4$) and Primary Secondary Amine (PSA) were obtained from Asa Scientific Pvt. ltd. Dhaka, Bangladesh.

2.2. Preparation of pesticide standard solution

The primary stock solutions of diazinon, dimethoate, chlorpyrifos, acephate, malathion, fenitrothion and quinalphos were prepared separately in acetonitrile at a concentration of 1000 mg/L and kept at -20°C until use. By putting the necessary volume of each individual stock solution in a 50 ml volumetric flask and bringing the mixture to volume with acetonitrile, a mixed standard solution of 50 mg/L in acetonitrile containing all the aforementioned pesticides was prepared. The mixed standard solution of 50 mg/L was converted into an intermediate mixed standard solution of 10 mg/L in acetonitrile. Then, acetonitrile 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

For this investigation, a total of 45 samples—15 each of yard-long beans, cauliflower, and hyacinth beans—were gathered. The samples were gathered from five neighborhood markets in the city of Gazipur: Joydebpur Bazar, Harinal Bazar, BIDC Bazar, Pubail Bazar, and Luxmipura Bazar. From each market, three samples of yard long beans, cauliflower, and hyacinth beans were taken. For all the chosen vegetables, there were a total of 1 Kg of samples per vegetable. Each clean, transparent, airtight polyethylene bag used to collect the samples was carefully 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

The QuEChERS extraction and clean up technique modified by Prodhan *et al*. 2015 was used for the extraction and clean-up of samples. The fruit blender was used to completely grind the sample pieces. Weighted into a 50 mL polypropylene centrifuge tube was a representative 10-g fully homogenized sample. Acetonitrile (ACN), 10 mL, was then poured into the centrifuge tube. The centrifuge tube was correctly sealed and forcefully shaken by the vortex mixer for 30 s. The centrifuge tube was then filled with 1 g of NaCl and 4 g of anhydrous MgSO4 before being agitated for a minute by the vortex mixer. The extract was then centrifuged at 5000 rpm for 5 minutes. A 15 mL micro centrifuge tube containing 600 mg of anhydrous MgSO4 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 selected organophosphorus pesticides (chlorpyrifos, diazinon, dimethoate, acephate, malathion, fenitrothion and quinalphos) were quantified using a Gas Chromatograph (GC-2010 Shimadzu) connected to Flame Thermionic Detector (FTD). Helium was employed as a carrier and make-up gas in addition to the Rtx-OPPesticide2 capillary column, which had a 30 m length, 0.32 mm i.d., and 0.32 m film thickness. The flow across the column was 1.5 mL/min. The column oven's temperature was designed to start at 150 °C (1 minute hold) and rise to 220 °C at an incremental rate of 10 °C (2 min hold). The injector and detector's temperatures were set to 250 °C and 280 °C, respectively. The injection of samples was done using the spitting mode, and the split ratio 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 Discussions

3.1. Pesticide residues in hyacinth bean

The extracts of hyacinth bean samples were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. The amount of pesticide residues estimated in the hyacinth bean samples are presented in Table 1.

In order to determine the presence of residual pesticides from seven different pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos, and quinalphos), 15 samples of hyacinth bean were collected from five different markets in the city of Gazipur (Joydebpur, Harinal, BIDC, Laxmipura, and Pubail). Fifteen samples were collected, of which 2 samples (13% of the total samples) had pesticide residues and 13 samples (87% of the total samles) had no detectable pesticide residues. Compared to Parven et al., 2021, the current findings are comparable. They gathered 70 samples of yard long and hyacinth bean from the Bogura district of Bangladesh, where they discovered that 14% of the yard long bean samples had pesticide residues. The findings of Islam et al. (2019b), who discovered that 8 of the 65 examined eggplant samples (12.3% of the total number of samples) were contaminated with pesticide residues, complement the findings of this investigation.

One hyacinth bean sample (GMBe-05) out of the 15 samples taken from the Gazipur area contained dimethoate residue (0.074 mg/kg), which was above the EU-MRL. The chlorpyrifos residue (0.17 mg/kg) was found in

another sample (GMBe-11) taken from Pubail bazar, and it was similarly above the EU-MRL. There was no pesticide residues found in the samples taken from Joydebpur Bazar, BIDC Bazar, or Luxmipura Bazar.

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

Area of collection	Sample ID	Detected pesticide	Level of residues (mg/kg)	MRLs (mg/kg)
Joydebpur	GMBe-01	ND	-	-
	GMBe-02	ND	-	-
	GMBe-03	ND	-	-
Harinal	GMBe-04	ND	-	-
	GMBe-05	Dimethoate	0.074	0.02
	GMBe-06	ND	-	-
BIDC	GMBe-07	ND	-	-
	GMBe-08	ND	-	-
	GMBe-09	ND	-	-
Pubail	GMBe-10	ND	-	-
	GMBe-11	Chlorpyrifos	0.171	0.01
	GMBe-12	ND	-	-
Luxmipura	GMBe-13	ND	-	-
	GMBe-14	ND	-	-
	GMBe-15	ND	-	-

3.2. Pesticide residues in cauliflower

The extracts of cauliflower samples were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. The amount of pesticide residues estimated in the analyzed cauliflower samples are presented in Table 2.

Table 2. The level of residues (mg/kg) of different pesticides found in the analyzed cauliflower samples collected from Gazipur.

Area of collection	Sample ID	Detected pesticide	Level of residues (mg/kg)	MRLs (mg/kg)
Joydebpur	GMCF-01	ND		
	GMCF-02	ND		
	GMCF-03	Chlorpyrifos	0.120	0.05
Harinal	GMCF-04	ND		
	GMCF-05	ND		
	GMCF-06	ND		
BIDC	GMCF-07	ND		
	GMCF-08	ND		
	GMCF-09	ND		
Pubail	GMCF-10	ND		
	GMCF-11	ND		
	GMCF-12	ND		
Luxmipura	GMCF-13	Chlorpyrifos	0.058	0.05
	GMCF-14	ND		
	GMCF-15	ND		

Fifteen samples of cauliflower were taken from 5 different marketplaces in the city of Gazipur (Joydebpur, Harinal, BIDC, Laxmipura, and Pubail), and they were examined to see whether any residues of the selected pesticides (acephate,diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos) remained on them. 13 samples (of the entire number of samples) contained no detectable pesticide residues, whereas 2 samples (13% of the total number of samples) contained residues. The results of the present study are in good agreement with those of Akter et al. (2017), who discovered that 11 (22% of the total number of samples) of the 50 analyzed samples contained residues of diazinon, dimethoate, quinalfos, and chlorpyrifos in eggplant gathered from Mymensingh district of Bangladesh, of which, 2 had multiple pesticide residues and 5 contained

residues above the EU-MRLs. The findings of Hasan et al. (2017), which indicated that of the 50 tested samples, 10 samples (20%) had residues of dimethoate and quinalphos in hyacinth bean gathered from various marketplaces in the Dhaka area of Bangladesh, further complement the conclusions of this study.
Among 15 samples of cauliflower, one sample collected from Joydebpur bazar (GMBe-03) contained chlorpyrifos residue (0.120 mg/kg) at a level above the EU-MRL. Another sample collected from Pubail bazar (GMBe-13) also contained chlorpyrifos residue (0.058mg/kg) which was also above EU-MRL. The samples collected from Harinal Bazar, BIDC Bazar and Luxmipura Bazar did not contain any residues of the selected pesticides.

3.3. Pesticide residues in yard long bean

The extracts of yard long bean samples collected from different local markets of Gazipur city were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. The amount of pesticide residues estimated in the analyzed yard long bean samples 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 Gazipur.

Area of collection	Sample ID	Detected pesticide	Level of residues (mg/kg)	MRLs (mg/kg)
Joydebpur	GMLB-01	Chlorpyrifos	0.062	0.01
	GMLB-02	ND		
	GMLB-03	ND		
Harinal	GMLB-04	ND		
	GMLB-05	ND		
	GMLB-06	ND		
BIDC	GMLB-07	ND		
	GMLB-08	Chlorpyrifos	0.100	0.01
	GMLB-09	ND		
Pubail	GMLB-10	ND		
	GMLB-11	ND		
	GMLB-12	ND		
Luxmipura	GMLB-13	ND		
	GMLB-14	Chlorpyrifos	0.106	0.01
	GMLB-15	ND		

Fifteen samples of yard long bean collected from 5 different local markets of Gazipur city (Joydebpur, Harinal, BIDC, Luxmipura and Pubail) were analyzed to find out the presence of left over residue of seven selected pesticides (acephate,diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos. In 3 samples (20% of the samples) had pesticide residues among the 15 samples, and 12 samples (80% 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 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 15 samples of yard long bean, one sample collected from Joydebpur bazar (GMLB 01) contained chlorpyrifos residue (0.062 mg/kg) which was above EU-MRL. Another sample collected from BIDC bazar (GMBe-08) contained chlorpyrifos residue (0.100 mg/kg) which was also above the EU-MRL. Sample collected from Luxmipura (GMLB 14) also contained chlorpyrifos residue (0.106 mg/kg) which was above EU-MRL. The samples collected from Harinal Bazar, Pubail Bazar and Luxmipura Bazar did not contain any residues of the selected pesticides.

4. Conclusions

In this study, 45 vegetable samples from different local markets in Gazipur district (Joydebpur Bazar, Harinal Bazar, BIDC Bazar, Pubail Bazar, and Luxmipura Bazar) were analyzed for the quantification of seven organophosphorus pesticide residues to ensure that consumers are eating safe food. The samples included 15 each of hyacinth bean, cauliflower, and yard long bean. In the case of 15 analyzed samples of hyacinth beans, 2 samples (13% of the total) contained pesticide residues above the EU-MRL; in the case of 15 analyzed samples

of cauliflower, 2 samples (13% of the total) contained pesticide residues above the EU-MRL; and in the case of 15 analyzed samples of yard long beans, 3 samples (20% of the total) contained pesticide residues above the EU-MRL. The findings of the current study will assist the key stakeholders and policy makers in taking the required steps to reduce the amount of pesticide residue in vegetables.

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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], [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 final manuscript.

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