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DETERMINATION OF MAJOR ORGANOPHOSPHATE INSECTICIDE RESIDUES IN CABBAGE SAMPLES FROM DIFFERENT MARKETS OF DHAKA

Mohammed Ariful Islam[1*], Ahammad Ullah[1], Marzia Habib[1], Md Tazul Islam Chowdhury[1], Md Sirajul Islam Khan[1], Abdul Kaium[1,2], Mohammad Dalower Hossain Prodhan[3]

[1] *Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka, BANGLADESH*
[2] *Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, CHINA*
[3] *Pesticide Research and Environmental Toxicology Section, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur, BANGLADESH*

Corresponding author: Mohammed Ariful Islam; arif.sau.agch@gmail.com;
Department of Agricultural Chemistry Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, 1207, Dhaka, Bangladesh

ABSTRACT

Objective: The study was conducted to analyse 4 organophosphorus pesticide namely Chlorpyrifos, Diazinon, Fenitrothion and Quinalphos residues in cabbage. **Methods:** Between September 2016 and March 2017, 50 Cabbage samples were collected from 5 vegetables markets of Dhaka city, namely Rampura kacha bazar, Jatrabari krishi market, Kawran Bazar, Taltola Bazar and Mohammadpur Krishi Market. The collected samples were extracted and analyzed by QuEChERS based Gas Chromatography coupled with Flame Thermionic Detector (GC-FTD) method. **Result:** Total 6 samples (12%) contained pesticide residues and interestingly all of them were above the MRLs set by EC. Among the four organophosphorus insecticides tested only Chlorpyrifos and Diazinon were detected above the MRLs. **Conclusion:** This research represents a snapshot situation of contamination of pesticides in one of the common winter vegetables available in Dhaka City's local markets linked to consumer safety.

Keywords: QuEChERS, GC-FTD, Cabbage, Chlorpyrifos, Diazinon

1. Introduction

Fresh and edible herbaceous plants are consumed as vegetables. Vegetables contain all of the essential nutrients that is very important to construct and repair the body healthy. Their high carbohydrate, vitamin, and mineral contents are valued strongly. In the daily diet, vegetables were heavily correlated with improved liver health, good eye vision, and reduced the risk of heart diseases, chronic diseases like diabetes, and different types of cancer (Wargovich, 2000; Dias and Ryder, 2011). Various types of vegetables are available in Bangladesh. Edible roots, stems, leaves, fruits or seeds of vegetables are edible and contributes a great for a good diet (Robinson, 1990). Cabbage (Brassica oleracea) is an outstanding source of vitamin C, K, B and folate, which can supply more than 20% each of these nutrients daily value in each serving (USDA, 2014). It also is a valuable source of carotene, different vitamins like: niacin, riboflavin, and minerals like: calcium, magnesium and iron (De Lannoy, 2001). Studies indicate that cabbage may have protective impacts against colon cancer. (Dinkova-Kostovaand and Kostov, 2012).

In spite of the benefits, vegetable production is beset with an insect attack. Usually a pest infestation reduces the market value of the product and, in some instances, complete crop failure. Therefore now a days, fermars are have no option but have to use many synthetic insecticides to manage insect pests and ensure their production. These insecticides can contaminate water bodies, air and soil. (Joel, 1994). Therefore, concern about the environmental pollution and its resulting impacts on human health are increasing worldwide. According to WHO, everyyear nearabout 0.2 millions peoples die and at least 3 million peoples are suffering in severe health issues every year due to pesticide poisoning (Barbara, 1993). One of the key way to control residues of pesticide by following a safe gape between the last or final application of pesticide and the time of harvest. This gap or withhold or waiting period is usually known as preharvest interval (PHI). Various factors like physico-chemical properties of pesticides, crops, cropping season, crop duration, intercultural practices affect the PHI (Prodhan et al., 2018). In addition to that different post-harvest treatment can also significantly able to reduce the PHI. These all pre and post-harvest management techniques to supply safe and sustainable food production is integrated in so called Good Agricultural Practices (GAP).

Pesticide residue monitoring is one of the essential tool for ensuring GAP. In order to analyses of multi-residue in fruit and vegetables, a number of analytical methods are used; ((Prodhan et al., 2016; Singh et al. 2012; Lehotay,2010; Dasika et al., 2012). , Reliable multi-residue analytical methods are needed to monitor pesticide residues nationally in agricultural products. Multi-residue techniques, which can simultaneously quantify residues of multiple analyses in a single run, are used for monitoring purposes. Different extraction and clean-up methods are used for different food matrices; among them, QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction techniques are most frequently used in the laboratories for testing fruits and vegetables. The accurate, fast, easy and inexpensive QuEChERS based multiresidue analytical method was introduced in 2003 (Anastassiades et al., 2003). More advanced and modified QuEChERS methods for the determination of multi pesticide residues in different sample matrices have been validated through follow-up research. (Lehotay et al., 2005). Therefore, in this study, QuEChERS extraction techniques with Gas Chromatography were used to quantify the organophosphorus pesticide residue in cabbage samples.

Now-a-days, pesticide residues problem in Bangladesh has become a big problem in consumer health safety issues. However, there are still a few references on pesticide residues in Bangladesh-grown vegetables. This research was designed to identify the amounts of multiple organophosphorus pesticides residue in the cabbage samples collected from different markets of Dhaka City and comparison with MRL established by EC of the identified pesticide residues (mg / kg).

2. Materials and Method

2.1. Study Location

The Cabbage samples were collected from from 5 vegetables markets of Dhaka city, namely Rampura kacha bazar, Jatrabari krishi market, Kawran Bazar, Taltola Bazar and Mohammadpur Krishi Market during September 2016 to March 2017. All the samples were transported to Pesticide Analytical Laboratory of BARI in labeled polyethylene bags and stored at -20℃ for further analysis.

Figure 1: Map showing the places of sample collection from the Dhaka city

2.2. Sample Collection and Preparation

Total 50 cabbage samples (10 samples from each market) were collected for this study. Every sample amount was 1 kg. To avoid any cross contamination each samples have been collected and marked properly in separate, transparent, clean, polyethylene bag. All the collected samples were transferred to the Pesticide Analytical Laboratory, of BARI on the day of collection. All the samples were chopped and mixed properly in labeled polyethylene bags and stored at -20℃ for further analysis

2.3. Chemicals and Reagent

All pure pesticide standards: Chlorpyrifos, Diazinon, Fenitrothion and Quinalphos(>99.6%) (Figure 1) were bought from Sigma-Aldrich Laborchemikalien (St Louis, MO, USA. GC grade methanol, acetone, acetonitrile, analytical grade NaCl, anhydrous MgSO4 and Primary Secondary Amine (PSA) were collected from Bangladesh Scientific Pvt. Ltd.

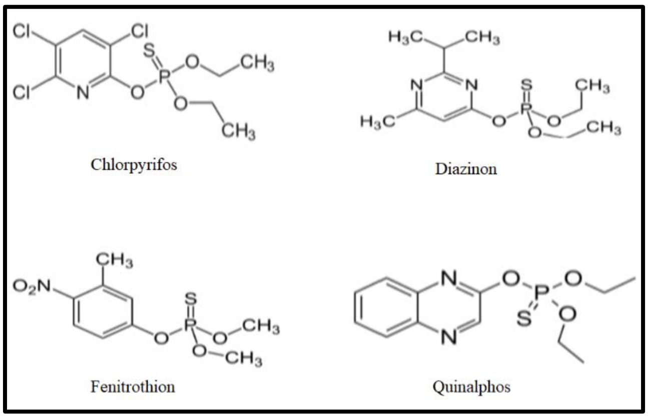

Figure 1: Chemical structures of the pesticides used in the present study

2.4. Preparation of Standard Solution

All of the standard stock solutions were made separately in acetone at 1000 ppm and stored until use at -20°C. 50 ppm mixed standards stock solution were made from all of the individual standard stock solution in 50 mL volumetric flask by adding appropirte amount of stock standards and acetone. Mixed intermediate stock solution of 10 ppm were prepared from this 50ppm mixed standard stock. Finally all the working satndards solution of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 ppm were prepared by using this Mixed intermediate stock solution. All the standard sock and working solutions were staored at -20°C.

2.5. Extraction and Clean up

The QuEChERS extraction method, now one of the most used extractions and cleaning methods in food matrices for pesticide residue assessment. This unique techniques was first introduced by Anastassiades and his co-workers (2003). In the present study, a modified QuEChERS extraction technique developed by Prodhan et al. (2015) was used. In brief, the sliced samples were grounded thoroughly using a homestead fruit blender. 10 gram of these homozenized sample was transferred into a 50 mL teflon centrifuged tube followed by adding of 10 mL of acetonitrile. Then vortex for 1 min and followed by adding extraction salt (4 g of anhydrous MgSO4 + 1 g of NaC). Then shake it for 2 minutes and followed by subsequently centrifuged at 5000 rpm for 5 minutes. Then 3 mL supernatant was transferred to a 15 mL centrifuge tube containing 600 mg MgSO4 anhydrous and 120 mg PSA. Againg the tube was vortex for 1 min, and centrifuged at 4000 rpm for 5 minutes. Finally, 1 ml of supernatant was filtered using a 0.2 µm PTFE filter and then transferred into a clean HPLC vial.

2.6. Detection and Quantification of Pesticide Residue

The concentrated extracts were analysed following the method described by Prodhan et al. (2009) using GC-2010 (Shimadzu) with Flame Thermionised Detector (FTD) detector. The capillary column was AT-1, 30 m long, 0.25 mm ID and 0.25 µm film thick. Helium was used as carrirer gas for FTD detector, Target pesticide was identified by was identified by peak retention times in pure standards peak to samples peak (Figure 2). The instrumental conditions are described in Tables 1 and 2.

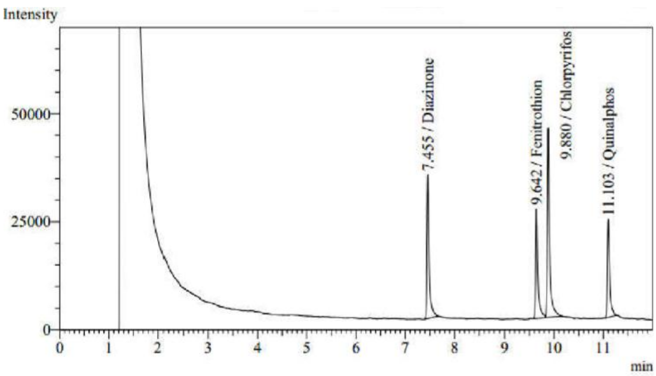

Figure 2: Typical chromatograms of four organophosphorus insecticide standards run by GC-FTD

Table 1: The Instrument parameters for GC-FID

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 2: Condition for column oven temperature for FTD

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

2.7. Calibration and Preparation

Different concentration level of standard solutions of each pesticide group were prepared and injected according to previously developed methods parameters prior to the injection of the sample extracts. The samples were calibrated against the five-pointed calibration curve of the standard pesticide solution (retention time, peak area, etc.). Every peak was distinguished by its time of retention. The GC software automatically displayed sample results in mg/kg.

3. Results and Discussion

Pesticide residue refers to the chemicals that may remain on or in food after they are applied to the plants (IUPAC, 1997). The MRLs of these residues in plants are often stipulated by regulatory bodies in most of the countries such as FDA in US and FSA in England. Some international bodies provide international standards like Codex Alimentarius, European Commission. Most to the list developed countries follow the standards given by these international bodies mainly because of insufficient laboratory facilities and budget. Therefore, it is important to regularly monitor the national market using international standards.

Table 3: The level of residues (mg/kg) of different pesticides found in the analyzed cabbage samples (N=50)

Sl. No.	Area of collection	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)*
1.	Rampura	Diazinon	0.12 ± 0.01	0.01
2.	Rampura	Chlorpyrifos	0.15 ± 0.02	0.01
3.	Rampura	Diazinon	0.07 ± 0.01	0.01
4.	Taltola Bazar	Diazinon	0.05 ± 0.01	0.01
5.	Taltola Bazar	Chlorpyrifos	0.06 ± 0.01	0.01
6.	Taltola Bazar	Chlorpyrifos	0.18 ± 0.02	0.01

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

The sample extracts of collected cabbage samples were analyzed by GC- FTD. Table 3 showing the number of pesticide residues found in the analyzed cabbage samples together with their corresponding MRLs. Out of the 50.0 samples, 6 samples (12% of the total samples) contained diazinon and chlorpyrifos residues and 38 samples (88% of the total) contained no detectable pesticide residue. But interestingly all the samples where pesticide residue was able to detect were above MRLs set by EC and were collected from two markets namely Rampura and Taltola Bazar, whereas samples from other three markets appear to be uncontaminated. Out of these 6 over residue samples, 3 contained Diazinon and 3 contains Chlorpyrifos. These contaminated samples contain 5-18 times higher amount of residues. These results are comparable to a previously conducted experiment by Rakibul et al. (2017). They have also collected 50 country bean samples from same markets of. Using the same extraction method to analyze seven different pesticides and have found that 10 samples (i.e. in 20% samples) contain pesticide residues in of which only 5 samples were above MRLs set by EC. However, the residue per cent of the analyzed samples looks lower of two studies conducted in Greece by one of our co-authors. Prodhan et al. (2016) have been detected three insecticides (chlorpyrifos, cypermethrin, and deltamethrin) and two fungicides (fluopicolide and propamocarb hydrochloride) in the cabbage collected from different markets of Thessaloniki, Greece.. Among the 132 analyzed samples residues were found in 41 (about 31% of the total samples) samples, of which, 2 samples contains multiple pesticides residues. In another experiment in the same region, four insecticides (chlorpyrifos, cypermethrin, deltamethrin and indoxacarb) has been detected in the cauliflower samples. There, among the 120 analyzed samples, 48 (40% of the total samples) were found to have pesticide residues (Prodhan et al 2016a). This comparatively high contamination in an EU country compare to an Asian country might be the due to the variations in the number samples analyzed, different degradation value due to climatic variation or may be due to the other pesticides which were considered on a study in Greece but not in Bangladesh.

4. Conclusion

As an overcrowded country, the major problem in Bangladesh is food security and malnutrition. Vegetables are one of the biggest vitamin and nutrition sources in our daily diet, but because of their limited supply, they contribute a very small portion of our daily consumption. In Bangladesh, insect pest infestation is the main barrier to vegetable manufacturing. a The use of different pesticides and other chemicals is becoming a prevalent agricultural practice by farmers, and a significant part of these pesticides are intercepted during application by the leaves of the plant. As a result, if the GAP is not properly maintained, residues of pesticides can remain in the vegetable. Consumers who frequently consume vegetables with high residual contamination may be affected by different types of foodborne diseases. In addition, some chronic illnesses may also lead to long-term pesticide-contaminated vegetable intake. However, this research area is still at its initial stage due to the absence of a well-equipped laboratory and skilled workforce in our country. This research represents a current snapshot of pesticide contamination situation in Dhaka City's local markets using an authentic and updated QuEChERS based GC-FTD analytical method in an accredited laboratory.

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