DETERMINATION OF PESTICIDE RESIDUES IN CABBAGE AND BITTER GOURD COLLECTED FROM SOME RETAIL MARKETS OF DHAKA CITY

BY

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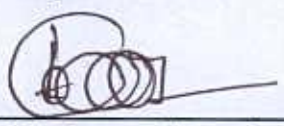

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CERTIFICATE

This is to certify that the thesis entitled **"DETERMINATION OF PESTICIDE RESIDUES IN CABBAGE AND BITTER GOURD COLLECTED FROM SOME RETAIL MARKETS OF DHAKA CITY"** submitted to the Department of Agricultural Chemistry, 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 **AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bona fide research work carried out by **AHAMMAD ULLAH**, Registration No. **10-03787** 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 this investigation has been duly acknowledged.

June, 2016
Dhaka, Bangladesh

Dr. Mohammad Dalower Hossain Prodhan
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and
Research Supervisor

Dedicated to

My

Beloved Parents

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All praises to the **"Almighty Allah"** Who enable the author to complete a piece of research work and prepare this thesis for the degree of Master of Science (M.S.) in Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207.

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The Author

DETERMINATION OF PESTICIDE RESIDUES IN CABBAGE AND BITTER GOURD COLLECTED FROM SOME RETAIL MARKETS OF DHAKA CITY

Abstract

The study was conducted to analyze four Organophosphorus (OP) and two Synthetic Pyrethroid (SP) pesticide residues in two common vegetables (cabbage and bitter gourd) collected from five different areas (Rampura, Kawran Bazar, Taltola Bazar, Jatrabari and Mohammadpur Krishi Market) of Dhaka city from September 2016 to November 2016. The collected samples were analyzed using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and Gas Chromatography (GC) coupled with Flame Thermionized Detector (FTD) and Electron Capture Detector (ECD) for the determination of pesticide residues in 50 samples of cabbage and 50 samples of bitter gourd. Among the analyzed 50 samples of cabbage, 13 samples (26%) contained residues of single compound namely chlorpyrifos, cypermethrin, diazinon and dimethoate, where 1 sample contained two pesticide residues that is diazinon and cypermethrin. Out of these 13 samples, 7 samples contained residue above the maximum residue limits (MRLs) and 5 samples below the MRLs. In 50 samples of bitter gourd, 12 samples (24%) contained residues of single compound namely cypermethrin, dimethoate, diazinon and chlorpyrifos, where 3 samples contained two pesticide residues. Among these 12 samples, 7 samples contained residue above the MRLs and 2 samples were below the MRLs. Diazinon and cypermethrin were detected in most of the contaminated cabbage and bitter gourd sample, while chlorpyrifos and dimethoate were also detected. This study reflects the overall scenario of pesticide contamination in vegetables especially in cabbage and bitter gourd available in the retail markets of Dhaka 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
ACH	Acetylcholine
APCI	Atmospheric Pressure Chemical Ionization
ASE	Accelerated solvent extraction
AOAC	Association of Official Analytical Chemists
BARI	Bangladesh Agricultural Research Institute
ChE	Cholinesterase
CSN	Committee for Standardization
DAS	Days After Spray
DLLME	Dispersive Liquid–Liquid Micro Extraction
DMCb	Dhaka Market Cabbage
DMBG	Dhaka Market Bitter Gourd
DV	Daily Value
d-SPE	dispersive solid phase extraction
ECD	Electron Capture Detector
EPA	Environmental Protection Agency
ESI	electrospray ionization
et al.	*et alibi* (and others)
etc.	*et cetra* (and so on)
ECD	Electron capture Detector
EU	European Union
FAO	Food and Agriculture Organization
FID	Flame Ionization Detector
FPD	Flame photometric Detector
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FTD	Flame Thermionized Detector
GAP	Good Agricultural Practices
GCB	Graphitized Carbon Black
GC-MS	Gas Chromatograph-Mass Spectrometry
HPLC	High Performance Liquid Chromatography

HRI	Hazard Risk Index
KMRL	Korean Maximum Residue Limits
LC-MS	Liquid Chromatography-Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MCS	Multiple Chemical Sensitivity Syndrome
MDQ	Minimum Detectable Quantity
MRL	Maximum Residue Limit
MRM	multiple reaction monitoring
NPD	Nitrogen-phosphorus Detector
NPTN	National Pesticides Telecommunications Network
NTE	Neuropathy Target Esterase
PDA	Photodiode Array detection
PDI	Potential Daily Intake
PSA	Primary Secondary Amine
PHI	Pre-Harvest Interval
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
SE	Solvent Extraction
SFE	Supercritical fluid extraction
SF	Supercritical Fluid
SIM	Selected Ion Monitoring
SRM	Selected Reaction Monitoring
SPE	Solid phase extraction
TOTAD	Through Oven Transfer Adsorption Desorption
TCD	Thermal conductivity Detector
UHPLC-MS/MS	Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry
WHO	World Health Organization

Chapter I

Introduction

CHAPTER I

INTRODUCTION

Vegetables are considered essential for well balanced diets since they supply vitamins, minerals, dietary fiber, and phytochemicals. In the daily diet, vegetables have been strongly associated with improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some forms of cancer. Vegetables make 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 (A, B1, B6, B9, C, E), minerals, dietary fiber and phytochemicals (Quebedeaux and Eisa, 1990; Craig and Beck, 1999; Wargovich, 2000; Dias and Ryder, 2011). Vegetables are the fresh and edible portions of herbaceous plants. They contain valuable food ingredients which can be successfully utilized to build up and repair the body. Vegetables are valuable in maintaining alkaline reserve of the body. They are valued mainly for their high carbohydrate, vitamin and mineral contents. There are different kinds of vegetables. They may be edible roots, stems, leaves, fruits or seeds. Each group contributes to diet in its own way (Robinson, 1990).

Cabbage (*Brassica oleracea*) is an excellent source of vitamin C and vitamin K, containing more than 20% of the Daily Value (DV) for each of these nutrients per serving (USDA, 2014). Cabbage is an important exotic vegetable grown in both small and large scales. It provides a source of livelihood to all individuals who are engaged in cabbage production from its cultivation till it gets to the final consumer (Asare-Bediako *et al.*, 2010). The cultivation of cabbage provides an excellent source of employment for both rural and urban dwellers through farming and gardening respectively. Cabbage has high nutritive value and it is used in the preparation of various kinds of dishes such as stews and salads (Norman, 1992). Cabbage is also a good source (10–19% DV) of vitamin B6 and folate, with no other nutrients having significant content per 100 gram serving. Cabbage is a good source of protein, carbohydrates, calcium, iron, carotene, thiamine, riboflavin, niacin, as well as vitamin C (De Lannoy, 2001). Basic research on cabbage phytochemicals is ongoing to

discern if certain cabbage compounds may affect health or have anti-disease effects. Such compounds include sulforaphane and other glucosinolates which may stimulate the production of detoxifying enzymes during metabolism (Dinkova-Kostovaand and Kostov, 2012). Studies suggest that cruciferous vegetables, including cabbage, may have protective effects against colon cancer.

Purple cabbage contains anthocyanins which are under preliminary research for potential anti-carcinogenic properties. Cabbage is also a source of indole-3-carbinol, a chemical under basic research for its possible properties (Wu *et al.*, 2010). In addition to its usual purpose as an edible vegetable, cabbage has been used historically as a medicinal herb for a variety of purported health benefits. The Ancient Greeks recommended consuming the vegetable as a laxative (Wright, 2001), and used cabbage juice as an antidote for mushroom poisoning(Decoteau, 2000), for eye salves, and for liniments used to help bruises health (Phillips and Henry,1827).

In spite of the enormous benefits of cabbage to the growth and development of humans, production of the crop is beset with insect pests attack. Pest infestation normally leads to reduction in market value and in some cases total crop failure. Synthetic insecticides have been widely used to control insect pests with a collage of risks. These insecticides may contaminate water bodies, air and the soil (Joel, 1994). There is therefore a growing concern in connection with environmental pollution and its resultant effects on the health of humans and animals arising from the continuous use of synthetic chemicals (pesticides) in cabbage production.

Bitter gourd (*Momordica charantia* L) is an important vegetable. Bitter gourd fruits are a good source of carbohydrates, proteins, vitamins, and minerals and have the highest nutritive value among cucurbits (Miniraj *et al.*, 1993; Desai and Musmade, 1998). The vitamin C content of Chinese bitter gourd varies significantly (440-780 mg kg^{-1} edible portion). Considerable variation in nutrients, including protein, carbohydrates, iron, zinc, calcium, magnesium, phosphorous, and ascorbic acid, has been observed in bitter gourd (Kale *et al.* 1991; Yuwai *et al.* 1991). Moreover, the crude protein content (11.420.9g kg^{-1}) of bitter gourd fruits is higher than that of tomato and cucumber (Xiang *et al.*, 2000).

This crop is attacked by many insect pests, among theseRed Pumpkin Beetle and Fruit fly are the major insectpests. Due to plant pests and diseases, 20 to 40 percent of the crop yields are reduced globally (FAOSTAT, 2012). To overcome these situations farmers are using pesticides. Pesticides play a key role to control the insect pests and diseases and hence protect and promote production (Prodhan *et al.*, 2015). On the other hand, pesticides create several adverse effects on human health and the environment (McIntyre, 1989; Hajslova and Zrostlikova, 2003; Fenik, *et al.*, 2011). These negative impacts of pesticides are increasing day by day in order to increase the uses of pesticides.

Besides, now a days food safety is a major concern to the consumers. But the percentage of food containing pesticide residues has increased in the last 10 years. In order to ensure the supply of safe food, pesticides should be used following Good Agricultural Practices (GAP). Monitoring of pesticide residues is the essential tool to ensure GAP. To monitor pesticide residues nationally in the commercial produce, reliable multi-residue analytical methods are required. Multi-residue methods, which allow the quantification of residues of different analytes at the same time in a single run, are used advantageously for monitoring purposes.

A survey on pesticide use in vegetables conducted in 1988 revealed that only about 15% and 6% of the farmers received information from the pesticide dealers and extension agents, respectively (Islam, 1999). In most of the cases, the farmers either forgot the instructions or did not care to follow those instructions and went on using insecticides at their own choice or experience. Some farmers believed that excess use of insecticides could solve the insect pests' problem. They did not follow the rule of economic threshold and economic injury level. Farmers use insecticides frequently without considering the level of infestation. They usually spray insecticides in their field indiscriminately even without thinking the economic return of their investment.

Pesticide being toxic can become a potential hazard to the manufacturers, the users, the public at large and the environment. Pesticide can produce negative impacts, both socially and economically (Antle and Pingali, 1994). Extensive use of pesticides has resulted in contamination of vital supplies, air, water, and food, the risk to humans may be short term as well as long term depending on the persistence of the pesticide and the exposure period.

It has been estimated by the World Health Organization (WHO) that about 20,000 people die each year from pesticide poisoning and at least 3 million people suffer acute health effects (Barbara, 1993). Pesticide residue in food has become a consumer safety issue and the consumer has the right to know how much pesticide get incorporated in the food he eats. The detection, identification and quantification of pesticide in the food we eat are a problem of increasing public interest. But still a few references are available on pesticide residues present in vegetables grown in Bangladesh.

Every pesticide has a withholding period, waiting period, lapse period or pre-harvest interval (PHI), which is defined as the number of days required to lapse, between the date of final pesticide application and harvest, for residues to fall below the tolerance level established for that crop or for a similar food items. The PHI differs from pesticide to pesticide and crop to crop. Food products become safe for consumption only after withholding period has lapsed. By this time, the pesticide residues get dissipated or degraded. However, the extent and rate of dissipation depends on the nature of the pesticide, crop, cultural practices and various environmental conditions under which the crop is grown or a treated commodity is stored (Handa *et al.*, 1999). Due to lack of education, the farmers of our country do not follow the prescribed dosages and use pesticides at any stage of the crop without any awareness of the residues and their ill effects on human health. The treated fruits and vegetables are picked/ harvested without taking into account the withholding period.

A number of analytical methods are used to determine multiple pesticide residues for fruits and vegetables (Anastassiades *et al.*, 2003; Prodhan *et al.*, 2016; Prodhan *et al.*, 2016a; Prodhan *et al.*, 2015; Prodhan *et al.*, 2015a; Schenck *et al.*, 2008; Singh *et al.* 2012; Dasika *et al.*, 2012; Lehotay,2010). Different extraction and clean-up methods are used for different food matrices; among them QuEChERS extraction techniques are widely used in the food testing laboratories. In 2003, the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method for pesticide residue analysis was introduced (Anastassiades *et al.*, 2003); it provides high-quality results in a fast, easy, inexpensive approach. Follow-up studies have further validated the method for more than 200 pesticides (Lehotay *et al.*, 2005), improved results for the remaining few problematic analytes (Lehotay *et al.*, 2005), and tested it in fat-containing matrices

(Lehotay *et al.*, 2005). Therefore QuEChERS extraction techniques along with Gas Chromatography were used in this study to determine selected pesticides in cabbage and bitter gourd.

Considering the aforesaid information the **objectives** of the present study were-

- To identify pesticide residues in cabbage and bitter gourd collected from different markets of Dhaka City.
- To quantify the level of detected pesticide residues (mg/Kg) remain in the selected vegetables collected from different markets of Dhaka City.
- To compare whether the levelof detected insecticide residues are above the Maximum Residue Limit (MRL) or not.

Chapter II
Review of Literature

CHAPTER II

REVIEW OF LITERATURE

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

2.1 Pesticides

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (EPA, 2014).The term pesticide includes herbicide, insecticide, insect growth regulator, nematicide, termiticide, molluscicide, piscicide, avicide, rodenticide, predacide, bactericide, insect repellent, animal repellent, antimicrobial, fungicide, disinfectant (antimicrobial), and sanitizer (Randall *et al.*, 2013).

According to the Food and Agriculture Organization, a pesticide is any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal diseases, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of

insects, arachnids, or other pests in or on their bodies. The term includes substances intended for use as plant growth regulators, defoliants, desiccants, or agents for thinning fruit or preventing the premature fall of fruits. It is also used for all substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport (FAOSTAT, 2002). Due to their wide use in agriculture, pesticides are the most investigated priority pollutants in agricultural products (Garrido Frenich *et al.*, 2008).

2.2 Pesticide classification

2.2.1 Organophosphate pesticides

Organophosphates affect the nervous system by disrupting, acetyl cholinesterase activity, the enzyme that regulates acetylcholine, a neurotransmitter. Most organophosphates are insecticides. They were developed during the early 19th century, but their effects on insects, which are similar to their effects on humans, were discovered in 1932. Some are very poisonous. However, they usually are not persistent in the environment.

2.2.2 Carbamate pesticides

Carbamate pesticides affect the nervous system by establishing an enzyme that regulates acetylcholine, a neurotransmitter. The enzyme effects are usually reversible. There are several subgroups within the carbamates.

2.2.3 Organochlorine pesticides

They were commonly used in the past, but many have been removed from the market due to their health and environmental effects and their persistence (e.g., DDT, chlordane, and toxaphene).

2.2.4 Pyrethroid pesticides

They were developed as a synthetic version of the naturally occurring pesticide pyrethrin, which is found in chrysanthemums. They have been modified to increase their stability in the environment. Some synthetic pyrethroids are toxic to the nervous system.

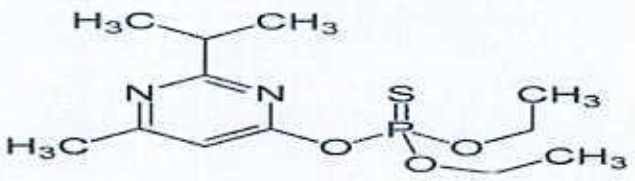
2.2.5 Neonicotinoid pesticides

Neonicotinoids area class of neuroactive insecticides chemically similar to nicotine. In the late 1990s neonicotinoids came under increasing scrutiny over their environmental impact and were linked in a range of studies to adverse ecological effects, including honey-bee colony collapse disorder (CCD) and loss of birds due to a

reduction in insect populations. In 2013, the European Union and a few non EU countries restricted the use of certain neonicotinoids (Cressey, 2013).Imidacloprid, of the neonicotanoid family, is the most widely used insecticide in the world (Yamamoto and Izuru, 1999).

2.3 Description of the selected pesticides

2.3.1 Diazinon



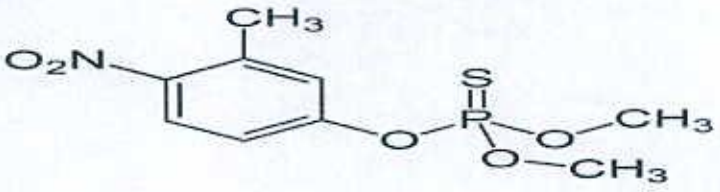
Structural formula of Diazinon

Diazinon (IUPAC name: *O,O*-Diethyl *O*-[4-methyl-6-(propan-2-yl) pyrimidin- 2-yl] phosphoro- thioate, INN - Dimpylate), a colorless to dark brown liquid, is a thiophosphoric acid ester developed in 1952 by Ciba-Geigy, a Swiss chemicalcompany (later Novartis and then Syngenta). It is a non-systemic organophosphate insecticide formerly used to control cockroaches, silverfish, ants, and fleas in residential, non-food buildings.Diazinon was heavily used during the 1970s and early 1980s for general-purpose gardening use and indoor pest control. A bait form was used to control scavenger wasps in the western U.S. Diazinon is used in flea collars for domestic pets in Australia and New Zealand. Residential uses of diazinon were outlawed in the U.S. in 2004 but it is still approved for agricultural uses. An emergency antidote is atropine (Robert *et al.*, 2003).

Diazinon is a contact insecticide which kills insects by altering normal neurotransmission within the nervous system of the insect. As mentioned above, diazinon inhibits the enzyme acetylcholinesterase (AChE), which hydrolyzes the neurotransmitter acetylcholine (ACh) in cholinergic synapses and neuromuscular junctions. This results in abnormal accumulation of ACh within the nervous system. Diazinon, although a thiophosphoric ester, shares a common mechanism of toxicity with other organophosphate insecticides such as chlorpyrifos, malathion and parathion, and is not very effective against the organophosphate-resistant insect populations. Symptoms of acute diazinon exposure develop in minutes to hours following exposure, depending of the exposure pathway. The initial symptoms of humans are nausea, dizziness, salivation, headache, sweating, lacrimation, and

rhinorrhea. The symptoms can progress to vomiting, abdominal cramps, diarrhea, muscle twitching, weakness, tremor, a lack of coordination and miosis. Intermediate syndrome generally occurs within 24–96 hours after exposure. Intermediate syndrome in humans is characterized by difficulty breathing and muscular weakness, often in the face, neck and proximal limb muscles. Cranial nerve palsies and depressed tendon reflexes have also been reported.

2.3.2 Fenitrothion

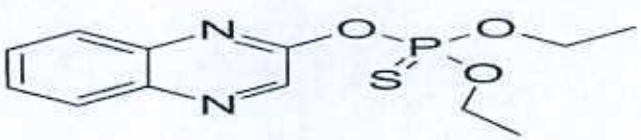


Structural formula of Fenitrothion

Fenitrothion (IUPAC name: O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate) is a phosphorothioate (organophosphate) insecticide; cheap and widely used worldwide.In experiments fenitrothion at sublethal doses affected the motor movement of marsupials (William *et al.*, 2008) and at acute dose levels it reduced the energy of birds (Malsha *et al., 2011).* In chronic (low) dose tests, unexpectedly only the lowest concentration (0.011 microgram/liter) of fenitrothion depressed the growth of an algae, though all of the chronic dose levels used were toxic in other ways to the algae (Ferrando, *et al.*, 1996).

Just half of fenitrothion's minimally effective dose altered the thyroid structure of a freshwater murrel (the snakehead fish).In an unusual demonstration of resistance to pesticides, 8% of insects in farm fields were found to carry a symbiotic gut microbe that can metabolize and detoxify fenitrothion; after in-vitro tests showed that the microbe significantly increased the survival of fenitrothion-treated insects (Kikuchi *et al.*, 2012).

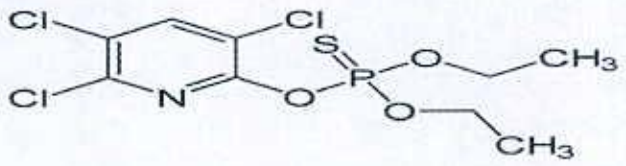
2.3.3 Quinalphos



Structural formula of Quinalphos

Quinalphos (IUPAC name: O, O-Diethyl O-2-quinoxalinyl phosphorothioate) Quinalphos is an organothiophosphate chemical chiefly used as a pesticide. It is a reddish-brown liquid. It is ranked 'moderately hazardous' in World Health Organization's (WHO) acute hazard ranking, use of quinalphos is either banned or restricted in most nations. Quinalphos, which is classified as a yellow label (highly toxic) pesticide in India, is widely used in the following crops: wheat, rice, coffee, sugarcane, and cotton.(Pesticideinfo).

2.3.4 Chlorpyrifos



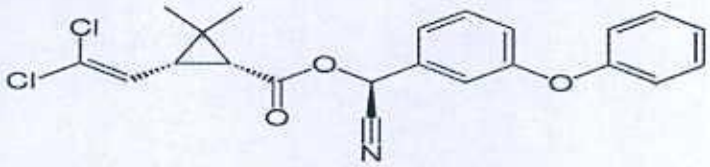
Structural formula of Chlorpyrifos

Chlorpyrifos (IUPAC name: O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) is a crystalline organophosphate insecticide, acaricide and miticide. It was introduced in 1965 by Dow Chemical Company and is known by many trade names, including Dursban. It acts on the nervous system of insects by inhibiting acetyl cholinesterase.Chlorpyrifos is moderately toxic to humans, and exposure has been linked to neurological effects, persistent developmental disorders and autoimmune disorders. Exposure during pregnancy retards the mental development of children, and most home use was banned in 2001 in the U.S. In agriculture, it is "one of the most widely used organophosphate insecticides" in the United States, according to the United States Environmental Protection Agency (EPA), and before being phased out for residential use was one of the most used residential insecticides.

In case of target organisms, Chlorpyrifos is a broad-spectrum insecticide which kills insects upon contact by affecting the normal function of the nervous system. Chlorpyrifos affects the nervous system by inhibiting the breakdown of acetylcholine (ACh), a neurotransmitter (Smegal, 2000). When insects are exposed, chlorpyrifos binds to the active site of the cholinesterase (ChE) enzyme, which prevents breakdown of ACh in the synaptic cleft. The resulting accumulation of ACh in the synaptic cleft causes overstimulation of the neuronal cells, which leads to neurotoxicity and eventually death. (Karanth and Pope, 2000; Toxicological Profile for Chlorpyrifos, 1997) Chlorpyrifos shares a common mechanism of toxicity with

other organophosphate insecticides such as malathion and parathion, thus, chlorpyrifos would not be effective against organophosphate-resistant insect populations. In case of non-target organisms, The mode of action of chlorpyrifos is similar for target and non-target organisms (Reigart and Roberts, 1999). Acetylcholine is found throughout the mammalian nervous system, including at cholinergic synapses in the central nervous system, the junction of post-ganglionic parasympathetic neurons in exocrine glands and smooth and cardiac muscles, at pre- and post-ganglionic neurons in the autonomic nervous system, at neuromuscular junctions of the somatic nervous system, and on the surface of red blood cells (Reigart and Roberts, 1999; Blodgett, 2006). Chlorpyrifos affects ChE levels differently in various systems throughout the body. Scientists have observed plasma and red blood cell ChE inhibition in experimental animals at doses lower than those required to cause ChE inhibition in the brain (Smegal, 2000). The physiological functions of the neuropathy target esterase (NTE) enzyme were studied in genetically altered mice, which lacked the NTE enzyme. The results demonstrated that NTE plays an essential role in placental development, blood vessel development and protein synthesis in the central nervous system.(Lotti and Moretto, 2005) Chlorpyrifos can inhibit NTE by binding to the active site of the enzyme. Inhibition of the NTE enzyme results in loss of myelin and degeneration of axon fibers of the peripheral and central nerves (Reigart and Roberts, 1999; Blodgett, 2006). Chlorpyrifos can cause permanent inhibition of the ChE or NTE enzymes, a process known as aging. Cleavage of an alkyl group from the chlorpyrifos residue produces a negative charge at the active site of the enzyme. This causes an unbreakable bond to form between the phosphorous atom on chlorpyrifos and the active site of the ChE or NTE enzyme (Blodgett, 2006; Lotti and Moretto, 2005). Chlorpyrifos also interacts with other enzymes, such as carboxylesterases and A-esterases. The functional role of these enzymes is not well understood, although they occur in many mammalian systems.

2.3.5 Cypermethrin



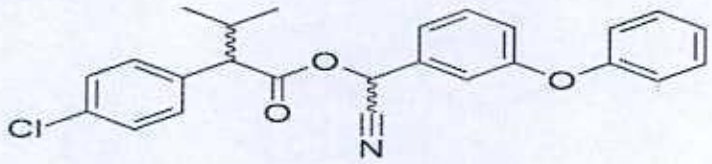
Structural formula of Cypermethrin

Cypermethrin (IUPAC name: [Cyano-(3-phenoxyphenyl) methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate)acts as a stomach and contact insecticide. It has wide uses in cotton, cereals, vegetables and fruit, for food storage, in public health and in animal husbandry. Its structure is based on pyrethrum, a natural insecticide which is contained in chrysanthemum flowers, but it has a higher biological activity and is more stable than its natural model. It was synthesised in 1974 and first marketed in 1977, by Shell (which has since sold their pesticide business to American Cyanamid.Cypermethrin is a synthetic pyrethroid used as an insecticide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes. It behaves as a fast-acting neurotoxin in insects. It is easily degraded on soil and plants but can be effective for weeks when applied to indoor inert surfaces. Exposure to sunlight, water and oxygen will accelerate its decomposition. Cypermethrin is highly toxic to fish, bees and aquatic insects, according to the National Pesticides Telecommunications Network (NPTN). It is found in many household ant and cockroach killers, including Raid and ant chalk.

Cypermethrin is classified by the World Health Organisation (WHO, 1985) as 'moderately hazardous' (Class II)(WHO, 1985). It interacts with the sodium channels in nerve cells through which sodium enters the cell in order to transmit a nerve signal. These channels can remain open for up to seconds, compared to the normal period of a few milliseconds, after a signal has been transmitted. Cypermethrin also interferes with other receptors in the nervous system. The effect is that of long-lasting trains of repetitive impulses in sense organs. Chronic symptoms after exposure to pyrethroids have now been reported (Müller-Mohnssen, 1995). Symptoms include brain and locomotory disorders, polyneuropathy and immuno-suppression, and resemble the multiple chemical sensitivity syndrome (MCS).Symptoms of poisoning include abnormal facial sensations, dizziness, headache, nausea, anorexia and fatigue, vomiting and increased stomach secretion. Cypermethrin is also a skin and eye

irritant. Normally, symptoms should disappear after some days but severely exposed patients additionally may suffer from muscular twitching, comata and convulsive attacks. In such cases, symptoms may persist for some weeks.

2.3.6 Fenvalerate



Structural formula of Fenvalerate

Fenvalerate (IUPAC name: (RS)-alpha-Cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate) is an insecticide. It is a mixture of four optical isomers which have different insecticidal activities. The 2-S alpha (or SS) configuration, known as esfenvalerate, is the most insecticidal active isomer. Fenvalerate consists of about 23% of this isomer.Based on fenvalerate similarities with deltamethrin, toxicity is probably due to effects on both peripheral and central nervous system caused by interference with sodium ion permeability in stimulated nerve membranes. The toxic signs in laboratory animals include restlessness, tremors, piloerection, choreo-athetosis and salivation (CS-syndrome). Fenvalerate is classififed as type II pyrethroid. Maximum residue limits have been recommended by the Joint FAO/WHO Meeting on Pesticide Residues. An acceptable daily intake (ADI) of 0-0.02 mg/kg b.w. was established for fenvalerate by JMPR in 1986.

Fenvalerate is an insecticide of moderate mammalian toxicity. In laboratory animals, central nervous system toxicity is observed following acute or short-term exposure. Fenvalerate has applications against a wide range of pests. Residue levels are minimized by low application rates. Fenvalerate is most toxic to bees and fish. It is found in some emulsifiable concentrates, ULV, wettable powders, slow release formulations, insecticidal fogs, and granules. It is most commonly used to control insects in food, feed, and cotton products, and for the control of flies and ticks in barns and stables. Fenvalerate does not affect plants, but is active for an extended period of time. Fenvalerate may irritate the skin and eyes on contact, and is also harmful if swallowed.

2.4 Instrument used for pesticide residues determination

For the determination of pesticide residues in foods different instrumental techniques are used. Gas Chromatography (GC), Gas Chromatography associated with Mass Spectrometry (GCMS), High Performance Liquid Chromatography (HPLC), and Liquid Chromatography associated with Mass Spectrometry (LC-MS) are the most commonly used techniques.

2.4.1 Liquid Chromatography-Mass Spectrometry

In recent years, LC-MS/MS has been used to determine pesticide residues in extracts of fruits and vegetables as it is an excellent technique which generally reduces the excessive cleanup steps, exhibits little chance of false-positive findings, and reduces the analysis time and cost (Hiemstra M. and Kok A de., 2007).

LC-MS is a powerful technique that has very high sensitivity, making it useful in many applications. Different mass analyzers are used in LC/MS, including single quadrupole, triple quadrupole, ion trap, time of flight mass spectrometry (TOF-MS). LC-MS/MS with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) source are used widely to analyze multiple pesticide residues from a wide variety of matrices (Prodhan *et al.* 2016; Prodhan *et al.* 2016a; Prodhan *et al.* 2015; Prodhan *et al.* 2015a; Garrido Frenich *et al.* 2004; Dasika *et al.* 2012; Camino-Sancheza *et al.* 2010; Caboni *et al.* 2008; Obana *et al.* 2003; Hiemstra M and Kok A. de. 2007; Jansson *et al.* 2004; Ferrer *et al.* 2005; Lucini and Molinari 2011; Satoshi *et al.* 2013; Hans *et al*, 2003; Pang 2006 and Fan *et al.* 2014). A quite number of pesticides can be analyzed by both GC-MS and LC-MS techniques. But, LC-MS was considered to cover a wider scope than GC-MS (Mol *et al.* 2008). LC-MS/MS with ESI and APCI source have improved the feasibility of the identification of pesticides of different chemical structures in food at concentrations comparable to those obtained by GC-MS (Pico *et al.* 2006).

2.4.2 Gas chromatography–mass spectrometry

In GC-MS, pesticides were identified by retention time and specific ions determined by selected ion monitoring (SIM) mode using the target and qualified ions. SIM mode provides adequate quantification at low levels as required for monitoring purposes but confidence in confirmation of identity is reduced if the selected ions are affected by matrix effect. Besides using the MS/MS it is possible to decrease the matrix effects,

may achieve a higher selectivity levels and lower detection limit (Hercegová *et al.*, 2007; Patel *et al.*, 2005). GC-MS/MS with triple quadrupole (Patel *et al.*, 2005, Garrido Frenich *et al.*, 2006) and ion trap mass spectrometers (Wang *et al.*, 2005) has been used for pesticide residue analysis on fatty food. Both acquisition mode, multiple reaction monitoring (MRM) (Patel *et al.*, 2005) and the selected reaction monitoring (SRM) (Garrido Frenich *et al.*, 2006) mode have been used to analyze multiple pesticide residues from food matrices. Using the MS/MS may overcome the problems arising from the chromatographic interference that occurred with GC-ECD (Garrido Frenich *et al.*, 2003). Several single and multiresidue methods using GC-MS have been developed for the analysis of pesticides from different classes (Akhlaghi *et al.*, 2013; Latif *et al.*, 2011; EL-Saeid & Selim 2013; Chauhan *et al.*, 2012; Hadian *et al.*, 2008; Chandra *et al.* 2012; Paramasivam and Chandrasekaran, 2012; Vidal *et al.*, 2004; Kabir *et al.*, 2007).

2.4.3 Gas Chromatography

A gas chromatograph (GC) is an analytical instrument that measures the content of various components in a sample. The analysis performed by a gas chromatograph is called gas chromatography. There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. Flame ionization Detector (FID) is feasible for most of the organic compounds. Thermal conductivity Detector (TCD) is a universal detector. Electron capture Detector (ECD) detector is used for halides, nitrates, nitriles, peroxides, anhydrides, organometallics etc. Nitrogen-phosphorus Detector (NPD) detector is normally used for Nitrogen, phosphorus and the Flame photometric Detector (FPD) detector are used for sulphur, phosphorus, tin, boron, arsenic, germanium, selenium and chromium. Till today, GC technique with different detectors are used for the quantification of pesticide residues from different food matrices (Prodhan *et al.*, 2010; Prodhan *et al.*, 2009; Panhwar and Sheikh , 2013; Latif *et al.* 2011; Bemph *et al.*, 2011; Srivastava *et al.*, 2011; Chandra *et al.*, 2010; Kabir *et al.*, 2007; Hajslova*et al.*, 1998).

2.4.4 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) has been used for manufacturing (e.g. during the production process of pharmaceutical and biological products), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the

components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (e.g. detecting vitamin D levels in blood serum) purposes. Now a day, HPLC are mostly used for the purity analysis of pesticides. But still it is also used for single pesticide residue analysis of different food matrices (Panhwar & Sheikh, 2013; Paranthaman *et al.*, 2012).

2.5 Extraction and Clean-up

To extract pesticide residues from the matrices different extraction and clean-up procedures are used in the food testing laboratories. Few of them are briefly described in below:

2.5.1 Supercritical fluid extraction (SFE)

This technique uses supercritical fluid (SF) as an extraction tool for "drawing out" the organic compounds from solid matrices. Commonly used for this purpose is CO_2, as it has relatively low critical temperature (31^0 C) and low critical pressure (73 kPa) (Atkins & De Paula, 2002), it is not reactive and is accessible in a high degree of purity at low cost. Changes in temperature and pressure at which the supercritical CO_2 is held will increase or decrease the "strength" of solvent and thus the selectivity of extraction performed. At constant temperature which exceeds critical temperature, the supercritical CO_2 will be able to extract analytes of low polarity at low pressure, and high polarity analytes at high pressure. SFE with CO_2 is usually performed at pressures that are not high enough to achieve efficient extraction of polar compounds. In such conditions, the supercritical CO_2 is a good extraction medium for non-polar compounds and moderately polar ones, such as PAHs, PCBs, organochlorine (OCPs) and organophosphorus (OPPs) pesticides, etc. The efficiency of supercritical CO_2 can be improved by adding small amounts of modifiers, which identity is often more important than their concentration, since the major role of a modifier is to interact with the sample matrix to promote desorption into the fluid. Some of the common solvents such as acetone (Valverde-García *et al.*, 1996; Kaihara *et al.*, 2002; Ono *et al.*, 2006) and methanol (Valverde-García *et al.*, 1996; Rissato *et al.*, 2005, 2005a) are now mostly used as modifiers. Besides CO_2, supercritical N_2O has been much in use as well, and it could be used both with and without modifiers.

2.5.2 Solid phase extraction (SPE)

SPE is one of the most commonly used sorbent techniques in analyzing pesticide residues. This method is based on the omission of extracts containing target analytes through a column filled with the appropriate sorbent (which was previously conditioned by an appropriate solvent or solvent mixture), or passing of an appropriate solvent through the SPE column to which a suitable amount of sample was previously added. It is easy to operate, costs less, it has been automated and uses small amounts of solvent. SPE is the multifunctional techniques, since the purification and the concentration occur in the same step. Unfortunately, SPE has certain limitations, primarily related to lower yields (recovery), i.e. slightly lower sensitivity, in situations where there is "clogging" of the SPE column (blocking of the sorption centers by solid and oily components originating from the sample). The most commonly used SPE sorbents in pesticide residues determination are: reversephase octadecyl (C18), normal-phase aminopropyl (-NH_2) and primary-secondary amine (PSA), anion-exchanger three-methyl ammonium (SAX) and adsorbents such as graphitized carbon black (GCB). Normal-phase sorbents such as florisil ($MgSiO_3$), aluminum oxide (Al_2O_3) and silica (SiO_2) are usually used in combination with the previously mentioned sorbents. The SPE cartridge should be chosen depending on the physicochemical properties of pesticides that are searched for in a particular sample, and the nature of the sample matrix.

2.5.3 Accelerated solvent extraction (ASE)

Accelerated solvent extraction (ASE), also known as pressurized liquid extraction (PLE), is relatively new sample preparation technique, that uses small amounts of water and organic solvents, and is based on the extraction under elevated temperature (up to 200 0C) and pressure (up to 20 MPa) for short time periods, resulting in better extraction efficiency.

2.6 QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method

Despite mentioned disadvantages related to conventional solvent extraction (SE) methods, they are still the most popular methods for routine analysis. To overcome the SE drawbacks, new trends in pesticide residues analysis have appeared. A good example of this is the QuEChERS method (Anastassiades *et al.*, 2003). The authors questioned the conditions previously used for pesticide residues analysis, and through

extensive experiments and novel use of $MgSO_4$ for salting out extraction/partitioning and dispersive solid-phase extraction (d-SPE) for cleanup, they devised a highly streamlined sample preparation method with excellent results for a wide range of pesticides in many types of samples. The original procedure consists in the sample extraction by hand-shaking or vortex mixing with the 10 mL of acetonitrile (MeCN). Gram quantities of salts (4 g of $MgSO_4$ and 1 g of NaCl) are then added to the sample by mixing, to drive analytes partitioning between the aqueous residue and the solvent. After vortex mixing and centrifugation, clean-up and removal of residual water is performed using a d-SPE procedure (PSA adsorbent and anhydrous $MgSO_4$ are mixed with the sample extract), that requires less time than the traditional SPE and simultaneously removes residual water and many polar matrix components, such as organic acids, some polar pigments, and sugars. As a polar solvent, miscible with water, with sufficient dispersive (hydrophobic) properties to extract effectively both polar and non-polar pesticides, MeCN is chosen as the QuEChERS solvent. Use of this solvent in the QuEChERS method proved to be successful for extraction of several pesticides classes from different matrices (Anastassiades *et al.*, 2003; Asensio-Ramos *et al.*, 2010; Drozdzyński & Kowalska, 2009; Lehotay *et al.*, 2005, 2005a, 2005b; Rashid *et al.*, 2010; Shi *et al.*, 2010; Yang *et al.*, 2010, Prodhan *et al.*, 2016; Prodhan *et al.*, 2016a; Prodhan *et al.*, 2015; Prodhan *et al.*, 2015a;). Studies showed that some pesticides gave lower recoveries depending on pH of the matrix (Anastassiades *et al.*, 2007; Lehotay *et al.*, 2005, 2005a). Anastassiades *et al.* (2007) realized that buffering at pH=5 during extraction gave the optimum balance to achieve acceptably recoveries (>70%) for pH-dependent pesticides, independent of the matrix. On the other hand, Lehotay (2007) modified the method to use even stronger acetate buffering conditions. Both versions of methods went through extensive laboratory trials and successfully met statistical criteria for acceptability by independent scientific standards organizations. So the acetate-buffering version becomes AOAC Official Method 2007.01 (Lehotay, 2007) and the citrate-buffering version being named as Standard EN 15662 Method (www.cen.eu). There is an abundance of the QuEChERS applications for pesticides determination in different plant samples. Thus, for example, QuEChERS provides satisfactory results for determination of 229 pesticides in lettuce and orange (Lehotay *et al.*, 2005), 109 in rice (Thanh *et al.*, 2007), 160 in tomato, pear and orange (Kmellár *et al.*, 2008), 140 in cucumber and orange (Fernández Moreno *et al.*, 2008), 118 in vegetables juice (Nguyen *et al.*,2009),

138 in apples, bananas, pears, apple juice, peas, creamed corn, squash and carrots (Wang & Leung, 2009), 150 in tomato, strawberry, potato, orange, and lettuce (Koesukwiwat *et al.,* 2010), 300 in tomato, apple, lettuce, cucumber, carrot, mushroom, grapes, lemon, pepper, pear, potato and cabbage (Kmellár *et al.,* 2010), 69 in zucchini, melon, cucumber, tomato, garlic, lettuce and pepper (Camino-Sánchez et al., 2010), 46 in onion, spinach, potato, carrot, cucumber, cabbage and tomato, 150 in grapes (Afify *et al.*, 2010), 148 in onion, spinach, potato, carrot, peas and tomato (Wang *et al.*, 2010), 73 OPPs and carbamates in rice, tree nuts and citric fruits (Chung & Chan, 2010) and 14 OCPs in apricot, plum, cherry, nectarine, pear and apple (Cieślik *et al.*, 2011), 13 in eggplant (Prodhan *et al.*, 2015), 10 in melon (Prodhan *et al.*, 2015a), 7 in cabbage (Prodhan *et al.*, 2016) and 7 in cauliflower (Prodhan *et al.*, 2016a). Besides, QuEChERS has been successfully used for determination of metaflumizone (Dong *et al.,* 2009), azadyrachtin, spinosad, rotenone (Drozdzyński & Kowalska, 2009), oxadiargyl (Shi *et al.,* 2010) and 38 pesticides (Yang *et al.,* 2010) in soil samples. As a modified version, it was applied for OCPs (Rashid *et al.*, 2010) and OPPs determination in soil samples (Asensio-Ramos *et al.,* 2010). The QuEChERS advantages are the high recovery, accurate results, low solvent and glassware usage, less labor and bench space, lower reagent costs, and ruggedness. The main QuEChERS disadvantage is that the final extract must be concentrated to furnish the necessary sensitivity i.e. to achieve the desired limits of quantification (LOQ).

2.7 Pesticides residues

Pesticide residue refers to the pesticides that may remain on or in food after they are applied to food crops (IUPAC, 1997). The maximum allowable levels of these residues in foods are often stipulated by regulatory bodies in many countries. Exposure of the general population to these residues most commonly occurs through consumption of treated food sources, or being in close contact to areas treated with pesticides such as farms or lawns.

Many of these chemical residues, especially derivatives of chlorinated pesticides, exhibit bioaccumulation which could build up to harmful levels in the body as well as in the environment (Walter, 2009).Persistent chemicals can be magnified through the food chain and have been detected in products ranging from meat, poultry, and fish, to vegetable oils, nuts, and various fruits and vegetables (Chung and Chen, 2011).

2.7.1 Determination of Pesticide Residues in Food

Amelina and Andoralovb (2016) has been proposed a method for the simultaneous identification and determination of 111 pesticides from various classes in food by high performance liquid chromatography–high resolution time of flight mass spectrometry combined with simple and fast sample preparation technique. Possibility of the identification and determination of pesticides in drinking, natural, and ground waters without sample preparation has been demonstrated. A scheme of the identification and determination of the detected analytes using the standard addition method has been suggested. The limit of detection is 0.05 (0.1) μg/(L)kg. The relative standard deviation of the results of analysis does not exceed 0.1. The time of identification is 30–40 min

Jankowska *et al.* (2016) carried out a study on the dissipation of six fungicides in greenhouse-grown tomatoes with processing and health risk where evaluate the dissipation rate kinetics and estimate the behavior of selected pesticides after washing, peeling, simmering, and canning of tomato expressed as processing factor (PF). Two varieties (Marissa and Harzfeuer) were treated by six fungicides: azoxystrobin, boscalid, chlorothalonil, cyprodinil, fludioxonil, and pyraclostrobin at single and double dose and risk assessment defined as hazard quotient was performed. The QuEChERS method was used for sample preparation followed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The dissipation of fungicides approximately fitted to a first-order kinetic model, with half-life values ranging from 2.49 and 2.67 days (cyprodinil) to 5.00 and 5.32 days (chlorothalonil) for Marissa and Harzfeuer variety, respectively. Results from processing studies showed that treatments have significant effects on the removal of the studied fungicides for both varieties. The PFs were generally less than 1 (between 0.01 and 0.90) and did not depend on variety. The dietary exposure assessed based on initial deposits of application at single and double dose on tomatoes and concentration after each process with PF correction showed no concern to consumer health. Our results would be a useful tool for monitoring of fungicides in tomatoes and provide more understanding of residue behavior and risk posed by these fungicides.

Zhang *et al.* (2016) developed a rapid, efficient, and environmentally friendly method using quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method

combined with ionic liquid-based dispersive liquid-liquid micro extraction (QuEChERS-IL-DLLME) prior to high-performance liquid chromatography coupled with photodiode array detection (HPLC-PDA) has been developed for the determination of six triazole fungicides (triazolone, triadimenol, epoxiconazole, flusilazole, tebuconazole, and diniconazole) in various fruits (pear, apple, and grapefruit). And the proposed method was successfully applied for the determination of trace amounts of triazole fungicides in various fruits including pear, apple, and grapefruit.

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

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

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

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

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

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

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

Biziuk and Stocka (2015) conducted a research on "Multi Residue Methods for Determination of Currently used Pesticides in Fruits and Vegetables Using

QuEChERS Technique'. They discussed the extraction and determination of pesticide residues in fruit and vegetable samples, as are the techniques most commonly used in these processes. They also outlined the difficulties occurring at each stage in the analytical procedure.

Ishibashi *et al.* (2015) examined the screening method for multi-residue pesticide analysis, which is simple, quick, and accurate and has a reliable performance, is becoming increasingly important for food safety and international trade. This paper proposes a high-throughput screening methodology that enables the detection of multi-residue pesticides using supercritical fluid chromatography coupled to a high-performance benchtop quadrupole Orbitrap mass spectrometry (SFC/Q Exactive) and an automated library-based detection. A total of 444 chemicals covering a wide polarity range (log Pow from -4.2 to 7.7) and a wide molecular weight range (from 99.0 to 872.5) were analyzed simultaneously through a combination of high mass resolution (a value of $m/\Delta m$ = 70000), high mass accuracy (<5 ppm) with positive/negative polarity switching, and highly efficient separation by SFC. A total of 373 pesticides were detected in QuEChERS spinach extracts without dispersive solid phase extraction at the 10 $\mu g\ kg^{-1}$ level (provisional maximum residue limits in Japan). In conclusion, the developed analytical system is a potentially useful tool for practical multi-residue pesticide screening with high throughput (time for data acquisition, 72 samples per day; and time for data processing of 72 samples, approximately 45 min).

Portolés *et al.* (2015) was developed a method for the detection of pesticides in fruit and vegetables. The method was based on gas chromatography coupled to a hybrid quadrupole time-of-flight mass spectrometer with an atmospheric pressure chemical ionization source (GC-(APCI) QTOF MS). A non-target acquisition was performed through two alternating scan events: one at low collision energy and another at a higher collision energy ramp (MS (E)). In this way, both protonated molecule and/or molecular ion together with fragment ions were obtained in a single run. Validation was performed according to SANCO/12571/2013 by analysing 20 samples (10 different commodities in duplicate), fortified with a test set of 132 pesticides at 0.01, 0.05 and 0.20mg kg^{-1}. For screening, the detection was based on one diagnostic ion (in most cases the protonated molecule). Overall, at the 0.01mg kg^{-1} level, 89% of the 2620 fortifications made were detected. The screening detection limit for individual pesticides was 0.01mg kg^{-1} for 77% of the pesticides investigated. The possibilities

for identification according to the SANCO criteria, requiring two ions with a mass accuracy ≤ ±5 ppm and an ion-ratio deviation ≤ ±30%, were investigated. At the 0.01mg kg^{-1} level, identification was possible for 70% of the pesticides detected during screening. This increased to 87% and 93% at the 0.05 and 0.20mg kg^{-1} level, respectively. Insufficient sensitivity for the second ion was the main reason for the inability to identify detected pesticides, followed by deviations in mass accuracy and ion ratios.

Mukherjee *et al.* (2015) carried out a research on "Analytical method validation and comparison of two extraction techniques for screening of azoxystrobin from widely used crops using LC–MS/MS" where a simple analytical method was developed and validated in chilli, tomato, grape and mango fruits using liquid chromatography tandem mass spectrometry. The method comprised of extraction with ethyl acetate and cyclohexane mixture followed by d-SPE cleanup employing modified quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction method and quantified in LC–MS/MS using gradient elution. The method was validated in concentration ranging from 0.01 to 0.11 μg g^{-1}. The recovery of azoxystrobin in different crops was ranging from 84.36 to 95.64 % at three different concentration levels of analytes with relative standard deviation of 4–14 %. The global uncertainty was calculated at limit of quantification level i.e. 0.011 μg g^{-1}. The PHI values of azoxystrobin in chilli, tomato, grape and mango fruits were determined as 4.76, 3.90, 4.06 and 10.74 days respectively.

Hossain *et al.* (2015) were detected Organophosphorus pesticides, Diazinon and Chlorpyrifos in collected samples of different vegetables from Bogra district. Detectable amount of Diazinon was found in one bringal sample (BS-4 at 0.32 ppm) among ten samples. It was also detected in one Cucumber sample CS-5 (0.18 ppm) among ten samples and one Tomato sample (TS-3 at 0.57 ppm) among five samples. Chlorpyrifos was being found in one Bringal sample (BS-7 at 0.4 ppm). It was also detected in three Cucumber samples among ten samples. Prodhan *et al.*(2015) developed and validated a methodon the ''Determination of Multiple Pesticide Residue in Eggplant with Liquid Chromatography-Mass Spectrometry'' by adopting (*QuEChERS*) extraction and liquid chromatography triple quadrupole-mass spectrometry for the determination of ten insecticides and three fungicides in 72 fresh eggplant fruit samples collected from different market places in Thessaloniki, Greece. The method was validated by evaluating the accuracy, precision, linearity, LOD, and LOQ and the average recoveries of the selected pesticides ranged from 71.8 to 112 %

in four fortification levels of 0.01, 0.05, 0.1, and 0.5 mg/kg. The limit of detection (LOD) ranged from 0.001 to 0.003 mg/kg and the limit of quantification (LOQ) was 0.01 mg/kg, which was lower than the Maximum residue levels set by European Union (EU-MRLs). Among the 72 analyzed samples, 34 (47 % of the total no. of samples) had pesticide residues, of which, 5 had multiple pesticide residues and 29 had single pesticide residue. Only one sample contained residue above the EU-MRLs.

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

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

A method was developed by Corteas *et al.* (2013) for the determination 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.

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

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

and relative standard deviation (less than 20%) were within acceptable level except for dichlorvos, propamocarb, chlorothalonil, dichlofluanid, cyhalothrin and fenvalerate.

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

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

Mantzos *et al.* (2013) conducted a research work on "QuEChERS and solid phase extraction methods for the determination of energy crop pesticides in soil, plant and runoff water matrices" where QuEChERS and solid phase extraction (SPE) methods were applied for determining four herbicides (metazachlor, oxyfluorfen, quizalofop-p-ethyl, quinmerac) and one insecticide (α (±)-cypermethrin) in runoff water, soil, sunflower and oilseed rape plant matrices. Determination was performed using gas chromatography mass spectrometry (GC-MS), whereas high-pressure liquid chromatography mass spectrometry (HPLC-MS) was used for quinmerac. In all substrates linearity was evaluated using matrix-matched calibration samples at five

concentration levels (50–1000 ng L^{-1} for water, 5–500 μg kg^{-1} for soil and 2.5–500 μg kg^{-1} for sunflower or oilseed rape plant). Correlation coefficient was higher than 0.992 for all pesticides in all substrates. Acceptable mean recovery values were obtained for all pesticides in water (65.4–108.8%), soil (70.0–110.0%) and plant (66.1–118.6%), with intra- and inter-day RSD% below 20%. LODs were in the range of 0.250–26.6 ng L^{-1} for water, 0.10–1.8 μg kg^{-1} for soil and 0.15–2.0 μg kg^{-1} for plants. The methods can be efficiently applied for field dissipation studies of the pesticides in energy crop cultivations.

Stephen and Lam (2012) reported a novel approach for the detection, confirmation, and quantification of 15 selected pyrethroid pesticides, including pyrethins, and two metabolites of dithiocarbamates in foods by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS–MS). The proposed method makes use of a modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure that combines isolation of the pesticides and sample cleanup in a single step. Analysis of pyrethroids and dithiocarbamate metabolites was performed by UPLC–MS–MS operated with electrospray and atmospheric pressure chemical ionization, respectively. Two specific precursor–product ion transitions were acquired per target compound in multiple reactions monitoring (MRM) mode. Such acquisition achieved the minimum number of identification points according to European Commission (EC) document no. SANCO/10684/2009, thus fulfilling the EC point system requirement for identification of contaminants in samples. The method was validated with a variety of food samples. Calibration curves were linear and covered from 1 to 800 μg kg^{-1} in the sample for all target compounds. Average recoveries, measured at mass fractions of 10 and 100 μg kg^{-1} for pyrethroids and 5 and 50 μg kg^{-1} for dithiocarbamate metabolites, were in the range of 70–120% for all target compounds with relative standard deviations below 20%. Method limits of quantification (MLOQ) were 10 μg kg^{-1} and 5 μg kg^{-1} for pyrethroids and dithiocarbamate metabolites, respectively. The method has been successfully applied to the analysis of 600 food samples in the course of the first Hong Kong total diet study with pyrethroids and metabolites of dithiocarbamates being the pesticides determined.

Kanda *et al.* (2012) conducted a research uning Gas Chromatography on the extracts from soil, water and vegetable samples. In soil samples, the concentrations of

pesticide residues are lower than 20 µg/kg of dry material. For water samples, contamination levels vary from 0.02 to 1.1 µg/L of dry material with the highest levels for metalaxyl M (1.1 µg/L) and for dimethoate (1 µg/L). In vegetables, the concentrations measured are between 0.01 and 0.1 mg/kg of dry material. All these concentrations are affected by a positive factor of the maximum limits of residues.

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

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

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

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°C (2 min) to 200°C (15 min) at the rate of 4°C/min giving a total analysis time of 47 min. The detector and injector were respectively at temperatures of 300 and 225°C. The method was validated with respect to precision in terms of reproducibility of retention times and peak heights, linearity and minimum detectable quantity of the pesticides. Under the operated GC conditions, diuron eluted first while heptachlor epoxide was the last to elute. The chromatographic detector was more sensitive to endosulfan and endosulfan with Minimum Detectable Quantity (MDQ) of 0.002 ng. The detector was however, less sensitive to captan with MDQ of 0.08 ng. Margins of errors associated with the precision of the method in terms of reproducibility of 11 retention times yielded standard deviation in the range of 0.026-0.063.

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

Charan and Sharma (2010) monitored pesticide residues in vegetables to find out severity of such synthetic agrochemicals on human being. A total of 182 samples of six vegetables were collected for pesticide residue analysis from different agricultural fields of central Aravalli region, when they were ready for transportation to market. The analysis of samples for different pesticide residues were carried out on GCECD

and GC-NPD systems equipped with capillary columns by using a multiple residue method. About 40.11% of total analyzed samples were contaminated with different pesticide residues, among which 35.62% of total contaminated samples were exceeded the maximum residual limit (MRL) values.

Islam *et al.* (2009) reported a method based on High Performance Liquid Chromatography (HPLC) for the determination of pesticide residues in Cauliflower. Cauliflower sprayed with 4 different pesticides (diazinon, malathion, chlorpyrifos and cypermethrin) at recommended dose and double of recommended dose were analyzed for their residual contents. Limit of 1 was obtained. Calibration curves that constructeddetection of 0.02 mg kg for the analytes spiked into samples followed linear relationships with good correlation coefficients (R^2>0.990). In the analysis, from vegetables treated with diazinon and chlorpyrifos at recommended and double of recommended doses, residual amounts above respective MRL values were found.

Yamagami *et al.* (2009) conducted a research on "Multi-Residue Method for Determination of 85 Pesticides in Vegetables, Fruits and Green Tea by Stir Bar Sorptive Extraction and Thermal Desorption GC-MS" where they used a multi-residue method to determine five groups of 85 pesticides - chlorinated, carbamate, phosphorous, pyrethroid and others - in vegetables, fruits and green tea has been developed using stir bar sorptive extraction (SBSE) coupled to thermal desorption and retention time locked (RTL) GC-MS. Pre-extraction with methanol and dilution with water prior to SBSE (60 min) were performed. Dilution of methanol extract for SBSE was examined to obtain high sensitivity and to compensate the effect of adsorption to the glass wall of extraction vessel and to sample matrix for the compounds with high log Ko/w values (e.g. pyrethroid). The methanol extracts were diluted twofold and fivefold, and were simultaneously SBSE-enriched. The two stir bars were placed in a single glass thermal desorption liner and were simultaneously desorbed. The versatility of the method was exhibited by its good linearity (4-100 µg/kg, r^2>0.9900) for 66 pesticides and limit of detection (LOD: < 5 µg/kg) for most of the analytes. The method enables to determine pesticides at low µg/kg in tomato, cucumber, green soybeans, and spinach, grape and green tea.

Prodhan *et al.* (2009) undertook a research on the "Quantification of Organophosphorus and Organochlorine insecticide residues from fish sample using

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

Garrido Frenich *et al.* (2008) has been developed a rapid, simple, and sensitive multiresidue method for analysis of 53 pesticides in fruit and vegetables by ultra-performance liquid chromatography (UPLC) coupled to triple-quadruple tandem mass spectrometry (MS-MS). Prior to analysis, analytes were extracted by use of buffered QuEChERS (quick, easy, cheap, effective, rugged, and safe) methodology without further cleanup for non-fatty matrices. Chromatographic conditions were optimized in order to achieve a fast separation in multiple reactions monitoring (MRM) mode. Indeed, more than 50 pesticides can be separated in less than 10 min. Four common representative matrices (cucumber, orange, strawberry, and olive) were selected to investigate the effect of different matrices on recovery and precision. Mean recoveries ranged from 70 to 109% with relative standard deviations lower than 20% for all the pesticides assayed in the four selected matrices. The method has been applied to the analysis of 200 vegetable samples, and imidacloprid was the pesticide most frequently found, with concentrations ranging from 0.01 to 1.00 mg kg−1. This methodology combines the advantages of both QuEChERS and UPLC-MS-MS producing a very

rapid, sensitive, and reliable procedure which can be applied in routine analytical laboratories.

Nguyen *et al.* (2008) has been developed a rapid multi-residue method for the simultaneous determination of 156 pesticides in commercial watermelon. The method involves a liquid-liquid extraction using acetonitrile coupled with dispersive solid phase extraction cleanup. The extracted elution of pesticides was determined by gas chromatography with electron impact mass spectrometric detection in the selected ion monitoring mode (GCMS-SIM). Standards were prepared spiking blank watermelon samples to counteract the observed matrix effect. The method was validated by fortified at the level 0.020-0.120 mg/kg in watermelon. The average recoveries of all analytes were between 70% and 121%, and standard deviations were below 16%. The limit of quantitation (LOQ) for most compounds was below 0.005 mg/kg, which were lower than the maximum residue levels established by Korean legislations. The proposed method has been applied to the analysis of the 156 pesticide residues in commercial watermelon samples.

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

Ochiai *et al.* (2008) has been developed a multi-residue method to determine 85 pesticides - chlorinated, carbamate, phosphorous, pyrethroid and othersin 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, r^2 0.9900) for 66 pesticides and limit of detection (LOD: < 5 μg/kg) for most of the analytes.

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

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

Fernández-Cruz *et al.* (2006) carried out a research on the "Residue levels of captan and trichlorfon in field-treated kaki fruits, individual versus composite samples, and after household processing" where the dissipation of residue levels of captan and trichlorfon in field-treated kaki crops was studied according to good laboratory practices to propose maximum residue limits (MRLs). Residue levels of captan and trichlorfon were analyzed by GC/MS and LC-MS/MS, respectively. Residue levels of captan and trichlorfon permitted one to propose MRLs in kaki of 3 and 5 mg kg^{-1}, respectively. The behavior of these residues was also studied after peeling and cooking, and in individual fruits versus composite samples. Residue levels of these compounds for individual fruits suggested that a variability factor up to three could be

set for the acute risk assessment. Levels of captan decreased by more than 90% after peeling and completely after cooking. Trichlorfon penetrates into the flesh in a proportion of 70% of the residue at the pre-harvest interval. Cooking resulted in a decrease of 27% of residue levels of trichlorfon.

Boulaid et al. (2005) carried out a research on the "Effect of household processing and unit-to-unit variability of pyrifenox, pyridaben, and tralomethrin residues in tomatoes" where the residue levels of pyrifenox, pyridaben, and tralomethrin were determined in unprocessed and processed tomatoes, grown in an experimental greenhouse, to evaluate the effect of three different household processes (washing, peeling, and cooking) and the "unit to unit" variability of these pesticides in tomatoes. The study was carried out on 11 greenhouse tomato samples collected during a 5 week period in which two successive treatments with the studied pesticides were applied. Residue levels in unprocessed and processed tomato samples were determined by means of ethyl acetate extraction and gas chromatography-electron capture detection determination. The washing processing factor results were 0.9±0.3 for pyridaben, 1.1±0.3 for pyrifenox, and 1.2±0.5 for tralomethrin, whereas the peeling processing factors were 0.3 ±0.2 for pyridaben and 0.0±0.0 for both pyrifenox and tralomethrin. The average loss of water in the tomato pure samples during the cooking process was approximately 50%; the cooking processing factors were 2.1±0.8 for pyridaben, 3.0±1.1 for pyrifenox, and 1.9±0.8 for tralomethrin. The unit-to-unit variability factors were determined on three different greenhouse samples analyzing 10 different units of unprocessed tomatoes from each sample. In all cases, the unit-to-unit variability factor results were within the range of 1.3-2.2.

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

all being within European Union regulations for food monitoring program. Finally, the methodology was applied to the analysis of two samples from an inter-laboratory exercise.

Ortelli *et al.* (2004) determine 74 pesticides commonly used in crop protection including mainly carbamateconazole, benzimidazole and pyrimidine fungicides and insecticides. Pesticides residues are extracted from the samples with ethyl acetate. Analysis is performed by liquid chromatography–electrospray ionization–tandem mass spectrometry. The method has been validated for various fruits and vegetables matrices. Good sensitivity and selectivity of the method are obtained with limits of quantification of 0.01 mg/kg in almost all cases. The method was applied very satisfactorily to routine analysis as a complement to traditional GC method. More than 2500 fruits and vegetables samples have been controlled, as a part of the pesticide monitoring program of the "Service de Protection de la Consummation" in Geneva.

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

Kumar and Hosmani (2001) conducted a research work on "Magnitude of the residue of carbofuran and 3-hydroxy carbofuran in/on rice in Brazil following furadan 50G

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

Gamon *et al.* (2001) carried out a research on "Multi-residue 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.

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.

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

Aguera *et al.* (2000) described a method (Splitless large- volume GC-MS injection for the analysis of organophosphorus and organochlorine pesticides in vegetables using a miniaturised ethyl acetate extraction) for the measurement of 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) undertook a research to monitor pesticide residues in Egyptian fruits and vegetables during 1995. Organophosphorus, dithiocarbamates and some synthetic pyrethroids pesticides, which were commonly used in Egypt for pest control, were monitored, as well as persistent organochiorines, which had been prohibited from use several years ago. Fruit and vegetable samples (397) were collected from 8 local markets and examined for 52 active ingredients. Of all analysed samples, 42.8% contained detectable residues, of which 1.76% exceeded their maximum residue limits (MRL's). The rates of contamination with the different pesticides were 0-86%. The most commonly detected residues were dithiocarbarnates as well as dicofol (15.1% of 397 samples), dimethoate (6.8%), tetradifon (4.5%), Malathion (3.3%), profenofos (2.8%), omethoate (2.3%), chlorothalonil (2.0%) and chiorpyrifos-methyl (1.5%). Among all samples, 22 strawberry samples (5.32%) contained 10 pesticide residues, 65 grape samples (15.73%) contained 11 pesticides residues and 62 tomato samples (15.01%) contained 13 pesticide residues. Cauliflower, onion and guava samples free from pesticides residues. Samples of carrot, and eggplant contained trace amounts of p, p'-DDT and p, p'-DDE residues. But in general, residues of DDT and HCH have disappeared almost completely from vegetables and fruits. Use of these pesticides in Egypt was completely prohibited by law in 1987.

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

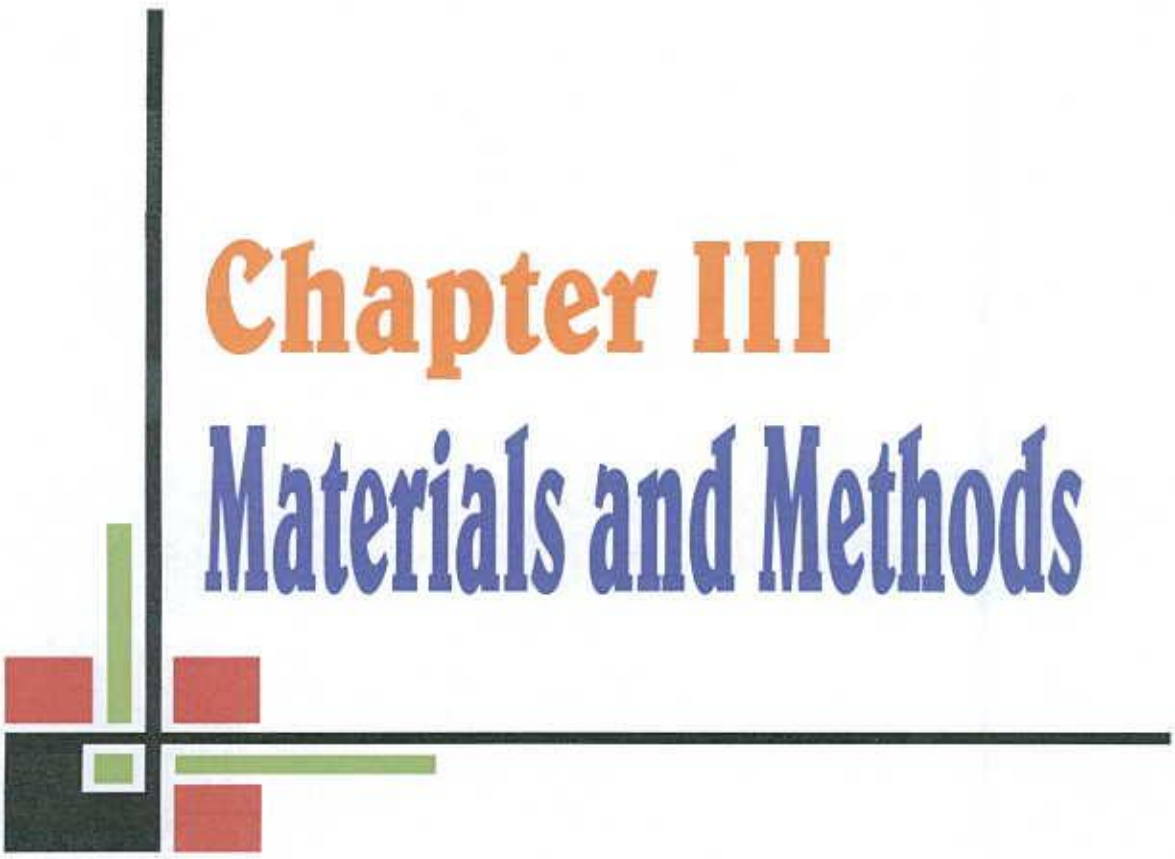
Rimkus *et al.* (1996) described that pesticide residue detection methods from food matrices mainly involve two preparation steps prior to the identification and quantification of pesticides: Extraction of target analytes from the bulk of the matrices and partitioning of the residues in an immiscible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

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

Frank *et al.* (1990) undertook a research on "Residues of insecticides and fungicides on Ontario-grown vegetables". The selected insecticides and fungicides were the classes of organophosphorus, synthetic pyrethroid, N-methyl carbamate insecticides and dithiocarbamate, dicarboximide and organochlorine fungicides. The estimation was done in 433 composite vegetable samples representing 16 commodities collected between 1986 and 1988 from farm deliveries to the market place Ontario, Canada. Commodities tested included eggplant, asparagus, carrots celery, cole crops, cucumbers, lettuce, onions, peppers, potatoes, radishes and tomatoes. In 64% of

samples, no pesticide residues were identified to the limits of detection which ranged from 0.005 to 0.05 mg/kg. These involved Diazinon and Parathion on celery and Chlarothalonil on peppers. Where as some commodities had no detectable residues.

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



Chapter III

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

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

3.1 Study area

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

Figure 1. Map showing the places of sample collection.

3.2 Sample collection

A total of 100 samples (50 cabbages and 50 bitter gourd) were collected for this study. Ten samples of cabbage and ten samples of bitter gourd were collected from each market.

Table 1. Sources and places of collection of cabbage samples

Area of collection	Sample ID	Source*
Rampura	$DMCb_{01}$	Manikganj
	$DMCb_{02}$	Saturia
	$DMCb_{03}$	Manikganj
	$DMCb_{04}$	Tangail
	$DMCb_{05}$	Narsingdi
	$DMCb_{06}$	Comilla
	$DMCb_{07}$	Manikganj
	$DMCb_{08}$	Meherpur
	$DMCb_{09}$	Savar
	$DMCb_{10}$	Jessore
Kawran Bazar	$DMCb_{11}$	Sripur
	$DMCb_{12}$	Singair
	$DMCb_{13}$	Manikganj
	$DMCb_{14}$	Tangail
	$DMCb_{15}$	Saturia
	$DMCb_{16}$	Jessore
	$DMCb_{17}$	Comilla
	$DMCb_{18}$	Jhenaidah
	$DMCb_{19}$	Savar
	$DMCb_{20}$	Comilla
Taltola Bazar	$DMCb_{21}$	Jessore
	$DMCb_{22}$	Singair
	$DMCb_{23}$	Meherpur
	$DMCb_{24}$	Manikganj
	$DMCb_{25}$	Singair
	$DMCb_{26}$	Chuadanga
	$DMCb_{27}$	Jhinaidah
	$DMCb_{28}$	Tangail
	$DMCb_{29}$	Meherpur
	$DMCb_{30}$	Jessore

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

DMCb= Dhaka Market Cabbage. * According to the retailer's opinion

Table 2. Sources and places of collection of bitter gourd samples

Area of collection	**Sample ID**	**Source***
Rampura	$DMBG_{01}$	Tangail
	$DMBG_{02}$	Narshingdi
	$DMBG_{03}$	Manikganj
	$DMBG_{04}$	Jessore
	$DMBG_{05}$	Manikganj
	$DMBG_{06}$	Comilla
	$DMBG_{07}$	Savar
	$DMBG_{08}$	Lalmonirhat
	$DMBG_{09}$	Bogra
	$DMBG_{10}$	Jessore
Kawran Bazar	$DMBG_{11}$	Meherpur
	$DMBG_{12}$	Meherpur
	$DMBG_{13}$	Manikganj
	$DMBG_{14}$	Savar
	$DMBG_{15}$	Rangpur
	$DMBG_{16}$	Bikrampur

	$DMBG_{17}$	Narayangonj
	$DMBG_{18}$	Basila
	$DMBG_{19}$	Saturia
	$DMBG_{20}$	Comilla
Taltola Bazar	$DMBG_{21}$	Jessore
	$DMBG_{22}$	Meherpur
	$DMBG_{23}$	Singair
	$DMBG_{24}$	Chuadanga
	$DMBG_{25}$	Singair
	$DMBG_{26}$	Jessore
	$DMBG_{27}$	Rajshahi
	$DMBG_{28}$	Hemaetrpur
	$DMBG_{29}$	Comilla
	$DMBG_{30}$	Savar
Jatrabari area	$DMBG_{31}$	Comilla
	$DMBG_{32}$	Jessore
	$DMBG_{33}$	Tangail
	$DMBG_{34}$	Comilla
	$DMBG_{35}$	Kurigram
	$DMBG_{36}$	Chuadanga
	$DMBG_{37}$	Comilla
	$DMBG_{38}$	Meherpur
	$DMBG_{39}$	Jessore
	$DMBG_{40}$	Manikganj
Mohammadpur Krishi Market	$DMBG_{41}$	Manikganj
	$DMBG_{42}$	Savar
	$DMBG_{43}$	Narayangonj
	$DMBG_{44}$	Nawgoan
	$DMBG_{45}$	Jamalpur
	$DMBG_{46}$	Natore
	$DMBG_{47}$	Tangail
	$DMBG_{48}$	Comilla
	$DMBG_{49}$	Manikganj
	$DMBG_{50}$	Narsingdi

DMBG= Dhaka Market Bitter Gourd. * According to the retailer's opinion

The amount of each sample was 1 Kg for all the vegetables. The samples were collected in clean transparent airtight polyethylene bag and each bag was properly labeled with sample number and sources. Sample was collected in individual polyethylene bag to avoid cross contamination.

3.3 Sample preparation for analysis

The samples were taken to the Pesticide Analytical Laboratory, Division of Entomology, BangladeshAgricultural Research Institute (BARI) on the day of

collection. The whole unit of each sample cut into small pieces and mixed properly. Clean air tight polythene bags were used to store chopped sample in refrigerator at -20°C until extraction and cleanup process started.

3.4 Chemicals and reagents

The standard of Diazinon, Fenitrothion, Quinalphos, Chlorpyrifos, Cypermethrin,andFenvalerate were obtained from Sigma-Aldrich Laborchemikalien (St Louis, MO, USA) via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. Standards of all the pesticides contained >99.6% purity.

Methanol, acetone, gradient grade acetonitrile (MeCN), 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. Centrifuge machine, Model: Sigma 3k 30, Germany (Plate 1)

b. Electric balance, Model: AY- 220, Shimadzu Corporation, Japan (Plate 2).

c. Vortex mixer, Model: Maxi max ii, USA (Plate 3)

d. Orbital shaker, Model: Rexmed, Sweden (Plate 4)

e. GC-2010, Shimadzu corporation, Japan (Plate 5)

Plate 1. Centrifuge Machine

Plate 2. Electric Balance

Plate 3. Vortex Mixer

Plate 4. Orbital Shaker

Plate 5. Gas Chromatograph (GC)

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

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

3.6 Preparation of pesticide standard solution

Pesticide standard stock solutions of Diazinon, Fenitrothion, Quinalphos, Chlorpyrifos, Cypermethrin and Fenvaleratewere 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 10mL 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). In this study, the QuEChERS extraction technique was used for the extraction and clean-up of samples which was modified by Prodhan *et al.* (2015). The chopped samples were grounded thoroughly with the fruit blender. A representative 10-g portion of thoroughly homogenized sample was weighted in a 50 mL polypropylene centrifuge tube. Then 10 mL of acetonitrile (MeCN) was added into the centrifuge tube. The centrifuge tube was closed properly and shaken vigorously for 30 s by the use of a vortex mixer. Then 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added into the centrifuge tube, and it was shaken immediately by the vortex mixer for 1 minute to prevent the formation of magnesium sulfate aggregates. Afterwards, the extract was centrifuged for 5 min at 5000 rpm. An aliquot of 3 mL of the MeCN layer was transferred into a 15 mL micro centrifuge tube containing 600 mg anhydrous $MgSO_4$ and 120 mg Primary Secondary Amine (PSA). Then it was thoroughly mixed by vortex for 30 s and centrifuged for 5 minutes at 4000 rpm. (Laboratory Centrifuges, Sigma-3K30, Germany). After centrifugation, 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 Detection and quantification of pesticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) with Flame Thermionized Detector (FTD) for the detection of four organophosphorus insecticides (Diazinon, Fenitrothion, Chlorpyrifos and Quinalphos) and two synthetic pyrethroids (Cypermethrin and Fenvelerate). 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 and it was Nitrogen for GC-ECD. The identification of

suspected pesticide was performed by peak retention times in samples to those of peaks in the pure analytical standards (Figure 2-4). The instrument conditions are described in Table 4 and Table 5.

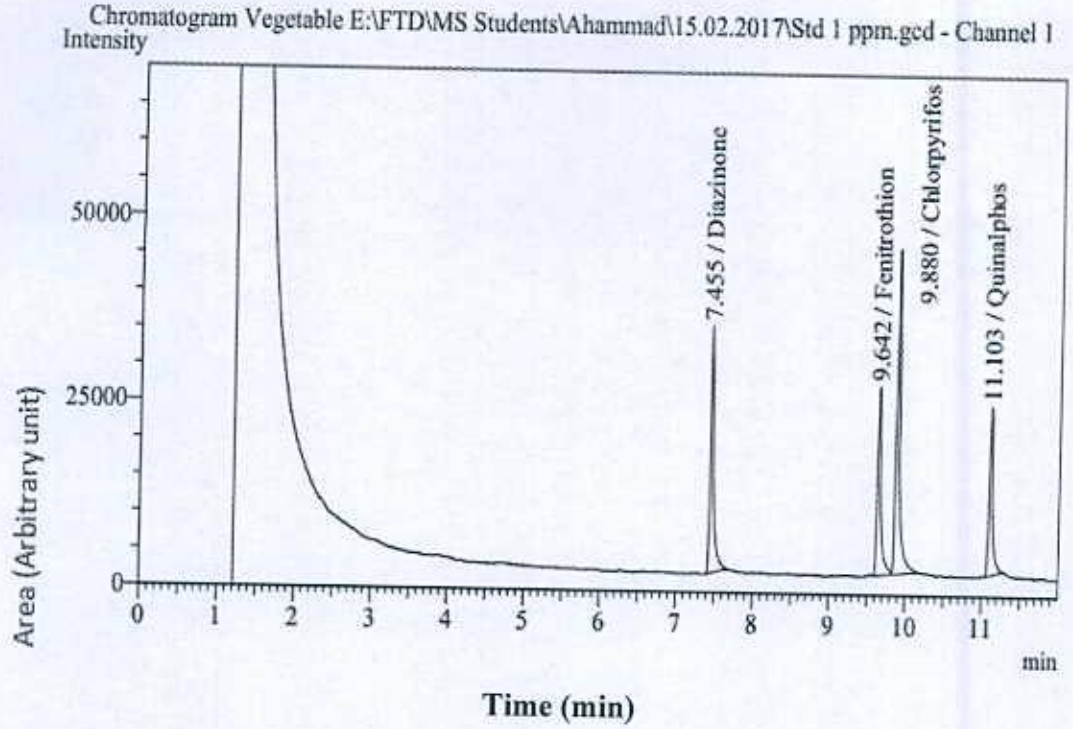


Figure 2. Typical Chromatograms of four organophosphorus insecticide (Diazinon, Fenitrothion, Chlorpyrifos and Quinalphos) standards run by GC-FTD.

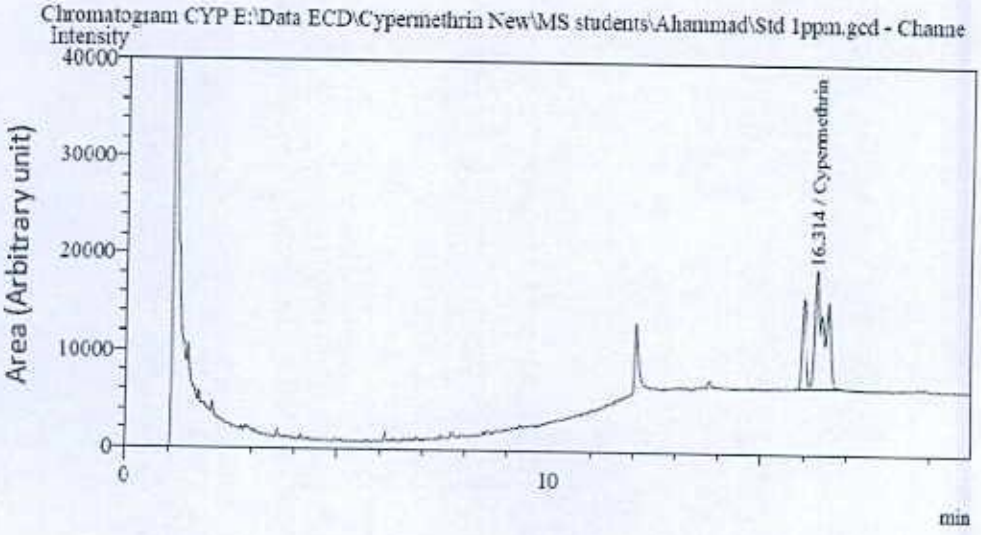


Figure 3. Typical Chromatograms of Cypermethrin standards run by GC-ECD.

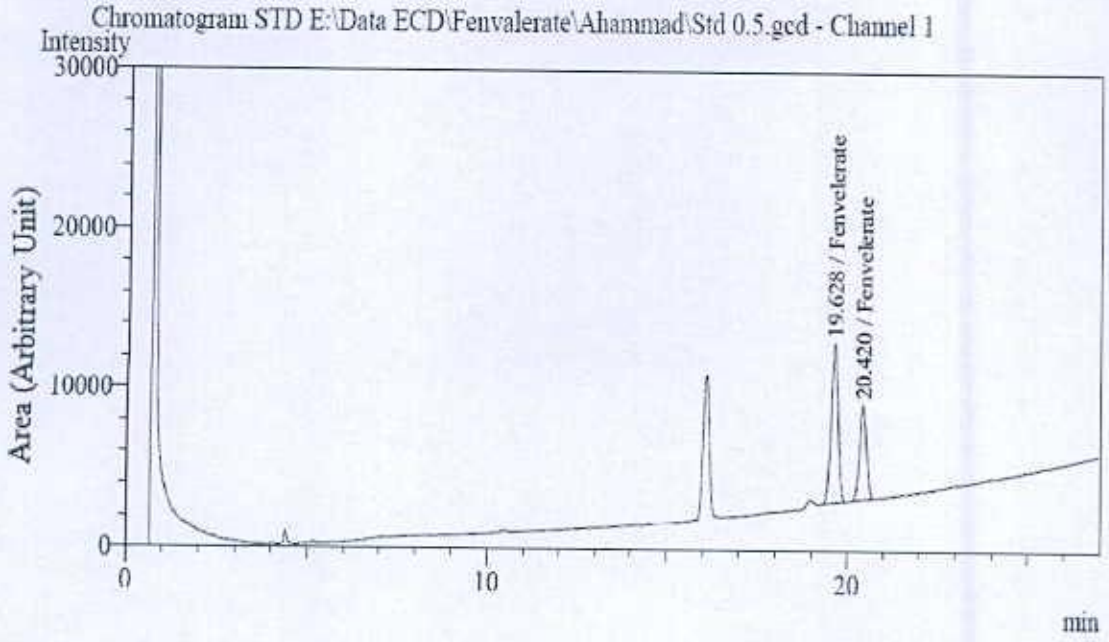


Figure 4. Typical Chromatograms of Fenvelerate standards run by GC-ECD.

Table 3. 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 4.Conditions for column oven temperature for FTD

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

Table 5. The instrument parameters for GC-ECD

Instruments	Conditions
Injection port SPL	Injection mode: split; temperature:280°C; flow control mode: linear velocity; split ratio: 10:0
Detector channel 1 ECD	Temperature:300°C; current: 0.50 Pa; make up flow: 30 mL/min;

Table 6.Conditions for column oven temperature for Fenvalerate Determination

Column oven	Rate	Temperature (°C)	Hold time (min)
Initial temperature:	-	160	0
160°C	10	230	0
	2	270	0

Table7.Conditionsforcolumnoven temperature for Cypermethrin Determination

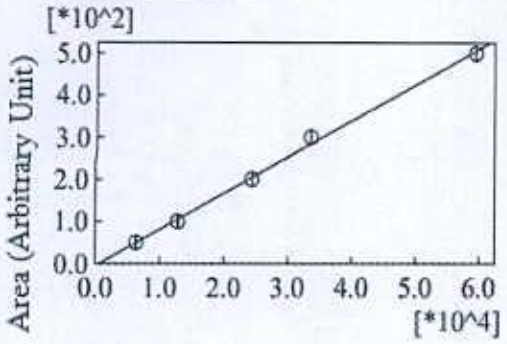
Column oven	Rate	Temperature(°C)	Hold time (min)
Initial temperature:	-	160	1
160°C	10	270	8

3.9 Calibration curve preparation

Prior to the injection of the sample extract, standard solutions of different concentrations of each pesticide group were prepared and injected with suitable instrument parameters. The samples were calibrated (retention time, peak area etc.) against five pointed calibration curve of standard solution of concerned pesticide (Figure 5-10). Each peak was characterized by its retention time. Sample results were expressed in mg/kg automatically by the GC software.

f(x)=8.58182478378e-003*x-4.76864750449
R=0.9987293676 R^2=0.997460349706
MeanRF:8.23177212761e-003 RFSD:4.50915039717e-004 RFRSD:5.47773957694
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



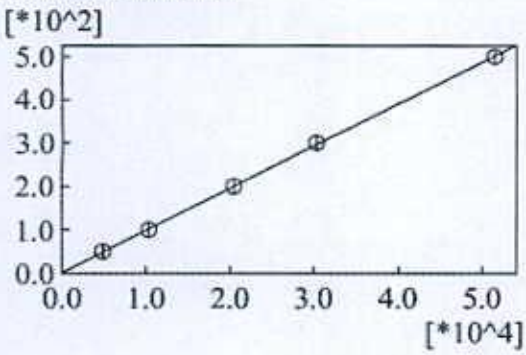
No.	Conc.	Area
1	50.000	6355
2	100.000	12850
3	200.000	24378
4	300.000	33704
5	500.000	59496

Conc. (µg/L)

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

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
Curve Type: Linear
ZeroThrough: Not through
Weighted Regression: None

External Standard



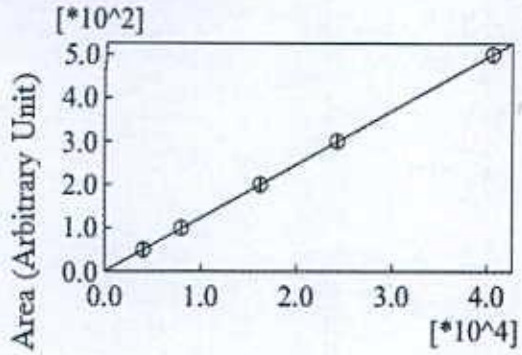
No.	Conc.	Area
1	50.000	4878
2	100.000	10347
3	200.000	20439
4	300.000	30352
5	500.000	51502

Conc. (µg/L)

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

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

External Standard

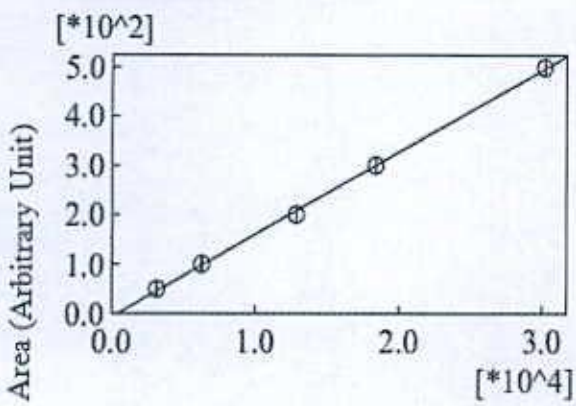


No.	Conc.	Area
1	50.000	4064
2	100.000	8014
3	200.000	16219
4	300.000	24288
5	500.000	40599

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

f(x)=1.66718969691e-002*x-6.50897807754
R=0.999643866504 R^2=0.999287859839
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	3162
2	100.000	6285
3	200.000	12871
4	300.000	18405
5	500.000	30208

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

f(x)=9.72861566278e-005*x+9.9950013555e-003
R=0.999966763424 R^2=0.999933527952
MeanRF:9.88583736873e-005 RFSD:9.19328503897e-007 RFRSD:0.929945000719
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

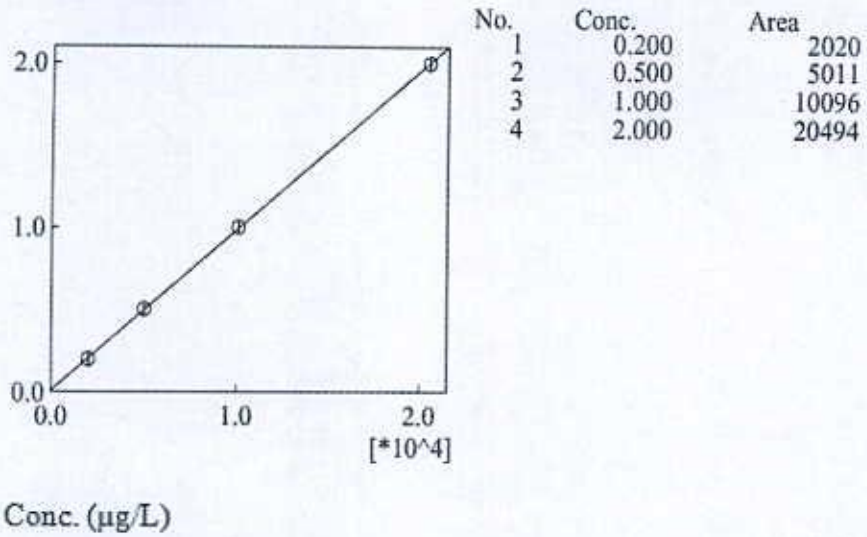


Figure 9. Calibration curve prepared for Cypermethrin made with different concentrations ranging from 0.2mg/L to 2mg/L.

f(x)=1.85256325579e-006*x+1.76302620759e-004
R=0.999989012827 R^2=0.999978025774
MeanRF:1.8508913558e-006 RFSD:1.18869074672e-008 RFRSD:0.642226105273
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

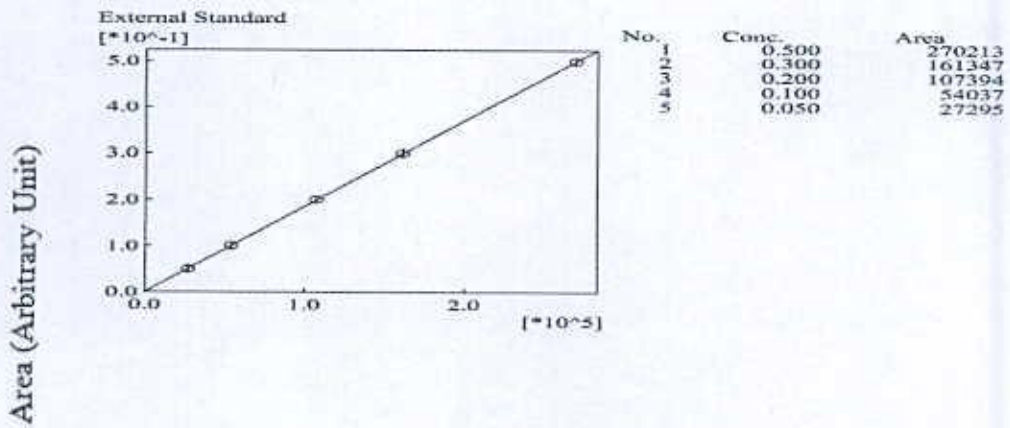
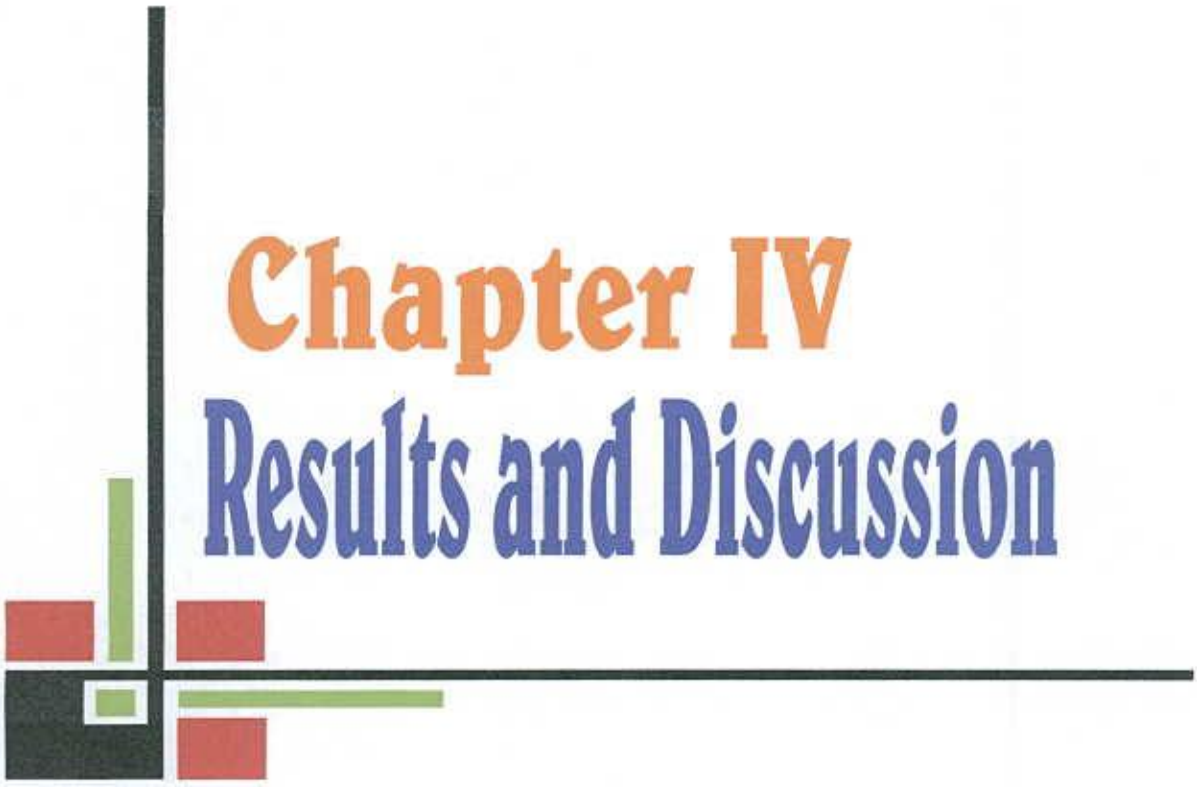


Figure 10. Calibration curve prepared for Fenvalerate made with different concentrations ranging from 50 µg/L to 500 µg/L.



Chapter IV
Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSIONS

100 vegetablesamples (cabbage and bitter gourd) were collected from 5 different markets (Rampura, Kawran Bazar, Taltola Bazar, Jatrabari areaand Mohammadpur Krishi Market) of Dhaka city to detect and quantify pesticide residues. The results obtained from this study are presented and described in this chapter using figures and tables.

4.1 Pesticide residues in cabbage

The concentrated extracts of cabbage samples collected from different markets were analyzed by GC-2010 (Shimadzu) with Flame Thermionized Detector (FTD) and Electron Capture Detector (ECD) with the pre-set parameters. Figure 11-24 shows the chromatograms of the injected extracts of cabbage sample containing detected pesticides.

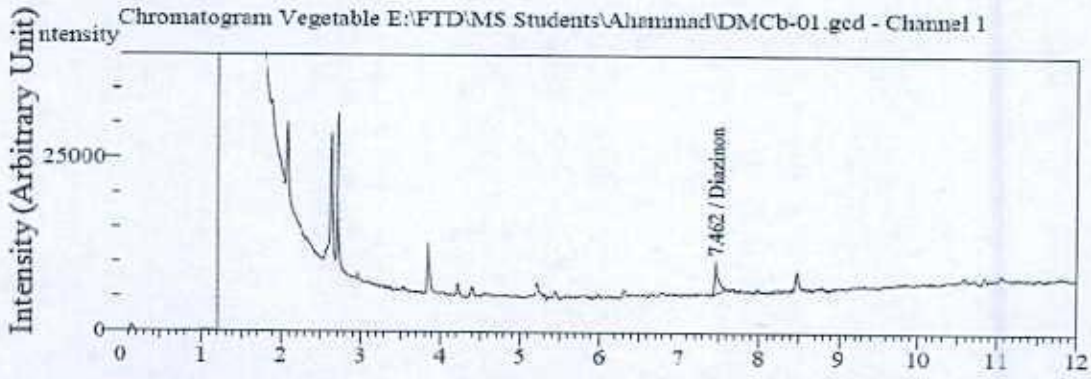


Figure 11. Chromatogram of Diazinon found in one of the cabbage marketed sample ($DMCb_{01}$) showing retention time.

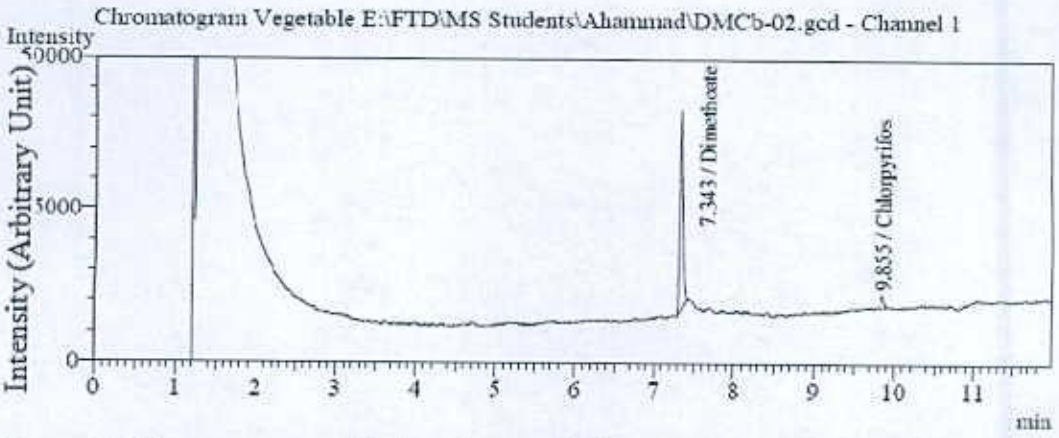


Figure 12. Chromatogram of Dimethoate and chlorpyrifos found in one of the cabbage marketed sample ($DMCb_{02}$) showing retention time.

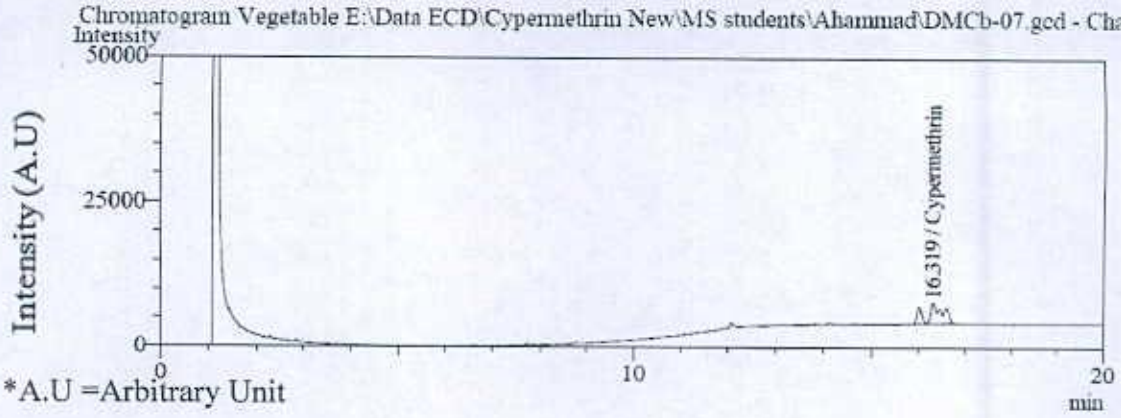


Figure 13. Chromatogram ofCypermethrin found in one of the cabbage marketed sample ($DMCb_{07}$) showing retention time.

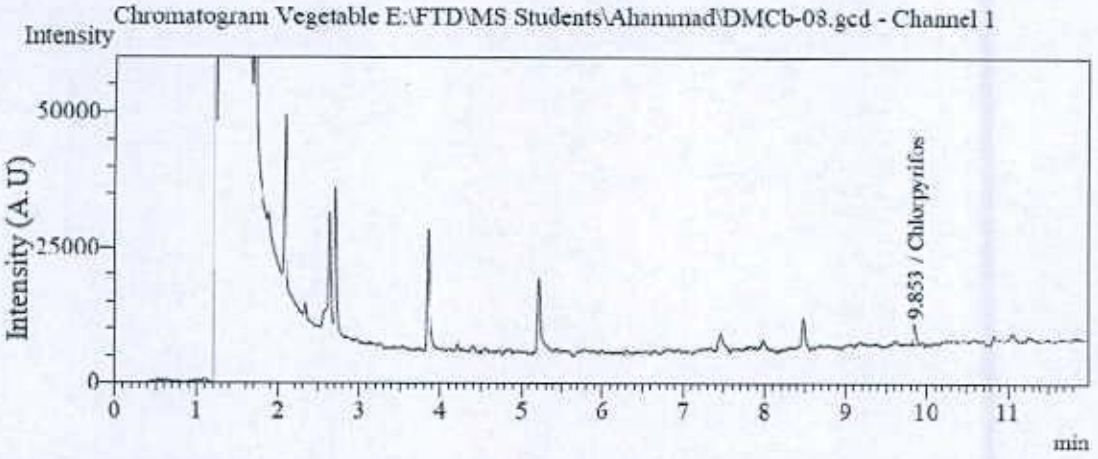


Figure 14. Chromatogram of Cypermethrin found in one of the cabbage marketed sample ($DMCb_{08}$) showing retention time.

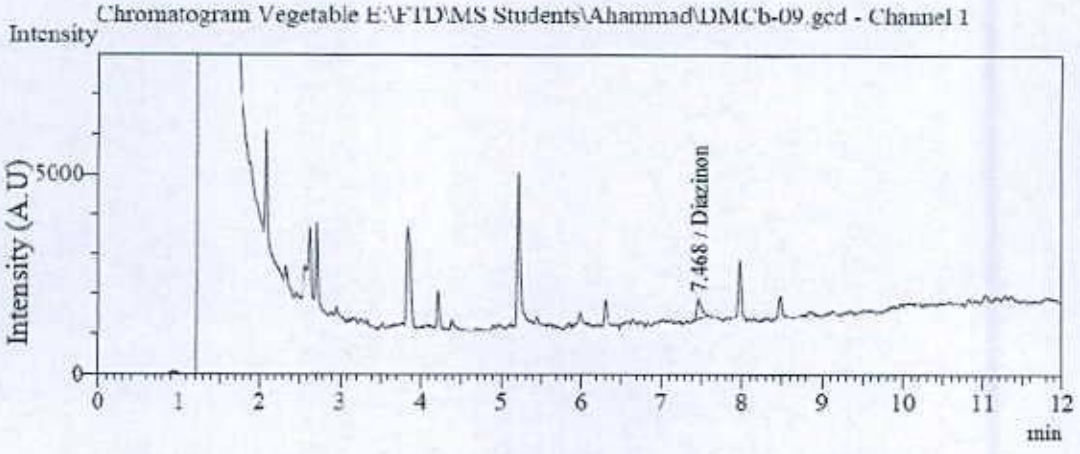


Figure 15. Chromatogram of Diazinon found in one of the cabbage marketed sample ($DMCb_{09}$) showing retention time.

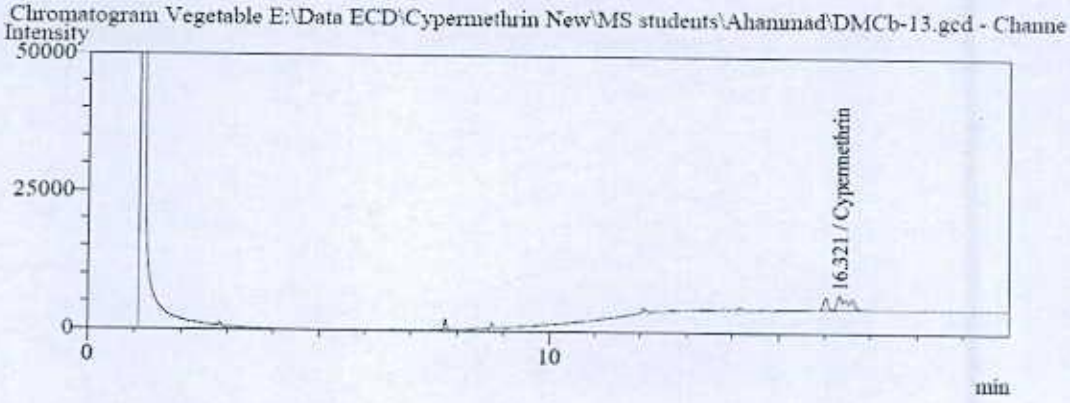


Figure 16. Chromatogram of Cypermethrin found in one of the cabbage marketed sample ($DMCb_{13}$) showing retention time.

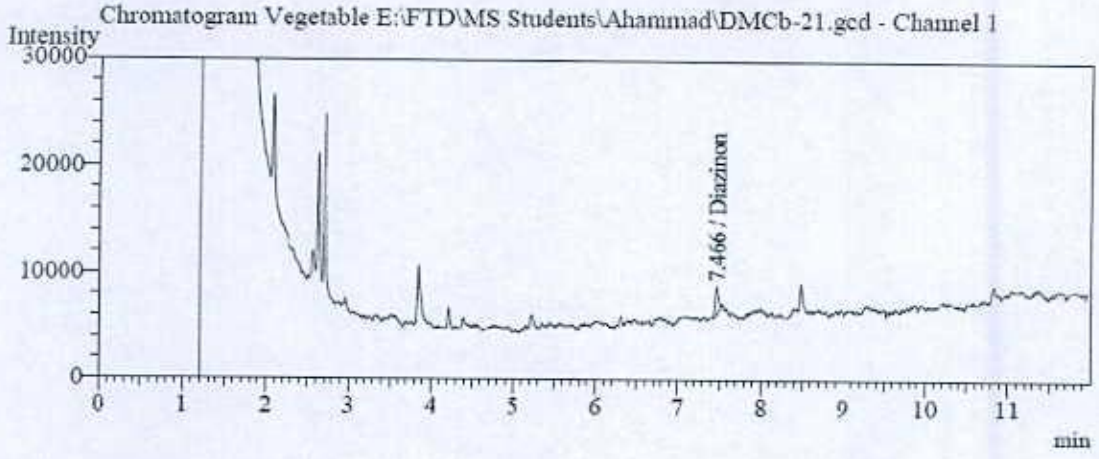


Figure 17. Chromatogram of Diazinon found in one of the cabbage marketed sample ($DMCb_{21}$) showing retention time.

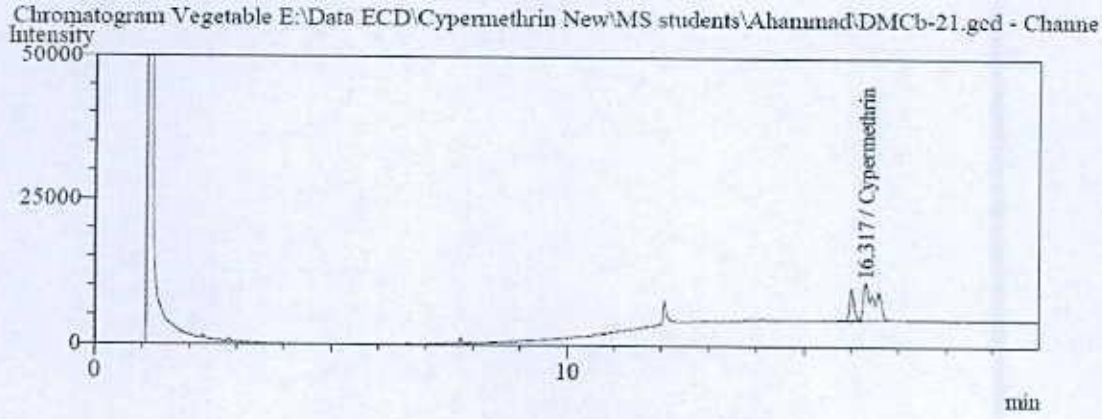


Figure 18. Chromatogram of Cypermethrin found in one of the cabbage marketed sample ($DMCb_{21}$) showing retention time.

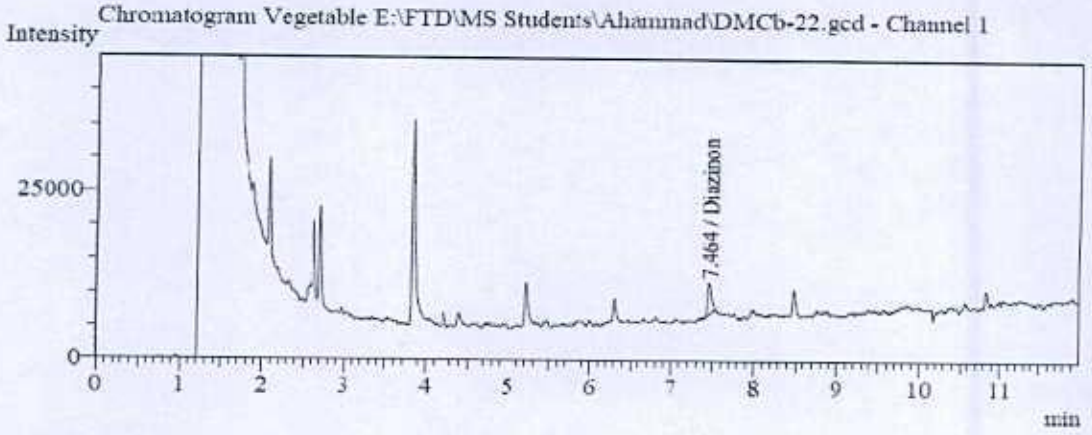


Figure 19. Chromatogram of Diazinon found in one of the cabbage marketed sample ($DMCb_{22}$) showing retention time.

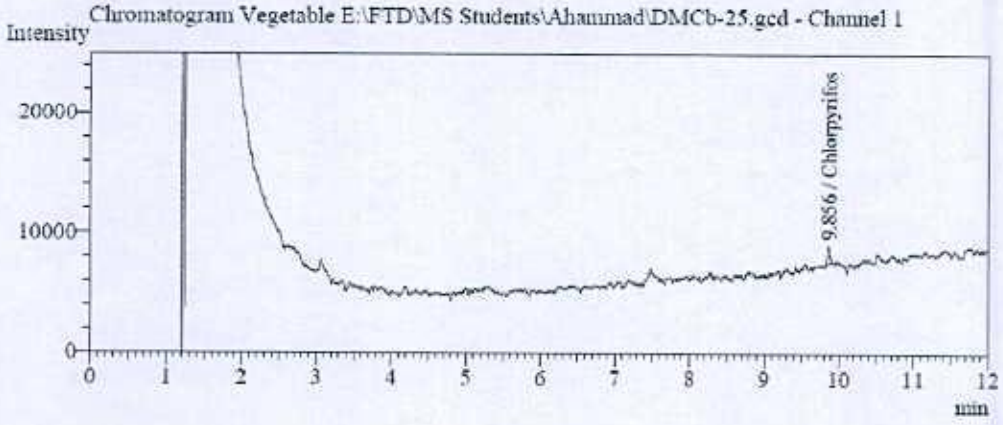


Figure 20. Chromatogram of chlorpyrifos found in one of the cabbage marketed sample ($DMCb_{25}$) showing retention time.

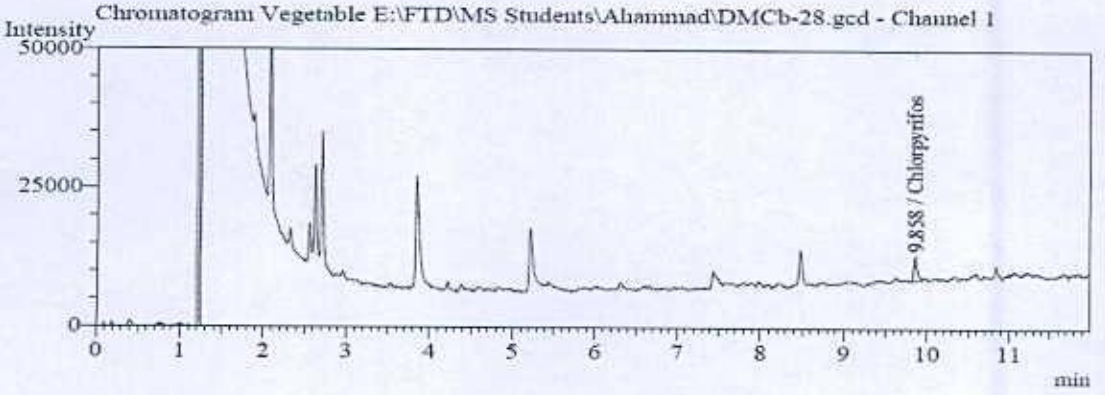


Figure 21. Chromatogram of chlorpyrifos found in one of the cabbage marketed sample ($DMCb_{28}$) showing retention time.

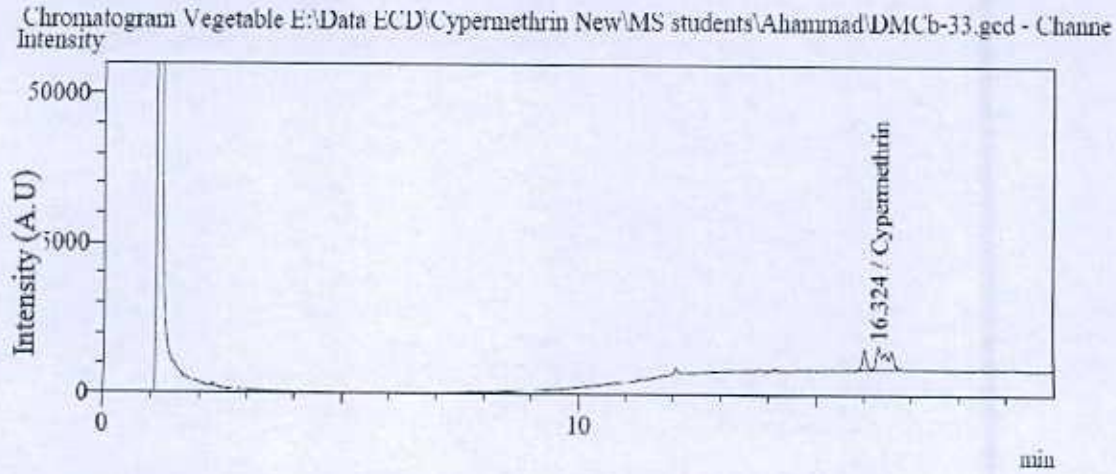


Figure 22. Chromatogram of Cypermethrin found in one of the cabbage marketed sample ($DMCb_{33}$) showing retention time.

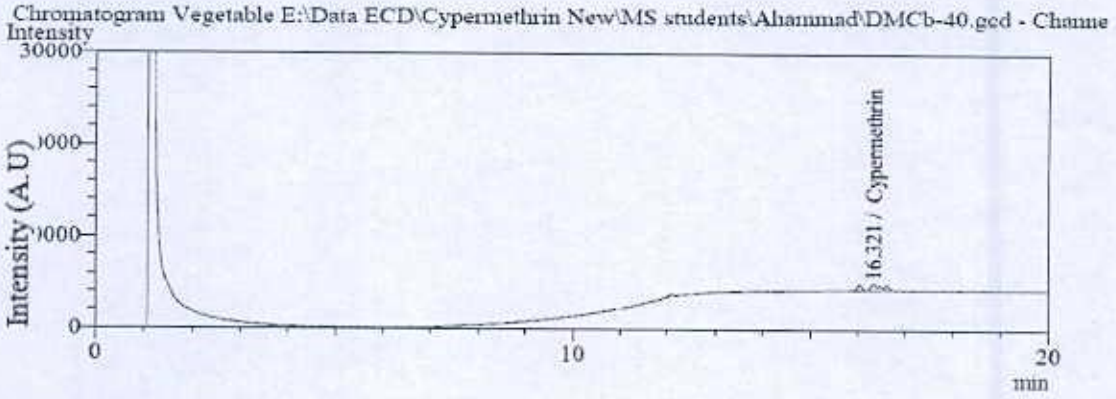


Figure 23. Chromatogram of Cypermethrin found in one of the cabbage marketed sample ($DMCb_{40}$) showing retention time.

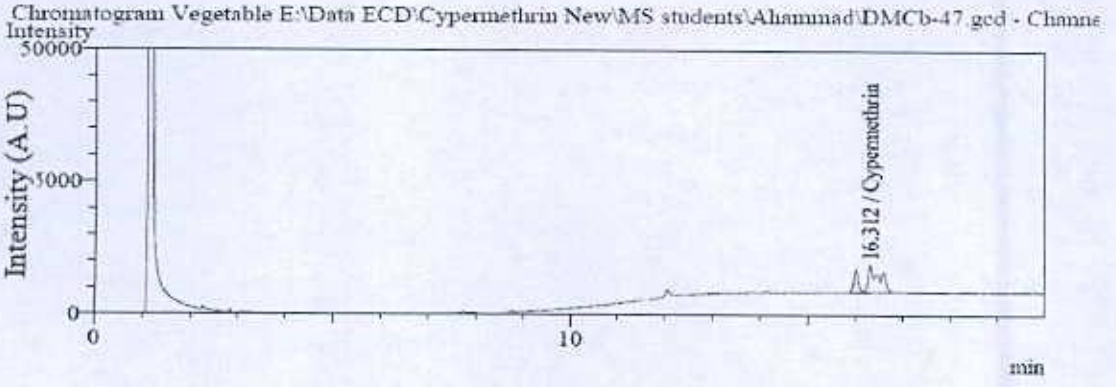


Figure 24. Chromatogram of Cypermethrin found in one of the cabbage marketed sample ($DMCb_{47}$) showing retention time.

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

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

Area of collection	Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)
Rampura	$DMCb_{01}$	Diazinon	0.12	0.01*
	$DMCb_{02}$	Dimethoate	1.34	0.02*
	$DMCb_{03}$	ND	-	-
	$DMCb_{04}$	ND	-	-
	$DMCb_{05}$	ND	-	-
	$DMCb_{06}$	ND	-	-
	$DMCb_{07}$	Cypermethrin	0.26	1.00*
	$DMCb_{08}$	Chlorpyrifos	0.15	0.01*
	$DMCb_{09}$	Diazinon	0.07	0.01*
	$DMCb_{10}$	ND	-	-
Kawran Bazar	$DMCb_{11}$	ND	-	-
	$DMCb_{12}$	ND	-	-
	$DMCb_{13}$	Cypermethrin	0.23	1.0*
	$DMCb_{14}$	ND	-	-
	$DMCb_{15}$	ND	-	-
	$DMCb_{16}$	ND	-	-
	$DMCb_{17}$	ND	-	-
	$DMCb_{18}$	ND	-	-
	$DMCb_{19}$	ND	-	-
	$DMCb_{20}$	ND	-	-
Taltola Bazar	$DMCb_{21}$	Diazinon Cypermethrin	0.05 0.42	0.01* 1.00*
	$DMCb_{22}$	Diazinon	0.10	0.01*
	$DMCb_{23}$	ND	-	-
	$DMCb_{24}$	ND	-	-
	$DMCb_{25}$	Chlorpyrifos	0.06	0.01*
	$DMCb_{26}$	ND	-	-
	$DMCb_{27}$	ND	-	-
	$DMCb_{28}$	Chlorpyrifos	0.18	0.01*
	$DMCb_{29}$	ND	-	-
	$DMCb_{30}$	ND	-	-
Jatrabari area	$DMCb_{31}$	ND	-	-
	$DMCb_{32}$	ND	-	-
	$DMCb_{33}$	Cypermethrin	0.30	1.00*
	$DMCb_{34}$	ND	-	-
	$DMCb_{35}$	ND	-	-
	$DMCb_{36}$	ND	-	-

	$DMCb_{37}$	ND	-	-
	$DMCb_{38}$	ND	-	-
	$DMCb_{39}$	ND	-	-
	$DMCb_{40}$	Cypermethrin	0.06	1.00*
Mohammadpur Krishi Market	$DMCb_{41}$	ND	-	-
	$DMCb_{42}$	ND	-	-
	$DMCb_{43}$	ND	-	-
	$DMCb_{44}$	ND	-	-
	$DMCb_{45}$	ND	-	-
	$DMCb_{46}$	ND	-	-
	$DMCb_{47}$	Cypermethrin	0.35	1.00*
	$DMCb_{48}$	ND	-	-
	$DMCb_{49}$	ND	-	-
	$DMCb_{50}$	ND	-	-

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

Fifty samples of cabbage collected from 5 different markets of Dhaka city (Rampura, Kawran Bazar, Taltola Bazar, Jatrabari and Mohammadpur Krishi Market) were analyzed to find out the content of left over residue of six pesticides (Diazinon, Fenitrothion, Quinalphos, Chlorpyrifos, Cypermethrin and Fenvalerate).

Out of 50 samples, 13 samples (26% of the total number of samples) contained detection amount of pesticide residues and 37 samples (74% 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). Who collected 42 samples of brinjal, cauliflower and country bean from fields and markets of Narsingdi district, Bandgladesh, and found 15 samples (above 68% of total samples) contained no residues of the sought pesticides.

Pesticide residue status in the samples of cabbage collected from Rampura area:

Out of ten cabbage samples collected from Rampura area, detectable amout of five samples ($DMCb_{01}$, $DMCb_{02}$, $DMCb_{07}$, $DMCb_{08}$ and $DMCb_{09}$) contained pesticide residues. Among them 2 samples ($DMCb_{01}$ and DMCb09) contained Diazinon at a level of 0.12 mg/kg and 0.07 mg/kg respectively, which were above the EU-MRL. $DMCb_{02}$ had multiple pesticides residue. These are Dimethoate and Chlorpyrifos at a level of 1.34 and 0.09 mg/kg, respectively, which were above the EU-MRL. $DMCb_{08}$ had chlorpyrifos pesticides residue at a level of 0.15, which were above the EU-MRL. $DMCb_{07}$ had cypermethrin pesticides residue at a level of 0.262, which were below the EU-MRL. (European Commission, 2005). The other 5 samples contain no detectable pesticide residues.

Pesticide residue status in the samples of cabbage collected from Kawran Bazar area:

Out of 10 sample only one sample ($DMCb_{13}$) contained residue of only Cypermethrin (0.23 mg/kg) and the other nine samples contained no detectable pesticide residues among the ten samples collected from Kawran Bazar area. The level of detected residue was 0.23 mg/kg, which was below the MRL (1.0 mg/kg). Other pesticides under study could be found out in cabbage collected from Kawran bazaar area.

Pesticide residue status in the samples of cabbage collected from Taltola Bazar area:

Among the ten samples of cabbage collected from Taltola Bazar, four samples ($DMCb_{21}$, $DMCb_{22}$, $DMCb_{25}$ and $DMCb_{28}$) contained pesticides residue. $DMCb_{21}$ had two pesticides residue. These were Diazinon and Cypermethrin at a level of 0.051 and 0.42 mg/kg, where the level of Diazinon residue was above MRL (0.01) and the level of Cypermethrin residue wasbelow MRL (1.0). One sample ($DMCb_{22}$) contained residue of Diazinon (0.01 mg/kg) which was above the EU-MRL (0.01 mg/kg). Another two sample ($DMCb_{25}$ and $DMCb_{28}$) of cabbage contained residue of Chlorpyrifos at a level of 0.063 and 0.18 mg/kg, respectively which were above the EU-MRL (0.01 mg/kg). The other 6 samples contain no detectable pesticide residues.

Pesticide residue status in the samples of cabbage collected from Jatrabari area:

Two samples ($DMCb_{33}$& $DMCb_{40}$) of cabbage contained residue of Cypermethrin among the ten samples collected from Jatrabari area.Thesample $DMCb_{33}$contained0.30 mg/kg and the other sample ($DMCb_{40}$) contained 0.06 mg/kg.Both the samples contained residue of cypermethrin below the Maximum Residue Limit (1.0 mg/kg). The other 8 samples contain no detectable pesticide residue.

Pesticide residue status in the samples of cabbage collected from Mohammadpur Krishi Market area:

Only one sample ($DMCb_{47}$) contained residue of Cypermethrin and the other nine samples contained no detectable pesticide residues among the ten samples collected from Mohammadpur Khrishi Market area. The level of detected residue of cypermethrin was 0.35 mg/kg, which was below the MRL (1.0 mg/kg).

4.2 Pesticide residues in bitter gourd

The concentrated extracts of bitter gourd samples collected from different markets of Dhaka city were analyzed by GC-2010 (Shimadzu) with Flame Thermoionized Detector (FTD) and Electron Capture Detector (ECD) with the pre-set parameters. Figure 25-37shows the chromatograms of the injected extracts of bitter gourd sample containing detected pesticides.

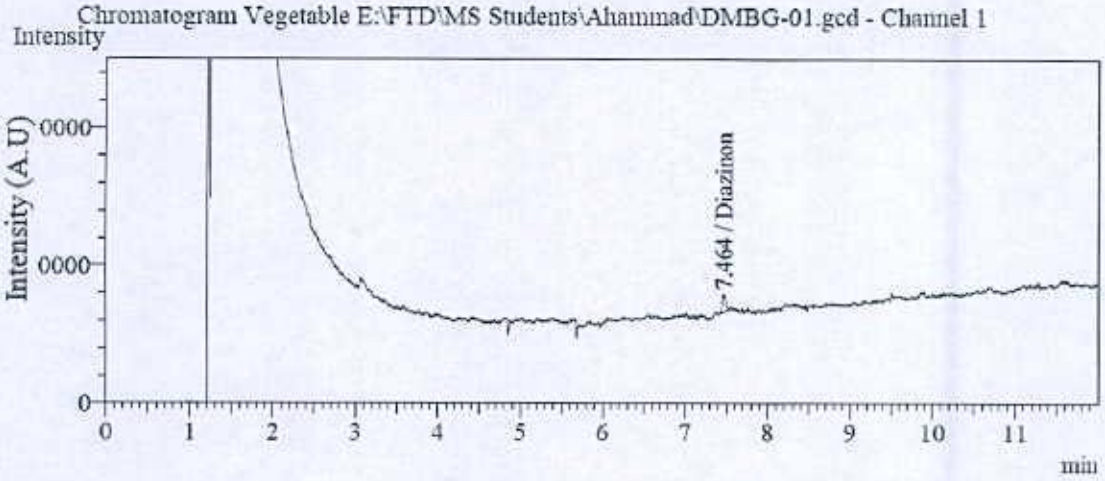


Figure 25. Chromatogram of Diazonon found in one of the bitter gourd marketed sample ($DMBG_{01}$) showing retention time.

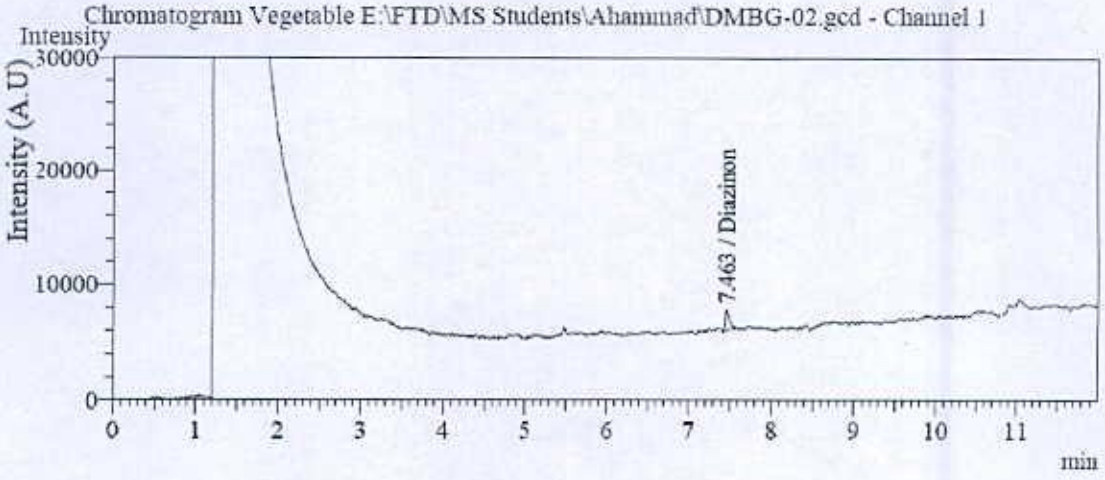


Figure 26. Chromatogram of Diazinon found in one of the bitter gourd marketed sample ($DMBG_{02}$) showing retention time.

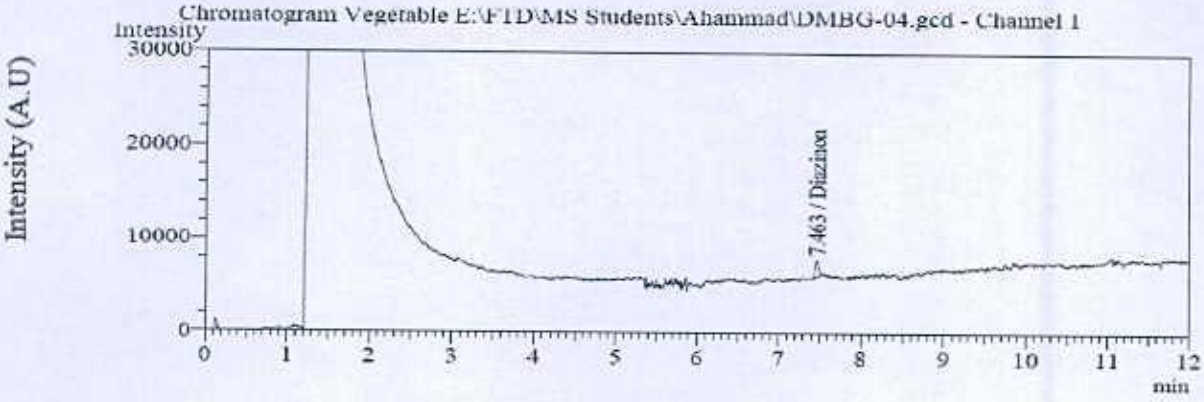


Figure 27. Chromatogram of Diazinon found in one of the bitter gourd marketed sample ($DMBG_{04}$) showing retention time.

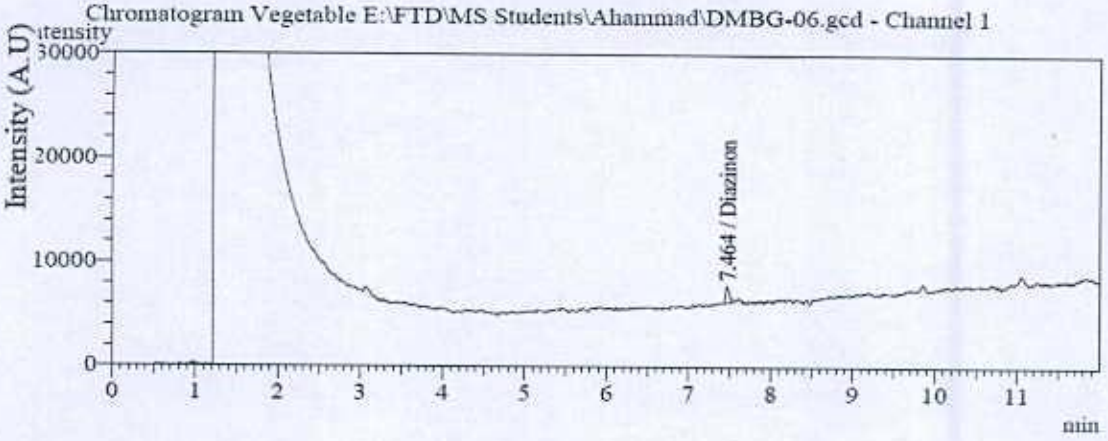


Figure 28. Chromatogram of Diazinon found in one of the bitter gourd marketed sample ($DMBG_{06}$) showing retention time.

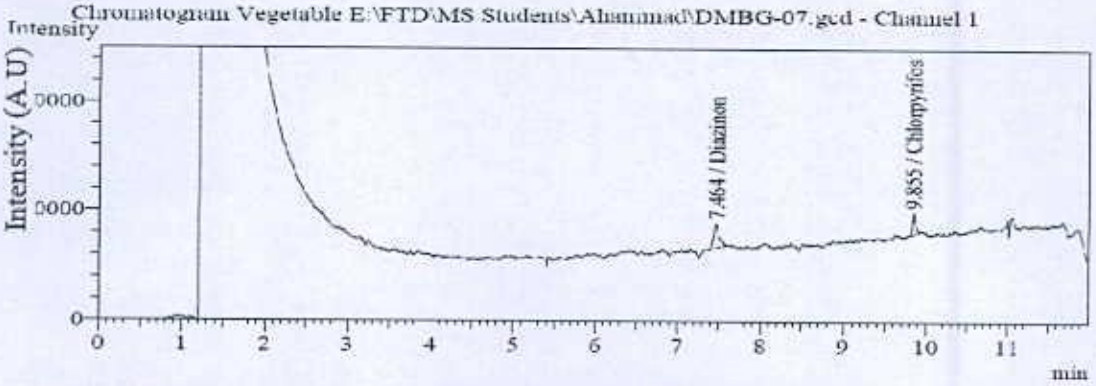


Figure 29. Chromatogram of Diazinon and Chlorpyrifos found in one of the bitter gourd marketed sample ($DMBG_{07}$) showing retention time.

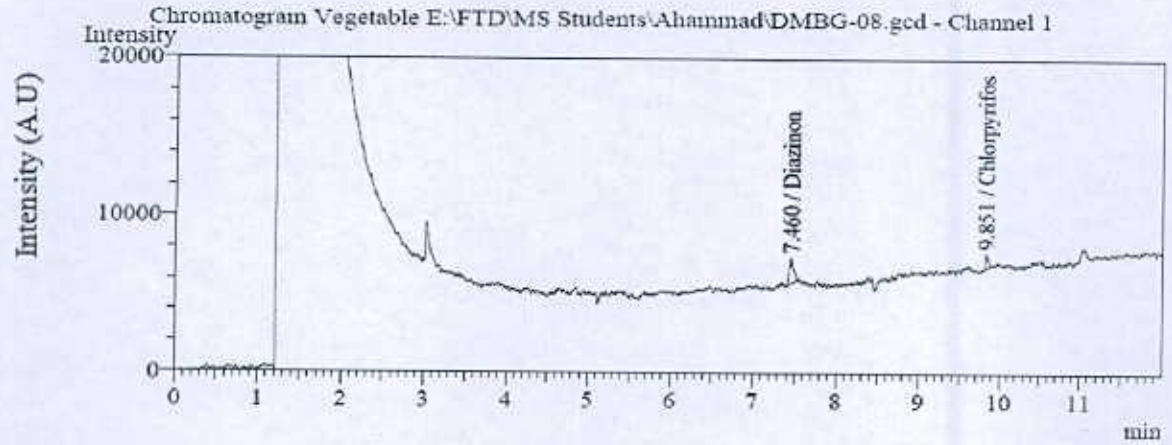


Figure 30. Chromatogram of Diazinon and Chlorpyrifos found in one of the bitter gourd marketed sample ($DMBG_{08}$) showing retention time.

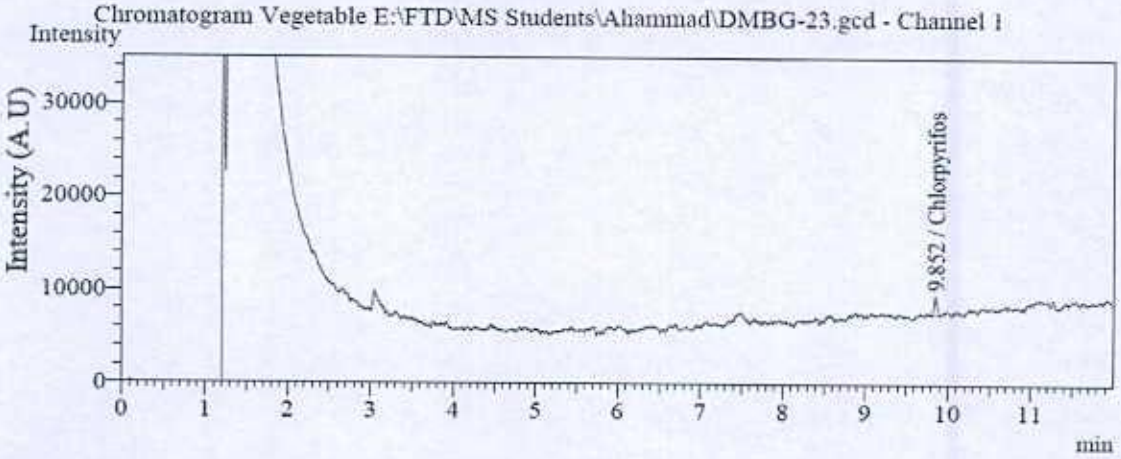


Figure 31. Chromatogram of Chlorpyrifos found in one of the bitter gourd marketed sample ($DMBG_{23}$) showing retention time.

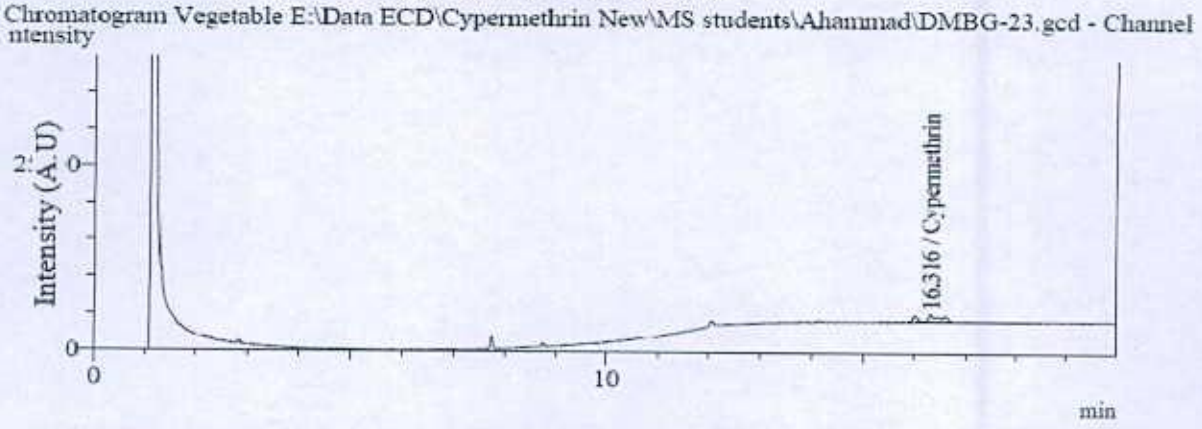


Figure 32. Chromatogram of Cypermethrin found in one of the bitter gourd marketed sample ($DMBG_{23}$) showing retention time.

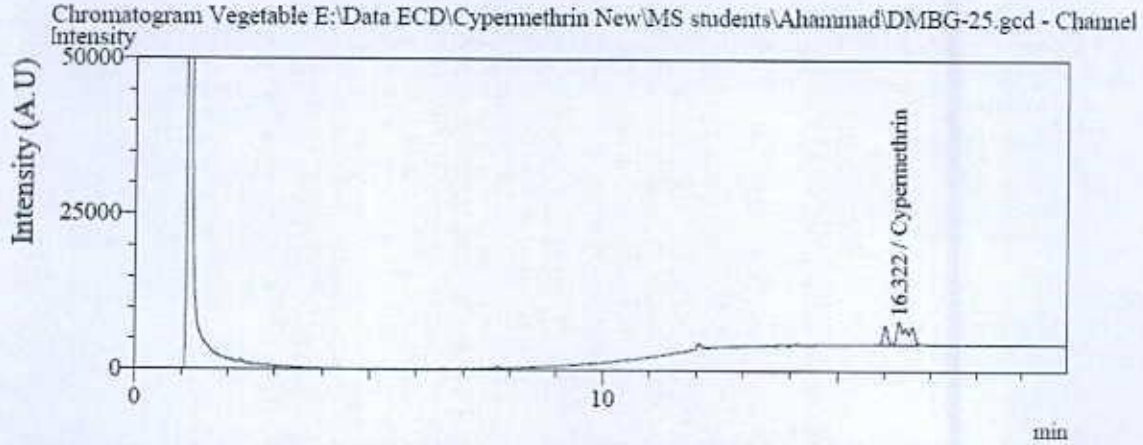


Figure 33. Chromatogram of Cypermethrin found in one of the Bitter Gourd marketed sample ($DMBG_{25}$) showing retention time.

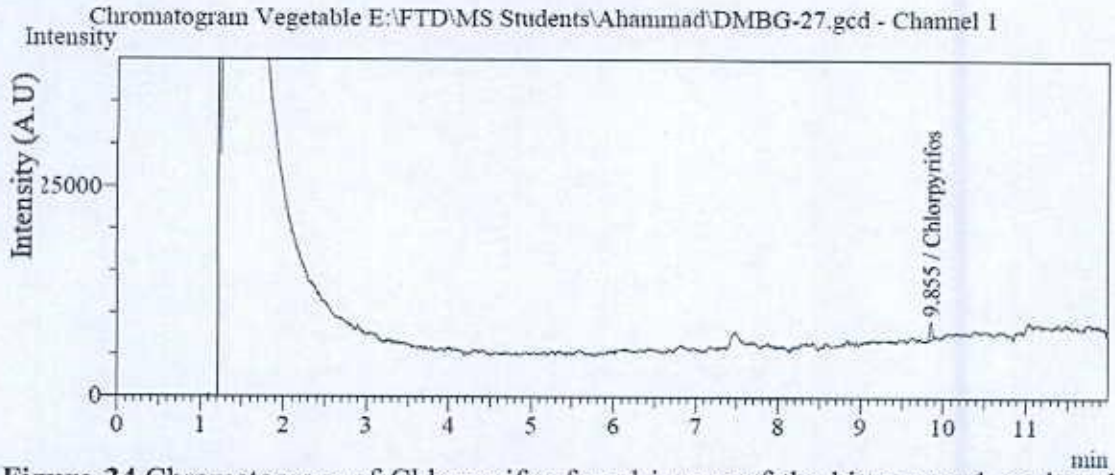


Figure 34. Chromatogram of Chlorpyrifos found in one of the bitter gourd marketed sample ($DMBG_{27}$) showing retention time.

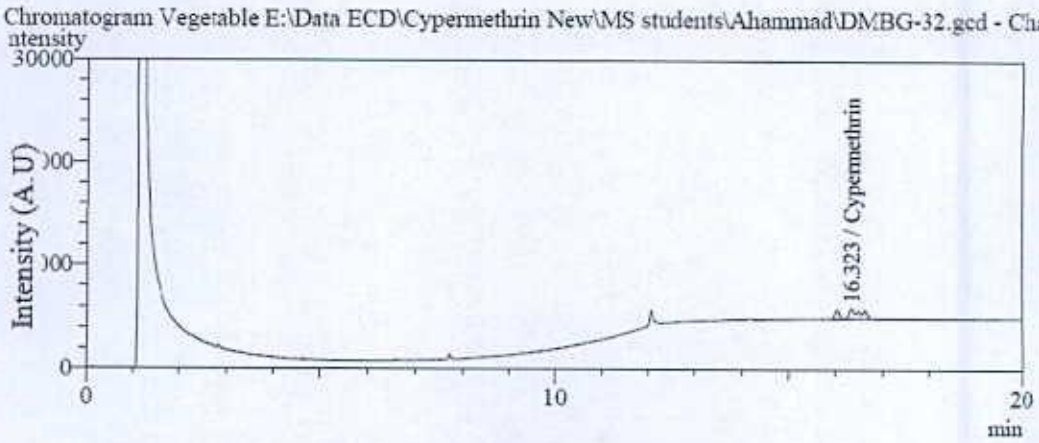


Figure 35. Chromatogram of Cypermethrin found in one of the bitter gourd marketed sample ($DMBG_{32}$) showing retention time.

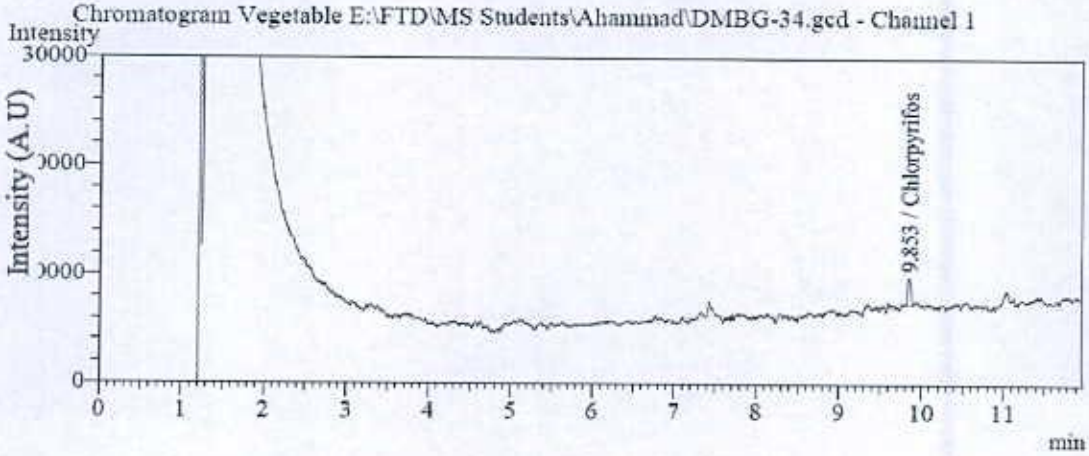


Figure 36. Chromatogram of Chlorpyrifos found in one of the bitter gourd marketed sample ($DMBG_{34}$) showing retention time.

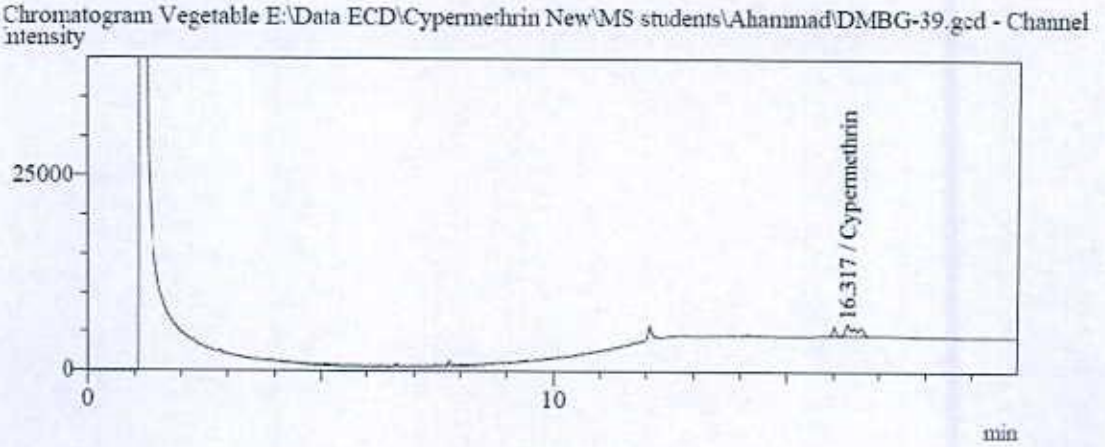


Figure 37. Chromatogram of Cypermethrin found in one of the bitter gourd marketed sample ($DMBG_{39}$) showing retention time.

The level of pesticide residues found in the analyzed bitter gourd samples and their maximum residue levels are presented in Table 9.

Table 9. The level of residues (mg/kg) of different pesticides found in the analyzed bitter gourd samples

Area of collection	Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)
Rampura	$DMBG_{01}$	Diazinon	0.02	0.01*
	$DMBG_{02}$	Diazinon	0.05	0.01*
	$DMBG_{03}$	ND	-	-
	$DMBG_{04}$	Diazinon	0.03	0.01*
	$DMBG_{05}$	ND	-	-
	$DMBG_{06}$	Diazinon	0.04	0.01*
	$DMBG_{07}$	Diazinon Chlorpyrifos	0.07 0.08	0.01* 0.05*
	$DMBG_{08}$	Diazinon Chlorpyrifos	0.04 0.02	0.01* 0.05*
	$DMBG_{09}$	ND	-	-
	$DMBG_{10}$	ND	-	-
Kawran Bazar	$DMBG_{11}$	ND	-	-
	$DMBG_{12}$	ND	-	-
	$DMBG_{13}$	ND	-	-
	$DMBG_{14}$	ND	-	-
	$DMBG_{15}$	ND	-	-
	$DMBG_{16}$	ND	-	-
	$DMBG_{17}$	ND	-	-
	$DMBG_{18}$	ND	-	-
	$DMBG_{19}$	ND	-	-
	$DMBG_{20}$	ND	-	-
Taltola Bazar	$DMBG_{21}$	ND	-	-
	$DMBG_{22}$	ND	-	-
	$DMBG_{23}$	Chlorpyrifos Cypermethrin	0.086 0.09	0.05* 0.20*
	$DMBG_{24}$	ND	-	-
	$DMBG_{25}$	Cypermethrin	0.34	0.20*
	$DMBG_{26}$	ND	-	-
	$DMBG_{27}$	Chlorpyrifos	0.07	0.05*
	$DMBG_{28}$	ND	-	-
	$DMBG_{29}$	ND	-	-
	$DMBG_{30}$	ND	-	-

Jatrabari	$DMBG_{31}$	ND	-	-
	$DMBG_{32}$	Cypermethrin	0.08	0.20*
	$DMBG_{33}$	ND	-	-
	$DMBG_{34}$	Chlorpyrifos	0.09	0.02*
	$DMBG_{35}$	ND	-	-
	$DMBG_{36}$	ND	-	-
	$DMBG_{37}$	ND	-	-
	$DMBG_{38}$	ND	-	-
	$DMBG_{39}$	Cypermethrin	0.13	0.20*
	$DMBG_{40}$	ND	-	-
Mohammadpur Krishi Market	$DMBG_{41}$	ND	-	-
	$DMBG_{42}$	ND	-	-
	$DMBG_{43}$	ND	-	-
	$DMBG_{44}$	ND	-	-
	$DMBG_{45}$	ND	-	-
	$DMBG_{46}$	ND	-	-
	$DMBG_{47}$	ND	-	-
	$DMBG_{48}$	ND	-	-
	$DMBG_{49}$	ND	-	-
	$DMBG_{50}$	ND	-	-

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

50 samples of bitter gourd collected from 5 different maarkets of Dhaka city (Rampura, Kawran Bazar, Taltola Bazar, Jatrabari and Mohammadpur Krishi Market) were analyzed to find out the presence of left over residues of six pesticides (Diazinon, Fenitrothion, Quinalphos, Chlorpyrifos, Cypermethrin and Fenvalerate).

Out of 50 samples, 12 samples (24% of the total number of samples) contained pesticide residues and 38 samples (76% of the total number of samples) contained no detectable residues of the pesticides under study. The present results can be compared to Islam *et al.* (2014). They 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 detectable residues of the sought pesticides.

Pesticide residue status in the samples of bitter gourd collected from Rampura area:

Out pf ten samples of bitter gourd collected from Rampura area, only six samples ($DMBG_{01}$, $DMBG_{02}$, $DMBG_{04}$, $DMBG_{06}$, $DMBG_{07}$ and $DMBG_{08}$) contained residue of pesticides. Ssamples ($DMBG_{01}$, $DMBG_{02}$, $DMBG_{04}$, and $DMBG_{06}$)contained residues of Diazinon and the levels were 0.02, 0.046, 0.03 and 0.04 mg/kg, respectively. These level of pesticide residues were higher the Maximum Residue Limit (0.01 mg/kg). Another two samples ($DMBG_{07}$ and $DMBG_{08}$) had two pesticide residues, where they contained Diazinon and Chlorpyrifos. The level of Diazinon residues were 0.07 and 0.042 mg/kg in the samples of $DMBG_{07}$ and $DMBG_{08}$. These were also above the MRL (0.01 mg/kg). On the other hand, the level of Chlorpyrifos resedues remain in the samples of $DMBG_{07}$ and $DMBG_{08}$ were 0.08 and 0.02 mg/kg. The residue level present in this two samples were lower than the MRL (0.2). The other 4 samples contain no detectable pesticide residues.

Pesticide residue status in the samples of bitter gourd collected from Kawran Bazar area:

Samples of bitter gourd collected from Kawran Bazar contained no residues of the pesticides under studied.

Pesticide residue status in the samples of bitter gourd collected from Taltola area:

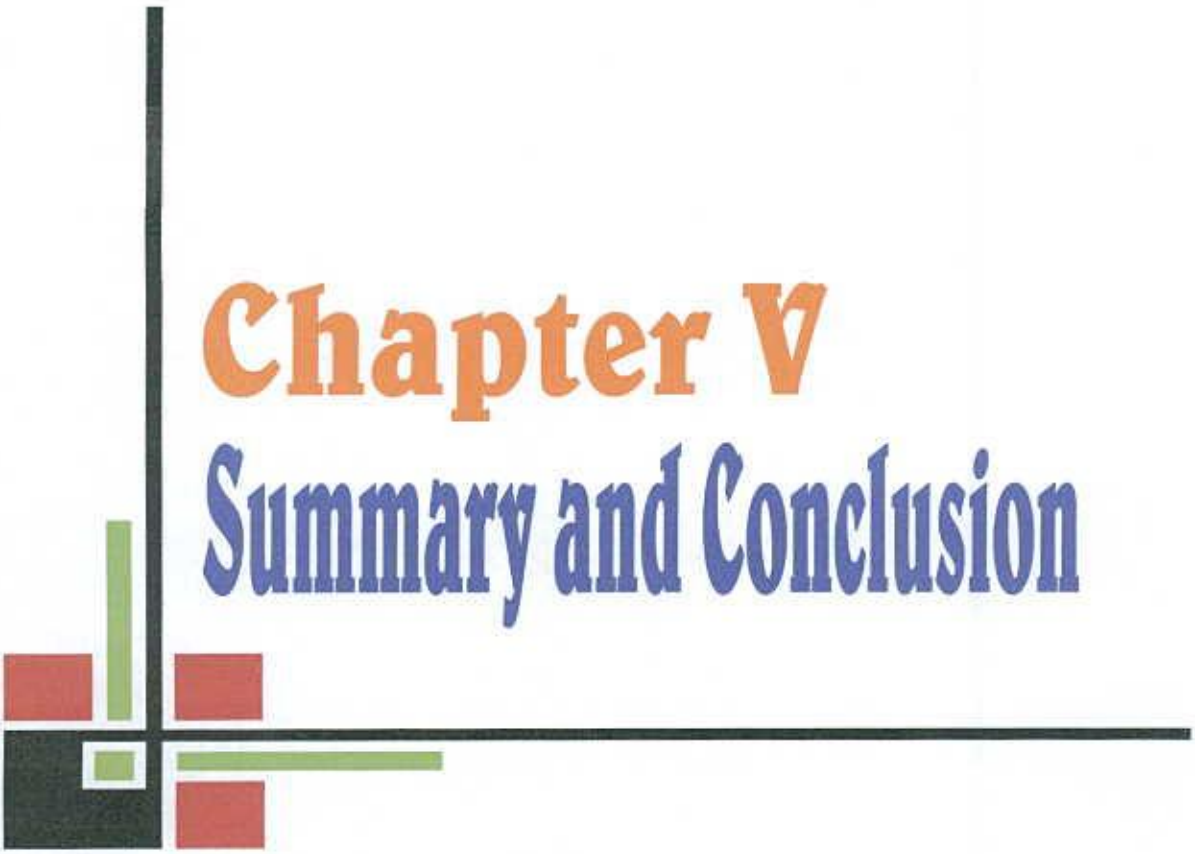
Among the ten samples of bitter gourd collected from Taltola Bazar, three sample ($DMBG_{23}$, $DMBG_{25}$and $DMBG_{27}$) contained residues of pesticides. The other 7 samples contain no detectable pesticide residues.$DMBG_{23}$and $DMBG_{27}$ contained residues of Chlorpyrifos, where the amount being 0.086 and 0.07 mg/kg, respectively and both of the levels were above MRL (0.05 mg/kg). On the other hand, DMBG23 and $DMBG_{25}$ samples contained, where the level of residue was 0.09 and 0.34 mg/kgcypermethrin, respectively. The samples of $DMBG_{23}$contained residue below the EU-MRL (0.2 mg/kg). The residue level in the sample of $DMBG_{25}$was higher the standard EU-MRL (0.2 mg/kg). The other 7 samples contain no detectable pesticide residue.

Pesticide residue status in the samples of bitter gourd collected from Jatrabari area:

Among the ten samples of bitter gourd collected from Jatrabari, three sample ($DMBG_{32}$, $DMBG_{34}$ and$DMBG_{39}$) contained residue of pesticides. Among them two sample ($DMBG_{32}$ and $DMBG_{39}$) had residue of Cypermethrin, where the amountwas 0.08 and 0.13 mg/kg, respectively. The level of residue of these two samples was lower than the MRL (0.2 mg/kg). On the other hand, DMBG34sample contained residue of chlorpyrifos the amount being 0.09 mg/kg and it was higher residues compared to the MRL (0.05 mg/kg) value.The other 7 samples contain no detectable pesticide residues.

Pesticide residue status in the samples of bitter gourd collected from Kawran Bazar and Mohammadpur Krishi Market area:

The ten samples of bitter gourd collected from Kawran Bazar and Mohammadpur Khrishi Marketarea contained no residues of the pesticides studied.



Chapter V

Summary and Conclusion

CHAPTER V

SUMMARY AND CONCLUSION

Being an overpopulated country food shortage and malnutrition are major problems of Bangladesh. Vegetables are one of the major part and sources of vitamin and others nutritional elements of our daily diet, but it contributes a very little portion of our daily intake because of its short supply. The main obstacle of vegetables production in our country is insect pest infestation. To enhance the vegetable production, protect the nutritional integrity of food, facilitate storage to assure year-round supplies, and provide attractive and appealing food products, farmers and growers have changed the way they produce vegetables.Use of different pesticides and other chemicals are becoming a common agricultural practice by the farmers, and a major portion of these pesticides are intercepted by the plant leaves during application.As a result, pesticide residues remain in the vegetable whichpose a threat to human body. Consumers, who intakes vegetables with high residual contamination in regular basis for long time will be affected by various types of food-borne diseases associated. Moreover, most chronic diseases e.g., cancer, kidney failure, heart attack etc. are the result of long term consumption of pesticide contaminated vegetables and other products.

Therefore, it is a prime need to detect and quantify pesticide residues in the food commodities. However, due to lack of well-equipped laboratory and skilled manpower in our country this area of research is still at initial stage. In recent years, pesticide analytical and residue research gained momentum in Bangladesh. Pesticide Analytical Laboratory, Division of Entomology, Bangladesh Agricultural Research Institute (BARI), Gazipur attained ability for the determination of commonly used pesticides in Bangladesh in major fruits and vegetables.

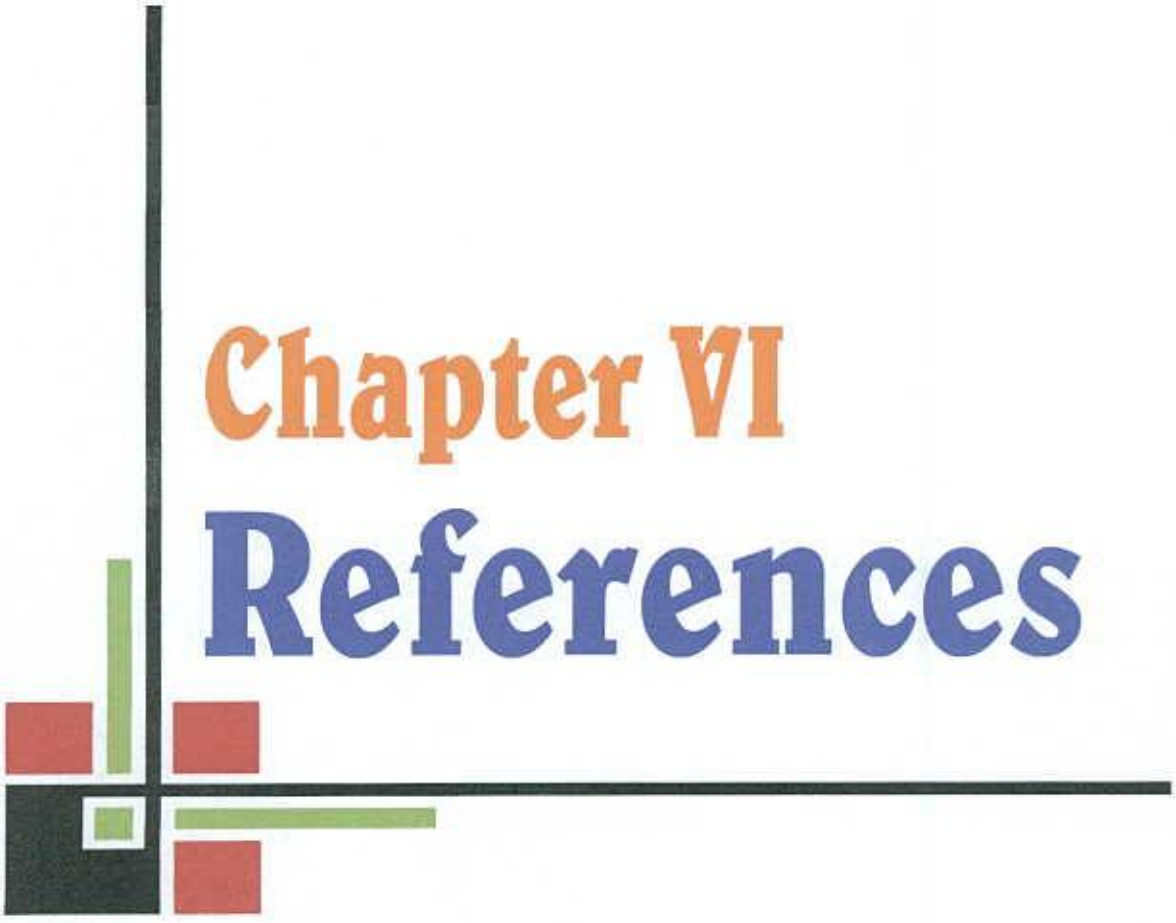
The purpose of this study was intended to identify and quantify the pesticide residue level present in the vegetables available in various local markets of Dhaka City. Regarding this, fifty samples of cabbage and fifty samples of bitter gourd were collected from five different locations (Rampura, Kawran Bazar, Taltola Bazar, Jatrabari area and Mohammadpur Krishi Market) of Dhaka City and carried to the Pesticide Analytical Laboratory, Division of Entomology, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The QuEChERS extraction

technique was applied for the extraction and cleanup of the collected sample. Gas chromatography associated with flame thermionized detector (FTD) and Electron Capture Detector ECD was used to identify and quantify the level of pesticide residues present in the extracted samples. Six most commonly used pesticides i.e. Diazinon, Fenitrothion, Chlorpyrifos, Quinalphos, Cypermethrin and Fenvelerate were considered for this study.

Among the 50 samples of cabbage, 13 samples (26% of the total number of samples) contained residues of Chlorpyrifos, Cypermethrin, Diazinon and Dimethoate, where 1 sample contained two pesticide residues. 7 samples were above the maximum residue limits (MRLs) and 5 samples were below the MRL. On the other hand 37 samples (74% of the total number of samples) contained no detectable pesticide residues of the sought pesticides.

Out of 50 samples of bitter gourd, 12 samples (24%) contained residues of Cypermethrin, Diazinon and Chlorpyrifos, where 3 samples contained multiple pesticide residues. 7 samples were above the MRL and 2 samples were below the MRL. And the other 38 samples (76% of the total number of samples) contained no detectable residues of the pesticide considered for this study.

At present days, pesticide residues in vegetables and other foods have become a prime concern and a safety issue for the consumers. This study will help to understand the residual contamination of studied vegetables in the study area and will help to increase public awareness as well.



Chapter VI
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CHAPTER VI

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