International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 11, No. 2, p. 22-31, 2017

RESEARCH PAPER OPEN ACCESS

Determination of pesticide residues in eggplant using modified QuEChERS Extraction and Gas chromatography

Mst. Afia Aktar*[1], Rehana Khatun[1], M. D. H. Prodhan[2]

[1]*Department of Environmental Science, Bangladesh Agricultural University, Mymensingh, Bangladesh*

[2]*Division of Entomology, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh*

Article published on August 30, 2017

Key words: Pesticide, Eggplant, Pesticide residue, QuEChERS extraction, GC-FTD.

Abstract

Food poisoning through pesticide contamination is a burning issue now a days all over the world. Peoples are suffering and dying in a large number from this problem every year. It is also a threat to the future generations. Foods like vegetables are being contaminated with pesticide for larger yield but the residual effect of this pesticide is devastating. So the study about the determination of pesticide residue in vegetables of a certain place is an important matter of public interest. This study reflects the overall scenario of pesticide residue contamination in eggplant available in the local markets of Mymensingh Sadar, Mymensinghfrom December 2015 to June 2016. 50 samples of eggplant were analyzed which were collected from 10 markets of Mymensingh Sadar, Mymensingh. A simple and efficient multiple pesticide residue analytical method (QuEChERS extraction) and gas chromatography-flame thermonized detector (GC-FTD) were used for the determination of pesticide residues. Among the 50 analyzed samples, 11 (22% of the total number of the samples) contained pesticide residues of diazinon, dimethoate, quinalfos, and chlorpyrifos, of which, 2 had multiple pesticide residues and 5 samples contained residue above the European Union maximum residue limit (EU-MRLs). Chlorpyrifos was detected as the most used pesticide in eggplant in the studied area. Eggplant samples collected from K.R market did not contain any residues of pesticides.

* **Corresponding Author:** Mst. Afia Aktar ✉ shomaevn123@gmail.com

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. Eggplant is one of the most important, inexpensive and popular vegetable crops in Bangladesh. It is grown extensively in Chaina, India, Bangladesh, Pakistan, Philpines, Japan, Indonesia, Turkry, Greece, Italy, France, USA and Mediterranean and Balkan countries (Bose and Som, 1986), Currently in Bangladesh, 7.8 percent of the land under vegetable crops, 64,208 hectares is dedicated to growing about 340,000 metric tones of eggplant per annum (Anon, 2016). The benefit of eggplant are well known. It helps to prevent colon cancer, reduce cholesterol level, helps in type 2 diabetes management, is very rich in antioxidant and also helps to control weight.

Eggplant is very susceptible to insect attack and disease infestitation from seeding is fruting stage and it cause a negative economic impact on its production. Due to plant pest and disease, 20-40% of crop yeild reduces globally (FAO, 2012). For protecting this vegetable from being attacked by pets and disease farmers sprayed pesticide frequently even everyday. For example, farmers spray pesticides 140 times during a cropping season of 180-200 days and 150 sprays in a crop season in eggplant (Annon, 2000).

The development of agriculture, the improvement of the food production technology and mainly the demands of the new markets for better products both in quality and quantity have been made among others with intensive uses of pesticides for ensuring productivity (Trevisan *et al.,* 2004). Pesticide being toxic can become a potential hazard to the manufacturers, the users, the public at large and the environment and it can produce negative impacts, both socially and economically (Antle and Pingali, 1994). 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.

The incidence of pesticides poisoning is increasing and it is estimated that about 5 million people die every year as a result of intentional, accidental and occupational exposure worldwide (Singh and Gupta, 2009). So, it has become an alarming issue to know what we are intaking day by day. The detection, identification, and quantification of pesticide in the food has become a matter of public interest. But very little references are available on the presence of pesticides in vegetables in Bangladesh (Khatoon *et al.,*2004). Therefore, this study was undertaken to identify and quantify pesticide residues in eggplant which are available in Mymensingh Sadar of Mymensingh district.

Materials and methods

Study area

The study area included major ten markets of Mymensingh Sadar, Mymensingh. Brinjals were collected from 10 markets: K.R market, Mesua bazar, Paurashava bazar, Shankipara bazar, Charpara bazar, Shambhuganj bazar, Sutiakhali bazar, Kewatkhali bazar, Khagdahar bazar and Chorkhai hat. These markets are famous for vegetables. The vegetables of these markets comes from different places covering a large area such as Char Gobadia, Char Nilakshmia, Kalibari char, Baganbari Char, Bobar Char, Bhabanipur, Gafargaon, Fulbaria, Ishwarganj, Paranganj, Char Khaircha, Char Gavindapur, Nuldug, Jailkhana road, Nandina, Joy bangla bazar, Konaparar Char,Jamalpur, Nurondi, Bidyagaon etc.

Sample collection

A total of 50 brinjal were collected for this study. Five samples of brinjal were collected from each market. Samples were collected in two steps.

On 14 December 2015, first 50 samples were collected from K.R market; Mesua bazar; Paurashava market; Shankipara bazar and Charpara bazaar. On 16 January 2016, the other 50 samples were collected from Shambhuganj bazar; Sutiakhali bazar; Kewatkhali bazar; Khagdahar bazar and Chorkhai hat. The sources of the vegetables were encoded from the shopkeepers (Table 1).

The amount of eggplant for each sample was 800gm and was collected in transparent clean air tight polyethylene bag and each bag was properly labeled with sample number and sources. Individual numbers of sample was collected in individual polyethylene bag to avoid cross contamination.

Chemicals and reagents

The standard ofchlorpyrifos, 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 methanol 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

In this study the QuEChERS extraction technique was used which was modified by Prodhan *et al.*,2015.The chopped samples were grounded thoroughly with the fruit grinder (Handmixer M-122, Bamix, Switzerland). 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 acetonitrile 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 thermonized detector (FTD) for the detection of acephate, dimethoate, diazinon, fenitrothion, malathion, chlorpyrifos and quinalphos. The capillary column was AT-1, length was 30m, ID was 0.25mm and film thickness was 0.25μm. 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 of peaks in the pure analytical standards (Fig. 1). The instrument conditions are described in Table 2 and Table 3.

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 three to four 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 which represented the concentration of the final volume injected. From this value, the actual amount of pesticide residue present in the sample was determined by using the following formula:

$$\text{Residue in sample}\left(\frac{mg}{kg}\right) = \frac{\text{conc. obtained in injected vol}\left(\frac{mg}{kg}\right) \times \text{amount of final vol. (l)}}{\text{amount of sample taken}}$$

Results and discussion

Identification of pesticides

After performing GC-FTD analysis it was confirmed that diazinon, chlorpyrifos, dimethoate, quinalphos were presented in the sample collected from Mesua bazar, Sutiakhali bazar, Charpara bazar and Kewatkhali (Eg-Mb$_{8}$, Eg-Su$_{39}$, Eg-Cb$_{22}$ and Eg-K$_{27}$).

Sample collected from Shambhuganj bazar (Eg-Sha$_{31}$) showed the peak of diazinon, chlorpyrifos and quinalphos.

Quantification of pesticide residues

The levels of pesticide residues found in the analyzed eggplant samples and their maximum residue levels are outlined in Table 4.

Fifty samples of eggplant from each of the ten markets (K.R market; Mesua bazar; Paurashava market; Shankipara bazar; Charpara bazar; Kewatkhali bazar; Shambhuganj bazar; Sutiakhali bazar; Khagdahar bazar and Chorkhai hat) were analyzed to find out the presence of the left over residue of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos.

Table 1. Sources and places of collection of eggplant samples.

Area of collection	Sample ID	Source
K.R. market	Eg-KR$_{1}$	Char Gobadia
	Eg-KR$_{2}$	Char Nilakshmia
	Eg-KR$_{3}$	Kalibari Char
	Eg-KR$_{4}$	Bobar Char
	Eg-KR$_{5}$	Bobar Char
Mesua bazar	Eg-Mb$_{6}$	Baganbari Char
	Eg-Mb$_{7}$	Bobar Char
	Eg-Mb$_{8}$	Baganbari Char
	Eg-Mb$_{9}$	Gafargaon
	Eg-Mb$_{10}$	Gafargaon
Paurashava market	Eg-Pm$_{11}$	Paranganj
	Eg-Pm$_{12}$	Paranganj
	Eg-Pm$_{13}$	Char Gavindapur
	Eg-Pm$_{14}$	Bobar Char
	Eg-Pm$_{15}$	Bobar Char
Shankipara bazar	Eg-Sb$_{16}$	Bobar Char
	Eg-Sb$_{17}$	Nandina
	Eg-Sb$_{18}$	Konapara Char
	Eg-Sb$_{19}$	Jamalpur
	Eg-Sb$_{20}$	Nurondi
Charpara bazar	Eg-Cb$_{21}$	Bobar Char
	Eg-Cb$_{22}$	Nandina
	Eg-Cb$_{23}$	Nandina
	Eg-Cb$_{24}$	Nandina
	Eg-Cb$_{25}$	Bobar Char
Kewatkhali	Eg-K$_{26}$	Bobar Char
	Eg-K$_{27}$	Bobar Char
	Eg-K$_{28}$	Char Nilakshmia

	Eg-K_{29}	Char Nilakshmia
	Eg-K_{30}	Bobar Char
Shambhuganj	Eg-Sha_{31}	Jamalpur
	Eg-Sha_{32}	Jamalpur
	Eg-Sha_{33}	Gafargaon
	Eg-Sha_{34}	Char Gobadia
	Eg-Sha_{35}	Char Nilakshmia
Sutiakhali	Eg-Su_{36}	Nandina
	Eg-Su_{37}	Baganbari Char
	Eg-Su_{38}	Char Nilakshmia
	Eg-Su_{39}	Char Nilakshmia
	Eg-Su_{40}	Bobar Char
Khagdahar	Eg-Kh_{41}	Bobar Char
	Eg-Kh_{42}	Gafargaon
	Eg-Kh_{43}	Gafargaon
	Eg-Kh_{44}	Char Gobadia
	Eg-Kh_{45}	Bobar Char
Chorkhai hat	Eg-Ch_{46}	Char Nilakshmia
	Eg-Ch_{47}	Baganbari Char
	Eg-Ch_{48}	Bobar Char
	Eg-Ch_{49}	Kalibari Char
	Eg-Ch_{50}	Bobar Char

Out of 50 samples, 11 samples (22% of the total number of samples) contained pesticide residues and 39 samples (78% of the total number of samples) contained no detectable residues of the sought pesticides (Table 4). Islam *et al.*, 2014 collected 42 samples of eggplant, cauliflower and country bean from fields and markets of Narshingdi district, Bangladesh, where they found 15 samples (above 68% of the total number of samples) contained no pesticide residues. Prodhan *et al.*, 2015 collected 72 samples of eggplant from different market places in Thessaloniki, Greece and found that 38 samples did not contain any pesticide residues. Hossain *et al.*, 2015 studied 25 samples of eggplant, cucumber and tomato which were collected from Savar bazar, Savar, Dhaka and found that only 8 samples contained with pesticide residues, of which 5 exceeded the maximum residue limits recommended by FAO/WHO. Farag *et al.*, 2011 collected 132 samples of fruits, vegetables, herbs and spices collected from Egyptian local markets and reported that only 72 samples were contaminated with pesticide residues which were higher than the present study.

Table 2. Conditions for 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 3. Conditions for column oven.

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

Samples collected from K.R market contained no detectable residues of the investigated pesticides. Among the five samples collected from Mesua bazar, two sample (Eg-Mb_8 and Eg-Mb_{10}) showed detectable residues of diazinon of 0.020 mg/kg and 0.023 mg/kg, which were below the maximum residue limits (0.5 mg/kg) according to FAO/WHO Codex Alimentarius Commission (Table 4). Islam *et al.*, 2014 collected 42 samples of eggplant, cauliflower and country bean from fields and markets of Narshingdi district, Bandgladesh and observed that the residue level of diazinon in eggplant was 0.5 mg/kg.

Table 3. Conditions for column oven.

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

Table 4. The level of residues of different pesticides in eggplants.

Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)
Eg-Mb8	Diazinon	0.020	0.5
Eg-Mb10	Diazinon	0.023	0.5
Eg-Pm13	Dimethoate	0.109	0.02
Eg-Sb16	Chlorpyrifos	0.163	0.5
Eg-Cb22	Dimethoate	0.054	0.02
Eg-K27	Chlorpyrifos	1.617	0.5
	Quinalphos	0.363	0.2
Eg-Sha31	Diazinon	0.0146	0.5
	Chlorpyrifos	0.045	0.5
	Quinalphos	0.018	0.2
Eg-Sha34	Chlorpyrifos	0.166	0.5
Br-Su39	Chlorpyrifos	0.895	0.5
Br-Kh45	Chlorpyrifos	0.093	0.5
Br-Ch48	Chlorpyrifos	0.083	0.5

Again, among five samples of Paurashava market, only one sample (Eg-Pm13) contained residues of dimethoate of about 0.109 mg/kg which was above the corresponding maximum residue limits (0.02 mg/kg) (Table 4). Among the 5 samples of Shankipara bazar, 0.163 mg/kg of chlorpyrifos was detected in one sample (Eg-Sb16) which was below the maximum residue limits (0.5 mg/kg)(Table 4). The presence of this pesticide residue in vegetables makes the safety of eggplant more venerable to public health. Even though, in Bangladesh eggplant usually did not eat raw, the stir fry or boil of eggplant could reduce this problem (Tanabe *et al.,* 2000).

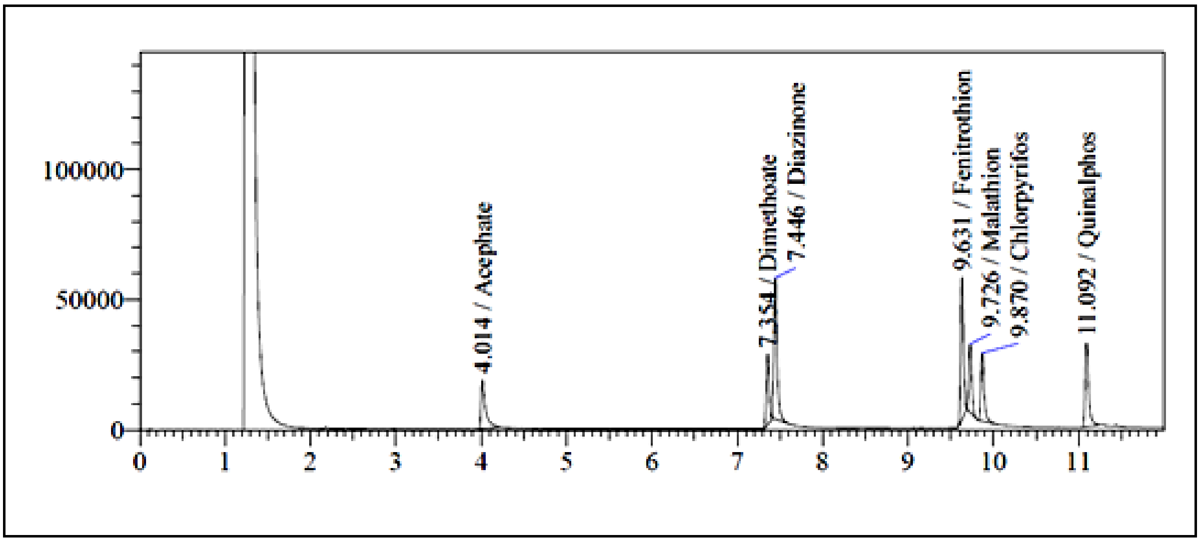

Fig. 1. Typical chromatogram showing standard peaks of sought pesticides.

In case of samples collected from Charpara bazar, only one sample (Eg-Cb_{22}) showed detectable residues of dimethoate of 0.054 mg/kg which was above the maximum residue limits (0.02 mg/kg) (Table 4). Prodhan *et al.* (2015) collected 72 samples of eggplant from different market places in Thessaloniki, Greece and reported that the residue level of dimethoate was 0.01 mg/kg in eggplant.

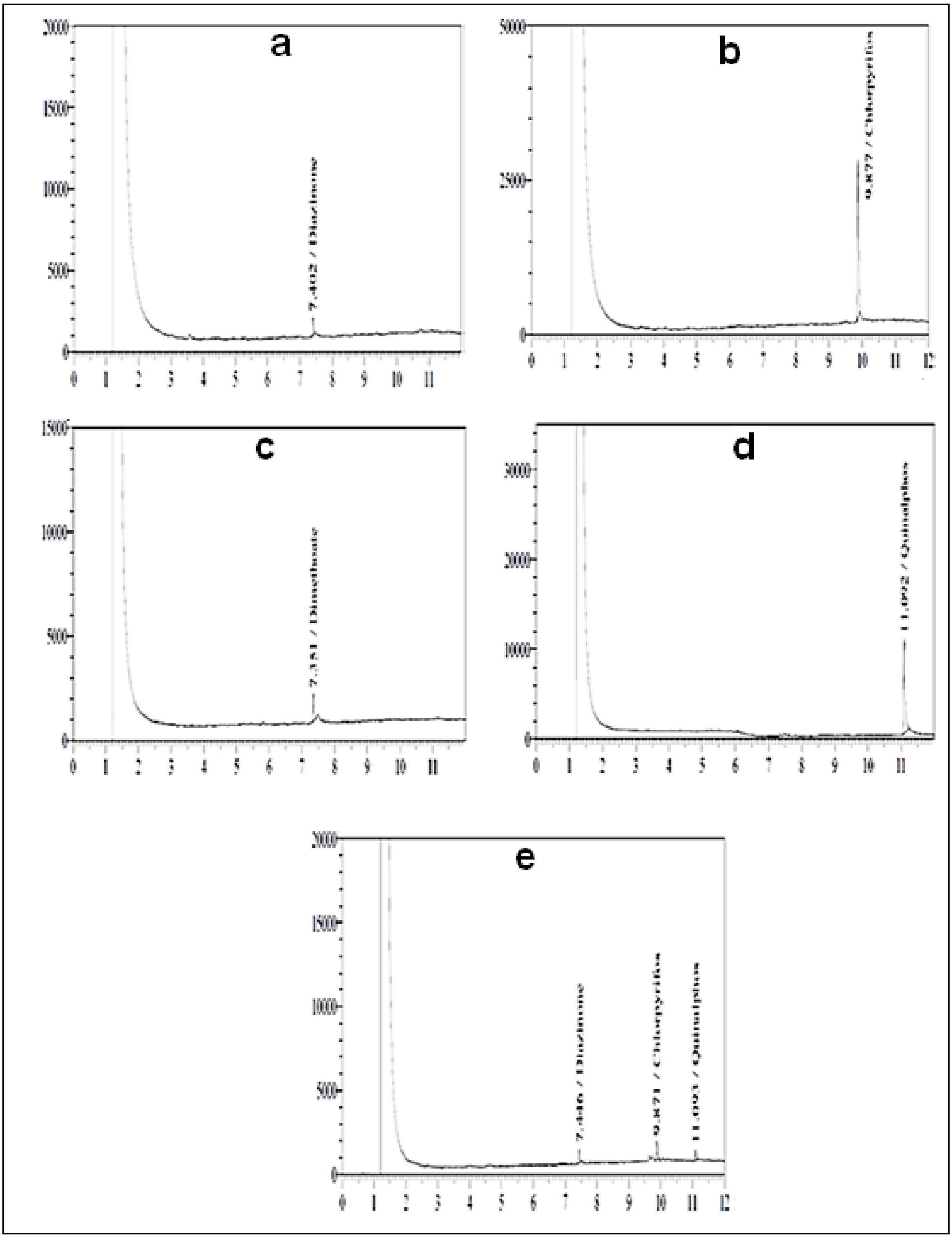

Fig. 2. Detected pesticides in eggplant samples (a) diazinon in Eg-Mb_8; (b) chlorpyrifos in Eg-Su_{39}; (c) dimethoate in Eg-Cb_{22}; (d) quinalphos in Eg-$K_{27}$and (e) diazinon, chlorpyrifos and quinalphos in Eg-Sha_{31}.

On the other hand, 1.617 mg/kg of chlorpyrifos and 0.363 mg/kg of quinalphos were detected in Eg-K_{27} which was collected from Kewatkhali bazar (Table 4). Both of the pesticides detected in this sample were above the maximum residue limits of 0.5 mg/kg and 0.2 mg/kg, respectively. Islam *et al.*, 2014 collected 42 samples of eggplant, cauliflower and country bean from fields and markets of Narshingdi district, Bangladesh and reported that the residues of quinalphos in eggplant were 0.016 mg/kg. Ahmed *et al.*, 2013 collected 75 samples of eggplant from farmer's field of Jessore, Gazipur and Rangpur and found multi product residues of three pesticides such as acephate, fenitrothion and quinalphos in eggplant where acephate residues were below the maximum residue limits and fenitrothion and quinalphos residues were above the MRLs.

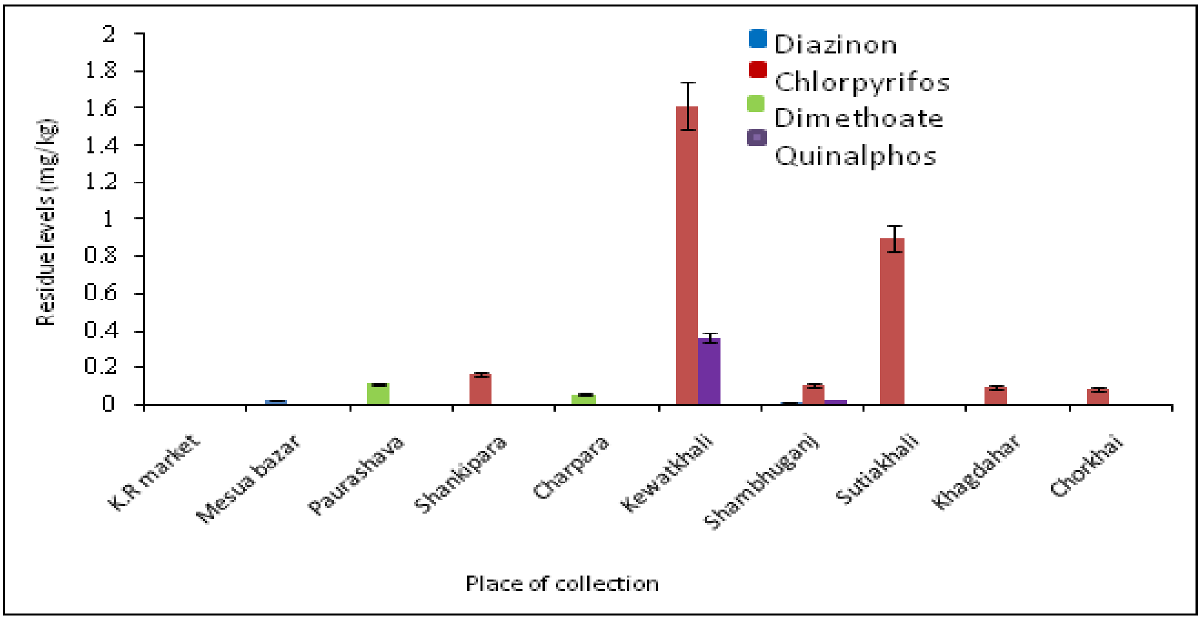

Fig. 3. Pesticides residue levels (mg/kg) in eggplants at different markets.

Diazinon, chlorpyrifos and quinalphos were found at the concentration of 0.0146 mg/kg, 0.045 mg/kg and 0.018 mg/kg in Eg-Sha_{31} sample collected from Shambhuganj bazar. All the three pesticide in Eg-Sha_{31} were below the maximum residue limits. In Eg-Sha_{34} sample which was also collected from Shambhuganj bazar contained detectable limits of chlorpyrifos of 0.166 mg/kg which was below the MRLs (0.5 mg/kg).

In Eg-Su_{39}, Eg-Kh_{45} and Eg-Ch_{48}eggplant samples collected from Sutiakhali bazar, Khagdahar bazar and Chorkhai hat chlorpyrifos were detected as 0.895 mg/kg, 0.093 mg/kg and 0.083 mg/kg, respectively.

Among which the sample of Sutiakhali bazar contained residues above the MRLs of 0.5 mg/kg. Hossain *et al.*, 2015 collected 15 samples of tomato, lady's finger and eggplant from Savar bazar, Savar, Dhaka and reported that, thechlorpyrifos residues were 1.03 mg/kg in eggplant.

Comparative analysis of residues in different sources of collection

Market wise pesticide residues in eggplant has given in Fig. 3. The figure revealed that pesticide residues varied from market to market.

Among the detected pesticide (diazinon, chlorpyrifos, dimethoate and quinalphos), the residues of chlorpyrifos was higher in the studied market compared to the other pesticides.

From Fig. 3, it is found that pesticide residues in eggplant were higher at Kewatkhali bazar followed by Sutiakhali bazar. Here, eggplant were collected from Bobar char and Char Nilakshmia.

On the other hand, eggplant collected from K.R market did not contain any pesticide residues. Although eggplants were brought from the same sources as of Kewatkhali bazar. The residue levels in eggplant collected from other markets were below the MRLs.

Although the sources are same, but residue differs from market to market. There might be some reason behind this. Firstly, perhaps some farmers brought their eggplants in market just after spraying while the others brought them after certain days of spraying pesticide within which the pesticide become degraded. Secondly, the farmers perhaps used pesticide which was not presented in our sought pesticides such as acephate, diazinone, malathion, dimethoate, chlorpyrifos, quinalphos and fenitrothion. Thirdly, the level of pesticide residue differ from field to field, plant to plant even a plant of different parts. This occurs specially when spraying pesticide, some vegetables do not get pesticide hindering by leaf and stem of the plant while the other receives pesticide. Prodhan *et al.,* 2016 conducted an experiment on cauliflower and found that the level of cypermethrin residue in one sample was 0.425 mg/kg, whereas another sample from the same field contained residue of cypermethrin level as 0.01 mg/kg.

Conclusion

Among the 50 analyzed samples, 11 (22% of the total number of the samples) contained pesticide residues of diazinon, dimethoate, quinalfos, and chlorpyrifos, of which, 2 had multiple pesticide residues and 5 samples contained residue above the European Union maximum residue limit (EU-MRLs). Chlorpyrifos was detected as the most used pesticide in eggplant in the studied area. Eggplant samples collected from K.R market did not contain any residues of pesticides.

Acknowledgement

All praises are due to the Almighty Allah, Who enabled the author to complete the present piece of research work successfully. The author would like to express her greatest gratitude to her supervisor, Professor Dr. Rehana Khatun, Department of Environmental Science, Bangladesh Agricultural University, Mymensingh for her guidance, criticism, encouragements and valuable suggestions throughout the tenure of the research work. The author humbly avails the opportunity to express her immense indebtedness, sincere appreciation to her co-supervisor, Dr. Mohammad Dalower Hossain Prodhan, Senior Scientific Officer, Division of Entomology, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh for his immense support, proper guidance, keen interest, continuous inspiration and ongoing advice and encouragement.

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