ANALYSIS OF PESTICIDE RESIDUES IN VEGETABLES COLLECTED FROM DIFFERENT MARKETS OF DHAKA

RAKIBUL HASAN

DEPARTMENT OF ENTOMOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

DECEMBER, 2015

ANALYSIS OF PESTICIDE RESIDUES IN VEGETABLES COLLECTED FROM DIFFERENT MARKETS OF DHAKA

BY

RAKIBUL HASAN

Registration No. : 10-04132

A Thesis

Submitted to the Department of Entomology
Sher-e-Bangla Agricultural University, Dhaka, in partial
fulfillment of the requirements
for the degree
of

MASTER OF SCIENCE
IN
ENTOMOLOGY

SEMESTER: JULY-DECEMBER, 2015

Approved by

Supervisor	**Co-Supervisor**
(Dr. Mohammad Dalower Hossain Prodhan) **Senior Scientific Officer** Entomology Division, Bangladesh Agricultural Research Institute Gazipur-1701, Bangladesh.	**(Dr. S M Mizanur Rahman)** **Associate Professor** Dept. of Entomology SAU, Dhaka-1207

(Dr. Mohammed Sakhawat Hossain)
Chairman
Examination Committee

DEPARTMENT OF ENTOMOLOGY
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

CERTIFICATE

This is to certify that the thesis entitled **“ANALYSIS OF PESTICIDE RESIDUES IN VEGETABLES COLLECTED FROM DIFFERENT MARKETS OF DHAKA”** submitted to the Department of Entomology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (M.S.)** in **ENTOMOLOGY**, embodies the result of a piece of bona fide research work carried out by **RAKIBUL HASAN**, Registration No. **10-04132** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dr. Mohammad Dalower Hossain Prodhan
Senior Scientific Officer
Entomology Division,
Bangladesh Agricultural Research Institute
and
Research Supervisor

December, 2015
Dhaka, Bangladesh

ACKNOWLEDGEMENT

All praises to the **“Almighty Allah”** who enable me to complete a piece of research work and prepare this thesis for the degree of Master of Science (M.S.) in Entomology.

He feels much pleasure to express his gratefulness, sincere appreciation and heartfelt liability to his venerable research supervisor **Dr. Mohammad Dalower Hossain Prodhan**, Senior Scientific Officer, Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur. for his scholastic guidance, support, uninterrupted encouragement, valuable suggestions and constructive criticism throughout the study period.

He also expresses his gratitude and thankfulness to reverend co-supervisor **Dr. S M Mizanur Rahman** and Chairman, **Dr. Mohammed Sakhawat Hossain,** Department of Entomology, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207 for their constant inspiration, valuable suggestions, cordial help, heartiest co-operation and supports throughout the study period.

The author would like to express his grateful thanks to all teachers of the Department of Entomology for their constructive suggestions and advice during the study period.

The author expresses heartfelt respect and sincere gratitude to **Dr. Syed Nurul Alam**, Chief Scientific Officer and Head, Entomology Division, BARI, Gazipur for his necessary and friendly help during the research work.

The author desires to express her cordial thanks to **Md. Kamal Hossain** and other office staff of Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur for their assistance and co-operation during the period of research work.

The author deeply acknowledges the profound dedication to his beloved Father, Mother, Sister and Brother for their moral support, steadfast encouragement and continuous prayer in all phases of academic pursuit from the beginning to the completion of study successfully.

Finally, the author is deeply indebted to his friends and well-wishers for their kind help, constant inspiration, co-operation and moral support which can never be forgotten.

December, 2015

Dhaka, Bangladesh **The Author**

ANALYSIS OF PESTICIDES RESIDUES IN VEGETABLES COLLECTED FROM DIFFERENT MARKETS OF DHAKA

Abstract

The study was conducted to analyze pesticide residues in three common vegetables (country bean, cauliflower and cucumber) collected from five different areas (Banani, Rampura, Mohammadpur, Kawran Bazar and Jatrabari) of Dhaka city from January 2016 to September 2016. The collected samples were analyzed using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and Gas Chromatography (GC) coupled with Flame Thermionized Detector (FTD) for the determination of pesticide residues in 50 samples of country bean, 30 samples of cauliflower and 20 samples of cucumber. 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). Out of 30 samples of cauliflower, 5 samples (16.67%) contained residues of Chlorpyrifos, Quinalphos, Acephate and Dimethoate, where 4 samples contained multiple pesticide residues. All the contaminated samples of cauliflower contained residue above MRL. On the other hand, among the 20 samples of cucumber, only 2 samples (10%) contained residue of Quinalphos and Chlorpyrifos, but the level of residue was below the MRL. Dimethoate was detected in most of the contaminated country bean sample, while Chlorpyrifos and Quinalphos were detected mostly in cauliflower and cucumber. This study reflects the overall scenario of pesticide contamination in vegetables specially in country bean, cauliflower and cucumber available in the local markets of Dhaka city, which will help the consumer to be aware of their health and safety.

TABLE OF CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
	ACKNOWLEDGEMENT	i
	ABSTRACT	ii
	TABLE OF CONTENTS	iii
	LIST OF TABLES	iv
	LIST OF FIGURES	v
	LIST OF PLATES	vii
	LIST OF ABBREVIATIONS	viii
1	**INTRODUCTION**	**1-6**
2	**REVIEW OF LITERATURE**	**7-30**
3	**MATERIALS AND METHODS**	**31-46**
	3.1 Study area	31
	3.2 Sample collection	33
	3.3 Sample preparation for analysis	36
	3.4 Chemicals and reagents	36
	3.5 Analytical Apparatus Required	36
	3.6 Preparation of pesticide standard solution	39
	3.7 Extraction and clean up	40
	3.8 Detection and quantification of pesticide residue in samples	41
	3.9 Calibration curve preparation	42
4	**RESULTS AND DISCUSSION**	**47-62**
	4.1 Pesticide residues in country bean	47
	4.2 Pesticide residues in cauliflower	55
	4.3 Pesticide residues in cucumber	59
5	**SUMMARY AND CONCLUSION**	**63-64**
6	**REFERENCES**	**65-82**

LIST OF TABLES

SERIAL NO.	TITLE	PAGE NO.
01	Sources and places of collection of Country bean samples	33
02	Sources and places of collection of Cauliflower samples	34
03	Sources and places of collection of Cumber samples	35
04	The instrument parameters for GC-FTD	42
05	Conditions for column oven temperature	42
06	The level of residues (mg/kg) of different pesticides found in the analyzed country bean samples	52
07	The level of residues (mg/kg) of different pesticides found in the analyzed cauliflower samples	57
08	The level of residues (mg/kg) of different pesticides found in the analyzed cucumber samples	61

LIST OF FIGURES

SERIAL NO.	TITLE	PAGE NO.
01	Map showing the places of sample collection	32
02	Typical Chromatograms of seven organophosphorus insecticide standards run by GC-FTD.	41
03	Calibration curve prepared for Dimethoate made with different concentrations ranging from 50 µg/L to 500 µg/L	43
04	Calibration curve prepared for Chlorpyrifos made with different concentrations ranging from 50 µg/L to 500 µg/L.	43
05	Calibration curve prepared for Acephate made with different concentrations ranging from 50 µg/L to 500 µg/L.	44
06	Calibration curve prepared for Quinalphos made with different concentrations ranging from 50 µg/L to 500 µg/L	44
07	Calibration curve prepared for Diazinon made with different concentrations ranging from 50 ug/L to 500 ug/L.	45
08	Calibration curve prepared for Fenitrothion made with different concentrations ranging from 50 µg/L to 500 µg/L.	45
09	Calibration curve prepared for Malathion made with different concentrations ranging from 50 µg/L to 500 µg/L.	46
10	Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB-3) showing retention time.	47
11	Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB-7) showing retention time	48
12	Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB-15) showing retention time.	48
13	Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB-22) showing retention time	49
14	Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB-28) showing retention time	49
15	Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB-34) showing retention time	50

LIST OF FIGURES (Cont'd)

SERIAL NO.	TITLE	PAGE NO.
16	Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB-39) showing retention time	50
17	Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB-41) showing retention time	51
18	Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB-45) showing retention time	51 52
19	Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB-49) showing retention time	
20	Chromatogram of Chlorpyrifos and Quinalphos found in one of the cauliflower marketed sample (DMCF$_{5}$) showing retention time	55
21	Chromatogram of Chlorpyrifos and Quinalphos found in one of the cauliflower marketed sample (DMCF$_{11}$) showing retention time	55
22	Chromatogram of Acephate, Chlorpyrifos and Quinalphos found in one of the cauliflower marketed sample (DMCF$_{19}$) showing retention time	56
23	Chromatogram of Chlorpyrifos and Quinalphos found in one of the cauliflower marketed sample (DMCF$_{24}$) showing retention time	56
24	Chromatogram of Dimethoate found in one of the cauliflower marketed sample (DMCF$_{29}$) showing retention time	57
25	Chromatogram of Quinalphos found in one of the cucumber marketed sample (DMCU$_{9}$) showing retention time	60
26	Chromatogram of Chlorpyrifos found in one of the cucumber marketed sample (DMCU$_{17}$) showing retention time	60

LIST OF PLATES

PLATE NO.	TITLE	PAGE NO.
01	Centrifuge machine	37
02	Electric balance	37
03	Homogenizer	37
04	Vortex mixer	37
05	Orbital shaker	38
06	Gas Chromatograph (GC)	38

LIST OF ABBREVIATIONS

ADI	Acceptable Daily Intake
AOAC	Association of Official Analytical Chemists
BARI	Bangladesh Agricultural Research Institute
CSN	Committee for Standardization
DAS	Days After Spray
DLLME	Dispersive Liquid–Liquid Micro Extraction
d-SPE	dispersive solid phase extraction
ECD	Electron Capture Detector
et al.	*et alibi* (and others)
etc.	*et cetra* (and so on)
EU	European Union
FAO	Food and Agriculture Organization
FTD	Flame Thermionized Detector
GC-MS	Gas Chromatograph-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
HRI	Hazard Risk Index
LC-MS	Liquid Chromatography-Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MDQ	Minimum Detectable Quantity
MRL	Maximum Residue Limit
PDI	Potential Daily Intake
PSA	Primary Secondary Amine
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RSM	Response Surface Methodology
RTL	Retention Time Locked
SAU	Sher-e-Bangla Agricultural University
SBSE	Stir Bar Sorptive Extraction
TOTAD	Through Oven Transfer Adsorption Desorption
UHPLC-MS/MS	Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry
WHO	World Health Organization

CHAPTER 1

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 play a significant role in human nutrition, especially as sources of phytonutriceuticals: vitamins (C, A, B1, B6, B9, E), minerals, dietary fiber and phytochemicals (Quebedeaux and Eisa, 1990; Craig and Beck, 1999; Wargovich, 2000; Dias and Ryder, 2011). A world vegetable survey showed that 402 vegetable crops are cultivated worldwide, representing 69 families and 230 genera (Kays and Dias 1995). 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 (Dias, 2011). Mostly, vegetable crops are cultivated under high pressure for achieving higher production.

Cauliflower is low in fat, high in dietary fiber, contains water and vitamin C, possessing a very high nutritional density. The warm and humid climatic condition of the country, increase use of modern high yielding varieties of crops and more use of chemical fertilizers are highly favorable for development and multiplication of pests and diseases. In Bangladesh context, the cauliflower growers have been using the pesticides frequently to have the higher yield. But the overdoses of pesticides make the residue problem, which might pollute our food and be harmful for our health. Cucumbers are scientifically known as *Cucumis sativus* and belong to the same botanical family as melons and squashes. Although, they have not received as much attention as other vegetables in terms of health benefits, but this widely-cultivated food provides a unique combination of nutrients, including antioxidant (vitamin C, beta-carotene, and manganese), anti-inflammatory and anti-cancer (Yang *et al*., 2006). The mean crude protein content of country bean is 17% with a range of 10% to 22% on a dry matter basis. Leaf crude protein varied from 14.3% to 38.5%, while the stem crude protein content range from 7.0% to 20.1%. Bean follows a familiar growth pattern as protein content drops with maturity (Milford and Minson 1968). It has been reported that some of the pesticides are being used on northern part of the country where pre-harvest time frame after application is not maintained. Additionally, the most alarming matter is that the frequent and indiscriminate use of pesticides causing severe health hazards to the consumers that may persist in amounts above prescribed Maximum Residue Limits (MRL).

Pesticides are widely used in agriculture to increase the yield, improve the quality, and extend the storage life of food crops (Fernandez-Alba and Garcia-Reyes 2008). The pesticides must undergo extensive efficacy, environmental, and toxicological testing to be registered by governments for legal use in specified applications. The applied chemicals and/or their degradation products may remain as residues in the agricultural products, which becomes a concern for human exposure. The problem becomes more acute if the xenobiotics are used close to harvest as well as during transit and in vegetable yards. In order to reduce the loss and maintain the quality of fruits and vegetables harvest, pesticides are used together with other pest management techniques during cropping to destroy pests and prevent diseases. One potential drawback associated with a shift toward more intensive vegetable production is the reliance of most vegetable producers on heavy application of pesticide (Hossain *et al*., 2000).

The use of pesticides has increased because they have rapid action, decrease toxins produced by food infecting organisms and are less labor intensive than other pest control methods (Gilden *et al.,* 2010). In Bangladesh, the application of pesticides has become unavoidable to uphold and improve the existing stage of harvest production by protecting the crop from pests. The climate of Bangladesh as being a subtropical, observes varying temperatures and humidity profile throughout the year, which brings a vast array of pests to be tackled. A number of pests are found to attack multiple crops and have been attained resistance from the prolonging application of common pesticides. Presently, it is estimated that almost 45% of the world crops have been destroyed by plant pests and diseases. Therefore, to meet the demand, it is essential to apply pesticides to protect the crops, both during development and their consequent storage and transportation.

Probably 2.5 million tons of pesticides are being applied globally each year and keep on rising with the passage of time (FAO/WHO 2002). Bangladesh has been using pesticides since 1950's in agriculture and public health. About 80 registered pesticides having 170 different trade names are commonly used in agriculture and public sectors in Bangladesh. The indiscriminate use of pesticides has created very serious health and environmental problems in many developing countries. Worldwide, one to five million farm workers are estimated to suffer pesticide poisoning every year. Most of the poisonings take place in rural areas of developing countries, where safeguards typically are inadequate or lacking altogether. Although developing countries use 25% of the world's production of pesticides, they experience 99% of the deaths due to pesticide poisoning (WHO 2004). The World Health Organization (WHO 2005) and United Nations Environmental Program have estimated one to five million cases of pesticide poisoning among agricultural workers each year with about 20,000 fatalities, mostly reported from developing countries (Pimental *et al.,* 1992).

Pesticides create several adverse effects not only on the human health, but also on the environment. Pesticide being toxic can become a potential hazard to the manufacturers, the users, the public at large and the environment. Pesticide 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 harmful pesticides are dissolved in our water system and ultimately enter into the human ecosystem, fishes and many other animals and cause severe damage to their health (Khandakar 1990). The sole reliance on pesticide applications has shown many side effects and limitations (Aktar *et al*., 2009). At present, inappropriate and irrational use of pesticides for the control of insect pest and diseases of vegetables is the common practice in Bangladesh (Kabir *et al*., 1996). In Bangladesh it has been reported that farmers sprayed insecticides quite frequently even every day in vegetables which are severely attacked by insect pests (Anon. 2000). Serious consequences of indiscriminate overuse and misuse of pesticides results in a disruption in the agro-ecosystem, human health hazard and environmental pollution (MacIntyre *et al.,* 1989).

In Bangladesh, farmers have no idea about the pesticide residues in the food as well as their ill effect on human health and the environment. In Bangladesh, whimsical spray of insecticides and selling of vegetables after one to two days of spray application are assumed to be a normal practice. No insecticide is available, whose retention period which is less than three to five days (Rahman 1999). The present pattern of pesticide usage in Bangladesh particularly in vegetables led to assume that majority of marketed vegetables contain pesticide residue more than Maximum Residue Limit (MRL) (Kabir 2007).

As crop production and pesticides are closely related and their left over residues might or might not persist in the environment that should be carefully examined and monitored. Modern pesticide residue analysis in developed countries is focusing more and, more on subtle problems, such as looking for very low concentrations of pesticides in the environment. This complicated and expensive equipment is being used. The detection and monitoring of pesticide residues, particularly in vegetable and fruits is being done in regular fashion in many countries (Kumari *et al*., 2004), but in Bangladesh it is just on the way to start. However, knowledge of the withholding period becomes important, even for less persistent pesticides, specifically in fruits and vegetables since these crops are harvested shortly after pesticide application. The problem of food contamination with pesticide residues is a cause of concern for almost everyone and everywhere. Pesticide residues above the Maximum Residue Limit (MRL) in the crop at harvest are a globally and nationally cause of great concerns. The gravity of the problem of residues is augmented by untimely, uneconomical and unscrupulous spraying of pesticides. These residues make food commodities hazardous for consumption and export and they also pollute the environment (FAO/WHO 1996). Maclntyre *et al.* (1989) reported that low level exposure of food products containing pesticide residues to consumers over time might cause cancer, teratogenesis, genetic damage and suppression of the immune system. Pesticide residues in food have become a consumer’s safety issue and the consumers have the right to know how much pesticide gets incorporated in the food they eat. The detection and quantification of pesticides in the food are a problem of increasing public interest. For that matter, in many countries, integrated pest management (IPM) system of agriculture is being encouraged as part of good agricultural practice (GAP). Besides, consumers are showing more and more interest in organic agricultural commodities.

As a safety measure for the consumers, many developed countries have set Maximum Residue Limit (MRL) based on the Acceptable Daily Intake (ADI) and Potential Daily Intake (PDI) that should not be exceeded for a food item to be considered safe for consumption (MacIntyre *et al*., 1989). In Bangladesh, since harvesting and selling of vegetables are done without perplexing for the pre-harvest interval, pesticide residue levels in such vegetables would undoubtedly be above Maximum Residue Limit. In Bangladesh, most of the growers are illiterate and they are not able to read and understand what is written on the label of pesticides. They mostly depend on ill motive pesticide dealers/retailers of their respective locations who have no clear idea about insect pests and pesticides but usually recommend insecticides that create serious problems for public health and the environment.

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 detection, identification, and quantification of pesticide in the food are becoming the public interest. Different scientists (Karanth, 2000; Dasika *et al.,* 2011; Hrouzkova and Matisova, 2011**)** analyzed pesticide residues in fruit and vegetables in India, Spain, China, Japan and other developed country. But very little references are available on the presence of pesticides in vegetables in Bangladesh (Islam 2014, Khatoon *et al.,* 2004). 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 (Kabir *et al*., 2007; Kabir *et al*., 2008; Prodhan *et al.,* 2009; Prodhan *et al.,* 2010; Islam, 2014). 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 with the following objectives:

OBJECTIVES

- To identify different pesticides present in the selected vegetables available in the markets of Dhaka City.
- To measure the amount of detected pesticide residues (mg/Kg) remain in the selected vegetables.
- To compare the amount of detected pesticide residues (mg/Kg) found in the selected vegetables with the Maximum Residue Limit (MRL).

CHAPTER 2

REVIEW OF LITERATURE

In this chapter attempt has been made to review literatures for updating the knowledge regarding the current status of research and information about determination of pesticide residues in fruits and vegetables. Available and accessible sources of information have been thoroughly reviewed and summarized with critical comments as appropriately as possible. Despite the fact that there have been limited source of information, most of the relevant information available in and around Bangladesh was collected and reviewed. It is revealed that most of the information on the aspects searched as mentioned above are mostly available from research station and information of farmers' field condition are scanty. However, a significant number of study-reports on insecticides residues in vegetable crops conducted under farmers' field conditions are available. The studies on the quantification of detected insecticides residues below or above the Maximum Residue Limit (MRL) of vegetables in Bangladesh are rarely reported. With this background, the information collected from different sources have been reviewed and presented below:

Zhang *et al.* (2016) developed a rapid, efficient, and environmentally friendly method using quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method combined with ionic liquid-based dispersive liquid-liquid micro extraction (QuEChERS-IL-DLLME) prior to high-performance liquid chromatography coupled with photodiode array detection (HPLC-PDA) has been developed for the determination of six triazole fungicides (triazolone, triadimenol, epoxiconazole, flusilazole, tebuconazole, and diniconazole) in various fruits (pear, apple, and grapefruit). And the proposed method was successfully applied for the determination of trace amounts of triazole fungicides in various fruits including pear, apple, and grapefruit.

Andraščíková and Hrouzková (2016) developed a fast, efficient, and simple method for determination of pesticide residues in pumpkin seeds by combining QuEChERS and dispersive liquid–liquid micro extraction (DLLME) followed by gas chromatography and mass spectrometry (GC-MS). The developed and validated method was successfully applied for the extraction and determination of pesticide residues in 16 real samples with 2 positive findings below maximum residue limits (MRL). Limits of detection (LODs) of the proposed method are below the MRLs established by the European Union.

Prodhan *et al.* (2016) have been detected three insecticides (chlorpyrifos, cypermethrin and deltamethrin) and two fungicides (fluopicolide and propamocarb hydrochloride) in the cabbage samples collected from different market places in Thessaloniki, Greece. Among the 132 analyzed samples, 41 (31% of the total no. of samples) had pesticide residues, of which, 2 had multiple pesticide residues and 39 had single pesticide residues.

Prodhan *et al.* (2016) have also been detected four insecticides (chlorpyrifos, cypermethrin, deltamethrin and indoxacarb) in the cauliflower samples collected from different market places in Thessaloniki, Greece. Among the 120 analyzed samples, 48 (40% of the total no. of samples) were found to have pesticide residues.

Park *et al.* (2016) investigated a total of 230 pesticide residues in 8496 samples of leafy vegetables (e.g.brassica lee ssp. namai, leafy lettuce, spinach, perilla leaves, crown daisy, marshmallow, aster scaber, pimpinella brachycarpa and Chinese chive). The result showed that among 8496 samples, 61 different pesticides were detected in 890 samples, of which 118 samples exceeded the Korean maximum residue limits (KMRLs).

Rai *et al.* (2016) conducted a research using quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction method combined with dispersive liquid-liquid micro extraction (DLLME) for the quantitative determination of 36 multiclass, multiresidue pesticides (13 organochlorines, 11 organophosphates, and 12 synthetic pyrethroids) in different vegetables and fruits without primary and secondary amine (PSA) cleanup step followed by gas chromatography-mass spectrometry (GC-MS) analysis. The samples collected from Lucknow City, India, were analyzed for the presence of pesticides and only three pesticides β-cypermethrin, λ-cyhalothrin, and chlorpyrifos were found to have value above PFA-1954/CODEX-MRL values.

Zanella *et al.* (2016) conducted a research on different extraction procedures based on the QuEChERS method for the multi-residue determination of pesticides in orange juice by ultra high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS). After choosing preliminary conditions, an experimental design was carried out with the variables of C18, PSA, NaOH and CH_3COONa to optimize the sample preparation step. The validation results of the method were satisfactory, since the method presented recoveries between 70% and 118%, with RSD lower than 19% for spike levels between 10 and 100 µg/L. The method limit of detection (LOD) and limit of quantification (LOQ) ranged from 3.0 to 7.6 µg/L and from 4.9 to 26 µg/L respectively. The method developed was adequate for the determination of 74 pesticide residues in orange juice.

Prodhan *et al.* (2015) determine seven insecticides (chlorpyrifos, dimethoate, deltamethrin, thiamethoxam, thiacloprid, pirimicarb and indoxacarb) and three fungicides (azoxystrobin, fluopicolide and propamocarb hydrochloride) in 122 fresh melon samples which was collected from different market places in Thessaloniki, Greece They found the average recoveries of the selected pesticides ranged from 82% to 106% with RSDr ≤6% in four fortification levels of 0.01, 0.05, 0.1 and 0.2 mg/kg and the correlation coefficient (R2) was ≥0.997 for all the selected pesticides The LOD values ranged from 0.001 to 0.003 mg/kg, and the LOQ was determined at 0.01 mg/kg for all the analytes. Among the 122 analysed samples, 32 (26% of the total no. of samples) were found to have pesticide residues.

Marek Biziuk and Jolanta Stocka (2015) conducted a research on ‘‘Multi residue Methods for Determination of Currently used Pesticides in Fruits and Vegetables Using QuEChERS Technique’. They discussed the extraction and determination of pesticide residues in fruit and vegetable samples, as are the techniques most commonly used in these processes. They also outlined the difficulties occurring at each stage in the analytical procedure.

Anastassiades *et al.* (2003) described the quick, easy, cheap, effective, rugged, low solvent consumption, wide pesticide range (Polar, pH – dependent compounds) and safe method for pesticide residues in food as an example of a method that takes advantage of the powerful features of nearly universal selectivity and high sensitivity of modern GC- and LC-MS(/MS) instruments. The QuEChERS approach has been extensively validated for hundreds of pesticide residues in many types of foods, and has become Association of Analytical Communities (AOAC) Official Method 2007 (Lehotay *et al.,* 2007). The QuEChERS method has several advantages over most traditional methods of analysis. High recoveries (greater than 85%) are achieved for a wide polarity and volatility range of pesticides, including notoriously difficult analytes. Very rugged because extract clean up is done to remove organic acids. The most common approach is to use matrix-matched calibration standards. However, it can be difficult to find a blank matrix from which to prepare the calibration standards and compensation from one sample to another (even for the same matrix) may not be the same. A method of standard additions in the sample extract may be an alternative approach.

Nowadays the QuEChERS technique, is widely used for the extraction and clean-up of the extracts of fruit and vegetable matrices (Schenck and Hobbs 2004; Cajka *et al.,* 2005; Lehotay *et al.,* 2005; Liu *et al*., 2005; Aysal *et al*., 2007; Banerjee *et al.,* 2007; Bolanos *et al.,* 2007; Lehotay *et al.,* 2007; Mol *et al.,* 2007; Paya *et al*., 2007; Wang *et al.,* 2007; Banerjee *et al.,* 2008; Fernandez Moreno *et al.,* 2008; Hayama and Takada, 2008; Ji *et al.,* 2008; Kirchner *et al.,* 2008; Kmellar *et al.,* 2008; Kovalczuk *et al*., 2008; Kruve *et al.,* 2008; Mezcua *et al.,* 2008; Nguyen *et al.,* 2008a; Romero-Gonzalez *et al*., 2008; Schenck *et al*., 2008; Walorczyk 2008; Banerjee *et al.,* 2009; Cunha *et al.,* 2009; Huskova *et al*., 2009; Li *et al.,* 2009; Schenck *et al.,* 2009; Zhang *et al.,* 2009; Gilbert-Lopez *et al.,* 2010; Lehotay*,* 2010; Chandrasekaran, 2012; Dasika *et al.,* 2012; Paramasivam and Chadrasekaran, 2012; Singh *et al.,* 2012; Mantzos *et al.,* 2013; Prodhan *et al.,* 2015). This technique was modified by several authors (AOAC Official methods, 2007.1; the European Committee for Standardization (CSN) Standard Method, CSN EN 15662, 2008). This technique is gaining popularity day by day compared to the other existing techniques as it has a lot of advantages. The most importants are the high analyte recoveries, the low organic solvent consumption, and the low cost per sample.

Hossain *et al.* (2015) studied that among Organophosphorus pesticides, Diazinon and Chlorpyrifos were detected in collected samples from Bogra district. Detectable amount of Diazinon was found in one Bringal sample (BS-4 at 0.32 ppm) among ten Bringal samples. It was also detected in one Cucumber sample CS-5 (0.18 ppm) among ten Cucumber samples and one Tomato sample (TS-3 at 0.57 ppm) among five

Tomato samples. Chlorpyrifos was being found in one Bringal sample (BS-7 at 0.4 ppm). It was also detected in three Cucumber samples among ten Cucumber samples. The IDs of Chlorpyrifos positive were CS-2 (0.05 ppm), CS-7 (0.02 ppm)

Prodhan *et al.* (2015) conducted a research on ‘‘Determination of Multiple Pesticide Residue in Eggplant with Liquid Chromatography-Mass Spectrometry’’ by adopting (QuEChERS) extraction and liquid chromatography triple quadrupole-mass spectrometry was developed and validated for the determination of ten insecticides and three fungicides in 72 fresh eggplant fruit samples collected from different market places in Thessaloniki, Greece. The method was validated by evaluating the accuracy, precision, linearity, LOD, and LOQ and the average recoveries of the selected pesticides ranged from 71.8 to 112 % with precision in case of repeatability (RSDr) ≤16.2 % in four fortification levels of 0.01, 0.05, 0.1, and 0.5 mg/kg. They found the linearity about ≥0.997 for the entire selected pesticides.The limit of detection (LOD) ranged from 0.001 to 0.003 mg/kg and the limit of quantification (LOQ) was 0.01 mg/kg, which was lower than the Maximum residue levels set by European Union (EU-MRLs). Among the 72 analyzed samples, 34 (47 % of the total no. of samples) had pesticide residues, of which, 5 had multiple pesticide residues and 29 had single pesticide residue. Only one sample contained residue above the EU-MRLs.

Satpathy *et al.* (2014) conducted a research on ‘‘Development and Validation of Multi-Residue Analysis of 82 Pesticides in Grapes and Pomegranate as per the Requirements of the European Union (EU) and Codex Alimentarius Using GC-MS/MS with Compound Based Screening’’. They validated the (QuEChERS) multi-residue method for the extraction of 82 pesticides belonging to various chemical classes from grapes and pomegranate (commodities with high sugar and low lipid contents). They found that matrix-matched calibration results have demonstrated good reproducibility, robustness and linearity and spiking levels for the recovery experiments as 0.005, 0.01 and 0.1 mg/kg for GC-MS/MS analyses. They also found the mean recoveries mostly ranged between 70 and 110 % (91% on average), and RSD were generally below 12% (7.3% on average).For all compounds LODs were 0.001 to 0.005 mg/kg and LOQs were 0.005 to 0.020 mg/kg.

Islam *et al.* (2014) conducted a survey on three major vegetables like eggplant, cauliflower, and country bean total 42 samples collected from fields and market by

Gas Chromatography (GC) with Flame Thermionized Detector (FTD) and Electron Capture Detector (ECD). On the basis of questionnaires, under their supervision 23 farmers were interviewed. They record 8.33 to 45.00 percent farmers in two selected locations of Narsinghdi to apply different pesticides every day and in some cases even twice in a day on vegetables. They found out of 42 samples, 27 had pesticide residue. Among these 27 samples, 14 samples had pesticide residues above the Maximum Residue Limit (MRL). The detected pesticides were Diazinon, Malathion, Quinalphos, Fenitrothion, Cypermethrin, Fenvalerate and Propiconazole.

Hossain *et al.* (2013) carried out a research on “Health Risk Assessment of Pesticide Residues via Dietary Intake of Market Vegetables from Dhaka, Bangladesh” where they used gas chromatography with a photo diode array detector (HPLC-PDA) to determine six organophosphorus (chlorpyrifos, fenitrothion, parathion, ethion, acephate, fenthion), two carbamate (carbaryl and carbofuran) and one pyrethroid (cypermethrin) pesticide residues in twelve samples of three common vegetables (tomato, lady’s finger and brinjal). Pesticide residues ranged from below detectable limit (<0.01) to 0.36 mg/kg. Acephate, chlorpyrifos, ethion, carbaryl and cypermethrin were detected in only one sample, while co-occurrence occurred twice for fenitrothion and parathion. Apart from chlorpyrifos in tomato and cypermethrin in brinjal, all pesticide residues exceeded the maximum residue limit (MRL). Hazard risk index (HRI) for ethion (10.12) and carbaryl (1.09) was found in lady’s finger and tomato, respectively.

A method was developed by Corteas *et al.* (2013) for the determination in vegetables of organophosphorus pesticides. Pesticide residues are extracted from samples with a small amount of ethyl acetate and anhydrous sodium sulfate. Analyses are performed by large volume GC injection using the through oven transfer adsorption desorption (TOTAD) interface. The calculated limits of detection for each pesticide injecting 50 μL of extract which is much lower than the maximum residues levels (MRLs). Repeatability studies yielded a relative standard deviation lower than 10% in all cases. The method was applied to the analysis of eggplant, lettuce, pepper, cucumber, and tomato.

A research was carried out by Panhwar and Sheikh (2013) to analyze the effect of traditional food processing on the reduction of pesticide residues in cauliflower through GC-μECD and HPLC. The results revealed that the residual level of pesticides in unwashed unprocessed cauliflower samples are beyond their recommended MRLs i.e Bifenthrin, endosulfan, profenofos, emamectin benzoate, imidacloprid and diafenthiuron and the respective values were 0.151, 0.671, 0.172, 1.04, 1.011 and 0.052ppm, respectively which is far above their respective MRLS set by FAO i.e. 0.05, 0.5, 0.05, 0.5, 0.4 and 0.02ppm. The results of the present study showed that, the plain washing and detergent washing reduced the fat soluble pesticides in the average of 28% and 48%, respectively whereas average of water soluble pesticides was found 40% and 55%, respectively. Plain washing followed by frying reduced the fat soluble residues more (up to 98%) as compared to water soluble pesticides (91%). Sun drying (up to 93% for fat soluble and 96% for water soluble pesticide), dehydration (up to 84% for fat soluble and 87% for water soluble pesticide) and blanching (up to 72% for fat soluble and 79% for water soluble pesticide).

Akan *et al.* (2013) found organophosphorus pesticide residues (dichlorvos, diazinon, chlorpyrifos, and fenitrothion) in some vegetables (spinach, lettuce, cabbage, tomato and onion) and soil samples from different depths within Alau Dam and Gongulong agricultural areas in Borno State, Nigeria. Samples collection and preparation were carried out using standard procedures. The concentrations of all the pesticides in the vegetables and soil samples were determined using GC equipped with electron capture detector (ECD). The highest concentrations of diclorvos, diazinon, chlorpiryfos and fenithrothion in the Alau Dam and Gongulong agricultural areas were observed in the leaf of tomato, while the lowest concentrations were observed in the root of spinach. The concentrations of all the pesticides in the soil samples were observed to be higher at a depth of 21-30cm, while the lowest concentrations were observed at a depth of 0-10cm. The concentrations of all the organophosphorus pesticides in the vegetables and soil samples from the two agricultural areas were observed to be at alarming levels, much higher than the maximum residue limits (MRLs) and acceptable daily intake values (ADIs) set for vegetables and soil by the Cordex 2009. The occurrence of pesticides in the vegetables and soil samples is a major threat to human that depends on these vegetables as food. Hence, the need for continuous monitoring is recommended so as to regulate the used of this pesticide in the study areas.

A research was conducted by Cho *et al.* (2013) on the "Evaluation of QuEChERS Method for the Determination of Pesticide Residues Using GC/NPD and GC/ECD" where the modified QuEChERS method was evaluated for rapid determination of pesticide residue in spinach by gas chromatography-nitrogen phosphorus detector and electron capture detector. They selected fifty GC amenable pesticides and found that the detector response linear with determination coefficient higher than 0.995.They also found that the LODs for most compound ranged between 0.001 and 0.1µg/g and about 90% of the compound had LODs less than 0.05 µg/g. The recoveries 80-120% and relative standard deviation (less than 20%) were within acceptable level except for dichlorvos, propamocarb, chlorothalonil, dichlofluanid, cyhalothrin and fenvalerate.

A research was carried out by Milhome *et al.* (2013) on the "Validation and Uncertainty of the method for multiresidue analysis of 35 pesticides in melon using Gas Chromatography Coupled to Quadropole Mass Spectrometry (GC-QP/MS)" and determined various validation parameters such as (selectivity, linearity, LOD, LOQ, accuracy and precision) according ABNT NBR 14029:2005. The recoveries rate for all the pesticide they studied was from 63-117% with RSD lower than 15% in the concentration range of 0.05-0.20mg/kg. They also found the LOQ for most compounds were below the MRLs established in Brazil.

Neetu (2013) studied the magnitude of contamination of DDT pesticides in vegetables, pulses and cereals which were brought for sales to the consumer in the local markets of Sahibabad and Ghaziabad, most of the collected samples were found to be contaminated with residues of DDT. In some of detected samples DDT exceeded the limit of tolerance prescribed by WHO and FAO.

A research work was conducted by Islam *et al.* (2013) on the "Analysis of Pesticide Residue in Vegetables Collected from Local Market by Using GC Technique" where they detect and quantify the presence of pesticide residues in Cucumber, Spinach, and Brinjal available in local market of Mymensingh sadar upazila. They found that among the studied 9 samples, only 3 samples responded to two remarkable elusions. Mancozeb 64% + Symoxanil 8% residues occurred in only one Cucumber sample which was collected from seshmore BAU, the quantity of the Mancozeb 64% + Symoxanil 8% residue was about 50 ppm. On the other hand, out of 3 spinach samples, 1 of them showed presence of imidachloprid residues. But sample from BAU Sesh More eluted a small area contained peak which was very minute level (less than 0.1 ppm). Cucumber sample from Seshmore, BAU showed a remarkable peak which was approximately 50 ppm level of Mancozeb 64% + Symoxanil 8% residue.

A research work was carried out by Cserhati, T. and Szogyi, M. (2012) on the ‘‘Chromatographic Determination of Pesticides in Food and Food Products’’. They operate chromatographic analysis of pesticides present in foods and food products which were collected and evaluates the results critically. They presented the employment of preconcentration and prepurification technologies, gas chromatography using ECD, NPD, MS and MS/MS detection methods, liquid chromatographic methodologies such as thin-layer chromatography, high performance liquid chromatographic methods. They mainly conducted their research on insecticides, herbicides, acaricides, organophosphorous and organochlorine compounds.

Chauhan (2012) found five commonly used pesticides on vegetables, namely endosulfan, carbendazim, chlorpyrifos, cypermethrin and imidacloprid using GC-ECD and HPLC UV-VIS type analytical techniques. Out of the five pesticides monitored, four of them were insecticides belonging to organochlorine, organophosphate, pyrethroidand nicotine based groups respectively and one was fungicide belonging to the benzimidazole group. The analysis revealed that most of the vegetables have endosulfan residues above MRL (maximum residue limit) values followed by carbendazim, chlorpyrifos, imidachloprid and cypermthrin respectively. Amongst the different vegetable samples cauliflower and tomato had carbendazim residues higher than the recommended MRL’s whereas cabbage had endosulfan contamination higher than the recommended MRL values.

Kanda *et al.* (2012) conducted a research uning Gas Chromatography on the extracts from soil, water and vegetable samples. In soil samples, the concentrations of pesticide residues are lower than 20 µg/kg of dry material. For water samples, contamination levels vary from 0.02 to 1.1 µg/L of dry material with the highest levels for metalaxyl M (1.1 µg/L) and for dimethoate (1 µg/L). In vegetables, the concentrations measured are between 0.01 and 0.1 mg/kg of dry material. All these concentrations are affected by a positive factor of the maximum limits of residues.

Dasika *et al.* (2012) carried out a research on “Pesticide residue analysis of fruits and vegetables” where they described an efficient and effective analytical method to screen pesticides in fruits and vegetable samples using liquid chromatography tandem mass spectrometry (LC-MS/MS).They used QuEChERS method with acetate buffering (AOAC Official Method 2007.01) for sample preparation, which has been previously shown to yield high-quality results for hundreds of pesticide residues in foods.

a research was conducted by Parveen *et al*. (2011) on the title named “Monitoring of Multi-residue Pesticide Residues in some fruits in Karachi, Pakistan” where they tested 120 sample of different fruits including apple, apricot, persimmon, chiku, citrus, grapes, guava, mango, papaya, peach, pulm and pomegranate procured from different selling point of Karachi. They analyzed the samples for multiple pesticide residue using GC/FID and HPLC/UV. They found and exceeding level of contamination that is 62.5% of samples contained residues of pesticide while 22% exceeded the maximum residue limit (MRL) according to FAO/WHO.

Farag *et al.* (2011) carried out a research on the ‘‘Monitoring of pesticide residues in some Egyptian herbs, fruits and vegetables’’. He collect One hundred thirty two samples of fruits, vegetables, herbs and spices from Egyptian local markets and analyzed for pesticide residues. He found that contamination with pesticide residues reached 54.55% while samples free from contamination reached 45.45%. He observed only one sample from 132 analyzed samples violated the Maximum Residue Limits (MRLs) of the Codex Committee. From the 132 analyzed samples, 72 samples (54.55%) were contaminated, from which 43.18% contaminated with residues from one pesticide residue, 6.06% with 2 residues and 5.3% with more than 2 residues.

A research work was conducted by Sahoo *et al*. (2011) on the ‘‘Development and Validation of QuEChERS Method for Estimation of Propamocarb Residues in Tomato (*Lycopersicon esculentum* Mill) and Soil’’. In his study an easy, simple and efficient analytical method was standardized and validated for the estimation of residues of propamocarb in tomato and soil. QuEChERS method included extraction of the sample with ethyl acetate and cleanup by treatment with PSA and graphitized carbon. Final clear extracts of ethyl acetate were concentrated under vacuum to almost dryness and reconstituted into hexane. The residues of propamocarb were estimated using gas chromatograph-mass spectrometry (GC-MS). They found that propamocarb presented a distinct peak at retention time of 8.962 min. and the consistent recoveries of propamocarb ranging from 87 to 92 percent were observed when they spiked the sample at 0.10, 0.50 and 1.00 mg·kg–1 levels. They also determined the limit of quantification (LOQ) of their method was 0.10 mg/kg.

Camino-Sánchez *et al.* (2011) used the QuEChERS extraction method and detectionwas done by GC-MS-MS for the quantification of 121 pesticide residues in samples of tomato, pepper, lettuce, cucumber, eggplant, zucchini, melon, watermelon and apple acquired from Spain.

Fernandes *et al.* (2011) reported pesticide residues in vegetables for the several extraction procedures (liquid extraction, single drop microextraction, microwave-assisted extraction, pressurized liquid extraction, supercritical fluid extraction, solid-phase extraction, solid-phase microextraction, matrix solid-phase dispersion, and stir bar sorptive extraction). A combination of techniques reported the use of new extraction methods and chromatography to provide better quantitative recoveries at low levels. The use of mass spectrometric detectors in combination with liquid and gas chromatography has played a vital role to solve many problems related to food safety.

Hrouzkova and Matisova (2011) conducted a research on "Fast gas chromatography and its use in pesticide residues analysis" linuron, folpet, chlordecone, prochloraz, cypermethrin and deltamethrin have the LOD values > 100 pg.ml-1 and LOQ values > 300 pg.ml-1. LODs and LOQs obtained in EI mode are at the level of ng.ml-1. For all analytes except linuron, dicofol and prochloraz, the LOQs were below 10 µg.kg-1, which is the MRL required for the pesticide residues in baby-food.

Afful *et al.* (2010) carried out a research on "Gas Chromatographic Methodology for the Determination of Some Halogenated Pesticides" where gas chromatography (GC) methodology has been validated for the determination of some halogenated pesticides. Complete separation of the pesticide prepared in ethyl acetate was achieved on Rtx - 1 column with dimension, 30mm x 0.25mm x 0.25mm. The GC equipped with electron capture detector was run using column temperature programmed from 80 ºC (2 min) to 200 ºC (15 min) at the rate of 4 ºC/min giving a total analysis time of 47 min. The detector and injector were respectively at temperatures of 300 and 225ºC. The method was validated with respect to precision in terms of reproducibility of retention times and peak heights, linearity and minimum detectable quantity of the pesticides. Under the operated GC conditions, diuron eluted first while heptachlor epoxide was the last to elute. The chromatographic detector was more sensitive to endosulfan and endosulfan with Minimum Detectable Quantity (MDQ) of 0.002 ng. The detector was however, less sensitive to captan with MDQ of 0.08 ng. Margins of errors associated with the precision of the method in terms of reproducibility of 11 retention times yielded standard deviation in the range of 0.026-0.063.

Schreiber and Wittrig (2010) carried out a research on “Enhanced LC/MS for the Quantitation and Identification of Pesticide in Food Sample” where he collected a variety of fruit and vegetable samples including apple, banana, carrot, cucumber, curry powder grapes, grapefruit, hazelnut, lemon, nectarine, orange, pear, raspberry, red pepper, raisin, salad, spinach and tomato from a supermarket and extracted using QuEChERs procedure. They injected the extracted sample into a liquid chromatography tandem mass spectrometry system where a total number of 12 pesticides were detected. They found 70-120% recovery for most of the pesticide with %CV<15%. They also found Methamidophos 130µg/kg, omithoate 42µg/kg, thiamethoxam 48 µg/kg, dimethoate 54µg/kg, clothianidin 14µg/kg, imadacloprid 2.4µg/kg, promamocarb 98µg/kg, carbyl 499µg/kg, metalaxyl 5.1µg/kg, myclobutanil 3.4µg/kg, spinosyn A 6.1µg/kg, spinosyn D 6.8 µg/kg.

Charan and Sharma (2010) monitored pesticide residues in vegetables to find out severity of such synthetic agrochemicals on human being. A total of 182 samples of six vegetables were collected for pesticide residue analysis from different agricultural fields of central Aravalli region, when they were ready for transportation to market. The analysis of samples for different pesticide residues were carried out on GCECD and GC-NPD systems equipped with capillary columns by using a multiple residue method. About 40.11% of total analyzed samples were contaminated with different pesticide residues, among which 35.62% of total contaminated samples were exceeded the maximum residual limit (MRL) values.

Islam *et al.* (2009) reported a method based on High Performance Liquid Chromatography (HPLC) for the determination of pesticide residues in Cauliflower. Cauliflower sprayed with 4 different pesticides (diazinon, malathion, chlorpyrifos and cypermethrin) at recommended dose and double of recommended dose were analyzed for their residual contents. Limit of 1 was obtained. Calibration curves that constructeddetection of 0.02 mg kg for the analytes spiked into samples followed linear relationships with good correlation coefficients (R2 >0.990). In the analysis, from vegetables treated with diazinon and chlorpyrifos at recommended and double of recommended doses, residual amounts above respective MRL values were found.

Yamagami *et al.* (2009) conducted a research on "Multi-Residue Method for Determination of 85 Pesticides in Vegetables, Fruits and Green Tea by Stir Bar Sorptive Extraction and Thermal Desorption GC-MS" where they used a multi-residue method to determine five groups of 85 pesticides - chlorinated, carbamate, phosphorous, pyrethroid and others - in vegetables, fruits and green tea has been developed using stir bar sorptive extraction (SBSE) coupled to thermal desorption and retention time locked (RTL) GC-MS. Pre-extraction with methanol and dilution with water prior to SBSE (60 min) were performed. Dilution of methanol extract for SBSE was examined to obtain high sensitivity and to compensate the effect of adsorption to the glass wall of extraction vessel and to sample matrix for the compounds with high log Ko/w values (e.g. pyrethroid). The methanol extracts were diluted twofold and fivefold, and were simultaneously SBSE-enriched. The two stir bars were placed in a single glass thermal desorption liner and were simultaneously desorbed. The versatility of the method was exhibited by its good linearity (4-100 μg/kg, r2 >0.9900) for 66 pesticides and limit of detection (LOD: < 5 μg/kg) for most of the analytes. The method enables to determine pesticides at low μg/kg in tomato, cucumber, green soybeans, and spinach, grape and green tea.

Prodhan *et al.* (2009) undertook a research on the "Quantification of Organophosphorus and Organochlorine insecticide residues from fish sample using simple GC technique" to develop the simple technique of quantification of organophosphorus and organochlorine insecticide residues from fish samples using Electron Capture Detector (ECD) and Flame Thermionic Detector (FTD) of Gas Chromatograph (GC). They collected sixty eight samples of fish (Rui, Shrimp & Others) from Dhaka, Khulna and Chittagong offices of Department of Fisheries including different fish export companies and supplied to Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur. They extracted and prepared all samples for injection using the standard protocols for residue analyses during August, 2008 to July, 2009. They also injected all samples in GC-ECD for the determination of organochlorine insecticides and in GC-FTD for the determination of organophosphorus insecticides. Their results revealed that among 68 samples, 13 had insecticide residues. For Dhaka, of six samples 1 had DDT residue. The level of detected residue was 0.28 ppm. For Chittagong, out of 23 samples 3 had Diazinon residue. The range of detected residue was 0.03-0.120 ppm. For Khulna, of 39 samples 9 had Diazinon residue. They found the range of detected residue was 0.04-0.205 ppm. Considering the average body weight (50 kg/person), 4 samples contained residues above MRL.

Nguyen *et al.* (2008b) undertook a research on the "Multi-residue Determination of 156 Pesticide in Watermelon by Dispersive Solid Phase Extraction and Gas Chromatography/Mass spectrometry" for a simultaneous determination of 156 pesticides in watermelon collected from market. They adopted gas chromatography with electron impact mass spectrometric detection in the selected ion monitoring mode. They found the limit of quantifications (LOQs) for most compounds was below 0.005mg/kg.

Kabir *et al.* (2008) reported residue of Diazinon and Carbosulfan in brinjal and Quinalphos in yard long bean and comparison between the detected residue level with maximum residue level (MRL) set by FAO and WHO(1970). Three supervised field trials (two for brinjal and one for yard long bean) were carried out sprayed with the field dose (1.5 ml/L of water) of Diazinon, Carbosulfan, and Quinaiphos. Samples were collected daily after spraying till residues were found. In case of Diazinon, left over residue was found upto 6 days after spray (DAS), and upto 3 DAS, the level of residue was above the MRL. Carbosulfan residue was detected till 7 DAS and the detected quantity of residue was above MRL upto 3 DAS. Left over residue of Quinalphos in yard long bean sample was detected upto 6 DAS and upto 4 DAS the level of residue was above the MRL.

Ochiai *et al.* (2008) has been developed a multi-residue method to determine 85 pesticides - chlorinated, carbamate, phosphorous, pyrethroid and others in vegetables, fruits and green tea using stir bar sorptive extraction (SBSE) coupled to thermal desorption and retention time locked (RTL) GCMS. Pre-extraction with methanol and dilution with water prior to SBSE (60 min) were performed. Dilution of methanol extract for SBSE was examined to obtain high sensitivity and to compensate the effect of adsorption to the glass wall of extraction vessel and to sample matrix for the compounds with high log Ko/w values (e.g. pyrethroid). The methanol extracts were diluted twofold and fivefold, and were simultaneously SBSE-enriched. The two stir bars were placed in a single glass thermal desorption liner and were simultaneously desorbed. The versatility of the method was exhibited by its good linearity (4-100 μg/kg, r2 0.9900) for 66 pesticides and limit of detection (LOD: < 5 μg/kg) for most of the analytes.

Butler *et al.* (2008) conducted a study to determine pesticide residue in vegetables by a new sample preparation method, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), and published recently as AOAC Method 2007.01.1 The sample preparation is shortened by using a single step buffered acetonitrile (MeCN) extraction and liquid-liquid partitioning from water in the sample by salting out with sodium acetate and magnesium sulfate (MgSO4).1 This technical note describes the application of the QuEChERS sample preparation procedure to analysis of pesticide residues in a lettuce matrix using gas chromatography/mass spectrometry (GC/MS) on the Thermo Scientific TRACE GC Ultr and Thermo Scientific DSQ single quadrupole mass spectrometer. Thermo Scientific Quan Lab Forms 2.5 software was used for data review and reporting. The MeCN extract is solvent exchanged to hexane/acetone for splitless injection with detection by electron ionization and selected ion monitoring (SIM). A calibration curve was constructed in iceberg lettuce and then the precision and accuracy of the analytical method were tested by preparing matrix spikes at 5 ng/g and 50 ng/g.

Fenoll *et al.* (2007) was developed an analytical multi-residue method for the simultaneous determination of various classes of pesticides in vegetables, pepper and tomato. Final determination was made by gas chromatography with nitrogen–phosphorus detection.

Ferrer *et al.* (2005) has been developed a new multi-residue methodology using liquid chromatography–time-of-flight mass spectrometry (LC TOF-MS) for the quantitative (routine) analysis of 15 pesticide residues. The analytical performance of the method was evaluated for different types of fruit and vegetables; pepper, broccoli, tomato, orange, lemon, apple and melon. The accurate mass measurements were compared in different matrices at significantly different concentration levels (from 0.01 to 0.5 mg/kg) obtaining accuracy errors lower than 2 ppm, which is well within the accepted limits for elemental confirmation. Instrumental limits of detection (LOD) were between 0.0005 and 0.03 mg/kg depending on the commodity and pesticide studied, all being within European Union regulations for food monitoring program. Finally, the methodology was applied to the analysis of two samples from an inter-laboratory exercise.

Colume *et al.* (2001) and Padron-Sanz *et al.* (2005) reported that the residues of pesticides especially on vegetables and possible risks of them on human health has become the prior subject of pesticide researchers who evaluate vegetable quality recently

Ortelli *et al.* (2004) hold a research on "Multi-residue analysis of 74 pesticides in fruits and vegetables by liquid chromatography-electrospray-tandem mass spectrometry" where they adopted liquid chromatography, electrospray ionization and tandem mass spectrometry for 2500 samples and they found that more than 30% of sample contained multiple pesticide residues, 12 different pesticide in grape sample but all concentrations found were below MRLs.

Gamon *et al.* (2001) determined the Pesticide residues in fruit and vegetables by gas chromatography/tandem mass spectrometry (GC/MS/MS). Electron impact (EI)/MS/MS and chemical ionization (CI)/MS/MS were developed for 80 compounds, including organochlorine, organophosphorus, organonitrogen, and pyrethroids, providing unambiguous spectral confirmation for these complex matrixes. Residues were extracted from samples with acetone followed by a mixture of dichloromethane petroleum ether. Two injections per sample were required for analysis of the entire pesticide list by EI/MS/MS and CI/MS/MS. Initial steps involving cleanup and concentration of extracts were eliminated. The excellent selectivity and good linearity allowed quantification and identification of low levels of pesticides in the most difficult matrixes. The method has been used for routine analysis of many vegetables.

Kumar and Hosmani (2001) conducted a research work on "Magnitude of the residue of carbofuran and 3-hydroxy carbofuran in/on rice in Brazil following furadan 50G insecticide treatment" where they treated rice plants with 3 broadcast application at the nursery (10 days before transplant), tillering and booting (25 and 89 days after transplanting, respectively) stages in India at maximum GAP rate of 2 kg AI/ha. Plant samples were harvested at 36 days PHI dried in the field for one day and under the sun for 4-6 hours for 3 days in a clean area. The grain was then separated from the straw by beaten on a wooden plank and analyzed. Carbofuran residue was 0.16 mg/kg.

Gamon *et al*. (2001) carried out a research on "Multiresidue Determination of Pesticides in Fruit and Vegetables by Gas Chromatography" where they determined the Pesticide residues in fruit and vegetables by gas chromatography/tandem mass spectrometry (GC/MS/MS). Electron impact (EI)/MS/MS and chemical ionization (CI)/MS/MS were 15 developed for 80 compounds, including organochlorine, organophosphorus, organonitrogen, and pyrethroids, providing unambiguous spectral confirmation for these complex matrixes. Residues were extracted from samples with acetone followed by a mixture of dichloromethane petroleum ether. Two injections per sample were required for analysis of the entire pesticide list by EI/MS/MS and CI/MS/MS. Initial steps involving cleanup and concentration of extracts were eliminated. The excellent selectivity and good linearity allowed quantification and identification of low levels of pesticides in the most difficult matrixes.

Colume *et al*. (2001) reported that Maximum Residue Levels (MRLs) are not exceeded if pesticides are applied according to appropriate agricultural techniques, but unconscious applications may lead to harmful remnants containing environmental pollution and possible health risks. Reductions frequently made in Maximum Remnant Levels (MRLs) accepted by the international institutions like EU and EPA and determination of levels by urgently creating purposive multi-residue methods are dramatical changes.

Aguera *et al*. (2000) described a method (Splitless large- volume GC-MS injection for the analysis of organophosphorus and organochlorine pesticides in vegetables using a miniaturised ethyl acetate extraction) for the measurement of only ten organophosphorus and organochlorine pesticides by GC-MS, but over the past decade, the number of pesticides typically included in methods has increased dramatically. The sample preparation techniques have also advanced to complement the analytical techniques depending on the types of analytes and matrices monitored.

Lehotay (2000) investigated 22 diverse pesticide residues in green bean, and carrot extracts by bench top gas chromatography. The targeted pesticides, some of which were incurred in the samples, included chlorpyrifos, azinphos-methyl, parathion-methyl, diazinon, terbufos, p,p'- DDE, endosulfan sulfate, carbofuran, carbaryl, propargite, bifenthrin, dacthal, trifluralin, metalaxyl, pendimethalin, atrazine, piperonyl butoxide, diphenylamine, vinclozolin, chlorothalonil, quintozene, and tetrahydrophthelimide (the breakdown product of captan). The analytical DSI method entailed the following steps: (i) blend 30 g sample with 60 mL acetonitrile for 1 min in a centrifuge bottle; (2) add 6 g NaCl and blend 30 s; (3) centrifuge for 1-2 min; (4) add 5 mL upper layer to 1 g anhydrous MgSO4 in a vial; and (5) analyze 11 NL extract, using DSI/GC/MS-MS. Sample cleanup is not needed because GC/MS-MS is exceptionally selective for the targeted analytes, and nonvolatile extracted matrix components do not contaminate the injector or the GC/MS-MS system. Average recoveries of the pesticides were 103 s 7% with relative standard deviations of 14 t 5% on average average and limits of detection were <2 ng/g for nearly all pesticides studied.

Salwa *et al.* (1999) undertook a research to monitor pesticide residues in Egyptian fruits and vegetables during 1995. Organophosphorus, dithiocarbamates and some synthetic pyrethroids pesticidcs, which were commonly used in Egypt for pest control, were monitored, as well as persistent organochiorines, which had been prohibited from use several years ago. Fruit and vegetable samples (397) were collected from 8 local markets and examined for 52 active ingredients. Of all analysed samples, 42.8% contained detectable residues, of which 1.76% exceeded their maximum residue limits (MRL's). The rates of contamination with the different pesticides were 0-86%. The most commonly detected residues were dithiocarbarnates as well as dicofol (15.1% of 397 samples), dimethoate (6.8%), tetradifon (4.5%), Malathion (3.3%), profenofos (2.8%), omethoate (2.3%), chlorothalonil (2.0%) and chiorpyrifos-methyl (1.5%). Among all samples, 22 strawberry samples (5.32%) contained 10 pesticide residues, 65 grape samples (15.73%) contained 11 pesticides residues and 62 tomato samples (15.01%) contained 13 pesticide residues. Cauliflower, onion and guava samples free from pesticides residues. Samples of carrot, and eggplant contained trace amounts of p, p'-DDT and p, p'-DDE residues. But in general, residues of DDT and HCH have disappeared almost completely from vegetables and fruits. Use of these pesticides in Egypt was completely prohibited by law in 1987.

Hura (1999) reported world public opinion has reached a highly sensitive position against allergen, mutagen and cancerogenic effects created by pesticide residues on soil, water and foods depending on extinction events occurred in bird species feeding with accumulated pesticide residue. Forbiddance of production and consumption of pesticides causing cancer has been recommended by World Health Organization (WHO) and International Cancer Research Institutions, some has been forbidden and production of some other has been decreased. Some among them are DDT, endosulphan, fenitrothion, fenthion, malathion, parathion and trifluralin.

Ahuja *et al.* (1998) conducted a research entitled "Monitoring of vegetables of insecticide residue contamination at harvest. Advances in IPM for horticultural crops" and reported that cauliflowers, cabbages, tomatoes, brinjal, okras, field beans and cucumbers were monitored for residues of GCH and its isomers, Endosulfan, Dimethoate, Monocrotophos, Quinalphos, Fenvalerate, Cypermethrin. The residues of alpha, beta, tau isomers of HCH, Endosulfan, monocrotophos, Quinalphos, Dimethoate were detected in most of the samples. However, the residues of Monocrotophos on tomatoes, brinjal and okras and those of Carbendazim on French beans were found to persist over the prescribed maximum residue limit values.

Rimkus *et al.* (1996) described that pesticide residue detection methods from food matrices mainly involve two preparation steps prior to the identification and quantification of pesticides: Extraction of target analytes from the bulk of the matrices and partitioning of the residues in an immiscible solvent and or clean up of the analytes from the matrix co-.extractives. Complex samples like meat and meat products need two step clean-up which combines different chromatographic techniques.

Dethe *et al.* (1995) carried out a research on "Insecticide residues in/on farm gate samples of vegetables"on the residues of commonlyy used pesticides in/on vegetables in India. Detectable levels or residues were observed in 33.3% of tomatoes (diazion, endosulfan, dimethoate and monocrotophos), 73.3% of eggplant (endosulfan, diazinon, cypermethrin, fenvalerate, quinalphos, dimethoate and monocrotophos), 14.3% of okras (endosulfan), and 88.9% of cabbage (endosulfan, fenvalerate, cypermethrin, dimethoate and monocrotophos). However, the levels of pesticide residues were lower than the maximum residue limits (MRL) prescribed.

FAO/WHO (1993) carried out a study on "Insecticide Residues in food. Part I-Residues" and reported that the trials were conducted in Canada (4), France (1) and the USA (23) using EC, WP and GR formulations. In the US trails a GR or EC pre-planting application at 4.4 Kg a.i/ha was followed by five foliar sprays at weekly intervals with WP or EC formulations at the rate of 0.55Kg a.i/ha, the other trials were with granules at 2.25 or 10 kg a.i/ha one month after planting. No residues of Diazinon in potatoes were detectable (<0.01 mg/kg) in any of the harvested samples except in three trials where residues of 0.01 mg/kg were found.

FAO/WHO (1993) conducted a research "Insecticide Residues in food. Part I-Residues" and reported that the trials were conducted in Germany (4), Switzerland (3), and the USA (2) using EC WP and GR formulations. The European trials were carried out with one or two applications after transplanting at 0.19 kg a.i/m row or 0.02 kg a.i/plant at an interval of 1-20 days. In US trials a GR or EC formulation was used before planting at an application rate of 4.4 kg a.1/ha followed by five foliar sprays at weekly intervals with WP or EC formulations at a rate of 0.55 kg a.i/ha. Residues of Diazinon in cabbage after one or two treatments ranged from <0.02 to 0.6 mg/kg 21-86 days after the last application. After pre-plan and foliar applications, residues of Diazinon 721 days after the last treatment varied between <0.01 and 1.8 mg/kg.

Ahmed (2001) reported that pesticide residues in food are a potential hazard, which has received much attention during the past 20 years. Extensive regulatory agencies have been created in developed countries to deal with pesticide residues in food. In many developing countries acceptable quantities of pesticide residues in food (tolerances) have not been established, however the guidelines developed by Food and Agriculture Organization and the World Health Organization (FAO/WHO) are generally followed. Because of the very small quantities of pesticide, which are permitted in food, elaborate analytical procedures are required. Some pesticide are relatively stable and since a considerable amount of the applied pesticide frequently ends up in the soil and in some cases bioaccumulation can occur to an extent, which causes damage to fish or birds.

Frank *et al.* (1990) undertook a research on "Residues of insecticides and fungicides on Ontario-grown vegetables" and studied that organophosphorus, synthetic pyrethroid and N-methyl carbamate insecticides and dithiocarbamate, dicarboximide and organochlorine fungicides. The estimation was done in 433 composite vegetable samples representing 16 commodities collected between 1986 and 1988 from farm deliveries to the market place Ontario, Canada. Commodities tested included eggplant, asparagus, carrots celery, cole crops, cucumbers, lettuce, onions, peppers, potatoes, radishes and tomatoes. In 64% of samples, no pesticide residues were identified to the limits of detection which ranged from 0.005 to 0.05 mg/kg. These involved Diazinon and Parathion on celery and Chlarothalonil on peppers. Whereas some commodities had no detectable residues.

Their *et al.* (1989) hold a research "Quality assurance in insecticide residue analysis" and reported that during the past few years' pesticide residues of the German Chemical Society has organized 6 laboratory performance tests in which numerous laboratories were involved. In these tests, the choice of analytical methods for the examination of fats or vegetable substance was free. Organochlorine pesticides at over 0.01 mg/kg were most readily identified, whereas in the analysis of organophosphorus residues often only the classic compound such as Parathion and Diazinon were reported. Many false positive results could have been avoided by using more accurate methods for confirmatory analysis. The quantitative results, however, were generally quite reliable. It can be concluded that the performance of a residue laboratory is not constant, and that it is necessary to assess regularly the quality of the results by participating in such inter laboratory tests.

Singh and Kalra (1989) conducted a research on the "Determination of residues of cypermethrin in brinjal fruits, leaves and soil.in Ludhiana, India" to determine the residues of Permethrin applied for the control of Leucinodes orbonalis on eggplant fruit. The compound was sprayed at a rate of 50g a.i/ha at fortnightly intervals. Initial deposits on fruit range from 1.3 to 0.7 mg/kg and maximum residue level 1, 2, 3 and 10 days after spraying was 0.34, 0.2, 0.11 and 0.07 mg/kg respectively. The half life on insecticides on fruits ranged from 2.1 to 3.0 days. The trans-isomers of Permethrin degraded slightly faster than the cis-isomers in leaves and fruits. A 1-day waiting period is recommended for consumption of fruits.

Sattar (1987) reported GC external standard analysis method for DDT and can also be applied for organophosphorus pesticides. Again, GC internal standard analysis method for organophosphorus pesticides universally established (Sattar, 1992).

Organophosphorus pesticides are the largely used pesticides in the world where these methods are extensively used for the detection of residues of one or more compounds from soils, crops and food materials (Sattar, 1992; Sattar, 1991; Sattar, 1990; Sattar, 1987 Sattar, 1985). This basic, applied and fundamental contribution is universal and can be treated as pioneer contribution in the history of environmental and pesticide sciences of Bangladesh. Here numerous solvent mixtures were established with recovery of 95-100% covering sensitivity of residues of different organophosphorus pesticides from soils, crops or food materials.

Sattar (1986) established GC external standard analysis method of five organochlorine and four DDT-type organochlorine pesticides were schematically described and their identical chloromatograms are listed against respective peaks of organochlorine pesticides and DDT-types pesticides. Different solvent systems were developed with residue recovery upto 100%. The method widely used for the detection of residue in soil lives (Sattar 1990; Sattar 1985; Sattar, 1983). This was the basic, applied and fundamental contribution in pesticide and environmental chemistry as well as pioneer wo'rk in environmental science of Bangladesh.

Sattar (1985) undertook a research where he used GC external standard and GC internal standard analysis methods to detect phenoxyherbicides, Here different solvent mixtures and clean up procedures were where sensitivity recorded to ppb levels. This technique is widely recommended to detect the residues of the compounds from soils, crops and food materials (Sattar and Paasivirta, 1979).

Devi and Manjunath (1985) found that Quinaiphos is a less persistent insecticide and dissipated on the fruits more quickly than Fenvalerate and Carbaryl. In the current study, the left over residue of Quinaiphos in the YLB sample had been detected upto 6 DAS, of which upto 4 DAS, the quantity of residue were above MRL. At the 0 DAS, the residue was 0.678 ppm and they were 0.552 ppm, 0.419 ppm, 0.306 ppm, and 0.2 14 ppm at 1 DAS, 2 DAS, 3 DAS, and 4 DAS, respectively. All these quantities were above MRL. The quantity decreased down to 0.164 ppm at 5 DAS and 0.087 ppm at 6 DAS. But they found the residue of Quinalphos (0.56 ppm) in brinjal above the tolerance level upto 10 DAS.

Sattar (1983) established GC external standard method with 4 chlorinated cresols compounds where 6 solvent systems were developed with recovery upto 100% and sensitivity upto pg (ppb) levels. The examples of chromatographic peaks are reported. Chlorinated cresols come to soils, crops, food materials through various sources and create hazard to human healths and other living bodies. This GC method development was the pioneer work as well as applied and fundamental contribution in the environmental sciences of Bangladesh. Even now, still no such method has been developed in Bangladesh. This method also widely applied for the detection of the residues of the compounds.

Sattar and Passivirta (1980) developed the detailed internal standard analysis method (procedure) of MCPA together its two metabolites 4-chlo-cresol and 5-chloro-3-methyl cathechol are schematically presented and the examples of chromatograms are reported as proof records. This was the universal applied fundamental contribution for the detection of the residues of MCPA and/or its metabolites in soils, crops and food, materials. Different solvent/solvent systems were developed including three cleanup procedures (Column, water, toluene-shaking and TLC) where shaking is mostly used. This internal standard procedure of Sattar and Paasivirta 1980 historically invented (lst times in the history of mankind and 1st time in the history of environmental/analytical chemistry of the world) the two metabolites of MCPA by GC, GC-MS, and NMR applications from foods and vegetables and soils where by applying of only MCPA compound, the recovery recorded up to three compounds like MCPA with its 2 metabolites.

Sattar *et al.* (1977) established detailed external standard procedure and analysis methods of MCPA and MCPA together with its two metabolites 4-chloro-o-cresol and 5-chloro-3methyl catechol are schernatically described and some examples of chromatograms (peaks) are presented. The method covered different solvent systems for extraction and three clean up procedures like column and water-toluene shaking. This was the excellent basic, applied and fundamental contribution and still largely using in pesticide, agriculture, environmental and analytical chemistry for minimizing residues and hazards and building of peace.

FAQ-WHO (1970) reported that the residue of Diazinon on/or in plants, in animal tissues, or even in the soil are not highly persistent. From this study, it was revealed that residue of Diazinon could be detected up to 6 DAS. The quantities of residue were above MRL upto 3 DAS and these were 2.228 ppm, 1.889 ppm, 1.675 ppm, and

0.761 ppm at 0, 1, 2 and 3 DAS, respectively. Samples of 4, 5, and 6 DAS contained 0.436 ppm, 0.396 ppm and 0.297 ppm. Diazinon residue, respectively, which are below MRL set by FAQ-WHO (1970). This results more or less agree with the observation of Geigy (1 956-67). He observed the Diazinon residue level after spraying the field dose, were < 0.1 ppm in Cabbage at 7 DAS, 0.4 ppm in Cauliflower at 5 DAS and < 0.1 ppm in Cucumber at 7 DAS. Adnan *et al.* (1987) found Diazinon residue above MRL upto 8 DAS in sweet pepper grown in green house.

CHAPTER 3

MATERIALS AND METHODS

The vegetable (country bean, cauliflower & cucumber) samples were collected from different markets of Dhaka City and carried to the Pesticide Analytical Laboratory, Entomology Division, BARI, Joydebpur, Gazipur for pesticide residue analysis during January 2016 to September 2016. From the collection of samples to the final analysis, all way required a number of processes which are described below.

3.1 Study area

The study area included major ten markets of Dhaka City. The area of Dhaka City is about 270 sq km, located at 23.42° North latitude and 90.22° East longitude with an elevation of 4 meter from the sea level. In this study, vegetables were collected from 5 markets of Banani, Rampura, Mohammadpur Krishi Market, Kawran Bazar and Jatrabari area.

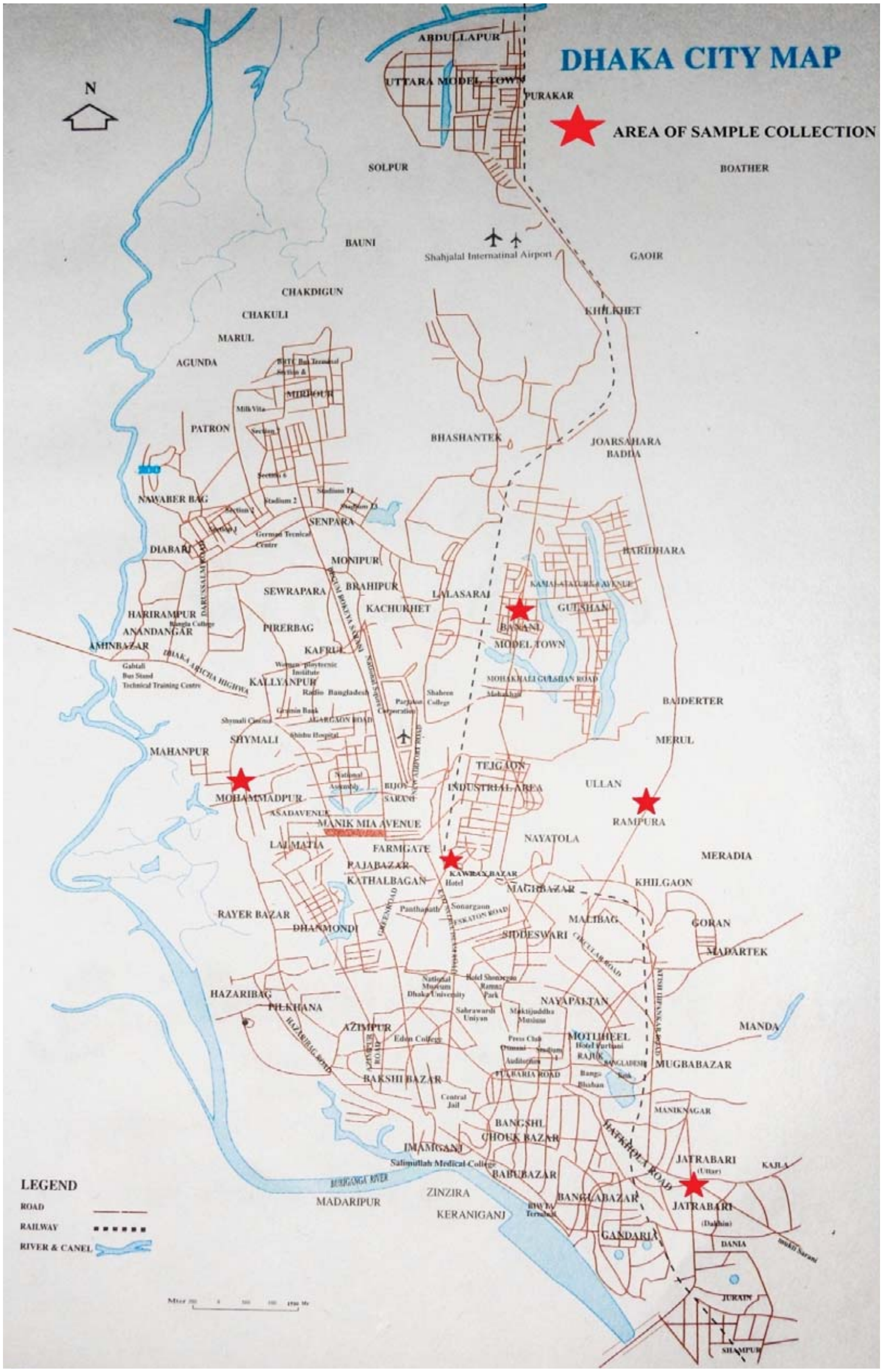


Figure 1. Map showing the places of sample collection.

3.2 Sample collection

A total of 100 samples (50 country bean, 30 cauliflower & and 20 cucumber) were collected for this study. Ten samples of country bean, six samples of cauliflower and four samples of cucumber were collected from each market.

Table 1. Sources and places of collection of Country bean samples

Area of collection	**Sample ID**	**Source***
Banani	DMB$_1$	Manikganj
	DMB$_2$	Saturia
	DMB$_3$	Manikganj
	DMB$_4$	Tangail
	DMB$_5$	Narsingdi
	DMB$_6$	Comilla
	DMB$_7$	Manikganj
	DMB 8	Meherpur
	DMB$_9$	Savar
	DMB$_{10}$	Jessore
Rampura	DMB$_{11}$	Sripur
	DMB$_{12}$	Singair
	DMB$_{13}$	Manikganj
	DMB$_{14}$	Tangail
	DMB$_{15}$	Saturia
	DMB$_{16}$	Jessore
	DMB$_{17}$	Comilla
	DMB$_{18}$	Jhenaidah
	DMB$_{19}$	Savar
	DMB$_{20}$	Comilla
Mohammadpur	DMB$_{21}$	Jessore
	DMB$_{22}$	Singair
	DMB$_{23}$	Meherpur
	DMB$_{24}$	Manikganj
	DMB$_{25}$	Singair
	DMB$_{26}$	Chuadanga
	DMB$_{27}$	Jhinaidah
	DMB$_{28}$	Tangail
	DMB$_{29}$	Meherpur
	DMB$_{30}$	Jessore

Table 1 (cont'd)

Jatrabari	DMB$_{31}$	Chuadanga
	DMB$_{32}$	Meherpur
	DMB$_{33}$	Jessore
	DMB$_{34}$	Manikganj
	DMB$_{35}$	Savar
	DMB$_{36}$	Srinagar
	DMB$_{37}$	Comilla
	DMB$_{38}$	Meherpur
	DMB$_{39}$	Saturia
	DMB$_{40}$	Jessore
Kawranbazar	DMB$_{41}$	Manikganj
	DMB$_{42}$	Meherpur
	DMB$_{43}$	Chuadanga
	DMB$_{44}$	Manikganj
	DMB$_{45}$	Comilla
	DMB$_{46}$	Singair
	DMB$_{47}$	Jessore
	DMB$_{48}$	Srinagar
	DMB$_{49}$	Comilla
	DMB$_{50}$	Savar

* According to the retailer's opinion

Table 2. Sources and places of collection of Cauliflower samples

Area of collection	**Sample ID**	**Source***
Banani	DMCF$_{1}$	Tangail
	DMCF$_{2}$	Narshingdi
	DMCF$_{3}$	Manikganj
	DMCF$_{4}$	Jessore
	DMCF$_{5}$	Manikganj
	DMCF$_{6}$	Jessore
Rampura	DMCF$_{7}$	Meherpur
	DMCF$_{8}$	Meherpur
	DMCF$_{9}$	Manikganj
	DMCF$_{10}$	Savar
	DMCF$_{11}$	Saturia
	DMCF$_{12}$	Comilla

Table 2 (cont'd)

Mohammadpur	DMCF$_{13}$	Jessore
	DMCF$_{14}$	Meherpur
	DMCF$_{15}$	Singair
	DMCF$_{16}$	Chuadanga
	DMCF$_{17}$	Comilla
	DMCF$_{18}$	Savar
Jatrabari	DMCF$_{19}$	Comilla
	DMCF$_{20}$	Jessore
	DMCF$_{21}$	Comilla
	DMCF$_{22}$	Meherpur
	DMCF$_{23}$	Jessore
	DMCF$_{24}$	Manikganj
Kawranbazar	DMCF$_{25}$	Manikganj
	DMCF$_{26}$	Savar
	DMCF$_{27}$	Tangail
	DMCF$_{28}$	Comilla
	DMCF$_{29}$	Manikganj
	DMCF$_{30}$	Narsingdi

* According to the retailer's opinion

Table 3. Sources and places of collection of Cumber samples

Area of collection	Sample ID	Source*
Banani	DMCU$_{1}$	Manikganj
	DMCU$_{2}$	Singair
	DMCU$_{3}$	Srinagar
	DMCU$_{4}$	Manikganj
Rampura	DMCU$_{5}$	Comilla
	DMCU$_{6}$	Tangail
	DMCU$_{7}$	Manikganj
	DMCU$_{8}$	Savar
Mohammadpur	DMCU$_{9}$	Comilla
	DMCU$_{10}$	Singair
	DMCU$_{11}$	Meherpur
	DMCU$_{12}$	Meherpur
Jatrabri	DMCU$_{13}$	Jessore
	DMCU$_{14}$	Chuadanga
	DMCU$_{15}$	Jessore
	DMCU$_{16}$	Meherpur
Kawranbazar	DMCU$_{17}$	Manikganj
	DMCU$_{18}$	Meherpur
	DMCU$_{19}$	Comilla
	DMCU$_{20}$	Manikganj

* According to the retailer's opinion

The amount of each sample was 1 Kg for all the vegetables. 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.

3.3 Sample preparation for analysis

The samples were 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.

3.4 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.

3.5 Analytical Apparatus Required

a. Centrifuge machine, Model: Sigma 3k 30, Germany (Plate 1)
b. Electric balance, Model: AY- 220, Shimadzu Corporation, Japan (Plate 2).
c. Homogenizer, Model: Ultraturax, IKA T18 basic, Germany (Plate 3)
d. Vortex mixer, Model: Maxi max ii, USA (Plate 4)
e. Orbital shaker, Model: Rexmed, Sweden (Plate 5)
f. GC-2010, Shimadzu corporation, Japan (Plate 6)

Plate 1. Centrifuge Machine

Plate 2. Electric Balance

Plate 3. Homogenizer

Plate 4. Vortex Mixer

Plate 5. Orbital Shaker

Plate 6. Gas Chromatograph (GC)

In addition to the above instruments the following accessories were also used:

- Scissors
- Measuring cylinder
- Conical flask
- Volumetric flask
- Tray
- Knife
- Spatula
- Funnel
- Test tube
- Micro pipette
- Aluminum foil
- Para film
- Centrifuge tube
- Glass vial etc.

3.6 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.

3.7 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 techniques was first introduced by Anastassiades et al. (2003), which is gaining popularity day by day compared to the other existing techniques such as Supercritical Fluid Extraction (SFE), Liquid-liquid extraction (LLE), Solid phase extraction (SPE), Solid phase micro extraction (SPME), Stir bar sorptive extraction (SBSE), and Microwave assisted extraction (MAE). The technique uses a single extraction in acetonitrile and requires a very small amount of (10-15 gm) sample. A large excess of salts or buffers are added to extract to aid in the extraction of both polar and non-polar pesticide. This sample initial step simultaneously extract the pesticide from the samples and prepares it for the next dispersive solid phase extraction (d-SPE), the salts and SPE sorbents chosen for the d-SPE step serve to remove residual water and further remove matrix interference from the sample. The resulting acetonitrile extract is typically analyzed directly by gas chromatography (GC), gas chromatography-mass spectrometry (GC/MS) or liquid chromatography tandem mass spectrometry (LC/MS/MS) with proper dilution (Anon, 2011).

In this study, the QuEChERS extraction technique was used for the extraction and clean-up of samples which was modified by Prodhan *et al.* (2015). 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.

3.8 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 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 (Figure 2). The instrument conditions are described in Table 4 and Table 5.

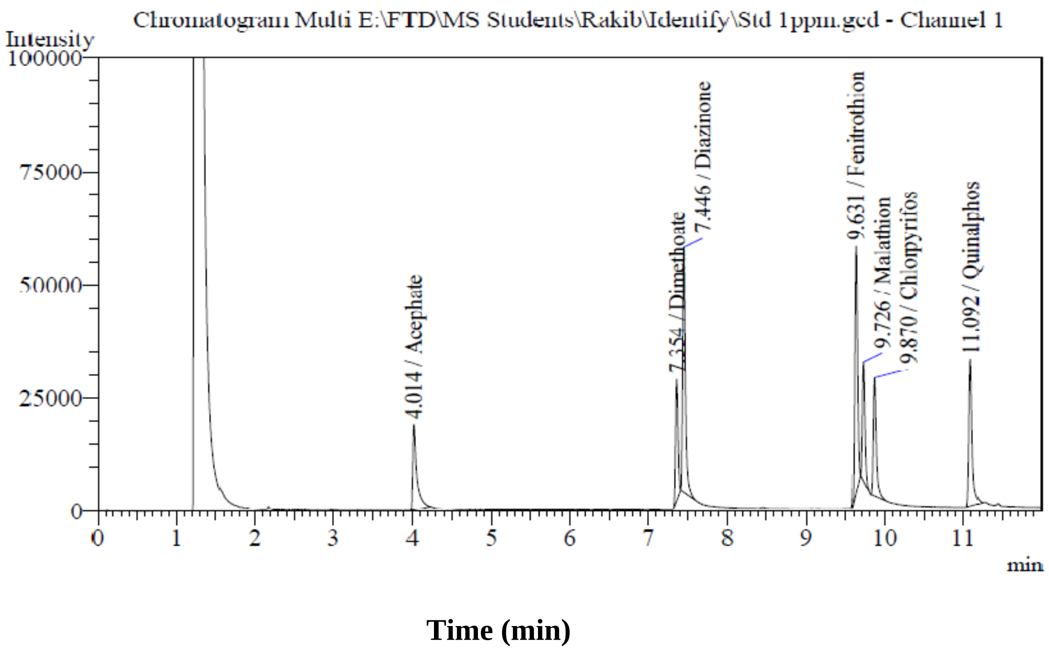


Figure 2. Typical Chromatograms of seven organophosphorus insecticide standards run by GC-FTD.

Table 4. The instrument parameters for GC-FTD

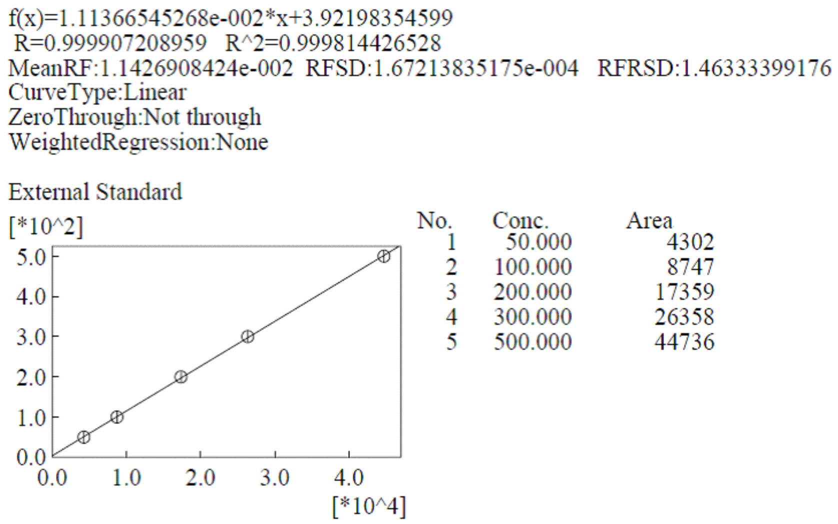
Instruments	Conditions
Injection port SPL	Injection mode: split; temperature:250°C; flow control mode: linear velocity; split ratio: 30:0
Detector channel 1 FTD	Temperature: 280°C; current: 1.00 Pa; H_2 flow: 1.5 ml/min; stop time: 10 min; make up flow: 30 ml/min; air flow: 145 ml/min

Table 5. Conditions for column oven

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

3.9 Calibration curve preparation

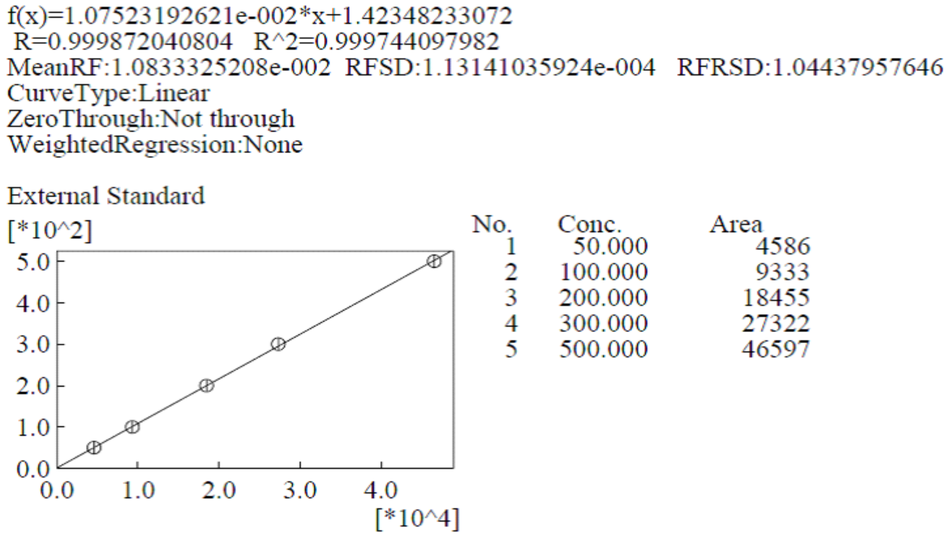
Prior to the injection of the sample extract, standard solutions of different concentrations of each pesticide group were prepared and injected with suitable instrument parameters. The samples were calibrated (retention time, peak area etc.) against five pointed calibration curve of standard solution of concerned pesticide (Figure 3-9). Each peak was characterized by its retention time. Sample results were expressed in mg/kg automatically by the GC software.



```
f(x)=1.11366545268e-002*x+3.92198354599
 R=0.999907208959   R^2=0.999814426528
MeanRF:1.1426908424e-002  RFSD:1.67213835175e-004   RFRSD:1.46333399176
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard
                                          No.     Conc.          Area
                                           1       50.000          4302
                                           2      100.000          8747
                                           3      200.000         17359
                                           4      300.000         26358
                                           5      500.000         44736
```

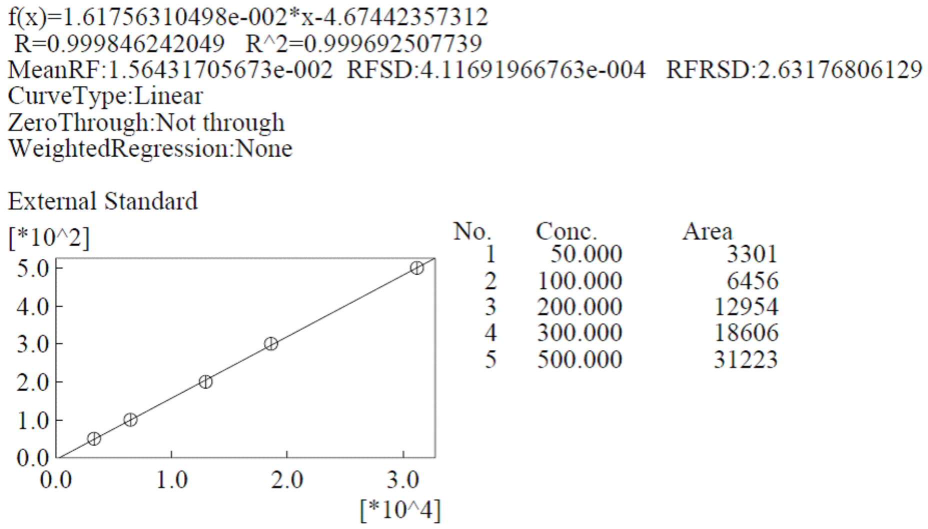
Figure 3. Calibration curve prepared for Dimethoate made with different concentrations ranging from 50 µg/L to 500 µg/L.



```
f(x)=1.07523192621e-002*x+1.42348233072
 R=0.999872040804   R^2=0.999744097982
MeanRF:1.0833325208e-002  RFSD:1.13141035924e-004   RFRSD:1.04437957646
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard
                                          No.     Conc.          Area
                                           1       50.000          4586
                                           2      100.000          9333
                                           3      200.000         18455
                                           4      300.000         27322
                                           5      500.000         46597
```

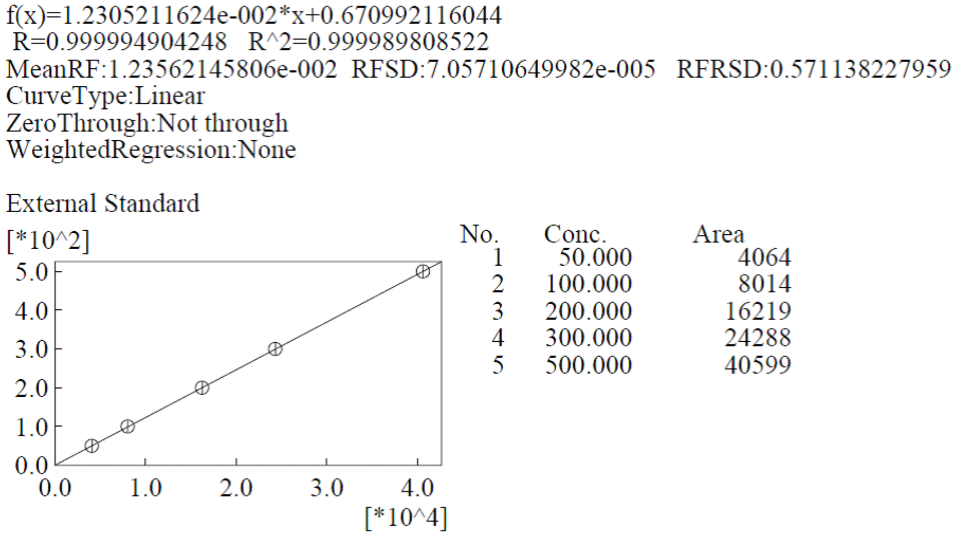
Figure 4. Calibration curve prepared for Chlorpyrifos made with different concentrations ranging from 50 µg/L to 500 µg/L.



```
f(x)=1.61756310498e-002*x-4.67442357312
 R=0.999846242049   R^2=0.999692507739
MeanRF:1.56431705673e-002  RFSD:4.11691966763e-004   RFRSD:2.63176806129
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard
                                       No.    Conc.        Area
                                        1      50.000       3301
                                        2     100.000       6456
                                        3     200.000      12954
                                        4     300.000      18606
                                        5     500.000      31223
```

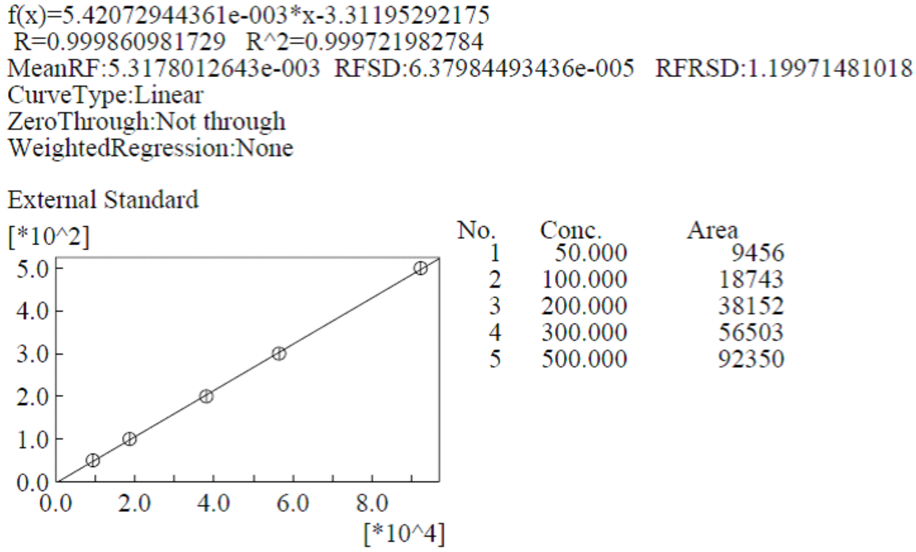
Figure 5. Calibration curve prepared for Acephate made with different concentrations ranging from 50 µg/L to 500 µg/L.



```
f(x)=1.2305211624e-002*x+0.670992116044
 R=0.999994904248   R^2=0.999989808522
MeanRF:1.23562145806e-002  RFSD:7.05710649982e-005   RFRSD:0.571138227959
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard
                                       No.    Conc.        Area
                                        1      50.000       4064
                                        2     100.000       8014
                                        3     200.000      16219
                                        4     300.000      24288
                                        5     500.000      40599
```

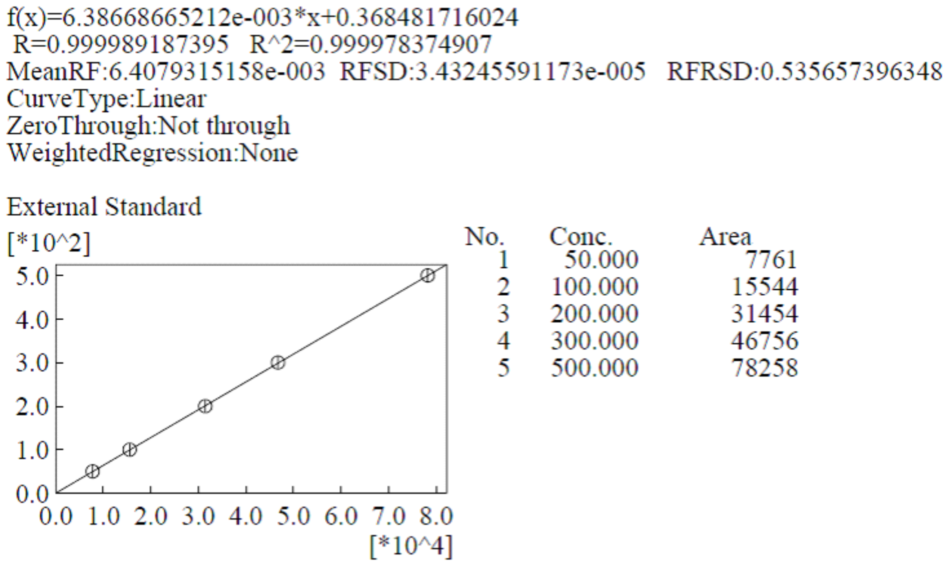
Figure 6. Calibration curve prepared for Quinalphos made with different concentrations ranging from 50 µg/L to 500 µg/L.



```
f(x)=5.42072944361e-003*x-3.31195292175
 R=0.999860981729   R^2=0.999721982784
MeanRF:5.3178012643e-003  RFSD:6.37984493436e-005   RFRSD:1.19971481018
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard
                                    No.    Conc.        Area
                                      1     50.000         9456
                                      2    100.000        18743
                                      3    200.000        38152
                                      4    300.000        56503
                                      5    500.000        92350
```

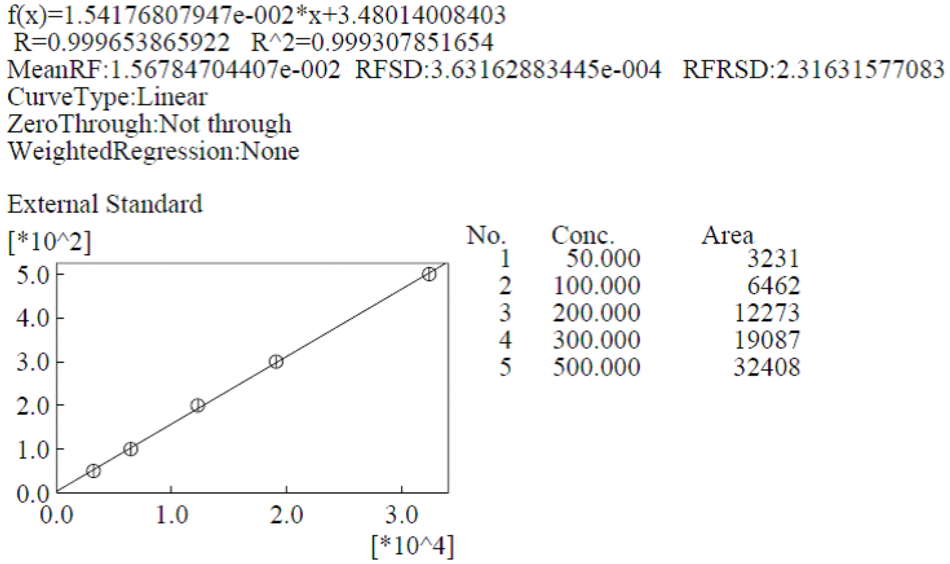
Figure 7. Calibration curve prepared for Diazinon made with different concentrations ranging from 50 µg/L to 500 µg/L.



```
f(x)=6.38668665212e-003*x+0.368481716024
 R=0.999989187395   R^2=0.999978374907
MeanRF:6.4079315158e-003  RFSD:3.43245591173e-005   RFRSD:0.535657396348
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard
                                    No.    Conc.        Area
                                      1     50.000         7761
                                      2    100.000        15544
                                      3    200.000        31454
                                      4    300.000        46756
                                      5    500.000        78258
```

Figure 8. Calibration curve prepared for Fenitrothion made with different concentrations ranging from 50 µg/L to 500 µg/L.



```
f(x)=1.54176807947e-002*x+3.48014008403
 R=0.999653865922   R^2=0.999307851654
MeanRF:1.56784704407e-002  RFSD:3.63162883445e-004   RFRSD:2.31631577083
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard
                                         No.    Conc.        Area
                                          1      50.000        3231
                                          2     100.000        6462
                                          3     200.000       12273
                                          4     300.000       19087
                                          5     500.000       32408
```

Figure 9. Calibration curve prepared for Malathion made with different concentrations ranging from 50 µg/L to 500 µg/L.

CHAPTER 4

RESULTS AND DISCUSSIONS

100 samples of vegetable (country bean, cauliflower & cucumber) were collected from 5 different markets of Dkaha city (Banani, Rampura, Mohammadpur, Kawran Bazar and Jatrabari) of Dhaka city to detect and quantify pesticide residues. The results obtained from this study are presented and described in this chapter using figures and tables.

4.1 Pesticide residues in country bean

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. Figure 10-19 shows the chromatograms of the injected extracts of country bean sample containing detected pesticides.

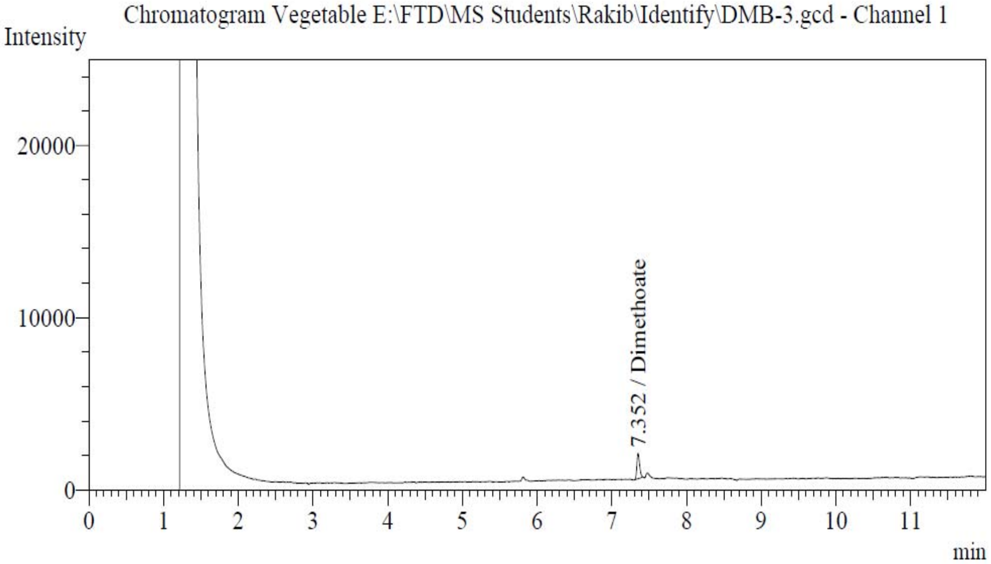


Figure 10. Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB_3) showing retention time.

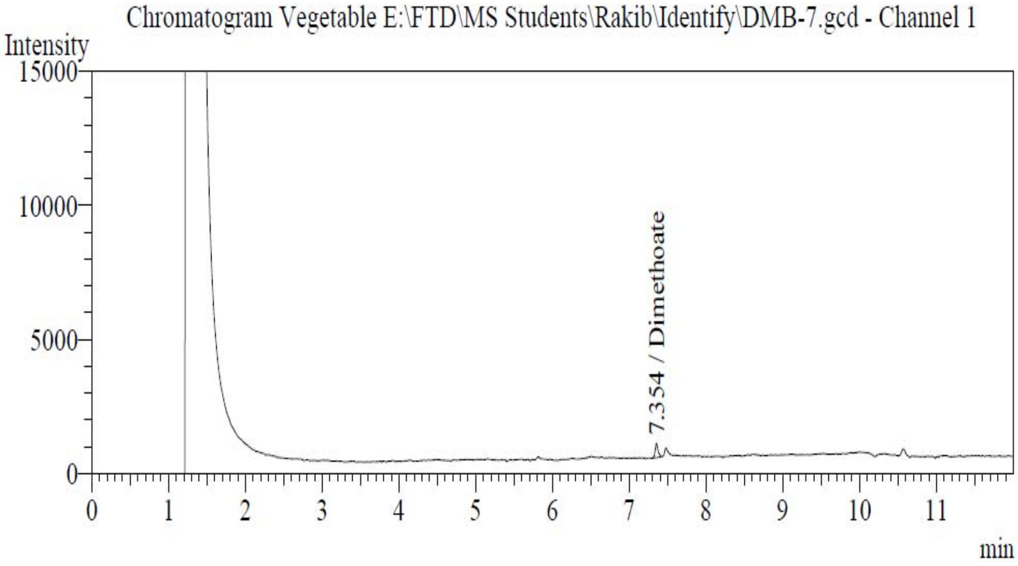


Figure 11. Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB_7) showing retention time.

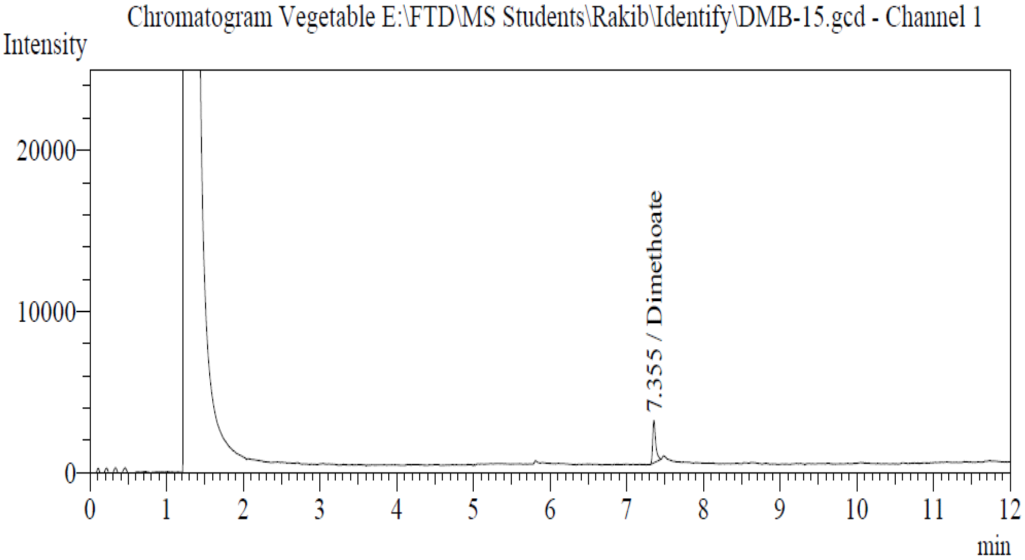


Figure 12. Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB_{15}) showing retention time.

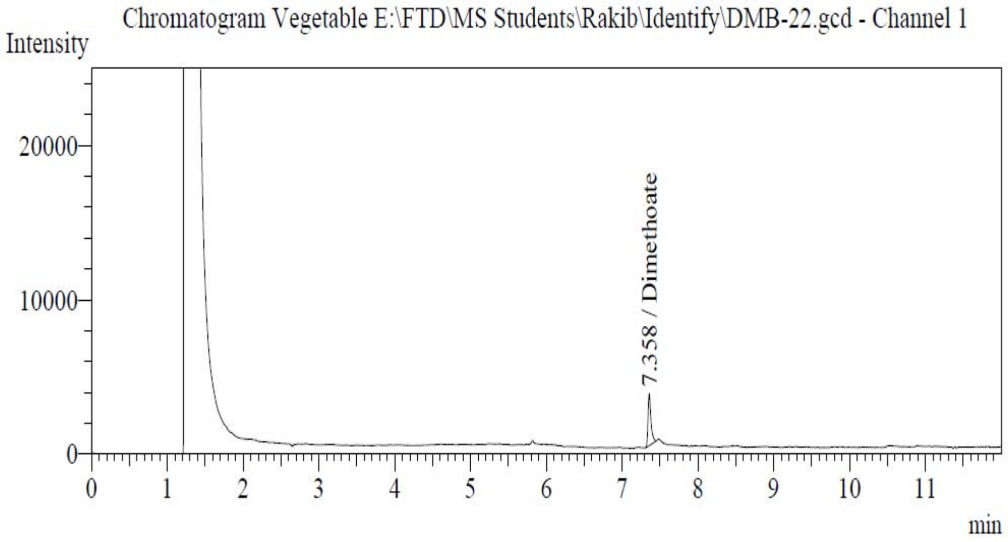


Figure 13. Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB_{22}) showing retention time.

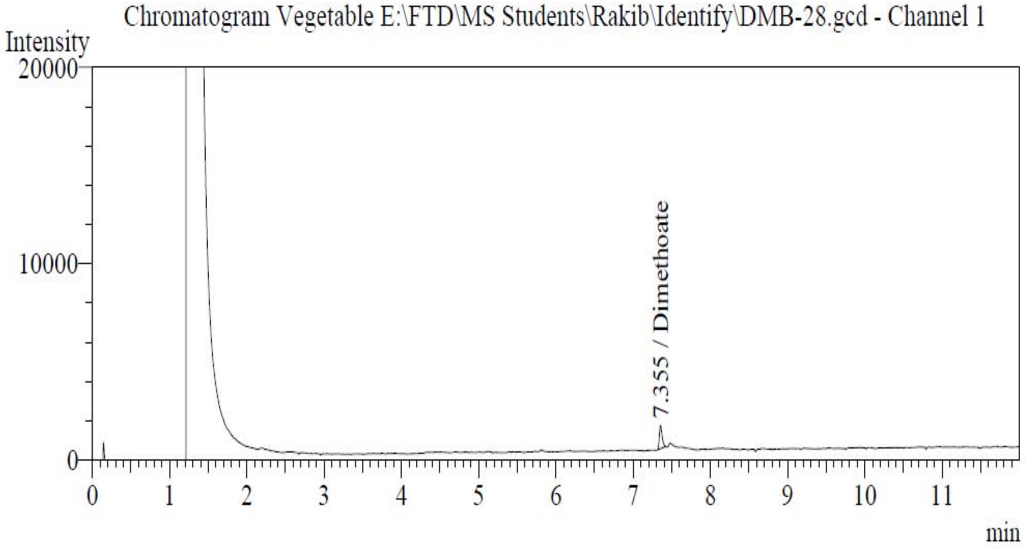


Figure 14. Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB_{28}) showing retention time.

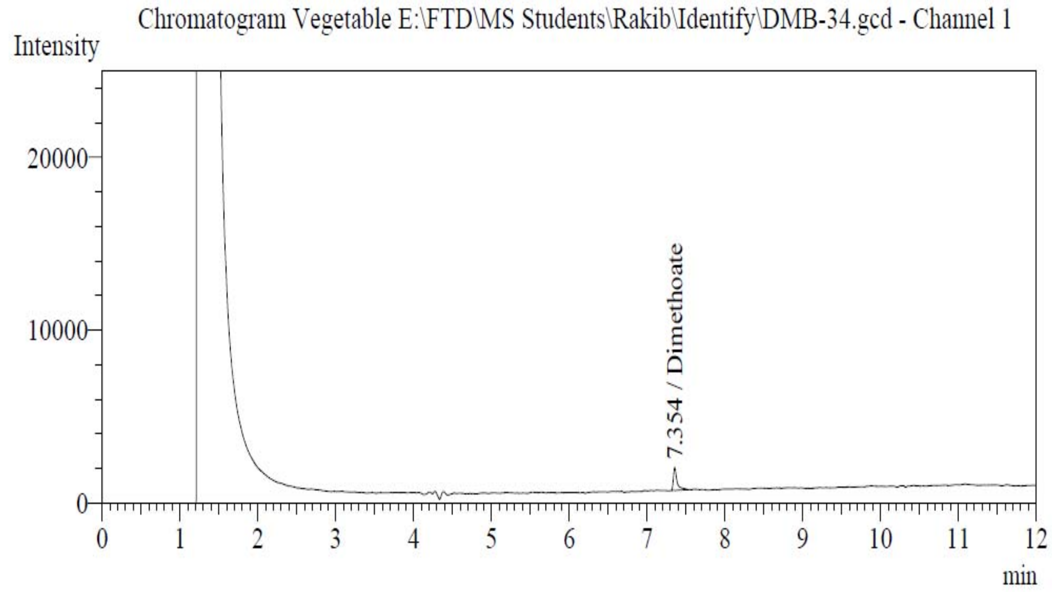


Figure 15. Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB_{34}) showing retention time.

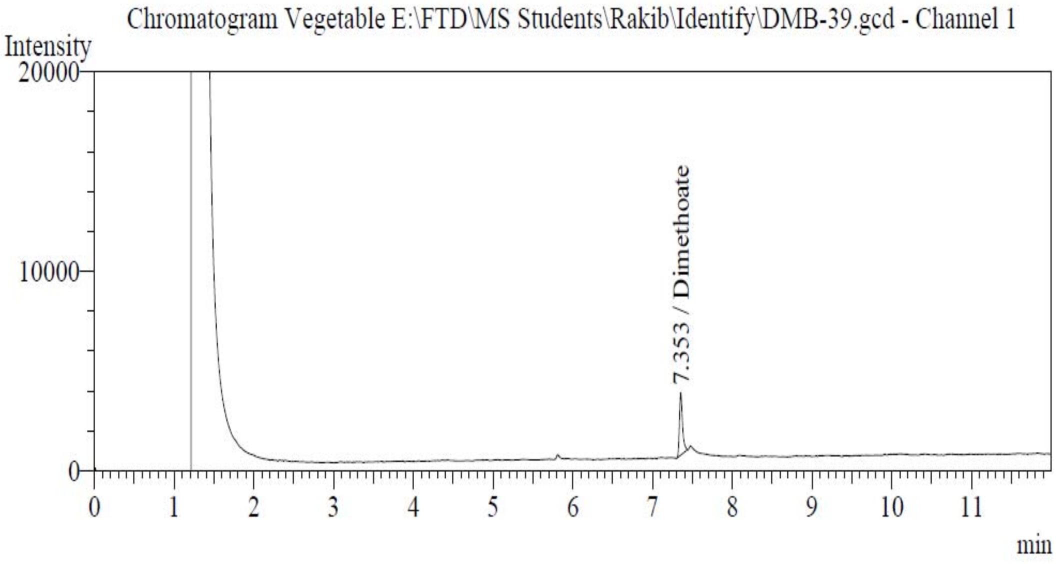


Figure 16. Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB_{39}) showing retention time.

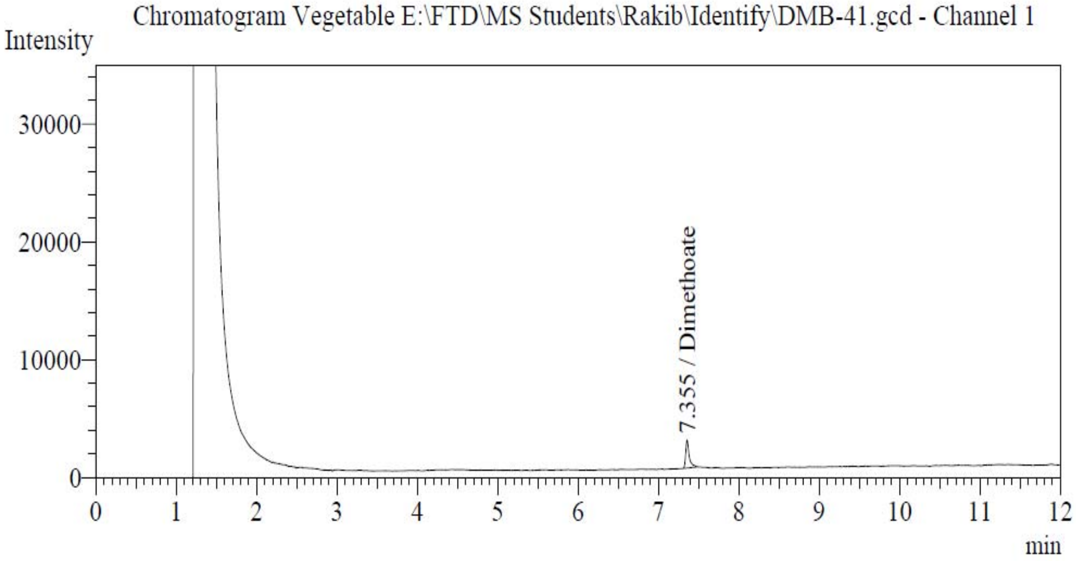


Figure 17. Chromatogram of Dimethoate found in one of the country bean marketed sample (DMB_{41}) showing retention time.

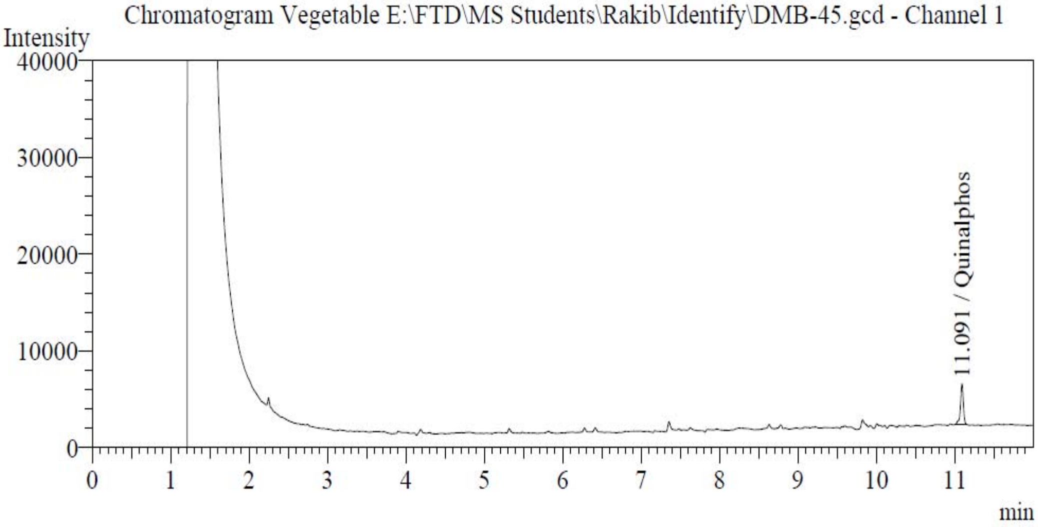


Figure 18. Chromatogram of Quinalphos found in one of the country bean marketed sample (DMB_{45}) showing retention time.

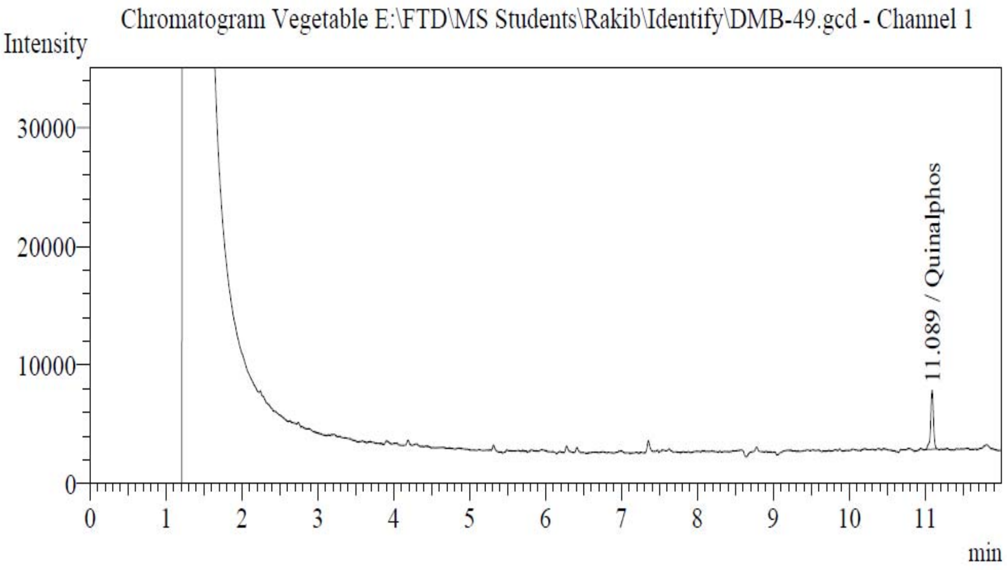


Figure 19. Chromatogram of Quinalphos found in one of the country bean marketed sample (DMB_{49}) showing retention time.

The level of pesticide residues found in the analyzed country bean samples and their maximum residue levels are outlined in Table 6.

Table 6. The level of residues (mg/kg) of different pesticides found in the analyzed country bean samples

Area of collection	**Sample ID**	**Name of detected pesticide**	**Level of residue (mg/kg)**	**MRLs (mg/kg)**
Banani	DMB_1	ND	-	-
	DMB_2	ND	-	-
	DMB_3	Dimethoate	0.018	0.02*
	DMB_4	ND	-	-
	DMB_5	ND	-	-
	DMB_6	ND	-	-
	DMB_7	Dimethoate	0.009	0.02*
	DMB 8	ND	-	-
	DMB_9	ND	-	-
	DMB_{10}	ND	-	-

Table 6 (cont’d)

Area of collection	Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)
Rampura	DMB$_{11}$	ND	-	-
	DMB$_{12}$	ND	-	-
	DMB$_{13}$	ND	-	-
	DMB$_{14}$	ND	-	-
	DMB$_{15}$	Dimethoate	0.069	0.02*
	DMB$_{16}$	ND	-	-
	DMB$_{17}$	ND	-	-
	DMB$_{18}$	ND	-	-
	DMB$_{19}$	ND	-	-
	DMB$_{20}$	ND	-	-
Mohammadpur	DMB$_{21}$	ND	-	-
	DMB$_{22}$	Dimethoate	0.093	0.02*
	DMB$_{23}$	ND	-	-
	DMB$_{24}$	ND	-	-
	DMB$_{25}$	ND	-	-
	DMB$_{26}$	ND	-	-
	DMB$_{27}$	ND	-	-
	DMB$_{28}$	Dimethoate	0.019	0.02*
	DMB$_{29}$	ND	-	-
	DMB$_{30}$	ND	-	-
Jatrabari	DMB$_{31}$	ND	-	-
	DMB$_{32}$	ND	-	-
	DMB$_{33}$	ND	-	-
	DMB$_{34}$	Dimethoate	0.011	0.02*
	DMB$_{35}$	ND	-	-
	DMB$_{36}$	ND	-	-
	DMB$_{37}$	ND	-	-
	DMB$_{38}$	ND	-	-
	DMB$_{39}$	Dimethoate	0.120	0.02*
	DMB$_{40}$	ND	-	
Kawran bazar	DMB$_{41}$	Dimethoate	0.013	0.02*
	DMB$_{42}$	ND	-	-
	DMB$_{43}$	ND	-	-
	DMB$_{44}$	ND	-	-
	DMB$_{45}$	Quinalphos	0.033	0.01*
	DMB$_{46}$	ND	-	-
	DMB$_{47}$	ND	-	-
	DMB$_{48}$	ND	-	-
	DMB$_{49}$	Quinalphos	0.045	0.01*
	DMB$_{50}$	ND	-	

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

Fifty samples of country bean collected from 5 different markets of Dhaka city (Banani, Rampura, Mohammadpur Krishi Market, 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. The present results can be compared to Islam *et al.* (2014). 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 & DMB_7) contained Dimethoate at a level of 0.018 mg/kg and 0.009 mg/kg respectively, which were below the EU-MRL (European Commission 2005). 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 Krishi Market, two samples (DMB_{22} & 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} & 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} & 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).

4.2 Pesticide residues in cauliflower

The concentrated extracts of cauliflower samples collected from different markets of Dhaka city were analyzed by GC-2010 (Shimadzu) with Flame Thermionized Detector (FTD) with the pre-set parameters. Figure 20-24 shows the chromatograms of the injected extracts of cauliflower sample containing detected pesticides.

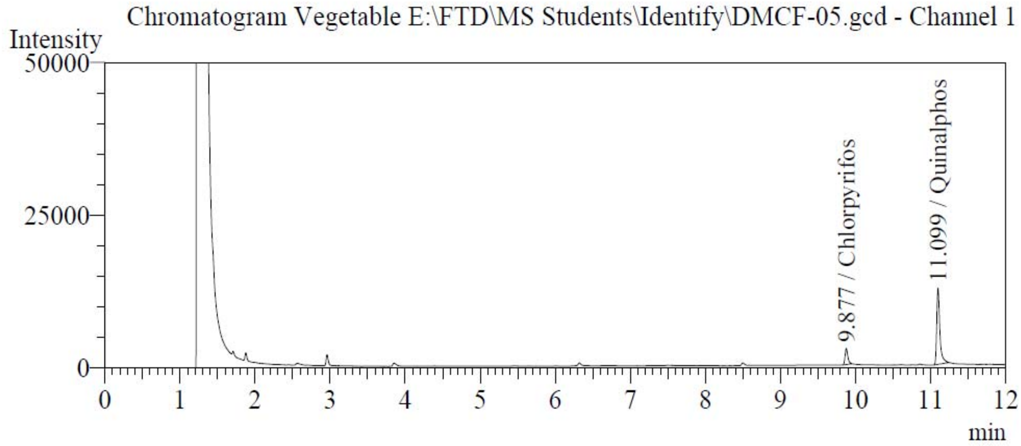


Figure 20. Chromatogram of Chlorpyrifos and Quinalphos found in one of the cauliflower marketed sample ($DMCF_5$) showing retention time.

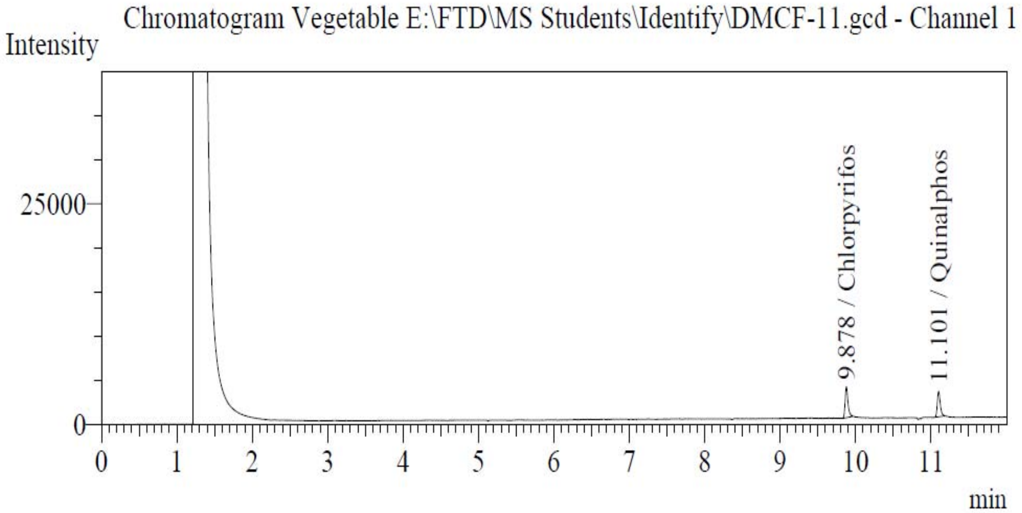


Figure 21. Chromatogram of Chlorpyrifos and Quinalphos found in one of the cauliflower marketed sample ($DMCF_{11}$) showing retention time.

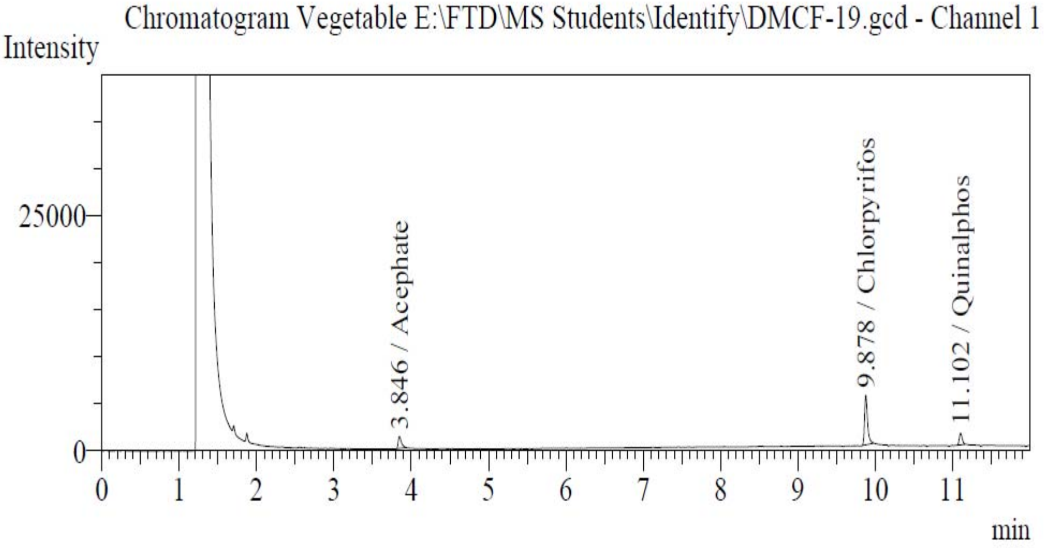


Figure 22. Chromatogram of Acephate, Chlorpyrifos and Quinalphos found in one of the cauliflower marketed sample (DMCF$_{19}$) showing retention time.

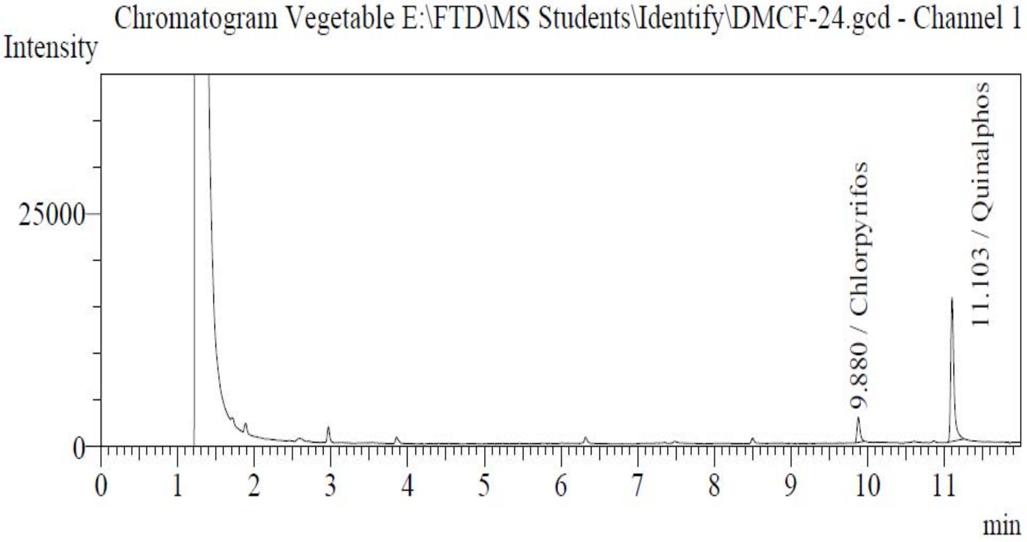


Figure 23. Chromatogram of Chlorpyrifos and Quinalphos found in one of the cauliflower marketed sample (DMCF$_{24}$) showing retention time.

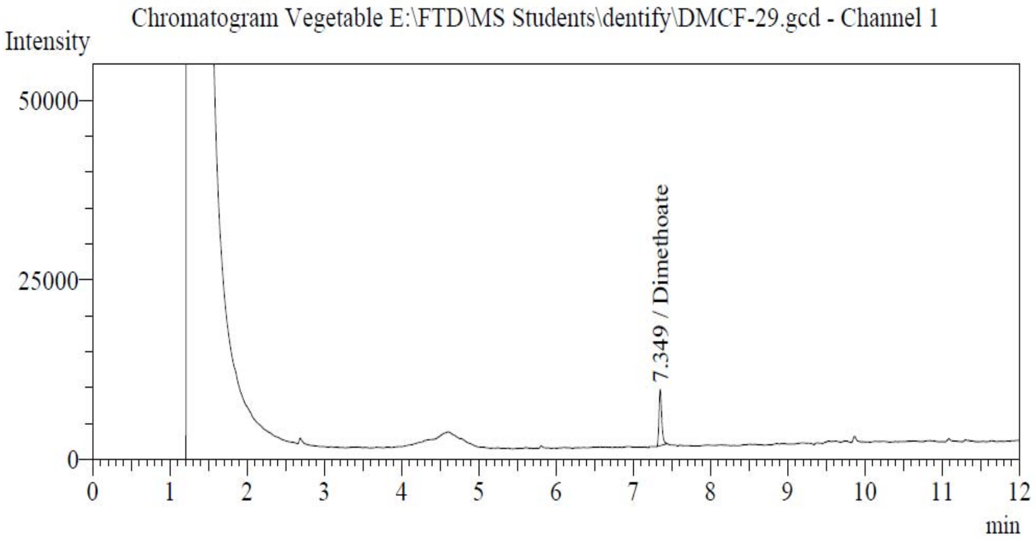


Figure 24. Chromatogram of Dimethoate found in one of the cauliflower marketed sample ($DMCF_{29}$) showing retention time.

The levels of pesticide residues found in the analyzed cauliflower samples and their maximum residue levels are presented in Table 7.

Table 7. The level of residues (mg/kg) of different pesticides found in the analyzed cauliflower samples

Area of collection	**Sample ID**	**Name of detected pesticide**	**Level of residue (mg/kg)**	**MRLs (mg/kg)**
Banani	$DMCF_1$	ND	-	-
	$DMCF_2$	ND	-	-
	$DMCF_3$	ND	-	-
	$DMCF_4$	ND	-	-
	$DMCF_5$	Chlorpyrifos Quinalphos	0.022 0.121	0.05* 0.01*
	$DMCF_6$	ND	-	-
Rampura	$DMCF_7$	ND	-	-
	$DMCF_8$	ND	-	-
	$DMCF_9$	ND	-	-
	$DMCF_{10}$	ND	-	-
	$DMCF_{11}$	Chlorpyrifos Quinalphos	0.038 0.046	0.05* 0.01*
	$DMCF_{12}$	ND	-	-

Table 7 (cont'd)

Area of collection	**Sample ID**	**Name of detected pesticide**	**Level of residue (mg/kg)**	**MRLs (mg/kg)**
Mohammadpur	DMCF $_{13}$	ND	-	-
	DMCF $_{14}$	ND	-	-
	DMCF $_{15}$	ND	-	-
	DMCF $_{16}$	ND	-	-
	DMCF $_{17}$	ND	-	-
	DMCF $_{18}$	ND	-	-
Jatrabari	DMCF $_{19}$	Acephate Chlorpyrifos Quinalphos	0.008 0.056 0.007	0.01* 0.05* 0.01*
	DMCF $_{20}$	ND	-	-
	DMCF $_{21}$	ND	-	-
	DMCF $_{22}$	ND	-	-
	DMCF $_{23}$	ND	-	-
	DMCF $_{24}$	Chlorpyrifos Quinalphos	0.03 0.296	0.05* 0.01*
Kawranbazar	DMCF $_{25}$	ND	-	-
	DMCF $_{26}$	ND	-	-
	DMCF $_{27}$	ND	-	-
	DMCF $_{28}$	ND	-	-
	DMCF $_{29}$	Dimethoate	0.06	0.02*
	DMCF $_{30}$	ND	-	-

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

30 samples of cauliflower collected from 5 different maarkets of Dhaka city (Banani, Rampura, Mohammadpur Krishi Market, Kawran Bazar and Jatrabari) were analyzed to find out the presence of left over residues of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos.

Out of 30 samples, 5 samples (16.67% of the total no. of samples) contained pesticide residues and 25 samples (83.33% of the total number of samples) contained no detectable residues of the sought pesticides. The present results can be compared to Islam *et al.* (2014). They were 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.

Six samples of cauliflower were collected from Banani area, among them only one sample ($DMCF_5$) contained multi residue of Chlorpyrifos (0.022 mg/kg) and Quinalphos (0.121 mg/kg), where the residue of Chlorpyrifos was below the MRL (0.05 mg/kg) and the residue of Quinalphos was above MRL (0.01 mg/kg).

Among the six samples of cauliflower collected from Rampura, one sample ($DMCF_{11}$) contained multi-residue of Chlorpyrifos and Quinalphos, where the residue of Chlorpyrifos was 0.038 mg/kg, which was below the MRL (0.05 mg/kg) and the residue of Quinalphos was 0.046 mg/kg, which was above MRL (0.01 mg/kg)

In case of six samples of cauliflower collected from Mohammadpur Krishi Market contained no residues of the sought pesticides.

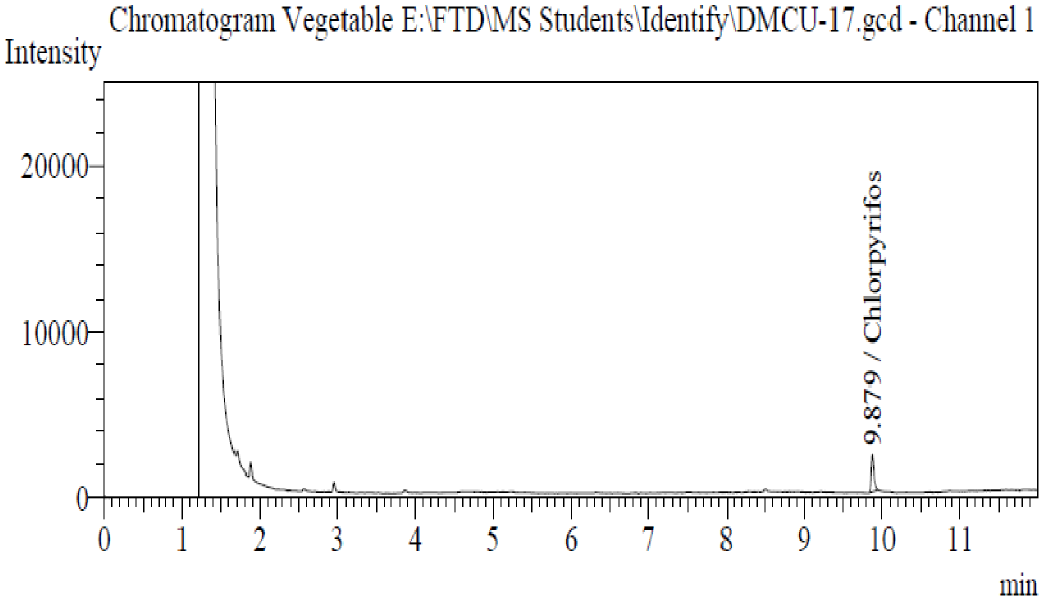
Among the six samples of cauliflower collected from Jatrabari area, one sample ($DMCF_{19}$) had multiple pesticide residues of Acephate (0.008 mg/kg), Chlorpyrifos (0.056 mg/kg) and Quinalphos (0.007 mg/kg), where the level of residue of Acephate and Quinalphos were below the MRL (0.01 mg/kg) and the level of residue of Chlorpyrifos was above MRL (0.05 mg/kg), and another sample ($DMCF_{24}$) had multiple residue of Chlorpyrifos and Quinalphos, where the level of Chlorpyrifos residue (0.03 mg/kg) was below the MRL (0.05 mg/kg) and the level of Quinalphos residue (0.296 mg/kg) was much higher than the MRL (0.01 mg/kg).

Only one sample ($DMCF_{29}$) contained residue of Dimethoate and the other five samples contained no detectable pesticide residues among the ten samples collected from Kawran Bazar. The level of detected residue was 0.06 mg/kg, which was above the MRL (0.02 mg/kg).

4.3 Pesticide residues in cucumber

The concentrated extracts of cucumber samples collected from different markets were analyzed by GC-2010 (Shimadzu) with Flame Thermonized Detector (FTD) with the pre-set parameters. Figure 25-26 shows the chromatograms of the injected extracts of cucumber sample containing detected pesticides.

Figure 25. Chromatogram of Quinalphos found in one of the cucumber marketed sample ($DMCU_9$) showing retention time.



marketed sample ($DMCU_{17}$) showing retention time.

The levels of pesticide residues found in the analyzed cauliflower samples and their maximum residue levels are outlined in Table 8.

Table 8. The level of residues (mg/kg) of different pesticides found in the analyzed cucumber samples

Area of collection	**Sample ID**	**Name of detected pesticide**	**Level of residue (mg/kg)**	**MRLs (mg/kg)**
Banani	$DMCU_1$	ND	-	-
	$DMCU_2$	ND	-	-
	$DMCU_3$	ND	-	-
	$DMCU_4$	ND	-	-
Rampura	$DMCU_5$	ND	-	-
	$DMCU_6$	ND	-	-
	$DMCU_7$	ND	-	-
	$DMCU_8$	ND	-	-
Mohammadpur	$DMCU_9$	Quinalphos	0.008	0.01*
	$DMCU_{10}$	ND	-	-
	$DMCU_{11}$	ND	-	-
	$DMCU_{12}$	ND	-	-
Jatrabri	$DMCU_{13}$	ND	-	-
	$DMCU_{14}$	ND	-	-
	$DMCU_{15}$	ND	-	-
	$DMCU_{16}$	ND	-	-
Kawranbazar	$DMCU_{17}$	Chlorpyrifos	0.04	0.05*
	$DMCU_{18}$	ND	-	-
	$DMCU_{19}$	ND	-	-
	$DMCU_{20}$	ND	-	-

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

20 samples of cucumber were collected from 5 different markets of Dhaka city (Banani, Rampura, Mohammadpur Krishi Market, Kawran Bazar and Jatrabari) were analyzed to find out the presence of left over residues of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos).

Out of 20 samples, 2 samples (10% of the total no. of samples) contained pesticide residues and 18 samples (90% of the total number of samples) contained no detectable residues of the sought pesticides. The present results can be compared to Islam *et al.* (2014). 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.

Among the four samples of cucumber collected from Banani, Rampura and Jatrabari contained no residues of the sought pesticides.

In case of four samples collected from Mohammadpur Krishi Market, one sample ($DMCU_9$) contained Quinalphos residue of 0.008 mg/kg, which is below the MRL (0.01 mg/kg).

One sample had Chlorpyrifos residue at a level of 0.04 mg/kg among the four samples collected from Kawran Bazar. Considering the maximum residue limit (0.05 mg/kg), the level of detected residue was below MRL.

CHAPTER 5

SUMMARY AND CONCLUSION

Vegetables are one of the most important source 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, thirty samples of Cauliflower and twenty samples of Cucumber 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.

Out of 30 samples of cauliflower, 5 samples (16.67% of the total number of samples) contained residues of Chlorpyrifos, Quinalphos, Acephate and Dimethoate, where 4 samples contained multiple pesticide residues. Among them, all of the contaminated samples (5) contained residue above MRL. And the other 25 samples (83.33% of the total number of samples) contained no detectable residues of the sought pesticide.

In case of 20 samples of cucumber, only 2 samples (10% of the total samples) contained residue of Quinalphos and Chlorpyrifos, but the detected residue level was below the maximum residue limit (MRL), and the rest 18 samples (90% of the total number of samples) contained no detectable pesticide residues.

Now 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 studied vegetables in the study area and will help to increase public awareness as well.

CHAPTER 6

REFERENCES

Ahmed, F.E. (2001). Analyses of pesticides and their metabolites in foods and drinks. *TrAC Trends in Analytical Chemistry*. **20**(11): 649-661.

Afful, S., Enimil, E., Blewu, B., Mantey, G.A. and Ewusie, E.A. (2010). Gas chromatographic methodology for the determination of some halogenated pesticides. *Res. J. Appl. Sci. Eng. Tech.* **2**(6): 592-595.

Agüera, A., Piedra, L., Hernando, M.D., Fernandez-Alba, A.R., Contreras, M. (2000). Splitless large-volume GC-MS injection for the analysis of organophosphorus and organochlorine pesticides in vegetables using a miniaturised ethyl acetate extraction. *Analyst*. **125**:1397–1402.

Ahuja, A.K., Soudamini, M., Debi, S., Awasthi, M.D., Mohapatra, S., Sharma, D., Reddy, P.P., Kumar N.K.K. and Verghese, A. (1998). Monitoring of vegetables of insecticide residue contamination at harvest, Advances in IPM for horticultural crops. Proc. of the 1st national symposium on pest management in horticultural crops: Environmental implications and thrusts, Bangalore, India, 15-17 Oct. 1997, pp.243-246.

Akan, J.C., Jafiya, L., Mohammed, Z. and Abdulrahman, F.I. (2013). Organophosphorus pesticide residues in vegetables and soil samples from alau dam and gongulong agricultural areas, Borno State, Nigeria. *Int. J. Environ. Monitoring and Analysis*. **1**(2): 58-64.

Aktar, M.W., Sengupta, D. and Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary Toxicol.* **2**: 1–12.

Anastassiades, M., Lehotay, S.J., Štajnbaher, D. and Schenck, F.J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solidphase extraction" for the determination of pesticide residues in produce. *J. AOAC. Int*. **86**: 412-431.

Andraščíková M. and S. Hrouzková. (2016). Fast preconcentration of pesticide residues in oilseeds by combination of QuEChERS with dispersive liquid–liquid microextraction followed by gas chromatography-mass spectrometry. *Food Anal. Methods.* **9**:2182–2193.

Anonymous. (2000). Annual Report- 1999-2000, Entomology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh.

Antle, J.M. and Pingali, P.L. (1994). Pesticides, productivity and farmer health: a Philippine case study, *Am. J. Agril. Econ.* **76**: 418-430.

Aysal, P., Ambrus, A., Lehotay, S.J. and Cannavan, A. (2007). Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. *J. Environ. Health Sci. B.* **42**(5):481-490.

Banerjee, K., Oulkar, D.P., Dasgupta, S., Patil, S.B., Patil, S.H., Savant, R. and Adsule, P.G. (2007). Validation and uncertainty analysis of a multi-residue method for pesticides in grapes using ethyl acetate extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A.* **1173**(1-2):98-109.

Banerjee, K., Oulkar, D.P., Patil, S.B., Jadhav, M.R., Dasgupta, S., Patil, S.H. and Adsule P.G. (2009). Multiresidue determination and uncertainty analysis of 87 pesticides in mango by liquid chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* **57**(10): 4068-4078.

Banerjee, K., Oulkar, D.P., Patil, S.B., Patil, S.H., Dasgupta, S., Savant, R. and Adsule, P.G. (2008). Single-laboratory validation and uncertainty analysis of 82 pesticides determined in pomegranate, apple, and orange by ethyl acetate extraction and liquid chromatography/tandem mass spectrometry. *J. AOAC Int.* **91**(6): 1435-1445.

Bolaños, P.P., Moreno, J.L., Shtereva, D.D., Frenich, A.G**.** and Vidal, J.L. (2007). Development and validation of a multiresidue method for the analysis of 151 pesticide residues in strawberry by gas chromatography coupled to a triple quadrupole mass analyzer. *Rapid Commun. Mass Spectrom.* **21**(14): 2282-2294.

Butler, J., Steiniger, D., and Phillips, E. (2008). Analysis of pesticide residues in lettuce using a modified quechers extraction technique and single quadrupole GC/MS, Retrieved from www.thermo.com. (Date: 12 Nov., 2013).

Cajka, T., Mastovská, K., Lehotay, S.J. and Hajslová, J. (2005). Use of automated direct sample introduction with analyte protectants in the gc-ms analysis of pesticide residues. *J. Sep. Sci.* **28**: 1048.

Camino-Sánchez, A., Zafra-Gómez, J., Ruiz-García, R., Bermúdez-Peinado, O., Ballesteros, A., Navalon and Vílchez, J.L. (2011). UNE-EN ISO/IEC 17025:2005 accredited method for the determination of 121 pesticide residues in fruits and vegetables by gas chromatography–tandem mass spectrometry. *J. Food Composition and Ana.* **24**: 427-440.

Charan, P.D. and Sharma, K.C. (2010). Monitoring of pesticide residues in farmgate vegetables of Central Aravalli region of Western India. *American-Eurasian J. Agril. Env. Sci.* **7** (3): 255-258.

Chauhan, S.S. (2012). Monitoring of pesticides residues in farmgate vegetables of Uttarakhand, India, *Wudpecker J. Agril. Res.* **1**(7):250 – 256.

Cho, T.H., Park, Y.H., Park, H.W., Hwang, L.H., Cho, I.S., Kim, H.J., Kim, M.J., Kim, M.S. and Chae, Y.Z. (2013). Evaluation of QuEChERS method for determination of pesticide residues using GC/NPD and GC/ECD. *The Korean J. of Pesticide Sci.* **17**(1):65-71.

Colume, A., Cardenas, S., Gallego, M. and Valcarcel, M. (2001). Semiautomatic multiresidue gas chromatographic method for the screening of vegetables for 25 organochlorine and pyrethroid pesticides. *Analytica Chemica Acta*. **436**: 153-162.

Corteas, J. M., Sanchez, R., Plaza, E.M.D., Villean, J. and Vaazquez A. (2013). Large volume GC injection for the analysis of organophosphorus pesticides in vegetables using the through oven transfer adsorption desorption (TOTAD) interface. *Int. J. Env. Monit. Anal.* **1**(2): 58-64.

Craig , W. and Beck, L. (1999). Phytochemicals: health protective effects. *Can. J. Dietetic Prac. Res.* **60**(2): 78-84.

Cunha, S.C., Fernandes, J.O., Alves, A. and Oliveira, M.B. (2009). Fast low-pressure gas chromatography-mass spectrometry method for the determination of multiple pesticides in grapes, musts and wines. *J. Chromatogr. A.* **1216**(1): 119-126.

Dasika, R., Tangirala, S. and Naishadham, P. (2012). Pesticide residue analysis of fruits and vegetables. *J. Environ. Chem. Ecotoxicol.* **4**(2): 19-28.

Dethe, M.D., Kale, V.D and Rane, S.D. (1995). Insecticide residues in/on farm gate samples of vegetables. *Pest Manag. Hort. Ecosystem.* **1**(1): 49-53.

Devi. L. S. and C.E. Manjunath. (1985). Current Research, University of Agricultural Sciences, Bangalore **14**(4/6):34-35.

Dias, J. S. and Ryder, E. (2011). World vegetable industry: production, breeding, trends. *Hort. Review*. **38**: 299-356.

Dias, J.S. (2011). World importance, marketing and trading of vegetables, *Acta Hort.* **921**: 153-169.

European Commission. (2005). EU pesticide residue MRLs. regulation no. 396/2005. http://ec.europa.eu/sanco_pesticides/public/index.cfm.

FAO (Food and Agricultural Organization) and WHO (World Health Organization). (1970). Evaluation of some pesticides in food, WHO, FOOD ADD, **71**:42.

FAO/WHO. (1996). Food Standards Program, 2nd Edn. Codex Alimentarius Commission, 1.

FAO/WHO. (2002). Global forum of food safety regulators. Marrakech, Morocco. [http://www.fao.org/DOCREP/MEETING/0 04/AB428E.HTM Agenda Item 4.2 a, GF/CRD Iran-1]. 28-30 January.

FAO/WHO. (1993). Insecticide Residues in Food. Part I-Residues. Food and Agriculture Organization (FAO). pp. 257-287.

Farag, R.S., Latif, M.S., El-Gawad, A.E. and Dogheim, S.M. (2011). Monitoring of pesticide residues in some Egyptian herbs, fruits and vegetables. Biochemistry Department, Faculty of Agriculture, Cairo University, Egypt. Agricultural Research Center, Ministry of Agricultural, Dokki, Egypt.

Fenoll, J., Hellin, P., Martinez, C.M., Miguel, M. and Flores, P. (2007). Multiresidue method for analysis of pesticides in pepper and tomato by gas chromatography with nitrogen–phosphorus detection. *Food Chem.* **105**: 711-719.

Fernandes, V.C., Valentina, F., Domingues, V.F., Mateus, N. and Matos. C.D. (2011). Determination of pesticides in fruit and fruit juices by chromatographic methods. An overview. *J. of Chromatogr. Sci.* **49** (9): 715-730.

Fernandez-Alba, A.R. and Garcia-Reyes, J.F. (2008). Large-scale multi-residue methods for pesticides and their degradation products in food by advanced LC-MS. Trac-Trend. *Anal. Chem.* **27** (11): 973-990.

Fernández Moreno, J.L., Garrido Frenich, A., Plaza Bolaños, P. and Martínez Vidal, J.L. (2008). Multiresidue method for the analysis of more than 140 pesticide residues in fruits and vegetables by gas chromatography coupled to triple quadrupole mass spectrometry. *J. Mass. Spectrom.* **43**(9): 1235-1254.

Ferrer, I., Reyes, J.F.G., Mezcua, M., Thurman, E. M. and Alba, A. (2005). Multi-residue pesticide analysis in fruits and vegetables by liquid chromatography–time-of flight mass spectrometry. *J. Chromatogr. A.* **1082**(1): 81-90.

Frank, R., Braun, H.E. and Ripley, B.D. (1990). Residues of insecticides and fungicides on Ontario-grown vegetables, 1986-88. Food Additives-and-Contaminants. **7**(4): 545-554.

Gamon, A., Leo, C. and Ten, A. 2001**.** Multiresidue determination of pesticides in fruit and vegetables by gas chromatography. *J. AOAC Int.* **84**(4): 342-354.

Gilbert-Lopez, B., Juan Garcia-Reyes, F., Lozano, A., Fernandez-Alba, A.R. and Molina-Diaz, A. (2010). Large-scale pesticide testing in olives by liquid chromatography–electrospray tandem mass spectrometry using two sample preparation methods based on matrix solid-phase dispersion and QuEChERS. *J. Chromatogr. A.* **1217**: 6022–6035.

Gilden, R.C., Huffling, K. and Sattler, B. (2010). Pesticides and health risks. *JOGNN*, **39**: 103-110.

Goring, H. (1977). Cytogenic effects of Metalaxyl on human and animal chromosomes. *Mutation Res.* **369**: 81-86.

Hayama, T. and Takada, M. (2008). Simple and rapid method for the determination of ethylenebisdithiocarbamate fungicides in fruits and vegetables using liquid chromatography with tandem mass spectrometry. *Anal. Bioanal. Chem.* **392**(5):969-976.

Hossain, M.I., Shively, G. and Mahmoud, C. (2000). Pesticide expenditure in a rice vegetable farming system: Evidence from Low-income Farms in Bangladesh. IPM-CRSP Working Paper, 120p.

Hossain, S. M., Hossain, M. A. and Rahman, M. A. (2013). Health risk assessment of pesticide residues via dietary intake of market vegetables from Dhaka, Bangladesh. *Bangladesh J. Pestic. Sci. and Environ.* **21**(7): 54-58.

Hossain, S., Chowdhury, M. A. Z., Alam, M.M., Islam, N., Rashid, M.H. and Jahan, I. (2015). Determination of pesticide residues in brinjal, cucumber and tomato using gas chromatography and mass spectrophotometry (GC-MS). *Bios. J. Org.* **1**(1): 1-16.

Hrouzkova, S. and Matisova, E. (2011). Fast gas chromatography and its use in pesticide residues analysis. In: Pesticides - Strategies for Pesticides Analysis. Stoytcheva, M. (ed.). ISBN. pp.131-154.

Huang, P.M. and Iskandar, I.K. (2000). Soils and Groundwater Pollution and Pemediation. Asia, Africa, and Ocenia. Lewis Publishers Washington, D.C. pp.196-231.

Hura, C. (1999). Risk assessment of pollution with pesticides in food in the Eastern Romania area (1996-1997). *Toxi. Letters*. **107**: 103-107.

Húsková, R., Matisová, E., Svorc, L., Mocák, J. and Kirchner, M. (2009). Comparison of negative chemical ionization and electron impact ionization in gas chromatography-mass spectrometry of endocrine disrupting pesticides. *J. Chromatogr. A* **1216**(24): 4927-4932.

Islam, M.W., Dastogeer, K.M.G., Hamim, I., Prodhan, M.D.H., Ashrafuzzaman, M. (2014). Detection and quantification of pesticide residues in selected vegetables of Bangladesh. *J. of Phytopath. and Pest Manag.* **1**(2): 17-30.

Islam, S., Afrin, N., Hossain, M. S. and Nahar, N. (2009). Analysis of some pesticide residues in cauliflower by high performance liquid chromatography. *American J. Environ. Sci.* **5** (3): 325-329.

Islam, Z., Islam, M.A. and Nahar, B.S. (2013). Analysis of pesticide residue in vegetables collected from local market by using GC technique. Department of Environmental Science. Bangladesh Agricultural University, Mymensingh.

Ji, F., Zhao, L., Yan, W., Feng, Q., Lin, J.M. (2008). Determination of triazine herbicides in fruits and vegetables using dispersive solid-phase extraction coupled with LC-MS. *J. Sep. Sci.* **31**(6-7): 961-968.

Kabir, K. H. (2007). Pesticide residues in agricultural produce and its safe use. A paper presented at the Symposium on "Safe use of Pesticide in Agriculture" held at BARC, Farmgate, Dhaka-1215 on October, 2007. pp. 32-39.

Kabir, K. H., Abdullah, M., Prodhan, M.D.H., Ahmed, M.S., and Alam, M.N. 2007. Determination of carbofuran residue in the samples of sugarcane and soil of sugarcane field. *The Agriculturist.* **5**(1& 2): 61-66.

Kabir, K. H., Rahman, M.A., Ahmed, M.S., Prodhan, M.D.H. and Akon, M.W. (2008). Determination of residue of diazinon and carbosulfan in brinjal and quinalphos in yard long bean under supervised field trial. *Bangladesh J. Agril. Res.* **33**(3): 503-513.

Kabir, K.H., Baksh, M.E., Rouf, F.M.A., Karim, M.A., Ahmed, A. (1996). Insecticide usage pattern on vegetables at farmers' level of Jessore region in Bangladesh: A survey finding. *Bangladesh J. Agril. Res.* **21**(2): 241-254.

Kanda, M., Boundjou, G.D., Wala, K., Gnandi, K., Batawila, K., Sanni, A. and Akpagana, K. (2012). Assessment of pesticide residues and trace element contamination in market gardens of Togo. *African J. Environ. Sci. and Tech.* **6**(10): 380-390.

Kays, S.J. and Dias, J.S. (1995). Common names of commer-cially cultivated vegetables of the world in 15 languages. *Economic Botany*. **49**(2): 115-152.

Khandakar, K. (1990). Population, agriculture and environmental degradation. An article published in the daily "The Bangladesh Observer" July 28, 1990.

Kirchner, M.R., Húsková, E., Matisová, J.M. (2008). Fast gas chromatography for pesticide residues analysis using analyte protectants. *J. Chromatogr. A.* **1186**: 271-280.

Kmellár, B., Fodor, P., Pareja, L., Ferrer, C., Martínez-Uroz, M.A., Valverde, A., Fernandez-Alba, A.R. (2008). Validation and uncertainty study of a comprehensive list of 160 pesticide residues in multi-class vegetables by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A.* **1215**(1-2): 37-50.

Kovalczuk, T., Lacina, O., Jech, M., Poustka, J., Hajslová, J. (2008). Novel approach to fast determination of multiple pesticide residues using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). *Food Addit. Contam. Part A.* **25**(4): 444-457.

Kruve, A., Künnapas, A., Herodes, K., Leito, I. (2008). Matrix effects in pesticide multi-residue analysis by liquid chromatography-mass spectrometry. *J. Chromatogr. A.* **1187**: 58-66.

Kumar, Y. and Hossmani, R.S. (2001). Magnitude of the residue of carbofuran and 3-hydroxy carbofuran in/on rice in India following furadan 3G insecticide treatment. p.309.

Kumari, B., Madan, V.K, Singh, J., Singh, S. and Kathpal, T.S. (2004). Monitoring of pesticidal contamination of farmgate vegetables, *Earth and Environ. Sci.* **90**(1-3): 65-71.

Lehotay, S.J. (2007). Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study. *J. AOAC Int.* **90**(2): 485-520.

Lehotay, S.J. (2000). Analysis of pesticide residues in mixed fruit vegetable extracts by direct sample introduction gas chromatography. *J. AOAC Int.* **83**(3): 312-314.

Lehotay, S.J., Hiemstra, M., van Bodegraven, P., de Kok, A. (2007). Validation of a fast and easy method for the determination of more than 200 pesticide residues in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. *J. AOAC Int.* **88**: 595-614.

Lehotay, S.J., Kyung, A.S., Kwon, H., Koesukwiwat, U., Fu, W., Mastovska, K., Hoh, E., Leepipatpiboon, N. (2010). Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *J. of Chromatogr. A.* **1217**: 2548-2560.

Lehotay, S.J., Mastovská, K., Yun, S.J. (2005a). Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrixes. *J. AOAC Int. L.* **88**(2): 630-638.

Lehotay, S.J., De Kok, A., Hiemstra, M., Van, B.P. (2005). Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. *J. AOAC Int.* **88**(2): 595-614.

Li, L., Li, W., Qin, D., Jiang, S., Liu, F. (2009). Application of graphitized carbon black to the QuEChERS method for pesticide multiresidue analysis in spinach. *J. AOAC Int.* **92**(2): 538-547.

Liu, M., Hashi, Y., Song, Y., Lin, J.M. (2005). Simultaneous determination of carbamate and organophosphorus pesticides in fruits and vegetables by liquid chromatography-mass spectrometry. *J. Chromatogr. A.* **1097**(1-2): 183-187.

Mantzos, N., karakitsou, A., Zioris, I., Leneti, E., Konstantinou, I. (2013). QuECHERS and soil phase extraction methods for the determination of energy crop pesticides in soil, plantand runoff water matrices. *Inter. J. Environ. Anal. Chem.* **93**(15): 1566-1584.

Marek Biziuk and Jolanta Stocka. (2015). Multiresidue methods for determination of currently used pesticides in fruits and vegetables using QuEChERS technique. *Int. J. Environ. Sci. and Development, IJESD.* **6**(1): 18-22.

Mezcua, M., Ferrer, C., García-Reyes, J.F., Martínez-Bueno, M.J., Albarracín, M., Claret M. and Fernández-Alba, A.R. (2008). Determination of selected non-authorized insecticides in peppers by liquid chromatography time-of-flight mass spectrometry and tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **22**(9): 1384-1392.

Milford, R. and Minson, D.J. (1968). The effect of age and method of haymaking on the digestibility and voluntary intake of the forage legumes *Dolichos lablab* and *Vigna sinensis*. *Aus. J. Exp. Animal Husbandry*. **8**: 409-418.

Milhome, M.A.L., Sousa, J.S., Andrade, G.A., Castro, R.C.D., Lima, L.K., Lima, C.G., and Nascimento, R.F. (2013). Validation and uncertainty of the method for multiresidue analysis of 35 pesticides in melon using gas chromatography coupled to quadropole mass spectrometry (GC-QP/MS). *J. Physics: conf. ser.* **575**:1-5.

Mol, H.G., Rooseboom, A., van Dam, R., Roding, M., Arondeus, K., Sunarto, S. (2007). Modification and re-validation of the ethyl acetate-based multi-residue method for pesticides in produce. *Anal. Bioanal. Chem.* **389**(6): 1715-1754.

Neetu, T. (2013). Determination of chlorinated pesticide in vegetables, cereals and pulses by gas chromatography in east national capital region, Delhi, India. *Res. J. Agri. Forestry Sci.* **1**(1): 27-28.

Nguyen, T.D., Lee, M.H. and Lee, G.H. (2008). Multi-residue determination of 156 pesticides in watermelon by dispersive solid phase extraction and gas chromatography/mass spectrometry. *Bull. Korean Chem. Soc*. **29**(12): 57-61.

Nguyen, T.D., Han, E.M., Seo, M.S., Kim, S.R., Yun, M.Y., Lee, D.M., Lee, G.H. (2008b). A multi-residue method for the determination of 203 pesticides in rice paddies using gas chromatography/mass spectrometry. *Anal. Chem. Acta.* **619**(1): 67-74.

Ochiai, N., Sasamoto, K., Kanda, H., Yamagami, T. and David, F. (2008). Multi-residue method for determination of 85 pesticides in vegetables, fruits and green tea by stir bar sorptive extraction and thermal desorption GC-MS. Retrieved from www.gerstel.de. (Date:16th Nov., 2013).

Ortelli, D., Edder, P. and Corvi, C. (2004). Multi-residue analysis of 74 pesticides in fruits and vegetables by liquid chromatography-electrospray-tandem mass spectrometry. *Analytic. Chemica. Acta.* **520**: 33-45.

Padron-Sanz, C., Halko, R., Sosa-Ferrera, Z. and Santana-Rodriguezb, J.J. (2005). Combination of microwawe assisted micellear extraction and liquid chromatography for the determination of organophosphorus pesticides in soil samples. *J. Chromatogr.* **1078**: 13-21.

Panhwar, A.A. and Sheikh, S.A. (2013). Assessment of pesticide residues in cauliflower through gas Chromatography-μecd and high 44 performance liquid Chromatography (hplc) analysis. *Int. J. Agril. Sci. Res. (IJASR),* **3**(1): 7-16.

Paramasivam, M, Chandrasekaran, S. (2012). Determination of fipronil and its major metabolites in vegetables, fruit and soil using QuEChERS and gas chromatography-mass spectrometry. *Intern. J. Environ. Anal. Chem.* **93**(11): 1203–1211.

Park, D.W., Kim, K.G., Choi, E.A.,Kang, G.R., Kim, T.S., Yang, Y.S., Moon, S.J., Ha, D.R., Kim, E.S. and Cho, B.S. (2016). Pesticide residues in leafy vegetables stalk and stem vegetables from South Korea: a long term study on safety and health risk assessment. *Food additives and Contaminants, Part A.* 33(1): 105-118.

Parveen, Z., Riazuddin, Iqbal, S., Khuhro, M.I., Bhutto, M.A. and Ahmed, M. (2011). Monitoring of multi-residue pesticide residues in some fruits in Karachi, Pakistan. *Pak. J. Bot.* **43**(4): 1915-1918.

Paya, P., Anastassiades, M., Mack, D., Sigalova, I., Tasdelen, B., Oliva, J., Barba, A. (2007). Analysis of pesticide residues using the quick easy cheap effective rugged and safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. *Anal. Bioanal. Chem.* **389** (6): 1697-1714.

Pimental, D., Acquay, H., Biltonen, M., Rice, P., Silva, M. and Nelson, J. (1992). Eenvironmental and economic costs of pesticide use. *Bioscience.* **42**: 750-760

Prodhan, M.D.H, Rahman, M.A., Ahmed, M.S. and Kabir, K.H. (2009). Quantification of organophosphorus and organochlorine insecticide residues from fish samples using simple GC technique. *Bangladesh J. Agriculturist*. **2**(2): 197-204.

Prodhan, M. D. H., Papadakis, E.N and Mourkidou, E.P. (2016). Analysis of Pesticide Residues and Their Variability in Cabbage Using QuEChERS Extraction in Combination with LC-MS/MS. *Food Anal. Methods*. **9**(12): 3470–3478.

Prodhan, M. D. H., Papadakis, E.N and Mourkidou, E.P. (2015). Analysis of pesticide residues in melon using QuEChERS extraction and liquid chromatography triple quadrupole mass spectrometry. *Int. J. Environ. Anal. Chem.* **95**(13): 1219-1229.

Prodhan, M. D. H., Papadakis, E.N and Mourkidou, E.P. (2016). Variability of pesticide residues in cauliflower units collected from a field trial, and market places in Greece. *J. Environ. Sci. Health*. **51**(9): 644-653.

Prodhan, M.D.H, Rahman, M.A., Ahmed, M.S. and Kabir, K.H. (2010). Pesticide residues in fish samples collected from different fish cultivation regions of Bangladesh. *SAARC J. Agri.* **8** (2): 53-64.

Prodhan, M. D. H., Papadakis, E.N and Mourkidou, E.P. (2015). Determination of multiple pesticide residues in eggplant with liquid chromatography-mass spectrometry. *Food Anal. Methods.* **8**: 229–235.

Prodhan, M.D.H., Rahman, M.A., Ahmed, M.S. and Kabir, K.H. (2009). Quantification of organophosphorus and organochlorine insecticide residues from fish samples using simple GC technique. *Bangladesh J. Agriculturist*. **2**(2): 197-204.

Quebedeaux, B. and Eisa H.M. (1990). Horticulture and human health: Contributions of fruits and vegetables, Proceedings of the 2nd International Symposium on Horticulture and Human Health. *Hort. Sci.* **25**: 1473-1532.

Rahman, A. M. (1999). Effect and residues of two selected insecticides applied for control of brinjal shoot and fruit borer in eggplant. An MS Thesis submitted to BSMRAU, Gazipur, Bangladesh, pp. 1-4

Rai, S., Singh, A.K., Srivastava, A., Yadav, S., Siddiqui, M.H., Mudiam, M.K.R. (2016). Comparative evaluation of quechers method coupled to DLLME extraction for the analysis of multiresidue pesticides in vegetables and fruits by gas chromatography-mass spectrometry. *Food Anal. Methods.* **9**: 2656-2669.

Rimkus, G.G., Rummler, M. and Nausch, I. (1996). Gel permeation chromatography-high performance liquid chromatography combination as an automated clean-up technique for the multiresidue analysis of fats. *J. Chromatogr. A.* **737**(1): 9-14.

Romero-GonzAlez, R., Garrido Frenich, A. and Martínez Vidal, J.L. (2008). Multiresidue method for fast determination of pesticides in fruit juices by ultra performance liquid chromatography coupled to tandem mass spectrometry. *Talanta.* **76**(1): 211-225.

Sahoo, S.K., Battu, R.S. and Singh, B. 2011. Development and validation of quechers method for estimation of propamocarb residues in tomato (*Lycopersicon esculentum* Mill) and soil. *American J. Anal. Chem.* **2**: 26-31.

Salwa, M., Dogheim, A., Gad A.S. and Ashraf, M.E. (1999). Monitoring pesticide residues in Egyptian fruit and vegetables in 1995. *J. Offic. Associ. Analyt. Chem.* **82** (4): 948-955.

Satpathy, G, Tyagi, Y.K. and Gupta, R.K. (2014). Development and validation of multi-residue analysis of 82 pesticides in grapes and pomegranate as per the requirements of the European Union (EU) and codex alimentarius using gc-ms/ms with compound based screening. *American J. Food Sci. and Tech.* **2**(2): 53-61.

Sattar, M.A. (1983). Analysis and residues of organochlorine pesticides in soil. D.Sc. Dissertation, Century Univ. Cal, p.131.

Sattar, M.A. (1987). GC of organophosphorus pesticides: influence of flow rate on retention time. *Bangladesh J. Pesticide, Sci. and Environ.* **3**(1): 33-35.

Sattar, M.A. (1991). Degradation of residues of aldrin, dieldrin in two Bangladesh soils. *J. Asia. Soc. Bangladesh Sci.* **17**(2): 145-153.

Sattar, M. A. and Passivirta, J. (1980). Fate of the herbicide MCPA in soil: analysis of the residues of MCPA by internal standard method. *Chemosphere*. **9**: 365-375.

Sattar, M.A. (1985). Improved analysis of phenoxy herbicides in soils by internal standard method by using a gas chromatography. *Bangladesh J. Pestici. Sci. and Environ.* **1**: 20-29.

Sattar, M.A. (1990). Effects of N-bearing salt on GC analysis of MCPA and metabolite in soil. *Pak J. Sci. Ind. Res*. **33**(5-6): 193-196.

Sattar, M.A. (1992). Gas chromatography of organophosphorus pesticide in soils by internal standard method. *Pak J. Sci. Ind. Res*. **35**(11): 442- 444.

Sattar, M.A. and Passivirta, J. (1979). Simultaneous determination of 4- chloro-2-methylphenoxyacetic acid and its metabolites in soil by gas chromatography. *Anal. Chem.* **51**: 598-602.

Sattar, M.A., Hattula, M.L., Lathipera, M. and Passivirta, I. (1977). Improved analysis method of MCPA from soil sample. *Chemosphere.* **1**: 747- 751.

Schenck, F., Wong, J., Lu, C., Li, J., Holcomb, J.R. and Mitchell, L.M. (2009). Multiresidue analysis of 102 organophosphorus pesticides in produce at parts-per-billion levels using a modified QuEChERS method and gas chromatography with pulsed flame photometric detection. *J. AOAC Int.* **92**(2): 561-573.

Schenck, F.J. and Hobbs, J.E. (2004). Evaluation of the quick, easy, cheap, effective, rugged, and safe (QuEChERS) approach to pesticide residue analysis. *Bull. Environ. Contam. Toxicol.* **73**: 24-30.

Schenck, F.J., Brown, A.N., Podhorniak, L.V., Parker, A., Reliford, M., Wong, J.W. (2008). A rapid multiresidue method for determination of pesticides in fruits and vegetables by using acetonitrile extraction/partitioning and solid-phase extraction column cleanup. *J. AOAC Int.* **91**(2): 422-438.

Schreiber, A. and Wittrig, R. (2010). Enhanced LC/MS for the quantitation and identification of pesticide in food sample. *Agro FOOD Industry Hi-tech.* **21**:18-22.

Singh, B., Kar, A., Mandal, K., Kumar, R. and Sahoo, S.K. (2012). Development and validation of QuEChERS method for estimation of chlorantraniliprole residue in vegetables. *J. Food Sci.* **77**(12): T208-215.

Singh, I.P. and Kalra, R.L. (1989). Dissipation of permethrin in brinjal fruits, leaves and soil. *Indian J. Entom.* **51** (3): 246-252.

Sinha, S.R. and Sharma, P.K. (2007). Efficacy of neonicotinoids against okra insect pest. *Pesticide Res. J.* **19**(1): 42-44.

Their, H.P., Stijve, T. and Specht, W. (1989). Quality assurance in insecticide residue analysis- experience gathered during inter laboratory tests. *Fresenius-zeitschriftfur-Analytische-chemie.* **3346**: 545-549.

Cserhati, T. and Szogyi, M. (2012). Chromatographic determination of pesticides in food and food products. Research Centre for Natural Sciences, Hungarian Academy of Sciences, H-1025, Budapest, Pusztaszeri u. 59-67, Hungary.

Walorczyk, S. (2008). Application of gas chromatography/tandem quadrupole mass spectrometry to the multi-residue analysis of pesticides in green leafy vegetables. *Rapid Commun. Mass Spectrom.* **22**(23): 3791-3801.

Wang, S., Xu, Y., Pan, C., Jiang, S., Liu, F. (2007). Application of matrix solid-phase dispersion and liquid chromatography-mass spectrometry to fungicide residue analysis in fruits and vegetables. *Anal. Bioanal. Chem.* **387**(2): 673-685.

Wargovich, M. J. (2000). Anticancer properties of fruits and vegetables. *Hort. Sci.* **35**: 573-575.

WHO (World Health Organization) (2004). Children are facing high risks from pesticide poisoning - Better protection and awareness rising needed say WHO/FAO/UNEP in a joint note. In: The 1st Ministerial Conference of the Rotterdam Convention, ended Friday, September 24

WHO (2005). The world health organization recommended classification of pesticides by hazard and guideline to classification. WHO, Geneva, Switzerland. World Health Organization. *Public Health Impact of Pesticides used in Agriculture*. WHO, Geneva, Switzerland **51**:86.

Yamagami, T., David, M. and Sandra, P. (2009). Multi-Residue Method for Determination of 85 Pesticides in Vegetables, Fruits and Green Tea by Stir Bar Sorptive Extraction and Thermal Desorption GC-MS. Retrieved from www.gerstel.de. (Date: 16th Nov., 2013).

Yang, R.Y., Tsou, S.C.S., Lee, T.C., Wu, W.J., Hanson, P.M., Kuo, G., Engle, L.M. and Lai, P.Y. (2006). Distribution of 127 edible plant species for antioxidant activities by two assays. *J. Sci. Food Agri.* **86**: 2395-2403.

Zanella, R., Tiele, M., Rizzetti, Kemmerich, M., Manoel, L., Martins, Osmar, D., Prestes, Martha, B. and Adaime. (2016). Optimization of a QuEChERS based method by means of central composite design for pesticide multiresidue determination in orange juice by UHPLC–MS/MS. *Food Chem.* **196**: 25–33.

Zhang, K., Wong, J.W., Hayward, D.G., Sheladia, P., Krynitsky, A.J., Schenck, F.J., Webster, M.G., Ammann, J.A. and Ebeler, S.E. (2009). Multiresidue pesticide analysis of wines by dispersive solid-phase extraction and ultrahigh-performance liquid chromatography-tandem mass spectrometry. *J. Agri. Food Chem.* **57**(10): 4019-4029.

Zhang, Y., Zhang, Y., Nie, J., Jiao, B. and Zhao, Q. (2016). Determination of triazole fungicide residues in fruits by quechers combined with ionic liquid-based dispersive liquid-liquid microextraction: optimization using response surface methodology. *Food Anal. Methods*. **9**: 3509–3519.