DETERMINATION OF PESTICIDE RESIDUES IN CAULIFLOWER, EGGPLANT AND YARD LONG BEAN COLLECTED FROM VARIOUS LOCAL MARKET OF MYMENSINGH

MD. MAHADI ALAM

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

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BY
MD. MAHADI ALAM

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Approved by:

Supervisor

_____

(Dr. Mohammad Dalower Hossain Prodhan)
Senior Scientific Officer
Entomology division, Bangladesh Agricultural Research Institute (BARI), Gazipur-1701, Bangladesh.

Co-Supervisor

_____

(Dr. S M Mizanur Rahman)
Professor
Department of Entomology
Sher-e-Bangla Agricultural University, Dhaka-1207

_____

Dr. Mst. Nur Mohal Akhter Banu
Chairman
Examination committe
Department of Entomology
Sher-e-Bangla Agricultural University, Dhaka-1207

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

CERTIFICATE

This is to certify that the thesis entitled **"DETERMINATION OF PESTICIDE RESIDUES IN CAULIFLOWER, EGGPLANT AND YARD LONG BEAN COLLECTED FROM VARIOUS LOCAL MARKET OF MYMENSINGH "** 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 **MD. MAHADI ALAM**, Registration No. **11-04437** 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.

June, 2017

Dhaka, Bangladesh

_____

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


Dedicated to
My
Beloved Parents

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Dhaka, Bangladesh **The Author**

DETERMINATION OF PESTICIDE RESIDUES IN CAULIFLOWER, EGGPLANT AND YARD LONG BEAN COLLECTED FROM VARIOUS LOCAL MARKET OF MYMENSINGH

Abstract

The study was conducted to analyze pesticide residues in three common vegetables (cauliflower, eggplant and yard long bean) collected from five local markets of Mymensingh city during November 2017 to January 2018. The collected samples were carried to the Pesticide Analytical Laboratory, Entomology Division of Bangladesh Agricultural Research Institute (BARI), Gazipur on the same sampling day. The collected samples were analyzed using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD) for the determination of pesticide residues in 30 samples of cauliflower, 30 samples of eggplant and 30 samples of yard long bean. Among the 30 analyzed samples of cauliflower, 5 samples (17% of the total number of samples) contained residues of dimethoate, chlorpyrifos and quinalphos, where 1 sample contained multiple residues and 3 samples contained residue above the maximum residue limits (MRLs). Out of 30 samples of eggplant, 5 samples (17% of the total number of samples) contained residues of chlorpyrifos, quinalphos, and dimethoate, where only one sample contained pesticide residues above MRL. On the other hand, among the 30 samples of yard long bean, 3 samples (10% of the total samples) contained residue of quinalphos, of which 2 samples contained pesticide residues above MRL. This study reflects the overall scenario of pesticide residue remain in cauliflower, eggplant and yard long bean collected from local markets of Mymensingh city, which will help the consumer to be aware of their health and safety.

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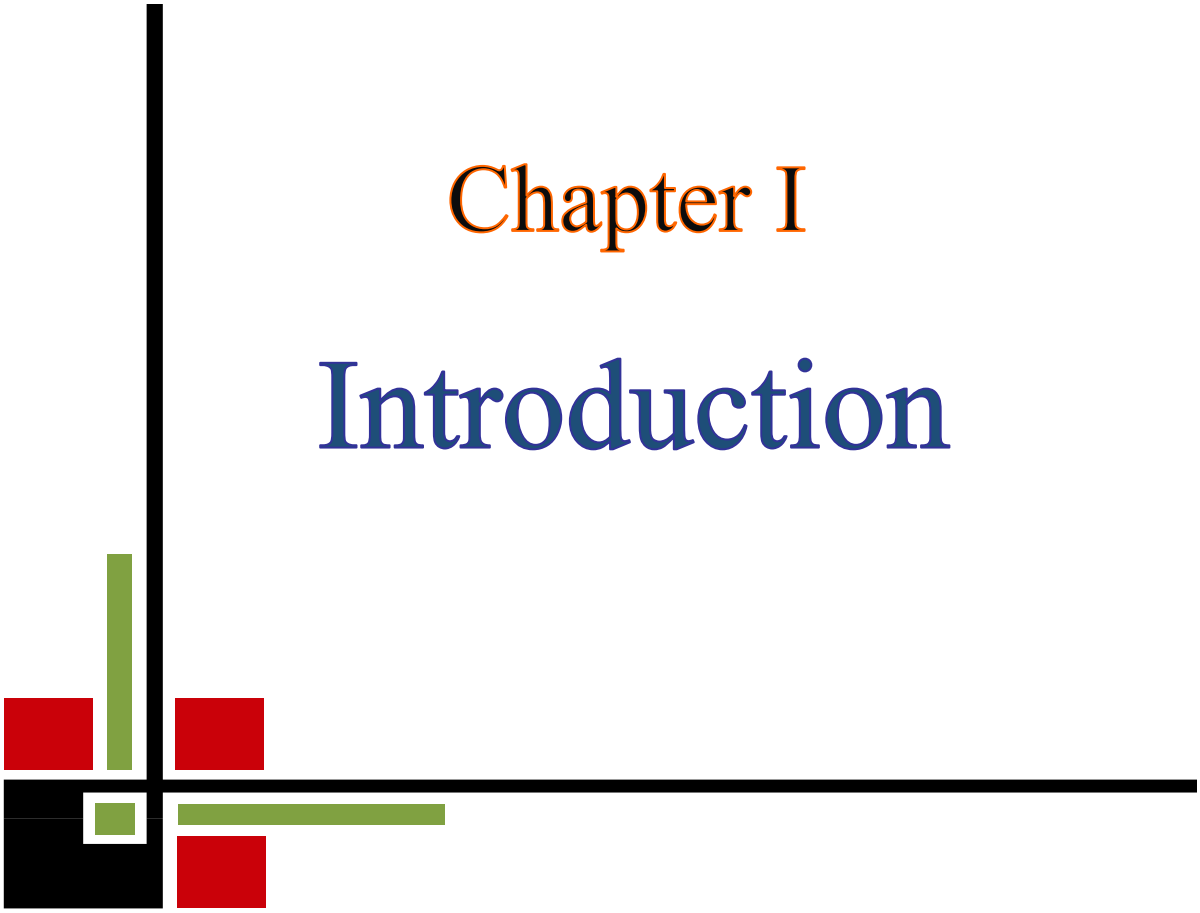
LIST OF ABBREVIATIONS

ADI	Acceptable Daily Intake
AOAC	Association of Analytical Communities
BARI	Bangladesh Agricultural Research Institute
CCD	Central Composite Design
CSN	Committee for Standardization
DAS	Days After Spraying
DLLME	Dispersive Liquid–Liquid Microextraction
d-SPE	dispersive solid phase extraction
ECD	Electron Capture Detector
et all	*et alibi* (and others)
etc	*et cetra* (and so on)
EU	European Union
FAO	Food and Agriculture Organization
FTD	Flame Thermionic 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 Quantifications
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 I

Introduction



CHAPTER I

INTRODUCTION

As an overpopulated country food shortage and malnutrition has become a general problem in Bangladesh. Not only in Bangladesh but also around the world the food demand is changing rapidly because of population growth, economic growth, rising income and rapid urbanization. Demand is changing away from traditional commodities towards high value food commodities like vegetables, fruits, spices, fish etc. In this regard, vegetable growing has become an important farming activity from the point of view of dietary fulfillment as well as economics returns (Aktar *et al.,* 2017). 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 (Craig and Beck, 1999; Wargovich, 2000; Dias and Ryder, 2011).

The major vegetables grown in our country are cabbage, cauliflower, tomato, brinjal, potato, radish, country bean, bottle gourd, pumpkin, bitter gourd, teasle gourd, ribbed gourd, ash gourd, okra, yard long bean, spinach etc (Hasan *et al.,* 2014). All these vegetables do not only meet our daily diet demand but also occupying a more or less significant position in earning foreign currency. Bangladesh earns 1456.33 million Tk. (USD 24.70 million) in the year 2003-2004 by exporting vegetables (Karim *et al.,* 2005).

Eggplant is one of the most common and popular vegetable throughout the entire tropical and subtropical regions of the world. It is grown extensively in China, India, Bangladesh, Pakistan, Philippines, Japan, Indonesia, Turkey, Greece, Italy, France, USA, and Mediterranean and Balkan countries. The benefits of eggplant are well known. It helps to prevent colon cancer, reduces cholesterol level, helps in the type 2 diabetes management, is very rich in antioxidant, and also helps to control weight (Dome, 2013). Cauliflower is a member of the cruciferous family of vegetables. It contains vitamins, fiber, and minerals and helps to prevent several diseases (Ambroson, C.B. *et al*., 2009).

Yard-long bean is rich in protein, vitamins A and C, and calcium; vitamin B and dry matter of green seeds contains 24.6% protein with high amount of lysine (Ferry, 1981). One of the major constraints for yard-long bean production in our country is the attack of insect pests (Anon, 2009). Among the insect pests, pod borer is considered the major pests of yard-long been in Bangladesh (Das, 1998 & Ali, 2006). In order to protect their crops from damages of pod borer, our farmers often apply traditional insecticides without having sufficient field control of this pest.

It is undoubtedly true that a negative economic impact on the production of vegetables is occurred by the insect pests and diseases. Due to plant pests and diseases 20 to 40 percent crop yields are reduced globally, besides the world will need to produce 60 percent more food for the over increasing world population by 2050 (FAO, 2012). To ensure this demand control of insect pests and diseases plays a key role. Till to date for the control of insect pests and diseases pesticides plays a vital role. It is studied that in advanced countries, the damage is less as compare to the developing countries. There is no doubt that due to the use of pesticides, production is increased day by day. Due to this reason, the pesticides are come in market on large scale. And their adverse effects are also increasing as their use is increased.

In Bangladesh, as in most developing countries, agriculture plays a key role in the overall economic performance of the country, not only in terms of its contribution to gross domestic product (GDP, 20.01%), but also as a major source of foreign exchange earnings, and in providing employment (47.3%) to a large segment of the population, particularly the poor (BER, 2010). Owing to the massive damage caused by pests to agricultural fields and crops, production often declines below the level of subsistence for farmers, which can eventually have adverse effects on the national economy. In the process of checking and killing pests over the years, pesticide application in Bangladesh has increased manifold from 758 metric tons in 1960 and 3028 metric tons in 1980 to over 19000 metric tons in 2000 (Kabir *et al*., 2008), and in 2008, the amount of pesticide applied in fields across the country rose to 48690 metric tons (BBS, 2008-2009). Insecticides, being the dominant item, account for 76% of the pesticides applied in a Year.

As many developing countries, most of the farmers in Bangladesh apply pesticide without knowing its actual requirements and/or effectiveness, and thus they apply very high frequencies of

pesticides in a cropping season. For example, farmers spray pesticides 140 times during a cropping season of 180-200 days and 150 sprays in a crop season in brinjal. More than 90% farmers of Bangladesh use pesticide unnecessarily, indiscriminately and excessively due to their ignorance and unconsciousness about the use (Anonymous, 2000). According to Pesticides Association of Bangladesh (2002-2003), pesticide use for growing vegetables was six times higher than the rice (1.12 kg/ha for vegetables while it was only 0.20 kg/ha in rice). Furthermore, farmers spray their vegetables 17-150 times per crop cycle. According to DAE, about 95 percent farmers do not wait for pre-harvest interval (PHI) after application of pesticides. The effective and responsible uses of pesticides bring significant positive contributions to the crop production. However, their irrational and unprotected uses are threatening our ecosystem, health and environment.

Pesticide residues are the deposits of active ingredient, its metabolites or breakdown products present in some component of the environment after its application, spillage or dumping. The applied chemicals and/or their degradation products may remain as residues in the agricultural products, which becomes a concern for human exposure (Dasika *et al*., 2012). In Bangladesh, farmers have no idea about 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 (Kabir, 2007).

Now pesticide residue in food has become a consumer's safety issue and the consumers have the right to know how much pesticide get incorporated in the food they eat. 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), Gazipur (Prodhan et al., 2018; Aktar *et al.,* 2017; Rasan et. al., 2017; Kabir *et al*., 2007; Islam *et al*., 2014; Kabir *et al*., 2008; Prodhan *et al.,* 2009; Prodhan *et al.*, 2010).

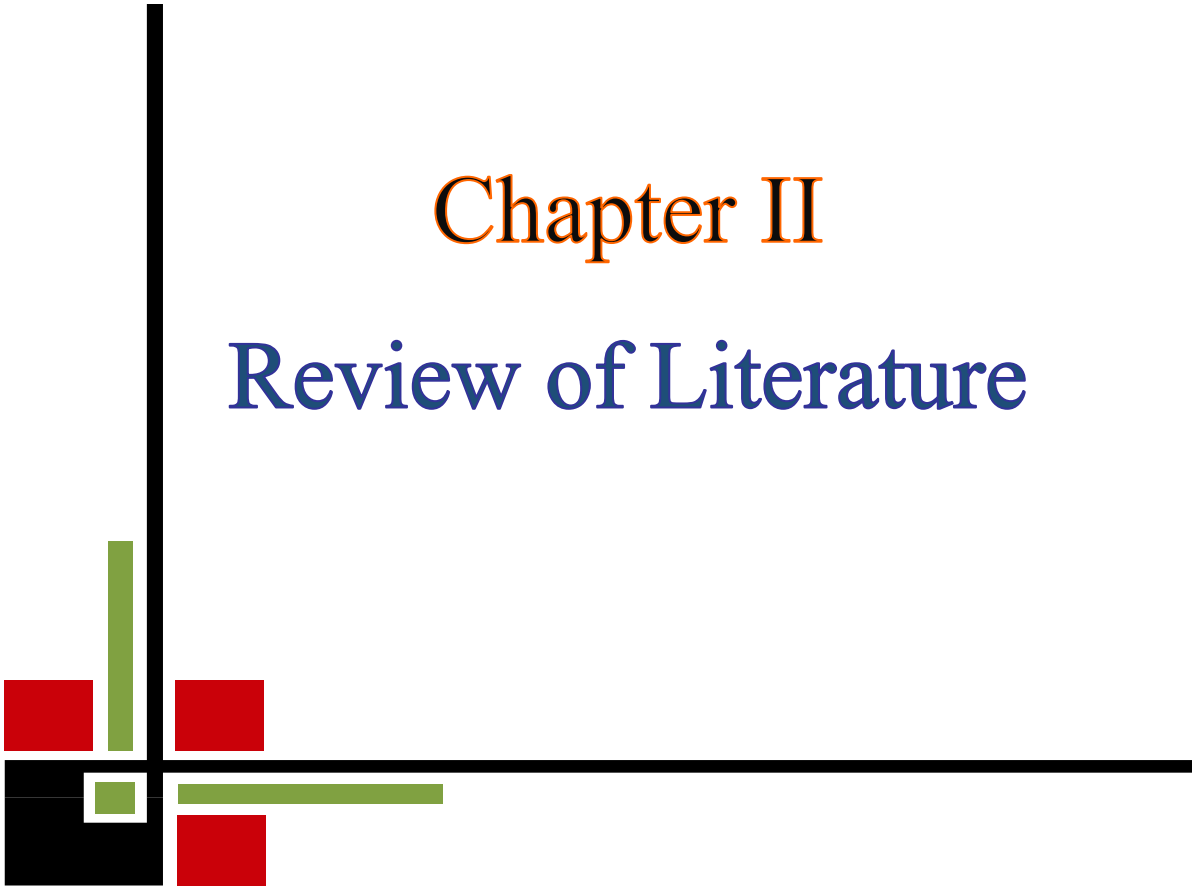
Mymensingh district of Bangladesh is very famous for vegetable cultivation and the farmers of Mymensingh are using pesticides indiscriminately to control the insect pests of vegetables. So, it is essential to find out the actual scenario of pesticide residues present in vegetables collected from Mymensingh district. Keeping this view, the present study was initiated with the following objectives:

OBJECTIVES

- To identify different pesticides present in eggplant, yard long bean and cauliflower collected from various local market of Mymensingh.
- To quantify the level of detected pesticide residues (mg/Kg) remain in the selected vegetables collected from various local market of Mymensingh.
- To compare the level of detected pesticide residues (mg/Kg) remain in the selected vegetables with the Maximum Residue Limit (MRL).

Chapter II

Review of Literature



CHAPTER II

REVIEW OF LITERATURE

An effort has been made to review the available literatures in order to extend our wisdom regarding the current status of research and information on pesticide residues remain in different food matrices. Although the review could not be made so comprehensive due to limited scope and facility, it is hoped that 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 also reported. With this background, the information collected from different sources have been reviewed and presented below-

Prodhan *et. al.* (2018) conducted an experiment to determine pre harvest interval (PHI) for quinalphos in eggplant, cabbage and yard long bean; malathion in eggplant, yard long bean and cauliflower; cypermethrin in tomato and yard long bean; and diazinon in eggplant and yard long bean depending on Maximum Residue Limit (MRL) set by FAO/ WHO. Five supervised field trials were conducted and sprayed with the field dose (2 ml/L of water) of each pesticide except cypermethrin (1 ml/L of water). Samples were collected at 0, 1, 3, 5, 7, 10, 12, 15 and 18 days after spray. The collected samples were analyzed using Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD) and Electron Capture Detector (ECD) for the determination of pesticide residues. The level of residues were above MRL up to 10 DAS for quinalphos in Cabbage, 7 DAS in Eggplant, 5 DAS in Yard long bean; for malathion 5 DAS in Yard long bean and Eggplant, 7 DAS in cauliflower; for diazinon 5 DAS in Yard long bean and Eggplant; and 3 DAS for cypermethrin in Yard long bean and Tomato.

Yu-Han Chiu *et al.* (2018) conducted a research on "Comparison of questionnaire-based estimation of pesticide residue intake from fruits and vegetables with urinary concentrations of

pesticide biomarkers" developed a pesticide residue burden score (PRBS) based on a food frequency questionnaire and surveillance data on food pesticide residues to characterize dietary exposure over the past year. They evaluated the association of the PRBS with urinary concentrations of pesticide biomarkers. Fruit and vegetable (FV) intake was classified as having high (PRBS≥4) or low (PRBS<4) pesticide residues for 90 men from the EARTH study. Two urine samples per man were analyzed for seven biomarkers of organophosphate and pyrethroid insecticides, and the herbicide 2,4-dichlorophenoxyacetic acid. They used generalized estimating equations to analyze the association of the PRBS with urinary concentrations of pesticide biomarkers. Urinary concentrations of pesticide biomarkers were positively related to high pesticide FV intake but inversely related to low pesticide FV intake. The molar sum of urinary concentrations of pesticide biomarkers was 21% (95% confidence interval (CI): 2%, 44%) higher for each one serving/day increase in high pesticide FV intake, and 10% (95% CI: 1%, 18%) lower for each one serving/day increase in low pesticide FV intake. Furthermore, intake of high pesticide FVs positively related to most individual urinary biomarkers.

Yolando Pico *et al*. (2018) conducted a research on" Target vs non-target analysis to determine pesticide residues in fruits from Saudi Arabia and influence in potential risk associated with exposure". The occurrence of pesticide residues in fruits was determined by a target method for 62 analytes and a wide scope screening method against a database of 500 pesticides. Limits of quantification (LOQs) were from 0.5 to 6.3 $\mu g\ kg^{-1}$ for the target method and from 0.5 to 119 $\mu g\ kg^{-1}$ for the non-target. Thirty samples: dates, apples, oranges, tangerines, lemons and grapefruits were selected because their high consumption, except lemons and grapefruits that were to cover all citrus fruits. Using the target method, 15 compounds (mostly insecticides and fungicides) out of 62 pesticides (organophosphorus, carbamates, pyrethroids, chloroacetanilides, triazines, triazoles, imidazoles, etc.) were detected. Residues were in 100% of the samples, 80% contained at or below maximum residue limits (MRLs), and 20% above. Non-target method identified several additional pesticides (cypronil, fludioxonil, boscalid and pyraclostrobin in apples).

Xiaomin Xu *et al. (*2018) conducted a survey on Four Groups of Cumulative Pesticide Residues in 12 Vegetables in 15 Provinces in China. Twenty-six pesticides were examined using gas chromatography–tandem mass spectrometry and liquid chromatography–tandem mass spectrometry in 2,169 samples of 12 typical vegetables collected from 15 provinces in China. The results showed that 908 (41.9%) samples were positive, with 133 (6.1%) samples exceeding the limit legal in China. Leafy vegetables showed higher positive rates than fruits and root vegetables. Organophosphorus, carbamate, pyrethroid, and triazine pesticides were found in 11.8, 7.7, 13.9, and 10.9% of the samples, respectively, which provided important information on current concentrations of cumulative assessment group pesticide residues for vegetables in China. Of the positive samples, a slight violation rate of 1.9% for the organophosphorus pesticide category exceeded China's maximum residue limits. Positive rates for chlorpyrifos in celery, pak-choi, and leeks were higher, but fewer exceeded China's maximum residue limits.

Akter *et al.* (2017) have been found pesticide residues in eggplant monitored in Mymensingh district and among the 50 analyzed samples, 11 (22% of the total number of the samples) contained pesticide residues of diazinon, dimethoate, quinalfos, and chlorpyrifos, of which, 2 had multiple pesticide residues and 5 samples contained residue above the European Union maximum residue limit (EU-MRLs). Chlorpyrifos was detected as the most used pesticide in eggplant in the studied area.

Hasan *et al*. (2017) have been detected two types of insecticides (Dimethoate and Quinalphos) in country bean samples collected from different market places of dhaka. Among the 50 analyzed samples of country bean, 10 samples (20%) contained residues of Dimethoate and Quinalphos, of which 5 samples were above the maximum residue limits (MRLs). Most of the contaminated samples (8 samples) contained residue of Dimethoate.

Prodhan M. D. H. *et al.* (2016) have 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).

Prodhan *et al.* (2016a) have been found 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.

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.

Yang *et al.* (2015) detected the residue levels of imidacloprid, acetamiprid, carbendazim, diflubenzuron, chlorbenzuron, phoxim, pyrimethanil, abamectin and iprodione in 117 samples of wax apple, starfruit and indian jujube which were collected from Hainan, Fujian, Guangdong and Guangxi province in Chaina using ultra performance liquid chromatography tandem mass spectrometry. They found carbendazim was the most frequently detected residue in 51 samples in the concentration range of 34.0-443.8 µg/kg. Diflubenzuron, phoxim, pyrimethanil and abamectin were not found in any sample. They also found that 29 samples contaminated with multiple pesticide residue.

Prodhan *et al.* (2015) found seven insecticides (chlorpyrifos, dimethoate, deltamethrin, thiamethoxam, thiacloprid, pirimicarb and indoxacarb) and three fungicides (azoxystrobin, fluopicolide and propamocarb hydrochloride) in the fresh melon samples which was collected from different market places in Thessaloniki, Greece. Among the 122 analyzed samples, 32 (26% of the total number of samples) were found to have pesticide residues.

Shida *et al.* (2015) analyzed 149 pesticide residues in apple, potato, cabbage and spinach using liquid chromatography coupled to hybrid quadruple time of flight mass spectrometry (LC-QTOF-MS). They found that out of 149 pesticides, 147 pesticide in apple, 145 in potato, 141 in cabbage and 131 in spinach. They also achieved calibration curve with satisfactory linearity in the concentration range of 0.002-0.1 µg/ml.

Hossain *et al.* (2015) analyzed pesticide residues in twenty five samples of brinjal, cucumber and tomato collected from different Upazilla of Bogra district, Bangladesh using Gas Chromatography and Mass Spectrophotometry (GC-MS). They found that out of twenty five samples eight samples were contaminated with pesticides residues, of which five samples exceeded the Maximum Residue Level (MRL) recommended by FAO /WHO.

Prodhan *et al.* (2015) undertaken an experiment to determine pesticide Residue in 72 fresh eggplant samples collected from different market in Thessaloniki, Greece with Liquid Chromatography-Mass Spectrometry by adopting QuEChERS extraction method. Among the 72 analyzed samples, 34 (47 % of the total number 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 (European Union- Maximum Residue Limits).

Pastor *et al.* (2015) investigated three spirocyclic tetronic/tetramic acid derivatives (spirotetramat, spiromesifen and spirodiclofen) and five neonicotinoid (thiamethoxam, chlotianidin, imidacloprid, acetamipirid and thiacloprid) in 25 samples of fruits and vegetables. The limit of detection (LOD) varied between 0.025 and 0.5 ng/g. They found that only thiamethoxam was detected in a lemon sample at a concentration close to the quantification limit and spirotetramat and spiromesifen at concentration between 11.6-54.5 ng/g.

Islam *et al.* (2014) conducted a survey on three major vegetables like eggplant, cauliflower, and country bean. A total of 42 samples collected from fields and market and analyzed by Gas Chromatography (GC) with Flame Thermionic 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.

Satpathy *et al.* (2014) conducted a research for the multi-residue analysis of 82 pesticides in grapes and pomegranate collected from a local market by QuEChERS extraction method and Gas Chromatography-tandem mass spectrometry (GC-MS/MS). They found 82 pesticides for all compound over the entire range of 0.005-0.1mg/kg except grapes in which they found 28 pesticides in 0.005mg/kg level.

A method was developed by Corteas *et al.* (2013) for the determination of organophosphorus pesticides in vegetables. 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.

Cho *et al.* (2013) conducted a research to evaluate pesticide residue in spinach by QuEChERS extraction method coupled with gas chromatography-nitrogen phosphorus detector and electron capture detector. They selected fifty GC amenable pesticides and found that the detector response linearly with determination coefficient higher than 0.995.They also found that the level of

detection (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.

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.

Hossain *et al.* (2013) conducted a research 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 tomato, lady's finger and brinjal in different markets of Dhaka using gas chromatography with a photo diode array detector (HPLC-PDA). 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).

Panhwar and Sheikh (2013) carried out a study to assess the pesticide residues of bifenthrin, endosulfan, profenofos, emamectin benzoate, imidacloprid and diafenthiuron in cauliflower through gas Chromatography-μECD and high 44 performance liquid Chromatography (HPLC) analysis. The results revealed that the residual level of pesticides in unwashed unprocessed cauliflower samples are beyond their recommended MRLs, the amount of bifenthrin, endosulfan, profenofos, emamectin benzoate, imidacloprid and diafenthiuron and the respective values were 0.151, 0.671, 0.172, 1.04, 1.011 and 0.052 ppm respectively.

Neetu (2013) conducted a research in order to investigate the chlorinated pesticide in vegetables, cereals and pulses by Gas Chromatography in East National Capital Region, Delhi, India. 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 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.

Prodhan M.D.H. *et al.* (2012) investigated insecticide residues in 94 samples of brinjal, hyacinth bean, cauliflower and yard long bean which were collected from farmers field and market of different regions like Barisal, Bogra, Chittagong, Comilla, Dhaka, Dinajpur, Gazipur, Jossore, Khagrachuri, Narsingdi, Rajshahi and Rangpur using GC-ECD and GC-FTD depending on the nature of insecticides. Results revealed that among 94 samples, 46 had insecticide residues and of them 18 had above MRL. Among 38 analyzed brinjal samples, 18 samples contained residue of Cypermethrin, Diazinon and Quinalphos; and only 8 had above MRL. They found Cypermethrin was frequent one which was found in 14 samples. Of 24 analyzed hyacinth bean samples, 15 had

the residue of Cypermethrin, Diazinon, Quinalphos, Fenotrothion and Malathion; and 7 had above MRL. Among the 16 analyzed cauliflower samples, 5 samples contained residue of Cypermethrin, Diazinon, Quinalphos and Malathion; and only 2 had above MRL. Among 16 samples of yard long bean, 8 samples contained residue of Cypermethrin, Diazinon, Quinalphos and Malathion; and only 2 had above MRL

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) estimated the residues of five commonly used pesticides (endosulfan, carbendazim, chlorpyrifos, cypermethrin and imidacloprid) in different vegetables collected from Uttarakhand, India. Out of the five pesticides, four of them were insecticides belonging to organochlorine, organophosphate, pyrethroid and 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 samples, cauliflower and tomato had carbendazim residues higher than the recommended MRL’s.

Kanda *et al.* (2012) conducted a research using 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.

Parveen *et al.* (2011) monitored pesticide residues in 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, Pakistan. They analyzed the samples for multiple pesticide residue using GC/FID and HPLC/UV. They found that 62.5% of samples contained residues of pesticide while 22% exceeded the maximum residue limit (MRL) according to FAO/WHO.

Bagyalakshmi *et al.* (2011) carried out a research on residue determination of Dimethoate in leafy vegetables (Spinach) using RPHPLC. They carried out the quantification using RP-HPLC for unprocessed (fresh) and processed (washed, washed and cooked) spinach samples. The extraction was carried out with benzene. They found that unprocessed spinach contains dimethoate residue which was slightly more than the tolerance limit, while the processed sample contains dimethoate residue within the tolerance limit. The washed samples (3 times under ordinary tap water) showed 28-50 % reduction while the cooked samples (boiling for 15 min) showed 58-71 % reduction compared to the unprocessed samples. They said that in case of greens, it should be washed three times with ordinary water and then cooked (cooked for fifteen minutes) so that the pesticide residue is very much reduced and it will be well within the tolerance limit as specified by EPA (Environmental Protection Agency).

Farag *et al.* (2011) estimated pesticide residues in some Egyptian herbs, fruits and vegetables. they collected One hundred thirty two samples of fruits, vegetables, herbs and spices from Egyptian local markets and analyzed for pesticide residues. They found that contamination with pesticide residues reached 54.55% while samples free from contamination reached 45.45%. 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 pesticide and 5.3% with more than 2 pesticide

Sahoo *et al.* (2011) estimated the propamocarb residues in tomato (*Lycopersicon esculentum* Mill) and soil using QuEChERS method and gas chromatograph-mass spectrometry (GC-MS). They found that propamocarb presented a distinct peak at retention time of 8.962 min. They also determined the limit of quantification (LOQ) of their method which was 0.10 mg/kg.

Camino-Sánchez *et al.* (2011) used the QuEChERS extraction method and detection was 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, microwaveassisted extraction, pressurized liquid extraction, supercritical fluid extraction, solidphase 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.

Schreiber and Wittrig (2010) conducted a research for the identification and quantitation of pesticide residues in apple, banana, carrot, cucumber, curry powder grapes, grapefruit, hazelnut, lemon, nectarine, orange, pear, raspberry, red pepper, raisin, salad, spinach and tomato from a supermarket by QuEChERS extraction method and liquid chromatography mass spectrometry (LC/MS). They injected the extracted sample into a liquid chromatography tandem mass spectrometry system where a total number of 12 pesticides was detected where the amount of methamidophos, omithoate, thiamethoxam, dimethoate, clothianidin, imadacloprid, promamocarb, carbyl, metalaxyl, myclobutanil, aspinosyn and dspinosyn were 130 µg/kg, 42 µg/kg, 48 µg/kg, 54µg/kg, 14µg/kg, 2.4µg/kg, 98µg/kg, 499µg/kg, 5.1µg/kg, 3.4µg/kg, 6.1µg/kg and 6.8 µg/kg, respectively.

Wang *et al.* (2010) investigated 148 pesticides in apple, banana, cantaloupe, orange, orange juice, carrot, corn, onion, pea, potato, spinach and tomato by adopting liquid chromatography electrospray ionization tandem mass spectrometry and ultra-high performance liquid chromatography electrospray ionization quadropole time of fight mass spectrometry. They found 81-110% recoveries of 95% of the pesticides and ≤20-95% intermediate precision of 97% pesticide. They also found ≤40% measurement uncertainty in case of 93% pesticide.

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.

Charan and Sharma (2010) detected pesticide residues in a total of 182 samples of six vegetables collected from different agricultural fields of central Aravalli region, India to find out the severity of synthetic agrochemicals on human being. They analyzed pesticide residues using 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% exceeded the maximum residual limit (MRL) values.

Yamagami *et al.* (2009) undertook a research to determine five groups of 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) GC-MS. They found the residual limit between 4-100 μg/kg for 66 pesticides.

Prodhan *et al.* (2009) estimated organophosphorus and organochlorine insecticide residues in fish sample using electron capture detector (ECD) and flame thermionic detector (FTD) of Gas Chromatograph (GC). They collected sixty eight samples of fish (Rui, Shrimp and others) from Dhaka, Khulna and Chittagong offices of Department of Fisheries including different fish export companies during August, 2008 to July, 2009. The results revealed that among 68 samples, 13 had insecticide residues. For Dhaka, out of six samples 1 had DDT residue which was 0.28 ppm. For Chittagong, out of 23 samples 3 had Diazinon residue ranged from 0.03-0.120 ppm. For Khulna, out of 39 samples 9 had Diazinon residue ranged from 0.04-0.205 ppm.

Islam *et al.* (2009) analyzed pesticide residues in cauliflower by high performance liquid chromatography. They sprayed cauliflower with 4 different pesticides (diazinon, malathion, chlorpyrifos and cypermethrin) at recommended dose and double of recommended dose. Calibration curves that constructed for the analytes spiked into samples followed linear

relationships with good correlation coefficients (R^2>0.990). In the analysis, residual amounts of diazinone and chlorpyrifos were above respective maximum residue limit (MRL) values.

Frenich *et al.* (2008) conducted a research for the analysis of 53 pesticides in 200 samples of cucumber, orange, strawberry and olive by using ultra performance liquid chromatography (UPLC) coupled to triple tandem mass spectrometry (MS-MS). They found the mean recoveries ranged from 70-109% with relative standard deviation less than 20%. They also found imidacloprid was mostly used pesticide ranged from .01-1.00 mg/kg.

Kabir *et al.* (2008) estimated the left over residue of Diazinon and Carbosulfan in brinjal and Quinalphos in yard long bean and made a comparison between the detected residue level with maximum residue level (MRL) set by FAO 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. They collected sample daily after spraying. They found that in case of Diazinon, left over residue was up to 6 days after spray (DAS), and upto 3 DAS, the level of residue was above the MRL. They also detected Carbosulfan residue remainded till 7 DAS and the detected quantity of residue was above MRL up to 3 DAS. Left over residue of Quinalphos in yard long bean sample was detected upto 6 DAS and up to 4 DAS the level of residue was above the MRL.

Nguyen *et al.* (2008) carried out a research for a simultaneous determination of 156 pesticides in watermelon collected from various markets of Korea using 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 0.005 mg/kg.

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.

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.

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) carried out a research for the quantitative analysis of 15 pesticide residues in pepper, broccoli, tomato, orange, lemon, apple and melon using liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS). They found residues ranged between 0.0005 and 0.03 mg/kg.

Khan and Ali (2005) estimated the residues of commonly used insecticides on fruits and vegetables grown in NWFP-Pakistan during crop season 2000, by using HPTLC. The initial residues of cypermethrin were 0.67 mg/kg. After 10 days, it was dissipated to 0.10 mg/kg, thus representing a loss of 85%. The samples did not contain any detectable residues 15 days after application. The year 2001, the initial residues of cypermethrin on tomato fruits were found to be 0.87 mg/kg which were reduced to 0.10 mg/kg after 15 days. The initial residue of chlorpyrifos 2.61 mg/kg degraded to 1.02 mg/kg 14 days after application. No residues were detected in the fruits 21 days after application.

Ortelli *et al.* (2004) analyzed 74 pesticides in 2500 samples of fruits and vegetables by liquid chromatography-electrospray-tandem mass spectrometry 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.

Lee (2001) conducted a research to investigate the magnitude of the residue of carbofuran and 3-hydroxy carbofuran in rice in Brazil following furadan 50G insecticide treatment in South Korea where plants were treated 2 or 3 from transplanting to milk-ripe stage. In one trials, total residues were 0.17 mg/kg and the two other trials, no residue was detected (<0.02 mg/kg) after 63 days of the last application.

Kumar and Hossmani (2001) examined the residue of carbofuran and 3-hydroxy carbofuran in 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. 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.

Colume *et al.* (2001) and Padron-Sanz *et al.* (2005) has been 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

Holihan (2001) conducted a research to investigate the magnitude of the residue of carbofuran and 3-hydroxy carbofuran in rice in Brazil following furadan 50G insecticide. A total of 9 trials were conducted, Total residues of Carbofuran in grain (Carbofuran and 3 hydroxy Carbofuran) were 0.10 and 0.12 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.

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.

Lehotay (2000) estimated 22 diverse pesticide residues in green bean and carrot extracts by bench top gas chromatography. The targeted pesticides which were incurred in the samples, included

chlorpyrifos, azinphos-methyl, parathion-methyl, diazinon, terbufos, 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). Average recoveries of the pesticides were 103 s 7% with relative standard deviations of 14 to 5% on 16 average.

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.

Salwa *et al.* (1999) analyzed organophosphorus, dithiocarbamates and some synthetic pyrethroids pesticide residues in 397 Egyptian fruits and vegetables collected from 8 local markets during 1995. 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 were free from pesticides residues. Samples of carrot and eggplant contained trace amounts of DDT and DDE residues.

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.

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." and 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 preplanting 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." and 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 2186 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.

Frank *et al.* (1990) conducted a research to investigate residues of insecticide (organophosphorus, synthetic pyrethroid N-methyl carbamate) and fungicide (dithiocarbamate, dicarboximide and organochlorine) in 433 composite samples of eggplant, asparagus, carrots celery, cole crops, cucumbers, lettuce, onions, peppers, potatoes, radishes and tomatoes in Ontario, Canada. In 64% samples, no pesticide residues were identified to the limits of detection which ranged from 0.005 to 0.05 mg/kg.

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.

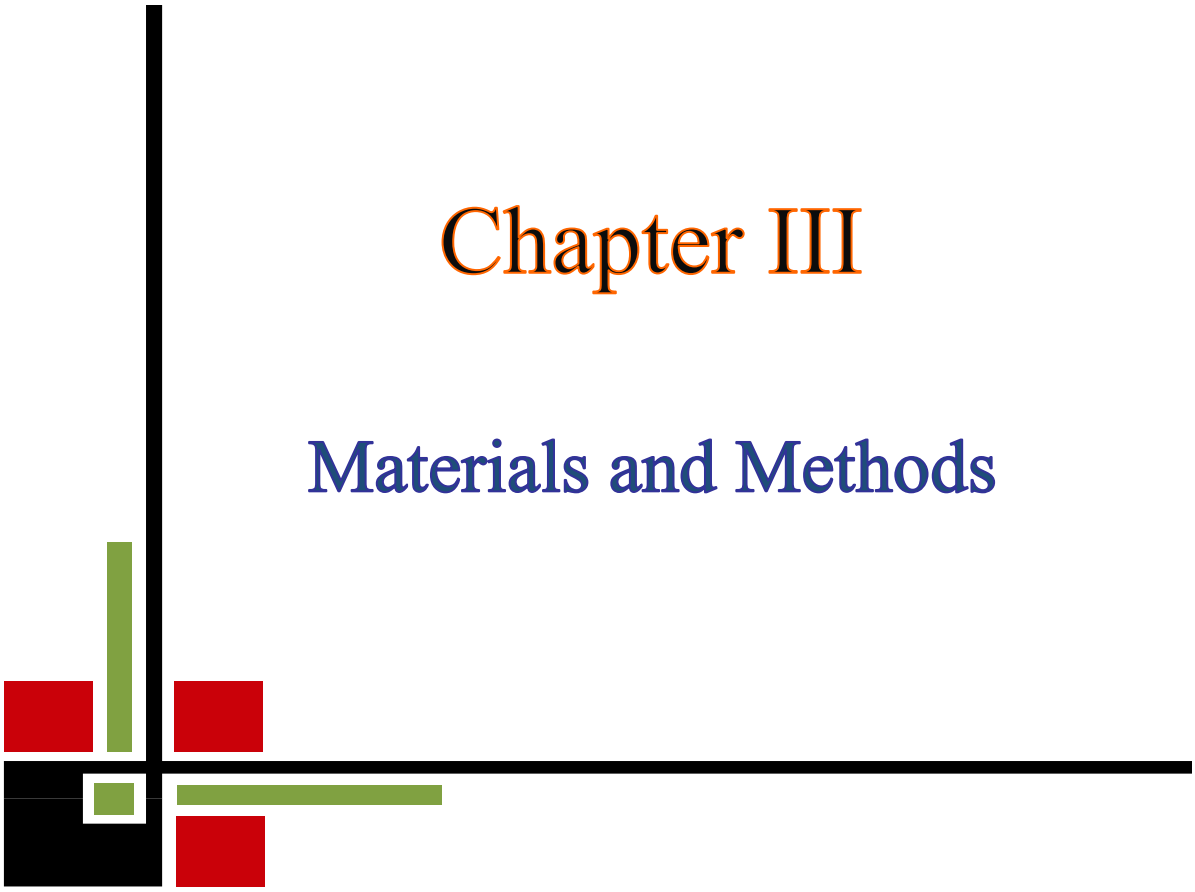
Sattar and Passivirta (1980) developed the detailed internal standard analysis method (procedure) of MCPA together its two metabolites 4-chlo-cresol and 5-chloro-3- methyl cathechol are schematically presented and the examples of chromatograms are reported as proof records. This was the universal applied 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.

FAO-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 III

Materials and Methods



Chapter III
Materials and Methods

The collected samples of vegetable (eggplant, cauliflower and yard long bean) from various local market of Mymensingh and carried to the Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute(BARI), Joydebpur, Gazipur for pesticide residue analysis during November, 2017 to January 2018. From sampling to final analysis, required procedures are described below.

3.1 Study area

The study area included major five markets of Mymensingh Sadar, Mymensingh. The area of Mymensingh district is about 4363.48sq km, located between 24º15´and 25º12´ north latitude and in between 90º04´ and 90 º 49´ east longitudes. Mymensingh Sadar is one of its 12 Upazilas and it is about 388.45 sq km. This upazila constitute of one municipality, 13 unions, 136 mouzas, and 174 villages and of 874452 populations. In this study, eggplant, yard long bean and cauliflower were selected to determine pesticide residues that were collected from 5 markets: Notun bazar, Mesua bazar, Sodesi bazar, Shankipara bazar and Mintu college bazar.

These bazars are famous for vegetables. The vegetables of these market comes from different places covers a large area such as Char Gobadia, Char Nilakshmia, Kalibari char, Banganbari char, Bobar char, Bhabanipur, Gafargaon, Fulbaria, Ishwarganj, Paranganj, Char Khairchaa, Char Gavindapur, Nuldug, Jailkhana road, Nandina, Joy Bangla bazar, Konaparar char, Jamalpur, Nurondi, Bidyagaon etc.

Figure 1: Map showing the places of sample collection in Mymensingh.

3.2 Sample collection

A total of 90 samples (30 cauliflower, 30 eggplants and 30 yard long bean) were collected for this study. Six samples of eggplant, six samples of cauliflower and six samples of yard long bean were collected from each market. The amount of each sample was 1 Kg for all the selected 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.

Table 1: Sources and places of collection of eggplant samples

Area of collection	Sample ID	Source
Shankipara Bazar	EP-Sb_1	Char gobadia
	EP-Sb_2	Char nilakshmia
	EP-Sb_3	Kalibari char
	EP-Sb_4	Bobar char
	EP-Sb_5	Paranganj
	EP-Sb_6	Kalibari char
Notun Bazar	EP-Nb_1	Banganbari char
	EP-Nb_2	Bobar char
	EP-Nb_3	Gafargaon
	EP-Nb_4	Jamalpur
	EP-Nb_5	Bobar char
	EP-Nb_6	Paranganj
Sodesi Bazar	EP-Sdb_1	Paranganj
	EP-Sdb_2	Char Gavindapur
	EP-Sdb_3	Nurondi
	EP-Sdb_4	Jamalpur
	EP-Sdb_5	Bobar char
	EP-Sdb_6	Paranganj
Mesua Bazar	EP-Mb_1	Bobar char
	EP-Mb_2	Nandina
	EP-Mb_3	Jamalpur
	EP-Mb_4	Bobar char
	EP-Mb_5	Paranganj
	EP-Mb_6	Kalibari char
Mintu College Bazar	EP-MCb_1	Bobar char
	EP-MCb_2	Nandina
	EP-MCb_3	Nandina
	EP-MCb_4	Bobar char
	EP-MCb_5	Char Gavindapur
	EP-MCb_6	Kalibari char

Table 2: Sources and places of collection of cauliflower samples

Area of collection	Sample ID	Source
Shankipara Bazar	$CF\text{-}Sb_1$ $CF\text{-}Sb_2$ $CF\text{-}Sb_3$ $CF\text{-}Sb_4$ $CF\text{-}Sb_5$ $CF\text{-}Sb_6$	Char nilakshmia Kalibari char Char gobadia Bobar char Char Gavindapur Kalibari char
Notun Bazar	$CF\text{-}Nb_1$ $CF\text{-}Nb_2$ $CF\text{-}Nb_3$ $CF\text{-}Nb_4$ $CF\text{-}Nb_5$ $CF\text{-}Nb_6$	Banganbari char Paranganj Bobar char Char Gavindapur Kalibari char Char gobadia
Sodesi Bazar	$CF\text{-}Sdb_1$ $CF\text{-}Sdb_2$ $CF\text{-}Sdb_3$ $CF\text{-}Sdb_4$ $CF\text{-}Sdb_5$ $CF\text{-}Sdb_6$	Char Gavindapur Nurondi Char nilakshmia Kalibari char Char gobadia Gafargaon
Mesua Bazar	$CF\text{-}Mb_1$ $CF\text{-}Mb_2$ $CF\text{-}Mb_3$ $CF\text{-}Mb_4$ $CF\text{-}Mb_5$ $CF\text{-}Mb_6$	Bobar char Paranganj Kalibari char Banganbari char Paranganj Bobar char
Mintu College Bazar	$CF\text{-}MCb_1$ $CF\text{-}MCb_2$ $CF\text{-}MCb_3$ $CF\text{-}MCb_4$ $CF\text{-}MCb_5$ $CF\text{-}MCb_6$	Gafargaon Jamalpur Bobar char Banganbari char Paranganj Bobar char

Table 3: Sources and places of collection of yard long bean samples

Area of collection	Sample ID	Source
Shankipara Bazar	YLB-Sb_1	Char nilakshmia
	YLB-Sb_2	Gafargaon
	YLB-Sb_3	Jamalpur
	YLB-Sb_4	Bobar char
	YLB–Sb_5	Paranganj
	YLB-Sb_6	Kalibari char
Notun Bazar	YLB-Nb_1	Bobar char
	YLB-Nb_2	Paranganj
	YLB-Nb_3	Kalibari char
	YLB-Nb_4	Jamalpur
	YLB-Nb_5	Bobar char
	YLB-Nb_6	Paranganj
Sodesi Bazar	YLB-Sdb_1	Char Gavindapur
	YLB-Sdb_2	Kalibari char
	YLB-Sdb_3	Char gobadia
	YLB-Sdb_4	Bobar char
	YLB-Sdb_5	Paranganj
	YLB-Sdb_6	Kalibari char
Mesua Bazar	YLB-Mb_1	Gafargaon
	YLB-Mb_2	Bobar char
	YLB-Mb_3	Nurondi
	YLB-Mb_4	Bobar char
	YLB-Mb_5	Paranganj
	YLB-Mb_6	Kalibari char
Mintu College Bazar	YLB-MCb_1	Bobar char
	YLB-MCb_2	Banganbari char
	YLB-MCb_3	Paranganj
	YLB-MCb_4	Jamalpur
	YLB-MCb_5	Bobar char
	YLB-MCb_6	Paranganj

3.3 Sample preparation for analysis

The collected samples were carried to the Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur 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 (St Louis, MO, USA) via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. Standards of all 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 used

a. Electric balance, Model: AY- 220, Shimadzu Corporation, Japan (Plate 1).

b. Vortex mixer, Model: Maxi max ii, USA (Plate 2)

c. Centrifuge machine, Model: Sigma 3k 30, Germany (Plate 3)

d. GC-2010, Shimadzu corporation, Japan (Plate 4)

e. Homogenizer, Model: Ultraturax, IKA T18 basic, Germany (Plate 5)

f. Orbital shaker, Model: Rexmed, Sweden (Plate 6)

Plate 1. Electric Balance

Plate 2. Vortex Mixer

Plate 3. Centrifuge Machine

Plate 4. Gas Chromatograph (GC)

Plate 5. Gas Chromatograph (GC)

Plate 6. Orbital Shaker

Plate 7. Chopping of collected sample

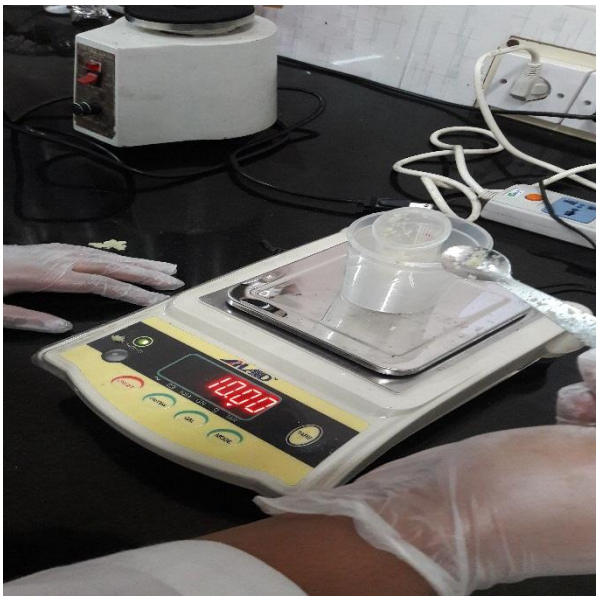


Plate 8. Weighing of sample and salt (NaCl and $MgSO_4$)

Plate 9. Adding acetonitrile (MeCN)

Plate 10. Shaking of sample

Plate 11. Centrifuging the sample

Plate 12. Weighing of PSA

Plate 13. Adding PSA in the sample extract

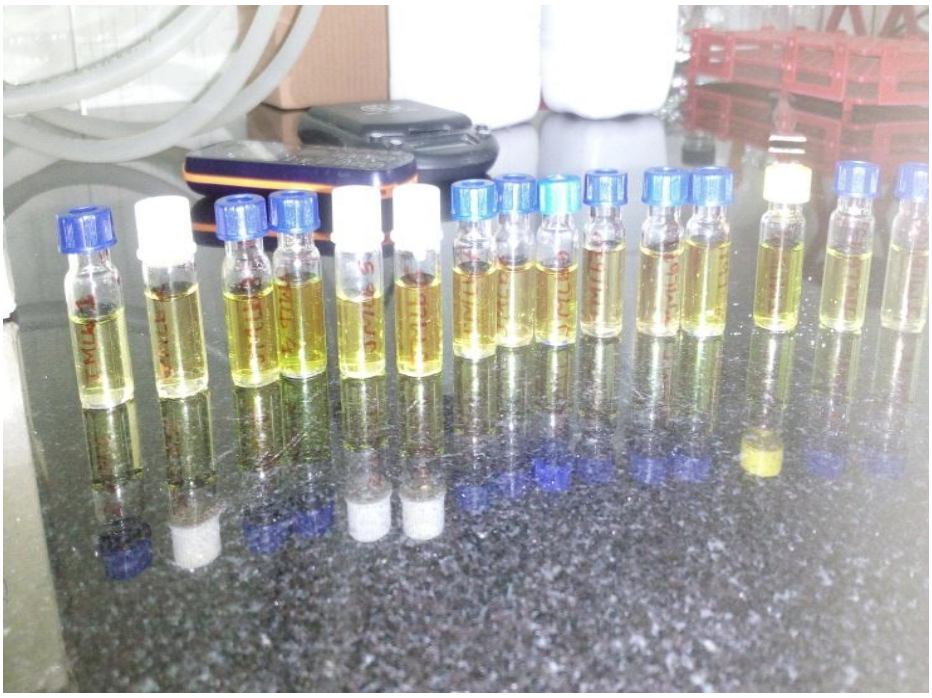


Plate 14. Sample extract ready for injection

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

3.6 Preparation of pesticide standard solution

Pesticide standard stock solutions of acephate, chlorpyrifos, 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 MgSO4 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 MgSO4 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 GC vial for injection.

3.8 Detetion and quantification of pesticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) with Flame Thermionic 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. The instrument conditions are described in Table 2 and Table 3.

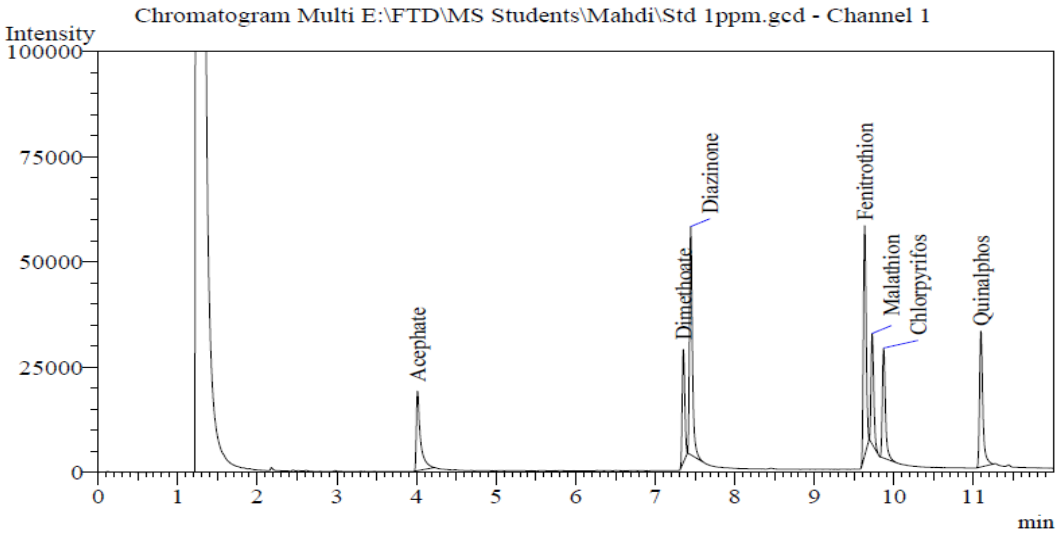


Figure 1: Typical chromatogram of seven organophosphorus insecticide standard run by GC-FTD.

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 temperature for FTD

Column oven	Rate	Temperature (^{0}C)	Hold time (min)
Initial temperature: 150°C	-	150	1
	10	220	2

3.9 Preparation of Calibration curve

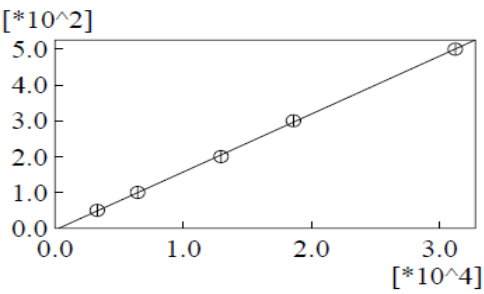
Prior to the injection of the sample extract, standard solutions of different concentrations of each pesticide group were prepared and injected with suitable instrument parameters. The samples were calibrated (retention time, peak area etc.) against five pointed calibration curve of standard solution of concerned pesticide (Figure 2-8). Each peak was characterized by its retention time. Sample results were expressed in mg/kg automatically by the GC software.

Calibration Curve - Analytical Line 1 - Channel 1

ID#:1 Name:Acephate

f(x)=1.61756310498e-002*x-4.67442357312
R=0.999987242049 R^2=0.999892507739
MeanRF:1.56431705673e-002 RFSD:4.11691966763e-004 RFRSD:2.63176806129
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



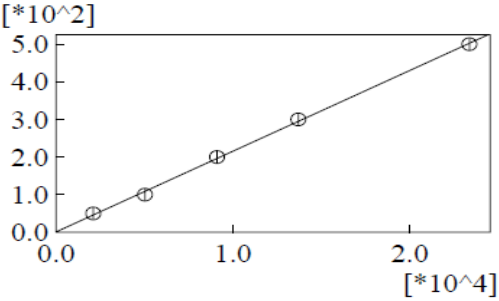
No.	Conc.	Area
1	50.000	3211
2	100.000	6906
3	200.000	13578
4	300.000	19584
5	500.000	32548

Figure 2: Calibration curve prepared for acephate made with different concentrations ranging from 50 µg/L to 500 µg/L.

ID#:2 Name:Dimethoate

f(x)=2.14476109468e-002*x+1.27409837292
R=0.999894212457 R^2=0.999926113376
MeanRF:2.17625864931e-002 RFSD:1.36709257517e-003 RFRSD:6.28184786586
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



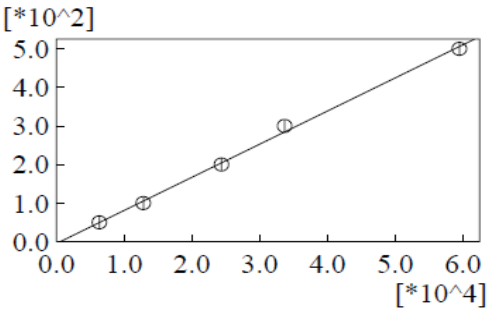
No.	Conc.	Area
1	50.000	2874
2	100.000	5247
3	200.000	9487
4	300.000	14587
5	500.000	22985

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

ID#:3 Name:Diazinone

f(x)=8.58182478378e-003*x-4.76864750449
R=0.9989993676 R^2=0.997890349706
MeanRF:8.23177212761e-003 RFSD:4.50915039717e-004 RFRSD:5.47773957694
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



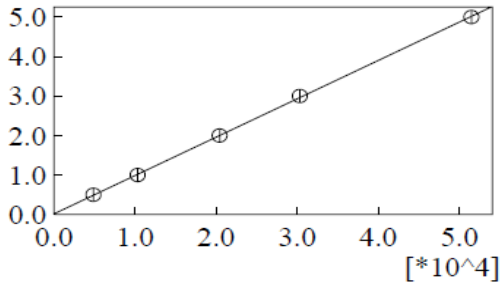
No.	Conc.	Area
1	50.000	6874
2	100.000	13214
3	200.000	23254
4	300.000	32354
5	500.000	58741

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

ID#:4 Name:Fenitrothion

f(x)=9.69984666697e-003*x+2.01882132141
R=0.999916224021 R^2=0.99983245506
MeanRF:9.85855366395e-003 RFSD:2.34530644934e-004 RFRSD:2.37895590903
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



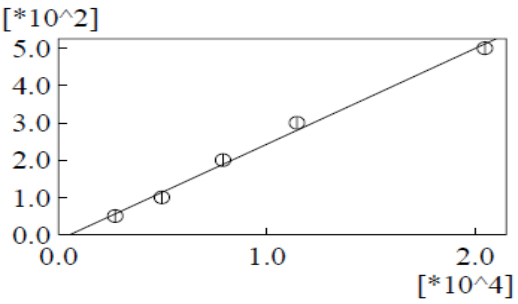
No.	Conc.	Area
1	50.000	4982
2	100.000	11425
3	200.000	21245
4	300.000	32301
5	500.000	50412

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

ID#:5 Name:Malathion

f(x)=2.5662119724e-002*x-13.8726059301
R=0.996793718496 R^2=0.993979245023
MeanRF:2.28770289157e-002 RFSD:3.45826768136e-003 RFRSD:15.116769289
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



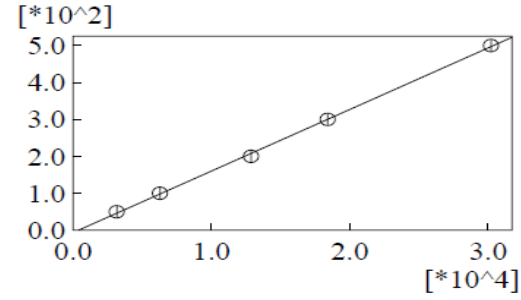
No.	Conc.	Area
1	50.000	2985
2	100.000	4475
3	200.000	7689
4	300.000	12543
5	500.000	21201

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

ID#:6 Name:Chlorpyrifos

f(x)=1.66718969691e-002*x-6.50897807754
R=0.999687866504 R^2=0.999299859839
MeanRF:1.60231310913e-002 RFSD:4.02450756721e-004 RFRSD:2.51168610197
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



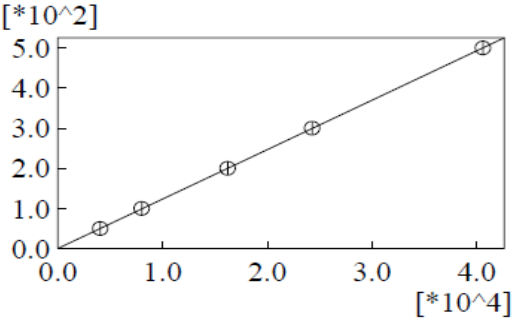
No.	Conc.	Area
1	50.000	3321
2	100.000	6684
3	200.000	13210
4	300.000	18987
5	500.000	31024

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

ID#:7 Name:Quinalphos

f(x)=1.2305211624e-002*x+0.670992116044
R=0.999994954248 R^2=0.999989878522
MeanRF:1.23562145806e-002 RFSD:7.05710649982e-005 RFRSD:0.571138227959
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard

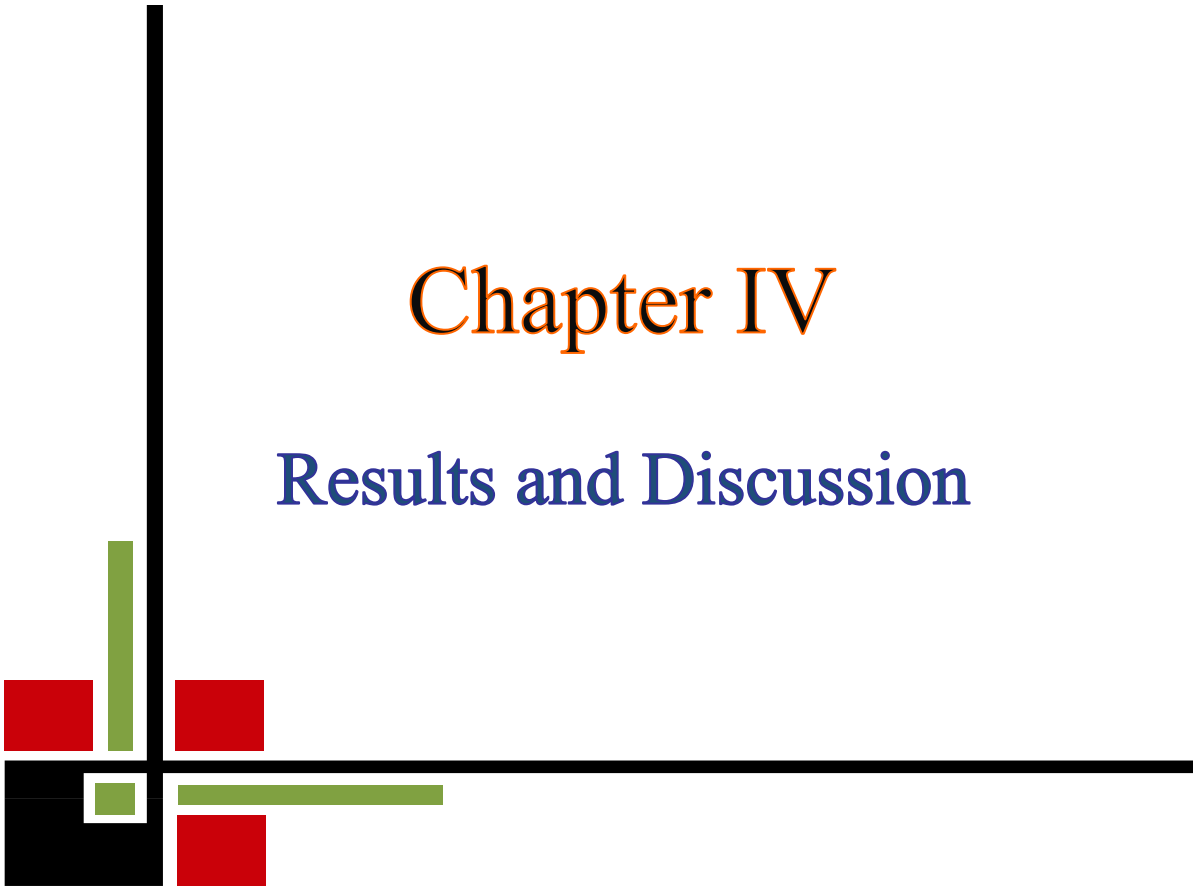


No.	Conc.	Area
1	50.000	4248
2	100.000	8325
3	200.000	16895
4	300.000	23985
5	500.000	41002

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

Chapter IV

Results and Discussion



CHAPTER IV

RESULTS AND DISCUSSIONS

90 samples of vegetable (cauliflower, eggplant & yard long bean) were collected from 5 different markets of Mymensingh sadar (Shankipara Bazar, Notun bazar, Sodesi bazar,Mesua Bazar and Mintu college bazar) of Mymensingh 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 cauliflower

The concentrated extracts of cauliflower samples collected from different markets of Mymensingh Sadar were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. Figure 9-13 shows the chromatograms of the injected extracts of cauliflower sample containing detected pesticides.

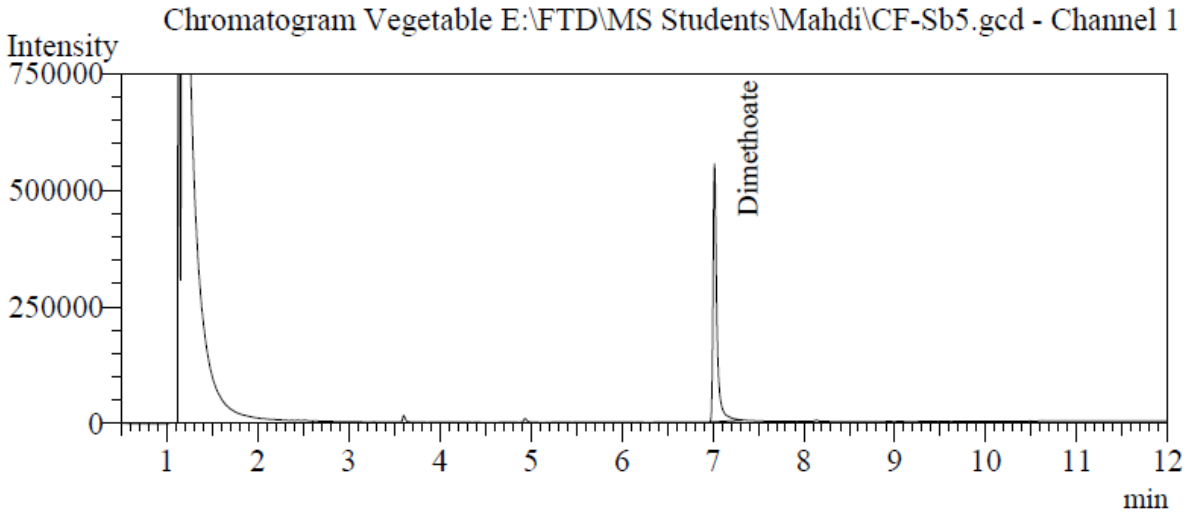


Figure 9: Chromatogram of dimethoate found in one of the cauliflower sample (CF-Sb_5).

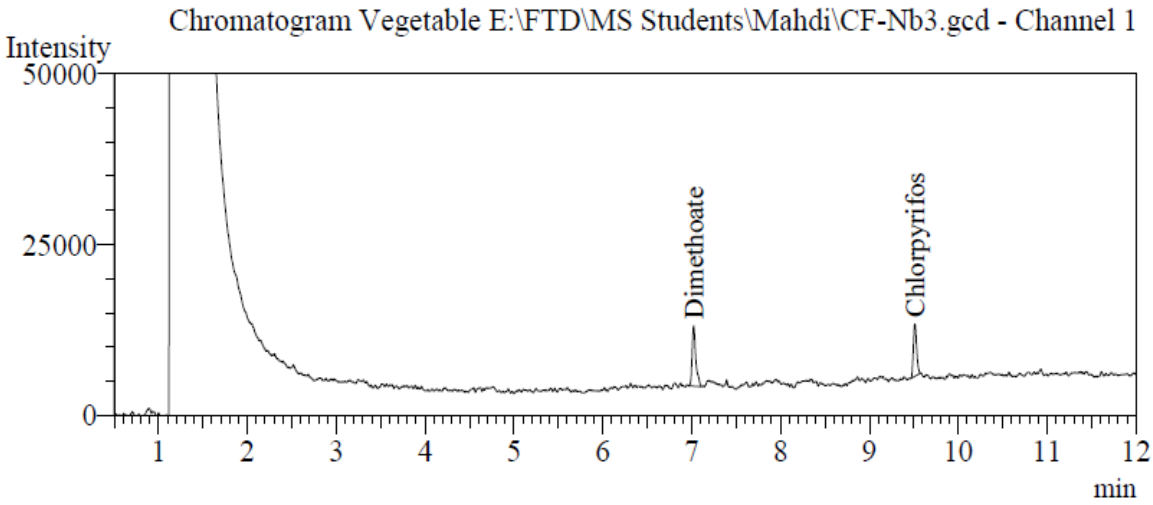


Figure 10: Chromatogram of dimethoate and chlorpyrifos found in one of the cauliflower sample ($CF\text{-}Nb_3$).

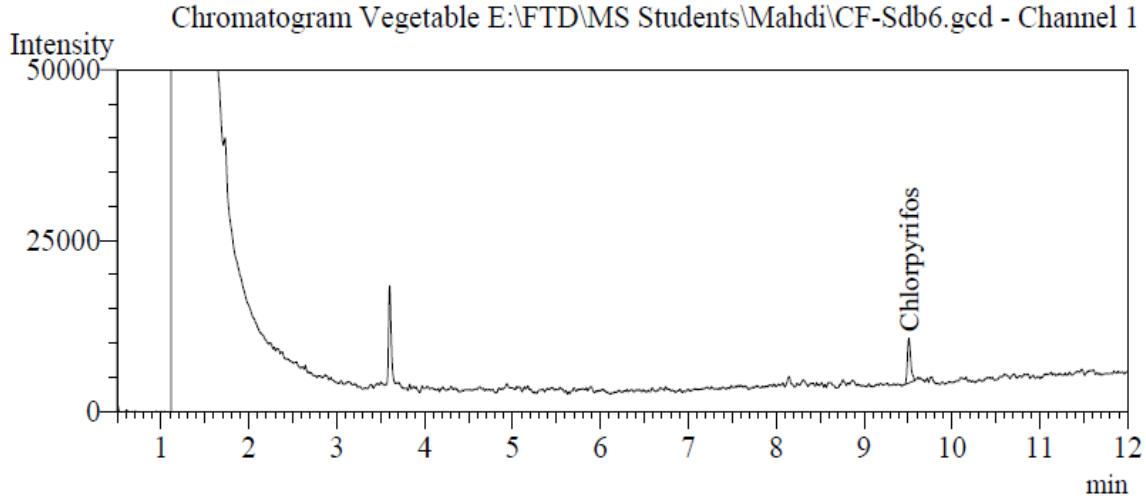


Figure 11: Chromatogram of chlorpyrifos found in one of the cauliflower sample ($CF\text{-}Sdb_6$).

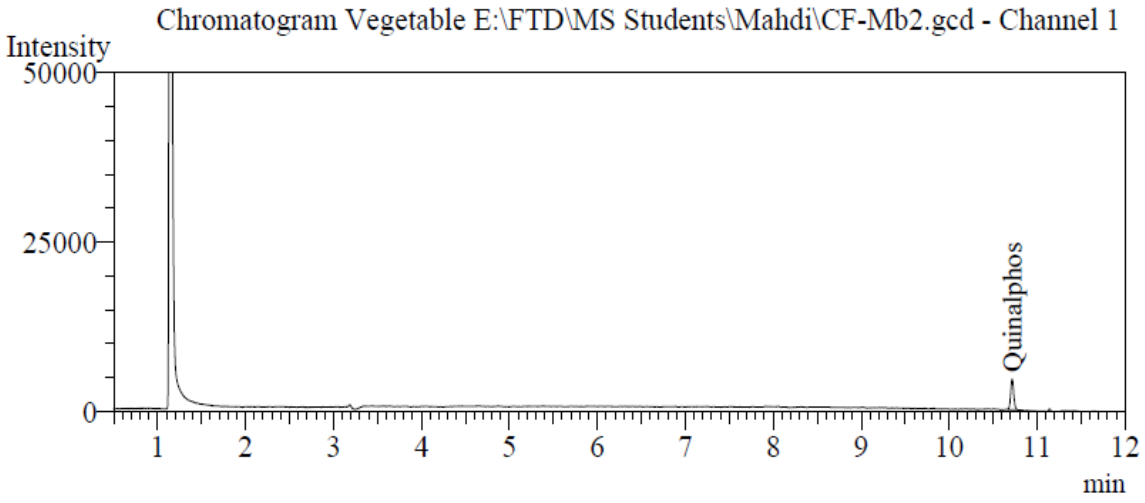


Figure 12: Chromatogram of quinalphos found in one of the cauliflower sample (CF-Mb_2).

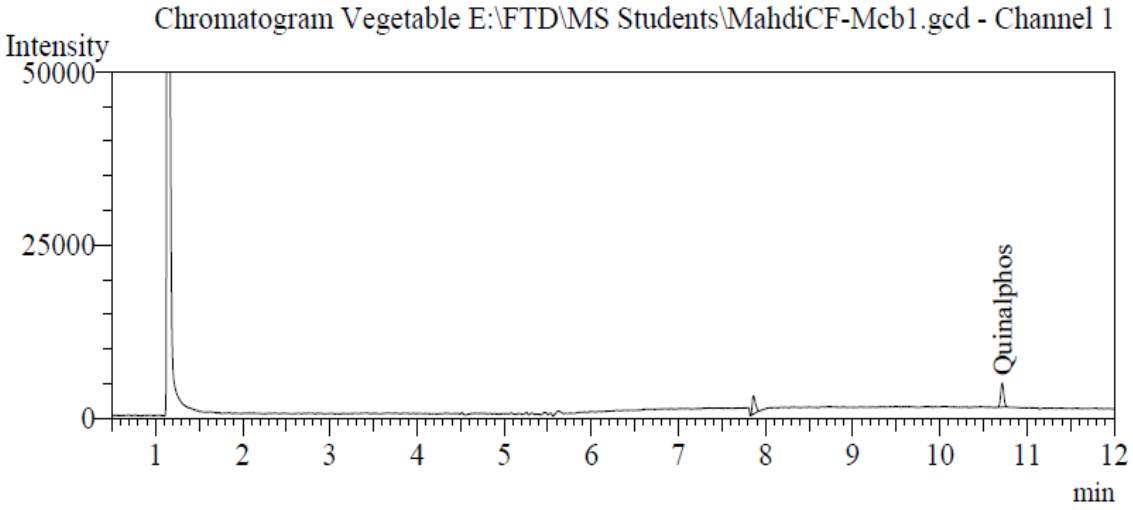


Figure 13: Chromatogram of quinalphos found in one of the cauliflower sample (CF- Mcb_1).

The level of pesticide residues found in the analyzed cauliflower 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 cauliflower samples.

Area of collection	**Sample ID**	**Name of detected pesticide**	**Level of residue (mg/kg)**	**MRLs (mg/kg)**
Shankipara Bazar	CF-Sb_1	ND	-	
	CF-Sb_2	ND	-	
	CF-Sb_3	ND	-	
	CF-Sb_4	ND	-	
	CF-Sb_5	Dimethoate	0.721	0.02*
	CF-Sb_6	ND	-	
Notun Bazar	CF-Nb_1	ND	-	
	CF-Nb_2	ND	-	
	CF-Nb_3	Dimethoate Chlorpyrifos	0.092 0.045	0.02* 0.05*
	CF-Nb_4	ND	-	
	CF-Nb_5	ND	-	
	CF-Nb_6	ND	-	
Sodesi Bazar	CF-Sdb_1	ND	-	
	CF-Sdb_2	ND	-	
	CF-Sdb_3	ND	-	
	CF-Sdb_4	ND	-	
	CF-Sdb_5	ND	-	
	CF-Sdb_6	Chlorpyrifos	0.036	0.05*
Mesua Bazar	CF-Mb_1	ND	-	
	CF-Mb_2	Quinalphos	0.025	0.01*
	CF-Mb_3	ND	-	
	CF-Mb_4	ND	-	
	CF-Mb_5	ND	-	
	CF-Mb_6	ND	-	
Mintu College Bazar	CF-MCb_1	Quinalphos	0.009	0.01*
	CF-MCb_2	ND	-	
	CF-MCb_3	ND	-	
	CF-MCb_4	ND	-	
	CF-MCb_5	ND	-	
	CF-MCb_6	ND	-	

*According to the EU Pesticide Database (European Commission 2015)

Thirty samples of cauliflower collected from 5 different markets of Mymensingh Sadar (Shankipara Bazar, Notun Bazar, Sodesi Bazar, Mesua Bazar, Mintu College Bazar) and were analyzed to find out the presence of left over residue of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos).

Out of 30 samples of cauliflower, 5 samples (17% of the total number of samples) contained pesticide residues and 25 samples (63% 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.

Six cauliflower samples were collected from Shankipara Bazar area, among them one samples (CF-Sb_5) contained dimethoate at a level of 0.721 mg/kg, which was above the EU-MRL (European Commission 2015). The other 5 samples contain no detectable pesticide residues.

From Notun Bazar, six samples were collected. Of which one sample (CF-Nb_3) contained multiple residues of dimethoate (0.092 mg/kg) and chlorpyrifos (0.045 mg/kg). But other five samples contained no detectable pesticide residues. The level of detected residue of dimethoate was above MRL (0.02 mg/kg) and the residue of chlorpyrifos was below MRL (0.05 mg/kg).

One sample (CF-Sdb_6) of cauliflower contained residue of chlorpyrifos (0.036 mg/kg) among the six samples collected from Sodeshi Bazar, which was below MRL (0.05 mg/kg). The other 5 samples contain no detectable pesticide residues.
One sample (CF-Mb_2) of cauliflower contained residue of quinalphos (0.025 mg/kg) among the six samples collected from Mesua Bazar, which was above MRL (0.01 mg/kg). The other 5 samples contain no detectable pesticide residues.

Among the six samples collected from Mintu College Bazar, one sample (CF-Mcb_1) contained residue of quinalphos (0.009 mg/kg) which was below the MRL (0.01 mg/kg).

4.2 Pesticide residues in Eggplant

The concentrated extracts of eggplant samples collected from different markets of Mymensingh Sadar were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. Figure 14-19 shows the chromatograms of the injected extracts of eggplant sample containing detected pesticides.

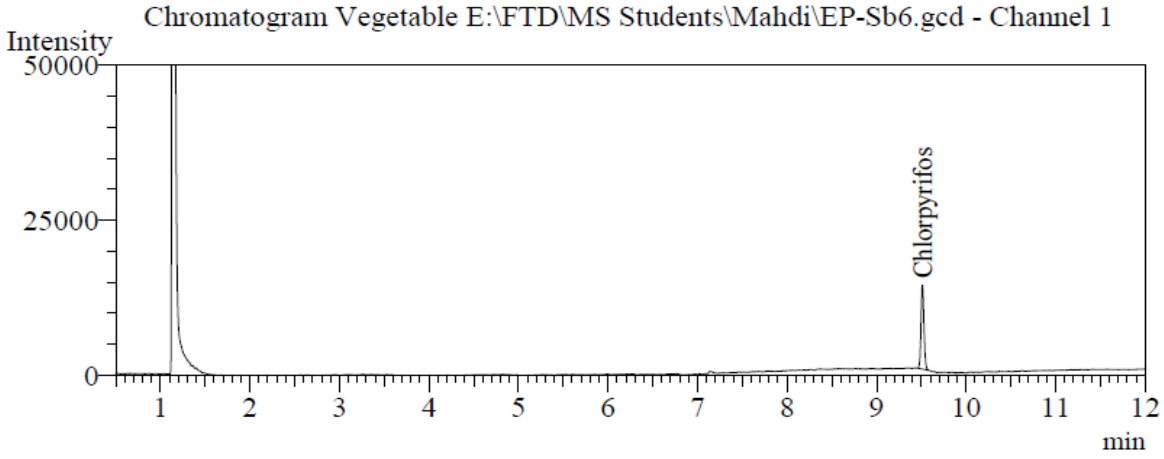


Figure 14: Chromatogram of chlorpyrifos found in one of the eggplant sample (EP-$Sb_{6)}$

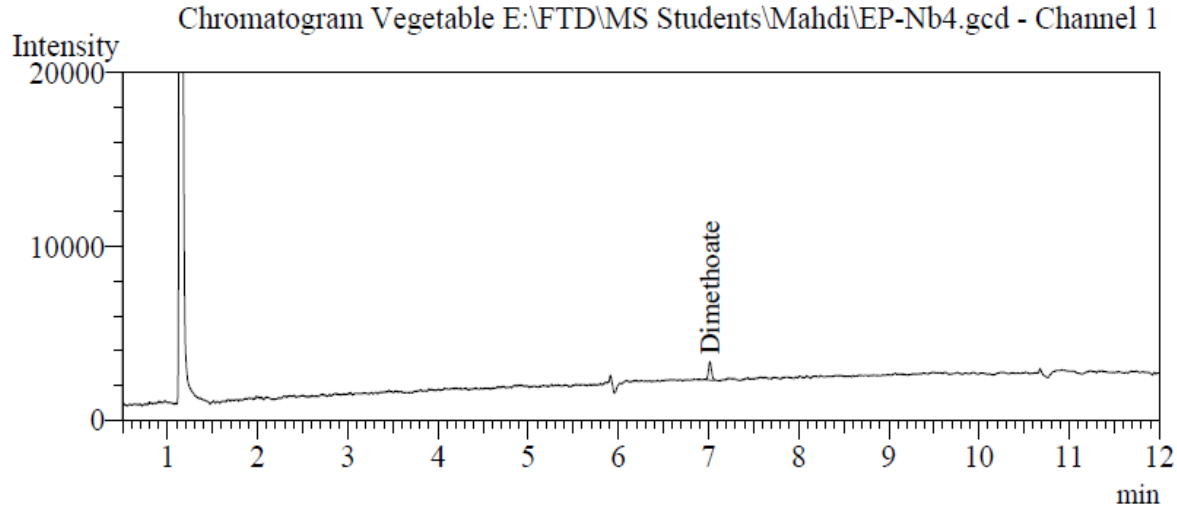


Figure 15: Chromatogram of dimethoate found in one of the eggplant sample (EP-Nb_4).

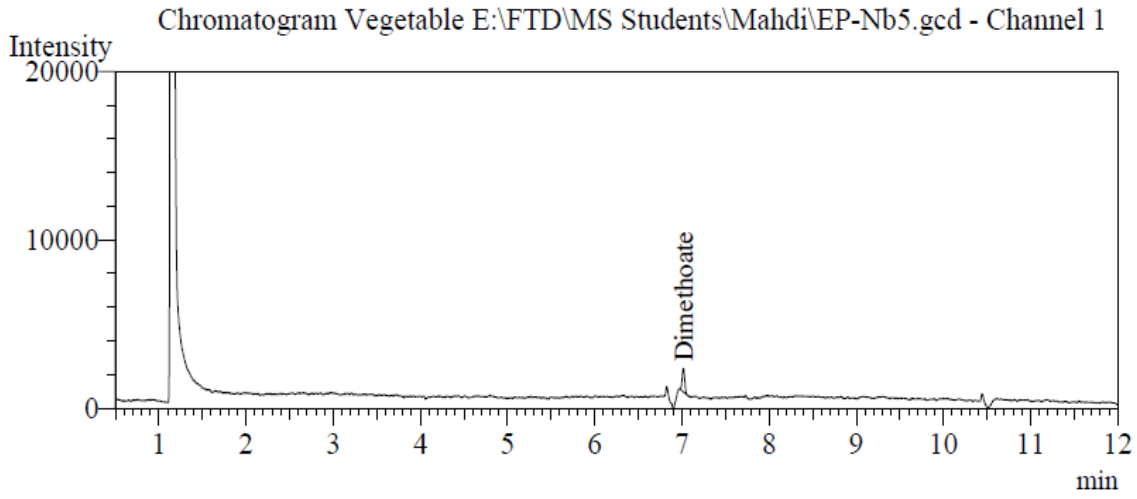


Figure 16: Chromatogram of dimethoate found in one of the eggplantr sample (EP-Nb_5).

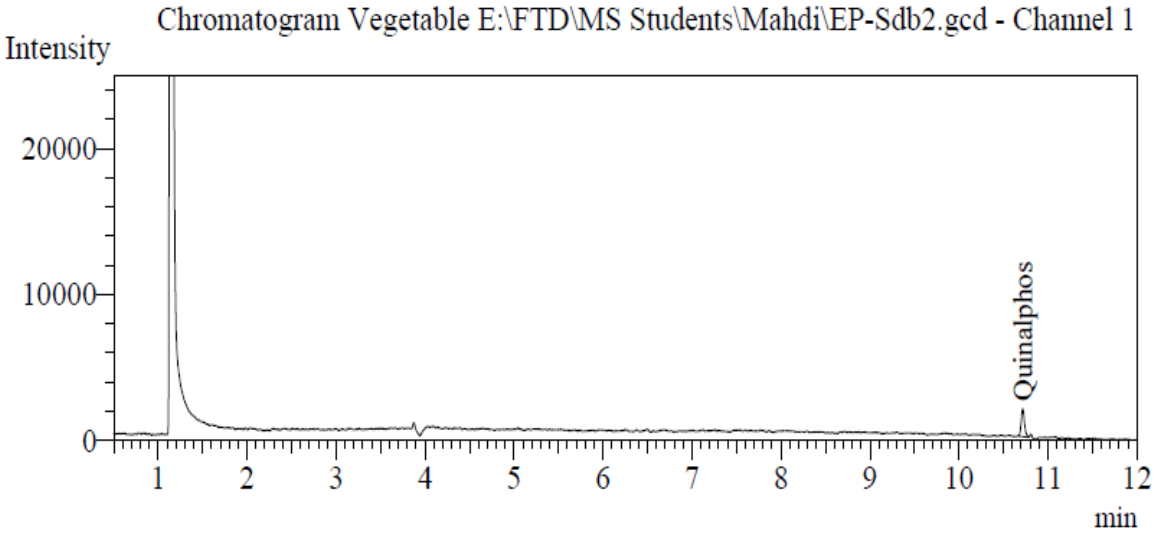


Figure 17: Chromatogram of quinalphos found in one of the eggplant sample (EP-Sdb_2).

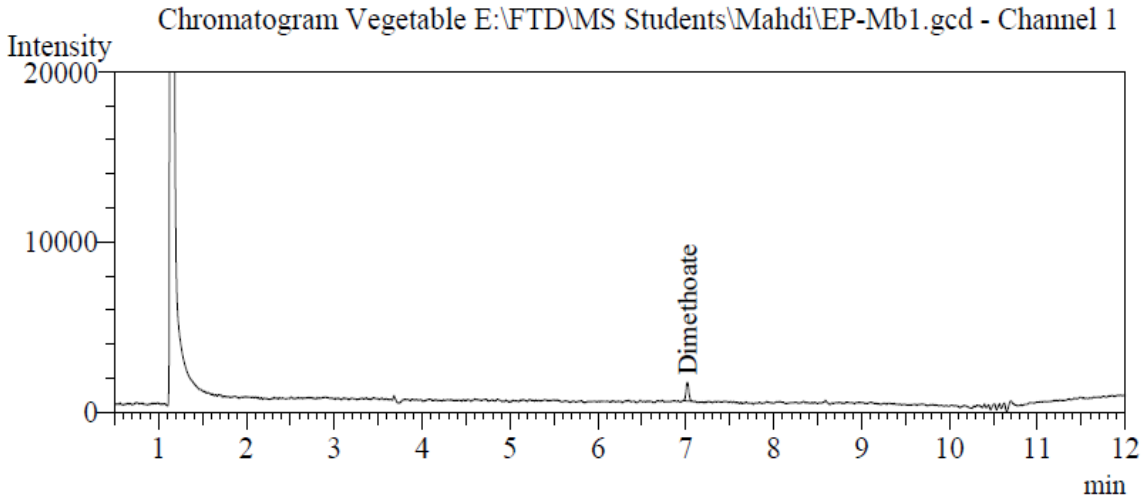


Figure 18: Chromatogram of dimethoate found in one of the eggplant sample(EP-Mb_1).

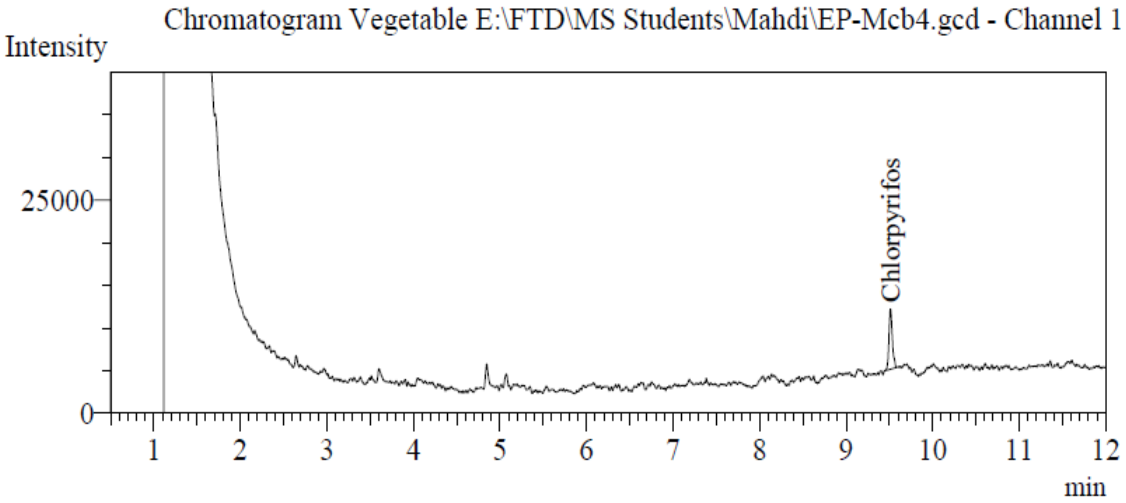


Figure 19: Chromatogram of chlorpyrifos found in one of the eggplant sample (EP-Mcb_4).

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

Area of collection	Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)
Shankipara Bazar	EP-Sb_1	ND	-	
	EP-Sb_2	ND	-	
	EP-Sb_3	ND	-	
	EP-Sb_4	ND	-	
	EP-Sb_5	ND	-	
	EP-Sb_6	Chlorpyrifos	0.173	0.40*
Notun Bazar	EP-Nb_1	ND	-	
	EP-Nb_2	ND	-	
	EP-Nb_3	ND	-	
	EP-Nb_4	Dimethoate	0.013	0.02*
	EP-Nb_5	Dimethoate	0.018	0.02*
	EP-Nb_6	ND	-	
Sodesi Bazar	EP-Sdb_1	ND	-	
	EP-Sdb_2	Quinalphos	0.042	0.01*
	EP-Sdb_3	ND	-	
	EP-Sdb_4	ND	-	
	EP-Sdb_5	ND	-	
	EP-Sdb_6	ND	-	
Mesua Bazar	EP-Mb_1	Dimethoate	0.017	0.02*
	EP-Mb_2	ND	-	
	EP-Mb_3	ND	-	
	EP-Mb_4	ND	-	
	EP-Mb_5	ND	-	
	EP-Mb_6	ND	-	
Mintu College Bazar	EP-MCb_1	ND	-	
	EP-MCb_2	ND	-	
	EP-MCb_3	ND	-	
	EP-MCb_4	Chlorpyrifos	0.108	0.40*
	EP-MCb_5	ND	-	
	EP-MCb_6	ND	-	

*According to the EU Pesticide Database (European Commission 2015)

Thirty samples of Eggplant collected from 5 different markets of Mymensingh sadar (Shankipara Bazar, Notun Bazar, Sodesi Bazar, Mesua Bazar, Mintu College Bazar) were analyzed to find out the presence of left over residues of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos).

Out of Thirty samples, 5 samples (17% of the total no. of samples) contained pesticide residues and 25 samples (83% of the total number of samples) contained no detectable residues of the sought pesticides.

Six samples of Eggplant were collected from Shankipara Bazar. Among them, only one sample (EP-Sb_6) contained residue of chlorpyrifos (0.173 mg/kg), which was below MRL (0.04 mg/kg). The other 5 samples contain no detectable pesticide residues.

Among the six samples of Eggplant collected from Notun Bazar, two samples (EP-Nb_4 and EP-N b_5) contained residue of dimethoate (0.013 mg/kg) and (0.018 mg/kg), respectively, which were below the MRL (0.02 mg/kg). The other 4 samples contain no detectable pesticide residues.

Six samples of Eggplant collected from Sodeshi Bazar. Of them, one sample (EP-Sdb_2) contained residues of quinalphos (0.042 mg/kg), which was above MRL (0.01 mg/kg). The other 5 samples contain no detectable pesticide residues.

Among the six sample of Eggplant collected from Mesua Bazar, one sample contained residue of dimethoate (0.017 mg/kg), which was below MRL (0.02 mg/kg). The other 5 samples contain no detectable pesticide residues.

Among the six samples collected from Mintu College Bazar, only one sample (EP-Mcb_4) contained residue of chlorpyrifos and the other five samples contained no detectable pesticide residues of the sought pesticides. The level of detected chlorpyrifos residue was 0.108 mg/kg, which was below MRL (0.04 mg/kg).

4.3 Pesticide residues in Yard Long Bean

The concentrated extracts of Yard Long Bean samples collected from different markets of Mymensingh sadar were analyzed by GC-2010 (Shimadzu) with Flame Thermonic Detector (FTD) with the pre-set parameters. Figure 20-22 shows the chromatograms of the injected extracts of yard long bean sample containing detected pesticides.

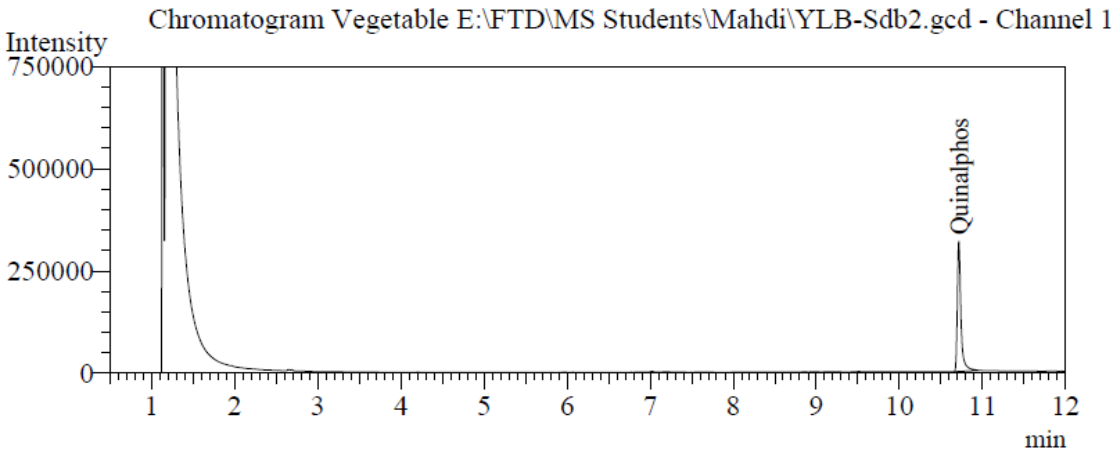


Figure 20: Chromatogram of quinalphos found in one of the yard long bean sample ($YLB\text{-}Sdb_2$).

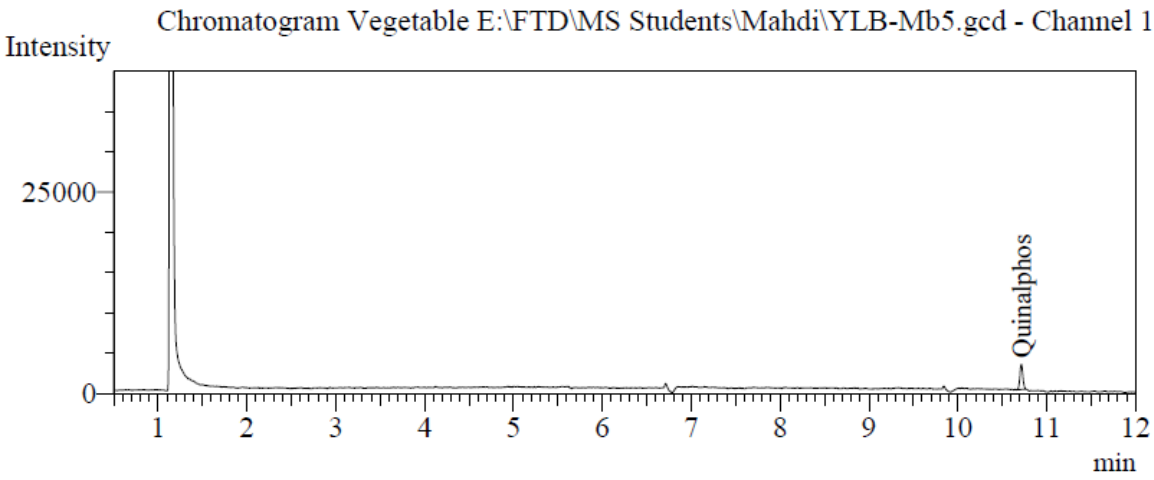


Figure 21: Chromatogram of quinalphos found in one of the yard long bean sample ($YLB\text{-}Mb_5$).

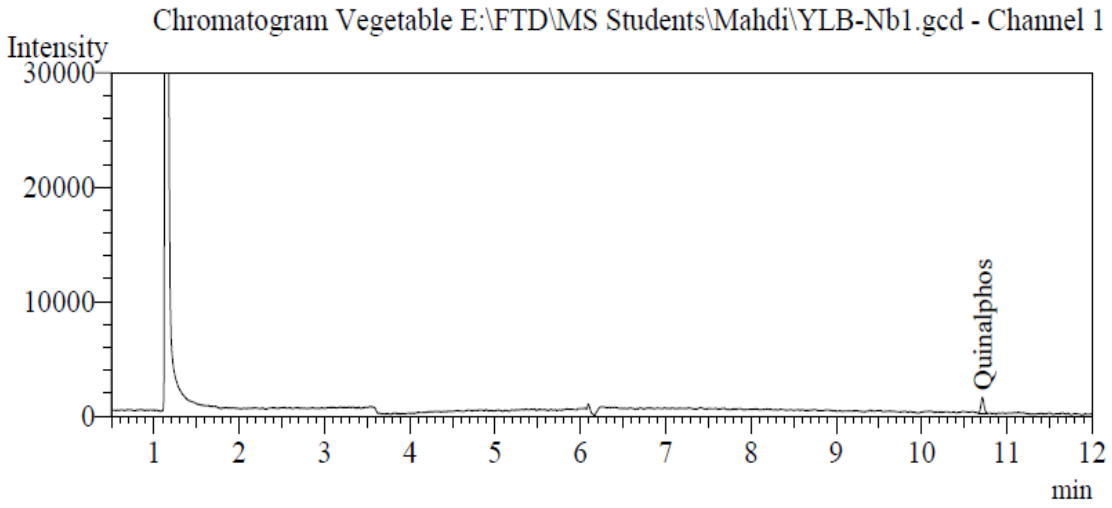


Figure 22: Chromatogram of quinalphos found in one of the yard long bean sample (YLB-Nb_1).

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

Area of collection	**Sample ID**	**Name of detected pesticide**	**Level of residue (mg/kg)**	**MRLs (mg/kg)**
Shankipara Bazar	YLB-Sb_1	ND	-	
	YLB-Sb_2	Quinalphos	0.562	0.01*
	YLB-Sb_3	ND	-	
	YLB-Sb_4	ND	-	
	YLB-Sb_5	ND	-	
	YLB-Sb_6	ND	-	
Notun Bazar	YLB-Nb_1	Quinalphos	0.008	0.01*
	YLB-Nb_2	ND	-	
	YLB-Nb_3	ND	-	
	YLB-Nb_4	ND	-	
	YLB-Nb_5	ND	-	
	YLB-Nb_6	ND	-	
Sodesi Bazar	YLB-Sdb_1	ND	-	
	YLB-Sdb_2	ND	-	
	YLB-Sdb_3	ND	-	
	YLB-Sdb_4	ND	-	
	YLB-Sdb_5	ND	-	
	YLB-Sdb_6	ND	-	
Mesua Bazar	YLB-Mb_1	ND	-	

	YLB-Mb_2	ND	-	
	YLB-Mb_3	ND	-	
	YLB-Mb_4	ND	-	
	YLB-Mb_5	Quinalphos	0.024	0.01*
	YLB-Mb_6	ND	-	
Mintu College Bazar	YLB-MCb_1	ND	-	
	YLB-MCb_2	ND	-	
	YLB-MCb_3	ND	-	
	YLB-MCb_4	ND	-	
	YLB-MCb_5	ND	-	
	YLB-MCb_6	ND	-	

*According to the EU Pesticide Database (European Commission 2015)

Thirty samples of yard long bean were collected from 5 different markets of Mymensingh Sadar (Shankipara Bazar, Notun Bazar, Sodesi Bazar, Mesua Bazar, Mintu College Bazar) and were analyzed to find out the presence of left over residues of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos).

Out of Thirty samples, 3 samples (10% of the total no. of samples) contained pesticide residues and 27 samples (90% of the total number of samples) contained no detectable residues of the sought pesticides.

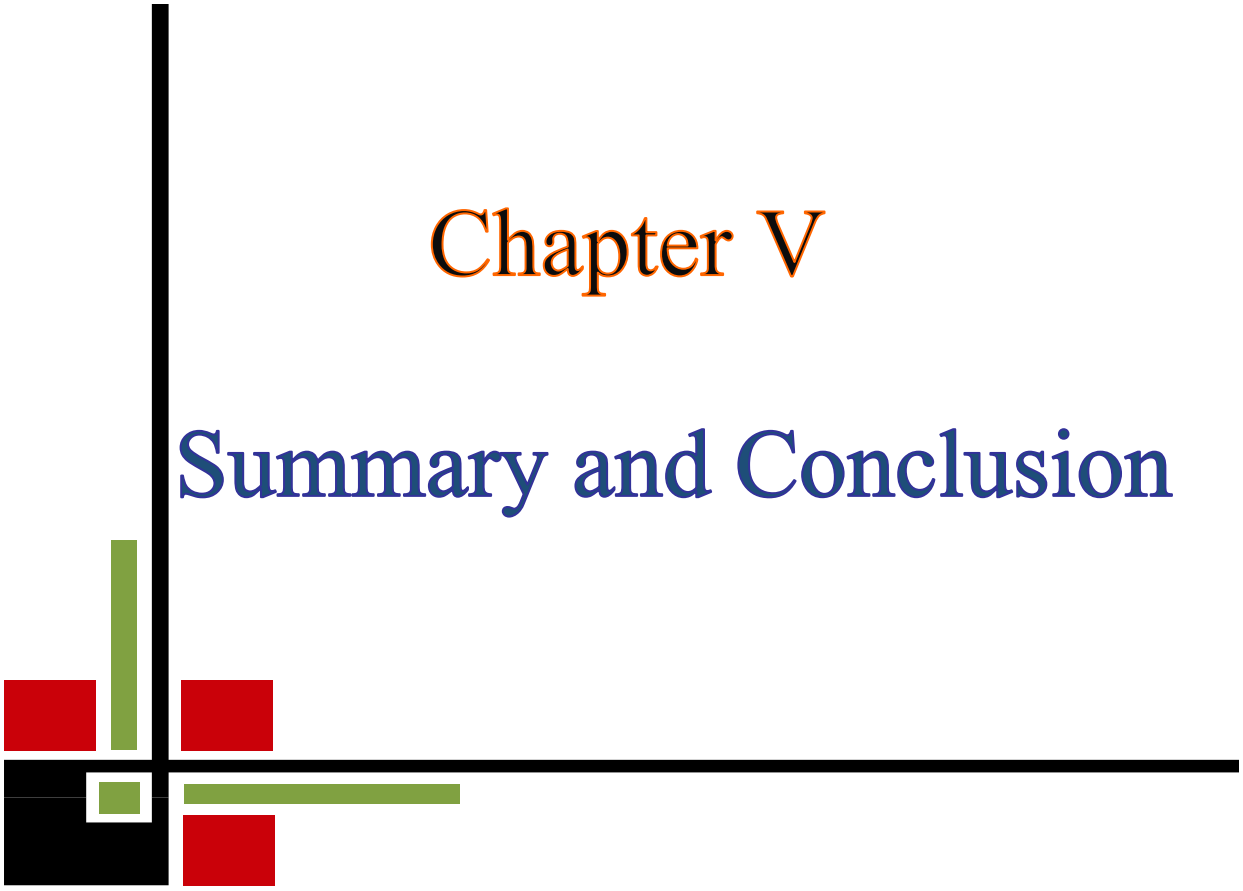
Among the six sample of Yard Long Bean one sample (YLB-Sb_2) from Shankipara Bazar contained pesticide residues of quinalphos (0.562 mg/kg) which was above MRL (0.01mg/kg). The other 5 samples contain no detectable pesticide residues.

One sample (YLB-Nb_1) contained residues of quinalphos (0.008 mg/kg) collected from Notun Bazar which was below MRL (0.01mg/kg). The other 5 samples contain no detectable pesticide residues.

The samples collected from Mintu College Bazar and Sodeshi Bazar contained no residues of the sought pesticides. However, one sample (YLB-Mb_5) collected from Mesua Bazar contained residues of quinalphos (0.024mg/kg) which was above MRL (0.01mg/kg).

Chapter V

Summary and Conclusion



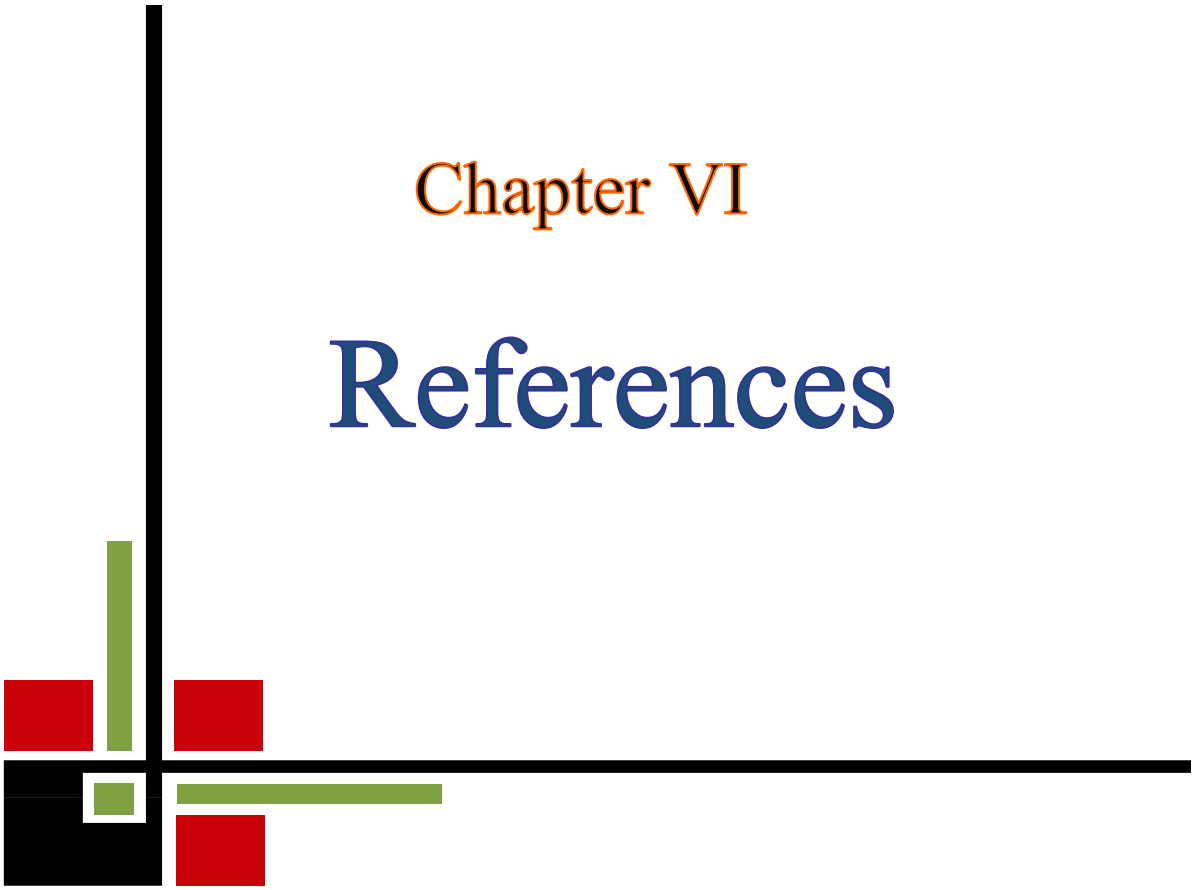
CHAPTER V

SUMMARY AND CONCLUSION

The purpose of this study was intended to identify and quantify the pesticide residue level present in the vegetables collected from various local markets of Mymensingh City. Regarding this, thirty samples of cauliflower, thirty samples of eggplant and thirty samples of yard long bean were collected from five different locations (Notun bazar, Mesua bazar, Sodesi bazar, Shankipara bazar, Mintu college bazar) of Mymensingh City and carried to the Pesticide Analytical Laboratory, Entomology Division, 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 thermionic 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 30 analyzed samples of cauliflower, 5 samples (17% of the total number of samples) contained residues of dimethoate, chlorpyrifos and quinalphos, where 1 sample contain multiple residues and 3 samples contained residue above the maximum residue limits (MRLs). Out of 30 samples of eggplant, 5 samples (17% of the total number of samples) contained residues of chlorpyrifos, quinalphos, and dimethoate, where 1 sample contained pesticide residue above MRL. On the other hand, among the 30 samples of yard long bean, 3 samples (10% of the total samples) contained residue of quinalphos, of which 2 samples contained pesticide residues above MRL.

Pesticide residues in vegetables and other foods have become a major concern and a safety issue for the consumers. The findings of the present study reflect the overall scenario of pesticide residue load in the selected vegetables collected from different markets of Mymensingh. The results of the present study indicate that the farmers of Mymensingh district are using chlorpyrifos, dimethoate and quinalphos indiscriminately. This study will help to increase public awareness as well.



Chapter VI

References

CHAPTER VI

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