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Determination of Organophosphorus Insecticide Residues in Country Bean Collected from Different Markets of Dhaka

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Abstract

The study was conducted to analyze pesticide residues in country bean collected from different areas of Dhaka city from January 2016 to September 2016. A simple and efficient multiple pesticide residue analytical method using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and Gas Chromatography (GC) coupled with Flame Thermionized Detector (FTD) were used for the determination of pesticide residues in 50 country bean samples. Among the 50 analyzed samples of country bean, 10 samples (20%) contained residues of Dimethoate and Quinalphos, of which 5 samples were above the maximum residue limits (MRLs). Most of the contaminated samples (8 samples) contain residue of Dimethoate. This study reflects the overall scenario of pesticide contamination in vegetables, especially in country bean collected from the local markets of Dhaka city, which will help the consumer to be aware of their health and safety.

Keywords: Country bean; Organophosphorus insecticide residue; QuEChERS extraction; GC-FTD

Introduction

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. It makes up a major portion of the diet of humans in many parts of the world and plays a significant role in human nutrition, especially as sources of vitamins, minerals, dietary fiber and phytochemicals [1-4]. A world vegetable survey showed that 402 vegetable crops are cultivated worldwide, representing 69 families and 230 genera [5]. According to the 2007 World Health Report unbalanced diets with low vegetable intake and low consumption of complex carbohydrates and dietary fiber are estimated to cause some 2.7 million deaths each year, and were among the top 10 risk factors contributing to mortality [6]. Country bean is one of the most important, inexpensive and popular vegetable crops in Bangladesh. Though it is commonly called country bean in Bangladesh, but it has a variety of names at different regions of the country like Sheem, Chhoi, Uri, Ushi, Deshi Sheem etc. Internationally also the crop has various other names e.g., hyacinth bean, bonavist bean, Dolichos bean, Indian bean, Egyptian kidney bean, Lima bean, faba bean etc. [7]. It is a rich source of essential vitamins which commonly grown during rainy through rabi seasons usually around the homestead by trailing its vine either on trees or by providing different kinds of supports. Despite of being a prospective crop, high incidences of insect pests have limited the crop to its low yield and poor quality. Farmers in our country face significant yield loss of beans every year due to severe attack of various insect pests. In general, insect pests cause enormous quantity of yield losses in every season and every year. Although no regular statistical records are kept, as per conservative estimate the yield loss in country bean due to insect pests is reported to be about 12-30% [8]. Country bean is attacked by nine different insect species and one species of mite [9]. Among these species, four species are considered as major pests and the rest of them as minor pest.

In order to reduce the loss and maintain the quality vegetables farmers use pesticides along with other pest management techniques during crop production to destroy pests and to prevent diseases. The use of pesticides has increased because they have rapid action to control the pests and diseases, and are less labor intensive than other pest control methods [10]. Pesticide being toxic can become a potential hazard to the manufacturers, the users and the environment. Pesticide can produce negative impacts, both socially and economically [11]. Extensive 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 has become a consumers' safety issue and the consumers have the right to know how much pesticide get incorporated in the food they eat. The identification and quantification of pesticide in the food are becoming the public interest. Several researchers [12-18] analyzed pesticide residues in fruit and vegetables in Greece, India, Spain, China, Japan and other developed country. A very little reference is available on the presence of pesticide residues in vegetables in Bangladesh [19]. However, up to now, in Bangladesh many research works on pesticide residues in vegetables and other matrices have been conducted in pesticide Analytical Laboratory, Entomology Division of Bangladesh Agricultural Research Institute (BARI), that have been published in the national journals [20-24]. Nevertheless, more research work is needed to find out the actual scenario of pesticide residues present in vegetables. Keeping this view, the present study was initiated to identify and measure the level of different pesticides present in country bean in the markets of Dhaka city and compare the level of detected pesticide residues (mg/Kg) with the Maximum Residue Limit (MRL).

Materials and Methods

Study area

The country bean samples were collected from different areas of Dhaka

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City (Banani, Rampura, Mohammadpur, Kawran Bazar and Jatrabari) and carried to the Pesticide Analytical Laboratory, Entomology Division, BARI, Joydebpur, Gazipur for pesticide residue analysis during January 2016 to September 2016.

Sample collection

A total of 50 samples of country bean were collected for this study. Ten samples were collected from each market. The amount of each sample was 1 Kg. 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 samples were then taken to the Pesticide Analytical Laboratory, Division of Entomology, Bangladesh Agricultural Research Institute (BARI) on the day of collection. 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 refrigerator at -20°C until extraction and cleanup process started.

Chemicals and reagents

The standard of Chlorpyrifos, Acephate, Diazinon, Dimethoate, Quinalphos, Malathion and Fenitrothion 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.

Preparation of pesticide standard solution

Pesticide standard stock solutions of Chlorpyrifos, Acephate, Diazinon, Dimethoate, Quinalphos, Malathion and Fenitrothion were prepared separately in acetone at a concentration of 1000 mg/L and stored at -20°C until use. A mixed standard solution of 50 mg/L in acetone 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 acetone. An intermediate mixed standard solution of 10 mg/L in acetone 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 acetone 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.

Extraction and clean up

QuEChERS extraction method is one of the latest extraction and clean up techniques for pesticide residue analysis in food matrices which is an anagram for Quick, Easy, Cheap, Effective, Rugged and Safe. This technique was first introduced by Anastassiades et al. [25]. For this study, QuEChERS extraction technique was used for the extraction and clean-up of samples which was modified by Prodhan et al. [14]. The chopped samples were grounded thoroughly with the fruit blender. A representative 10-g portion of thoroughly homogenized sample was weighted in a 50 mL polypropylene centrifuge tube. Then 10 mL of acetonitrile (MeCN) was added into the centrifuge tube. The centrifuge tube was closed properly and shaken vigorously for 30 s by the use of a vortex mixer. Then, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added into the centrifuge tube, and it was shaken immediately by the vortex mixer for 1 minute to prevent the formation of magnesium sulfate aggregates. Afterwards, the extract was centrifuged for 5 min at 5000 rpm. An aliquot of 3 mL of the MeCN 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 HPLC vial for injection.

Detection and quantification of pesticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) with Flame Thermionized Detector (FTD) for the detection of Acephate, Dimethoate, Diazinon, Fenitrothion, Malathion, Chlorpyrifos and Quinalphos. The capillary column was AT-1, length was 30 m, ID was 0.25 mm and film thickness was 0.25 µm. Helium was used as carrier and make up gas for FTD. The identification of suspected pesticide was performed by peak retention times in samples to those

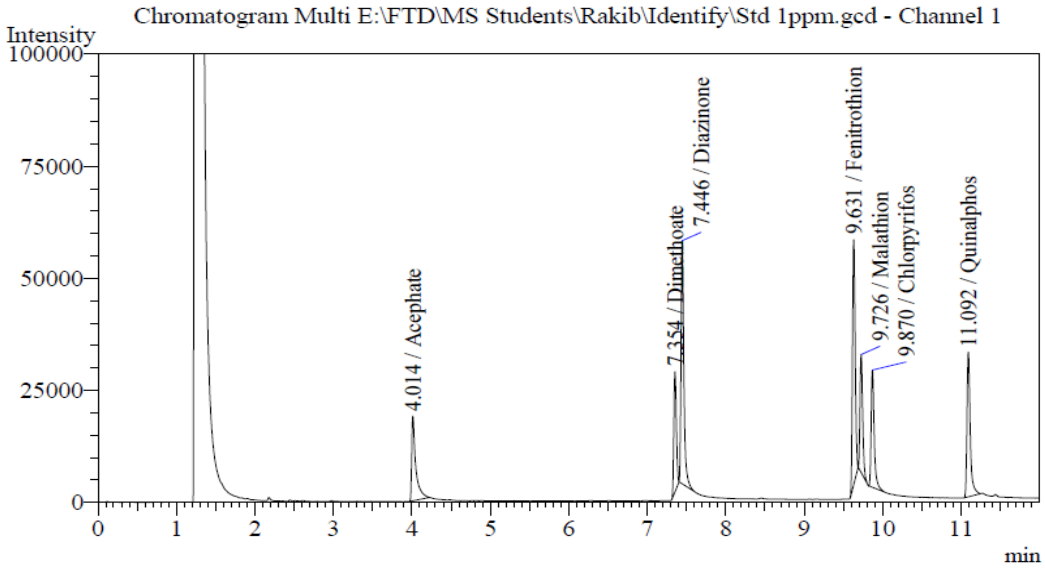

Figure 1: Typical Chromatograms of seven organophosphorus insecticide standards run by GC-FTD.

Instruments	Conditions
Injection port SPL	Injection mode: split; temperature:250°C; flow control mode: linear velocity; split ratio: 30:0
Detector channel 1 FTD	Temperature: 280°C; current: 1.00 Pa; H_2 flow: 1.5 ml/min; stop time: 10 min; make up flow: 30 ml/min; air flow: 145 ml/min

Table 1: The instrument parameters for GC-FTD.

Column oven	Rate	Temperature	Hold time (min)
Initial temperature: 150°C	**-10**	**150** **220**	**1** **2**

Table 2: Conditions for column oven.

Sample ID	Area of collection	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)*
DMB_3	Banani	Dimethoate	0.018	0.02
DMB_7	Banani	Dimethoate	0.009	0.02
DMB_{15}	Rampura	Dimethoate	0.069	0.02
DMB_{22}	Mohammadpur	Dimethoate	0.093	0.02
DMB_{28}	Mohammadpur	Dimethoate	0.019	0.02
DMB_{34}	Jatrabari	Dimethoate	0.011	0.02
DMB_{39}	Jatrabari	Dimethoate	0.120	0.02
DMB_{41}	Kawran bazar	Dimethoate	0.013	0.02
DMB_{45}	Kawran bazar	Quinalphos	0.033	0.01
DMB_{49}	Kawran bazar	Quinalphos	0.045	0.01

*According to the EU Pesticide Database (European Commission 2005)

Table 3: The level of residues (mg/kg) of different pesticides found in the analyzed country bean samples.

of peaks in the pure analytical standards (Figure 1). The instrument conditions are described in Tables 1 and 2.

Calibration curve preparation

Prior to the injection of the sample extract, standard solutions of different concentrations of each pesticide group were prepared and injected with suitable instrument parameters. The samples were calibrated (retention time, peak area etc.) against five-pointed calibration curve of standard solution of concerned pesticide. Each peak was characterized by its retention time. Sample results were expressed in mg/kg automatically by the GC software.

Results and Discussion

The concentrated extracts of country bean samples collected from different markets were analyzed by GC-2010 (Shimadzu) with Flame Thermionized Detector (FTD) with the pre-set parameters. The levels of pesticide residues found in the analyzed country bean samples and their maximum residue levels are outlined in Table 3. Fifty samples of country bean collected from 5 different markets of Dhaka city (Banani, Rampura, Mohammadpur, Kawran Bazar and Jatrabari) were analyzed to find out the presence of left over residue of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos. Out of 50 samples, 10 samples (20% of the total number of samples) contained pesticide residues and 40 samples (80% of the total number of samples) contained no detectable residues of the sought pesticides. 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.

Ten country bean samples were collected from Banani area, among them two samples (DMB_3 and DMB_7) contained Dimethoate at a level of 0.018 mg/kg and 0.009 mg/kg respectively, which were below the EU-MRL [26]. The other 8 samples contain no detectable pesticide residues. Only one sample (DMB_{15}) contained residue of Dimethoate (0.069 mg/kg) and the other nine samples contained no detectable pesticide residues among the ten samples collected from Rampura area. The level of detected residue was 0.069 mg/kg, which was above the MRL (0.02 mg/kg).

Among the ten samples of country bean collected from Mohammadpur, two samples (DMB_{22} and DMB_{28}) contained residue of Dimethoate at 0.093 mg/kg and 0.019 mg/kg, respectively. Considering the maximum residue limit (0.02 mg/kg), the level of detected residue of DMB_{22} was above the MRL and DMB_{28} was below the MRL. Two samples (DMB_{34} and DMB_{39}) of country bean contained residue of Dimethoate among the ten samples collected from Jatrabari, in which the level of residue of one sample (DMB_{34}) was 0.011 which was below the MRL (0.02 mg/kg) and the other sample contained 0.120 mg/kg Dimethoate residue which was much higher than the Maximum Residue Limit (0.02 mg/kg). In case of the ten samples collected from Kawran Bazar, one sample (DMB_{41}) contained residue of Dimethoate (0.013 mg/kg) which was below the MRL (0.02 mg/kg), and two samples (DMB_{45} and DMB_{49}) contained Qunalphos residue at 0.033 mg/kg and 0.045 mg/kg, respectively and the levels of detected residues of both samples were above the Maximum Residue Limit (0.01 mg/kg).

Conclusion

Vegetables are one of the most important sources of vitamin and nutrition in our country, but it contributes a very poor portion of our daily intake because of its low production. The main obstacle of vegetable production in our country is insect pest infestation. To increase the vegetable production use of different pesticides and other chemicals are become a common agricultural practice by the farmers, and a major portion of these pesticides are intercepted by the plant leaves during application. Vegetables such as brinjal, tomatoes, country beans, cabbage, cauliflower and cucumber may receive higher doses of pesticides. As a result, pesticide residues remain in the vegetables, even after they are washed and cooked. Consumers, who intakes vegetables with high residual contamination, are subjected to or affected by various types of food-borne diseases associated with pesticide contamination. Moreover, most chronic diseases e.g., cancer, heart attack etc. are the result of long term consumption of pesticide contaminated vegetables.

The purpose of this study was intended to identify and quantify the pesticide residue level present in the vegetable available in various local markets of Dhaka City. Regarding this, fifty samples of Country bean were collected from five different locations (Banani, Rampura, Mohammadpur, Jatrabari and Kawran Bazar) of Dhaka City and carried to the Pesticide Analytical Laboratory, Division of Entomology, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The QuEChERS extraction technique was applied for the extraction and cleanup of the collected sample. Gas chromatography associated with flame thermionized detector (FTD) was used to identify and quantify the level of pesticide residues present in the extracted samples. Seven most commonly used pesticides i.e., diazinon, acephate, chlorpyrifos, malathion, fenitrothion, dimethoate and quinalphos were selected for this study. Among the 50 samples of country bean, 10 samples (20% of the total number of samples) contained pesticide residues of Dimethoate and Quinalphos. Among these, 5 samples had residue levels above the maximum residue limits (MRLs). On the other hand, 40 samples (80% of the total number of samples) contained no detectable pesticide residues of the sought pesticides. Now a day's pesticide residues in vegetables and other foods have become a major concern and a safety issue for the consumers. This study will help to understand the residual contamination of country bean in the study area and will help to increase public awareness as well.

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