

**DETERMINATION OF ORGANOPHOSPHORUS PESTICIDE
RESIDUES IN CAULIFLOWER AND BITTER GOURD
COLLECTED FROM PATUAKHALI DISTRICT OF
BANGLADESH**

MEHIR CHANDRA PAUL



**DEPARTMENT OF AGRICULTURAL CHEMISTRY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207**

DECEMBER, 2020

**DETERMINATION OF ORGANOPHOSPHORUS PESTICIDE
RESIDUES IN CAULIFLOWER AND BITTER GOURD
COLLECTED FROM PATUAKHALI DISTRICT OF
BANGLADESH**

BY

MEHIR CHANDRA PAUL

Registration No.: 18-09178

A Thesis

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

**MASTER OF SCIENCE
IN
AGRICULTURAL CHEMISTRY**

SEMESTER: JULY-DECEMBER, 2020

Approved by

Supervisor

Co-Supervisor

Dr. Mohammad Dalower Hossain Prodhon
Senior Scientific Officer
Pesticide Research & Environmental Toxicology Section
Entomology Division
Bangladesh Agricultural Research Institute
Gazipur-1701, Bangladesh

Dr. Md. Sirajul Islam Khan
Professor
Department of Agricultural Chemistry
Sher-e-Bangla Agricultural University
Dhaka-1207, Bangladesh

Prof. Dr. Md. Tazul Islam Chowdhury
Chairman
Examination Committee



DEPARTMENT AGRICULTURAL CHEMISTRY
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

CERTIFICATE

*This is to certify that the thesis entitled “**DETERMINATION OF ORGANOPHOSPHORUS PESTICIDE RESIDUES IN CAULIFLOWER AND BITTER GOURD COLLECTED FROM PATUAKHALI DISTRICT OF BANGLADESH**” 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 **MEHIR CHANDRA PAUL**, Registration No. **18-09178** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Research supervisor

DECEMBER, 2020

Dhaka, Bangladesh

Dr. Mohammad Dalower Hossain Prodhan
Senior Scientific Officer

Pesticide Research & Environmental Toxicology Section
Entomology Division
Bangladesh Agricultural Research Institute
Gazipur-1701, Bangladesh.



*Dedicated to
My
Beloved Parents*

ACKNOWLEDGEMENT

All praises to the “**Almighty**” 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.

The author feels much pleasure to express his gratefulness, sincere appreciation and heartfelt liability to his venerable research supervisor **Dr. Mohammed Dalower Hossain Prodhan**, Senior Scientific Officer, Pesticide Research & Environmental Toxicology Section, Entomology Division, Bangladesh Agricultural Research Institute Gazipur-1701, Bangladesh for his scholastic guidance, support, uninterrupted encouragement, valuable suggestions and constructive criticism throughout the study period.

The author also expresses his gratitude and thankfulness to co-supervisor **Dr. Md. Sirajul Islam Khan**, Professor, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University Dhaka-1207, Bangladesh for his constant inspiration, valuable suggestions, cordial help, heartiest co-operation and supports through out the study period.

I also express gratitude and thankful to **Dr. Md. Tazul Islam Chowdhury**, Chairman, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, for his scholastic guidance, support, uninterrupted encouragement, valuable suggestions and constructive criticism throughout the study period.

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

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

The author deeply acknowledges the profound dedication to his beloved parents: **Nirmal Chandra Paul** and **Suchitra Rani Paul**, sisters and brothers for their moral support, steadfast encouragement and continuous prayer in all phases in all phases of academic pursuit from the beginning to the completion of study successfully

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

December, 2020
Dhaka, Bangladesh

The Author

DETERMINATION OF ORGANOPHOSPHORUS PESTICIDE RESIDUES IN CAULIFLOWER AND BITTER GOURD COLLECTED FROM PATUAKHALI DISTRICT OF BANGLADESH

Abstract

The present study was conducted to analyze seven organophosphorus (OP) pesticide residues in two common winter season vegetables (cauliflower and bitter gourd) collected from five different markets (Baga Bazar, Pirtola Bazar, Patuakhali New Market, Kalapara Bazar, Galachipa Bazar) of Patuakhali district during November 2019 to February 2020. The collected samples were analyzed using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD) for the determination of selected organophosphorus pesticide residues (malathion, diazinon, chlorpyrifos, quinalphos, acephate, fenitrothion and dimethoate) in 30 samples of cauliflower and 30 samples of bitter gourd. Among the 30 analyzed samples of cauliflower, 6 sample (20%) contained residue of diazinon, chlorpyrifos, dimethoate and quinalphos, where 1 sample contained multiple pesticide residues. All of the contaminated samples contained residue above the maximum residue limits (MRLs). In case of bitter gourd, among the 30 analyzed samples, 5 samples (16.66%) contained residue of diazinon, quinalphos, dimethoate and chlorpyrifos. Among these 5 contaminated samples, 4 samples contained residues above MRLs and 1 sample contained residue below MRLs. This study reflects a random scenario of pesticide contamination in vegetables especially in cauliflower and bitter gourd purchased from different retail markets of Patuakhali district, which will help the consumer to be aware of their health and safety.

TABLE OF CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
	ACKNOWLEDGEMENT	i
	ABSTRACT	ii
	TABLE OF CONTENTS	iii-iv
	LIST OF TABLES	v
	LIST OF FIGURES	vi-vii
	LIST OF PLATES	viii
	LIST OF ABBREVIATIONS	ix
I	INTRODUCTION	1-5
II	REVIEW OF LITERATURE	6-52
	2.1 Pesticides	6
	2.2 Classification of Pesticides	7
	2.2.1 Organophosphate Pesticides	7
	2.2.2 Organocarbamate Pesticides	7
	2.2.3 Organochlorine Pesticides	7
	2.2.4 Pyrethroid Pesticides	7
	2.2.5 Neonicotinoid Pesticides	7
	2.3 Description of selected pesticides	8
	2.3.1 Diazinon	8
	2.3.2 Dimethoate	8
	2.3.3 Fenitrothion	9
	2.3.4 Quinalphos	10
	2.3.5 Chlorpyrifos	11
	2.3.6 Acephate	11
	2.3.7 Malathion	13
	2.4 Instrument used for determination	14
	2.4.1 Liquid Chromatography-Mass Spectrometry	15
	2.4.2 Gas Chromatography-Mass Spectrometry	15
	2.4.3 Gas chromatography	16
	2.4.4 High Performance Liquid Chromatography	17

TABLE OF CONTENTS (cont'd)

CHAPTER NO.	TITLE	PAGE NO.
	2.5 Extraction and Clean-up	17
	2.5.1 Supercritical Fluid Extraction (SFE)	17
	2.5.2 Solid Phase Extraction (SPE)	18
	2.5.3 Accelerated Solvent Extraction (ASE)	18
	2.6 QuEChERS	
	(Quick, Easy, Cheap, Effective, Rugged and Safe) Method	19
	2.7 Pesticide Residue	21
	2.7.1 Acceptable Daily Intake or ADI	21
	2.7.2 Maximum Residue Level (MRL)	21
	2.7.3 Determination of Pesticide Residues in Food	21
III	MATERIALS AND METHODS	53-67
	3.1 Study area	53
	3.2 Sample collection	53
	3.3 Sample preparation for analysis	56
	3.4 Chemicals and reagents	57
	3.5 Analytical apparatus used	57
	3.6 Preparation of pesticide standard solution	61
	3.7 Extraction and clean up	61
	3.8 Detection and quantification of pesticide residue in samples	62
	3.9 Calibration curve preparation	63
IV	RESULTS AND DISCUSSION	68-79
	4.1 Pesticide residues in cauliflower	68
	4.2 Pesticide residues in bitter gourd	74
V	SUMMARY AND CONCLUSION	80-81
VI	REFERENCES	82-104

LIST OF TABLES

SERIAL NO.	TITLE	PAGE NO.
1	Sources and places of collection of cauliflower samples	55
2	Sources and places of collection of bitter gourd samples	56
3	The instrument parameters for GC-FTD	63
4	Conditions for column oven temperature for FTD	63
5	The level of residues (mg/kg) of different pesticides found in the analyzed cauliflower samples.	72
6	The level of residues (mg/kg) of different pesticides found in the analyzed bitter gourd samples	77

LIST OF FIGURES

SERIAL NO.	TITLE	PAGE NO.
1	District map of Patuakhali.	54
2	Typical chromatograms of seven organophosphorus insecticide standards run by GC-FTD.	62
3	Calibration curve prepared for acephate made with different concentrations ranging from 50 µg/L to 500 µg/L.	64
4	Calibration curve prepared for dimethoate made with different concentrations ranging from 50 µg/L to 500 µg/L.	64
5	Calibration curve prepared for diazinon made with different concentrations ranging from 50 µg/L to 500 µg/L.	65
6	Calibration curve prepared for fenitrothion made with different concentrations ranging from 50 µg/L to 500 µg/L.	65
7	Calibration curve prepared for malathion made with different concentrations ranging from 50 µg/L to 500 µg/L.	66
8	Calibration curve prepared for chlorpyrifos made with different concentrations ranging from 50 µg/L to 500 µg/L.	66
9	Calibration curve prepared for quinalphos made with different concentrations ranging from 50 µg/L to 500 µg/L.	67

LIST OF FIGURES (cont'd)

SERIAL NO.	TITLE	PAGE NO.
10	Chromatogram of chlorpyrifos found in one of the cauliflower sample (PaCf-03) collected from Baga Bazar.	68
11	Chromatogram of chlorpyrifos found in one of the cauliflower sample (PaCf-08) collected from Pirtola Bazar.	69
12	Chromatogram of diazinon found in one of the cauliflower sample (PaCf-13) collected from Patuakhali New Market.	69
13	Chromatogram of dimethoate found in one of the cauliflower sample (PaCf-18) collected from Patuakhali New Market.	70
14	Chromatogram of chlorpyrifos found in one of the cauliflower sample (PaCf-23) collected from Kalapara Bazar.	70
15	Chromatogram of quinalphos found in one of the cauliflower sample (PaCf-28) collected from Galachipa Bazar.	71
16	Chromatogram of chlorpyrifos found in one of the bitter gourd sample (PaBg-06) collected from Baga Bazar.	74
17	Chromatogram of diazinon found in one of the bitter gourd sample (PaBg-10) collected from Pirtola Bazar.	75
18	Chromatogram of diazinon found in one of the bitter gourd sample (PaBg-17) collected from Patuakhali New Market.	75
19	Chromatogram of dimethoate found in one of the bitter gourd sample (PaBg-24) collected from Kalapara Bazar.	76
20	Chromatogram of quinalphos found in one of the bitter gourd sample (PaBg-29) collected from Galachipa Bazar.	76

LIST OF PLATES

PLATE NO.	TITLE	PAGE NO.
1	Centrifuge Machine	57
2	Electric Balance	57
3	Vortex Mixer	58
4	Gas Chromatograph (GC)	58
5	Chopping of sample	59
6	Weighing of PSA	59
7	Adding acetonitrile	59
8	Extract Vortexing	60
9	Centrifuging of Extract	60
10	Filtration through PTFE filter	60
11	Sample extract ready for injection	60

LIST OF ABBREVIATIONS

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

CHAPTER I

INTRODUCTION

Bangladesh is an agricultural country and its economy highly depends on agriculture. It has an area of 1,47,570 sq. kilometer, only 0.31% of the total agricultural land in the world but 2.0 percent of total population in the world (Rasul and Thapa, 2004). As an overpopulated country food shortage and malnutrition has become a general problem in Bangladesh. Not only in Bangladesh but also around the world the food demand is changing rapidly because of population growth, economic growth, rising income and rapid urbanization. Demand is changing away from traditional commodities towards high value food commodities like vegetables, fruits, spices, fish etc. In this regard, vegetable growing has become an important farming activity from the point of view of dietary fulfillment as well as economics returns (Aktar *et al.*, 2017).

Vegetables are important components of the human diet since they provide essential nutrients that are required for most of the reactions occurring in the body. It makes up a major portion of the diet of humans in many parts of the world and play a significant role in human nutrition, especially as sources of phytonutriceuticals: vitamins (C, A, B1, B6, B9, E), minerals, dietary fiber and phytochemicals (Craig and Beck, 1999; Wargovich, 2000; Dias and Ryder, 2011). The major vegetables grown in our country are cabbage, cauliflower, tomato, brinjal, potato, radish, country bean, bottle gourd, pumpkin, bitter gourd, teasle gourd, ribbed gourd, ash gourd, okra, yard long bean, spinach etc (Hasan *et al.*, 2014). All these vegetables do not only meet our daily diet demand but also occupying a more or less significant position in earning foreign currency. Bangladesh earns 53344 million Tk. (USD 104.34 million) in the year 2003-2004 by exporting vegetables (NBR, Export Promotion Bureau (EPB) & Data Analysis by Mitul 2016, Hortex Foundation, Dhaka).

Cauliflower (*Brassica oleracea botrytis*), is a vegetable of the family of Brassicaceae. Normally only the head is eaten. The head is composed of a white inflorescence meristem. Cauliflower is an excellent source of vitamin B6, vitamin C, vitamin K, pantothenic acid and folate. It is a source of omega-3 fatty acids, biotin, phosphorus, and manganese. Furthermore, it is a good source of vitamin B1, B2, and B3, the

minerals potassium and magnesium, and protein. Raw white cauliflower provides calories, low in fat, carbohydrates, dietary fiber and protein.

It contains sulforaphane, a sulfur compound that has also been shown to kill cancer stem cells, thereby slowing tumor growth. Some researchers believe eliminating cancer stem cells may be key to controlling cancer. It also contains a wealth of anti-inflammatory nutrients to help keep inflammation in check, including indole-3-carbinol or I3C, an anti-inflammatory compound that may operate at the genetic level to help prevent the inflammatory responses at its foundational level. Cauliflower is a good source of choline, a B vitamin known for its role in brain development. Choline intake during pregnancy "super-charged" the brain activity of animals in utero, indicating that it may boost cognitive function, and improve learning and memory. It may even diminish age-related memory decline and your brain's vulnerability to toxins during childhood, as well as conferring protection later in life. For the warm and humid climatic condition of the country, increase use of modern high yielding varieties of crops. Also more use of chemical fertilizers is highly favorable for development and multiplication of pests and diseases. In Bangladesh circumstances, the cauliflower growers have been using pesticides frequently to have the higher yield. But the overdoses of pesticides make the residue problem, which might pollute our food and be harmful for our health. Cauliflower is also very susceptible to insect infestation like other vegetables. The main insects of cauliflower are diamond back moth, leaf webber, stem borer, tobacco caterpillar, aphids, painted bug etc.

Bitter melon (*Momordica charantia* L.) is an important vegetable. It is commonly called bitter in Bangladesh, but it is locally known as 'korola', 'ussay' etc. Bitter melon fruits are a good source of carbohydrates, proteins, vitamins, and minerals and have the highest nutritive value among cucurbits. Moreover, the crude protein content (11.42 g kg⁻¹) of bitter melon fruits is higher than that of tomato and cucumber (Gilden et al., 2010). The vitamin C content of Chinese bitter melon 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 melon (Kale et al. 1991; Yuwai et al. 1991). Moreover, the crude protein content (11.420.9g) of kg⁻¹ bitter melon fruits is higher than that of tomato and cucumber (Xiang et al., 2000).

This crop is attacked by many insect pests, among these Red Pumpkin Beetle and Fruit fly are the major insect pests. 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, *et al.*, 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.

It is undoubtedly true that a negative economic impact on the production of vegetables is occurred by the insect pests and diseases. Due to plant pests and diseases 20 to 40 percent crop yields are reduced globally, besides the world will need to produce 60 percent more food for the over increasing world population by 2050 (FAO, 2012). To ensure this demand control of insect pests and diseases plays a key role. Till to date for the control of insect pests and diseases pesticides plays a vital role. It is studied that in advanced countries, the damage is less as compare to the developing countries. There is no doubt that due to the use of pesticides, production is increased day by day. Due to this reason, the pesticides are come in market on large scale. And their adverse effects are also increasing as their use is increased. In many developing countries like Bangladesh, most of the farmers apply pesticide without knowing its actual requirements and/or effectiveness, and thus they apply very high frequencies of pesticides in a cropping season. For example, farmers spray pesticides 140 times during a cropping season of 180-200 days and 150 sprays in a crop season in cauliflower. More than 90% farmers of Bangladesh use pesticide unnecessarily, indiscriminately and excessively due to their ignorance and unconsciousness about the use (Anonymous, 2000). According to Pesticides Association of Bangladesh (2002-2003), pesticide use for growing vegetables was six times higher than the rice cultivation (1.12 kg/ha for vegetables while it was only 0.20 kg/ha in rice). Furthermore, farmers spray their vegetables 17-150 times per crop cycle. According to DAE, about 95 percent farmers do not wait for pre-harvest interval (PHI) after application of pesticides. The effective and responsible uses of pesticides bring significant positive contributions to the crop production. However, their irrational and unprotected uses are threatening our ecosystem, health and environment. Pesticide

residues are the deposits of active ingredient, its metabolites or breakdown products present in some component of the environment after its application, spillage or dumping. The applied chemicals and/or their degradation products may remain as residues in the agricultural products, which becomes a concern for human exposure (Islam *et al.*, 2019). In Bangladesh, farmers have no idea about pesticide residues in the food as well as their ill effect on human health and the environment. In Bangladesh, whimsical spray of insecticides and selling of vegetables after one to two days of spray application are assumed to be a normal practice. No insecticide is available, whose retention period which is less than three to five days (Rahman 1999). The present pattern of pesticide usage in Bangladesh particularly in vegetables led to assume that majority of marketed vegetables contain pesticide residue more than Maximum Residue Limit (Kabir, 2007).

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

Now pesticide residue in food has become a consumer's safety issue and the consumers have the right to know how much pesticide get incorporated in the food they eat. Up until now, in Bangladesh several research works on pesticide residues in vegetables and other matrices have been conducted in pesticide Analytical Laboratory, Entomology Division of Bangladesh Agricultural Research Institute (BARI), Gazipur (Prodhan *et al.*, 2018; Aktar *et al.*, 2017; Hasan *et al.*, 2017; Kabir *et al.*, 2007; Islam *et al.*, 2014; Kabir *et al.*, 2008; Prodhan *et al.*, 2009; Prodhan *et al.*, 2010). However, there is no study available on the quantification of pesticide residues

in the major vegetables collected from Patuakhali district of Bangladesh. Therefore, it is essential to find out the actual scenario of pesticide residues remain in vegetables collected from Patuakhali district. Keeping this view, the present study was initiated with the following objectives:

OBJECTIVES:

- To identify pesticide residues in cauliflower and bitter gourd collected from different markets of Patuakhali district.
- To quantify the levels of detected pesticide residues (mg/kg) remain in the selected vegetables collected from different markets of Patuakhali district.
- To compare whether the level of detected pesticide residues is above the maximum residue limit (MRL) or not.

CHAPTER II

REVIEW OF LITERATURE

In this chapter challenge has been made to assess literatures for updating the information regarding the existing status of research and knowledge about the determination of pesticide residues in 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 has 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 pesticides residues in vegetable crops conducted under farmers' field conditions are available.

2.1 Pesticides

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (EPA, 2006). The term pesticide includes herbicide, insecticide, insect growth regulator, nematocide, 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 Classification of Pesticides

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 Organocarbamate 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 are a 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 neonicotinoid 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

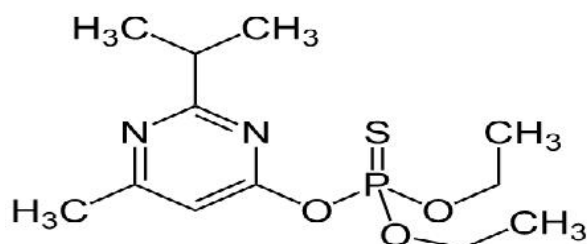


Fig:Structural formula of Diazinon

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 chemical company (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. Dimethoate

Dimethoate is a widely used organophosphate pesticide. Like other organophosphates, dimethoate is an acetylcholinesterase inhibitor which disables cholinesterase, an enzyme essential for central nervous system function. It is a contact insecticide and also acts through ingestion. It is readily absorbed and distributed throughout plant tissues, and is degraded relatively rapidly (Dauterman *et al.*, 1960).

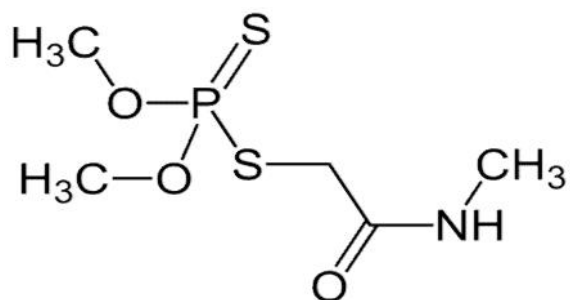


Fig: Structural formula of Dimethoate

The CAS No. of dimethoate is 60-51-5 and the IUPAC name is O, O-dimethyl 9-[2-(methylamino)-2-oxoethyl] dithiophosphate (EPA 2006). Dimethoate is a colorless crystalline solid with a camphor-like (mercaptan) odor (Worthing, 1987). It will breakdown rapidly when heated to temperatures above 80° C. creating the possibility of explosion. It should never be heated above 35 degrees C. Thermal decomposition may release hazardous and toxic fumes of dimethylsulfide, methyl mercaptane, carbon monoxide, carbon dioxide, phosphorus pentoxide and nitrogen oxides (Meister, 1992). Dimethoate is possibly carcinogenic (Hayes, 1982; Hallenbeck and Cunningham, 1985). An increase in mammary tumors was reported in rats given oral doses of 511 or 30 mg/kg dimethoate for 511 to 627 days (Hayes and Laws, 1990). Dimethoate is

available in aerosol spray, dust, emulsifiable concentrate and ULV concentrate formulations (Hayes and Laws, 1990; Meister, 1992).

2.3.3 Fenitrothion

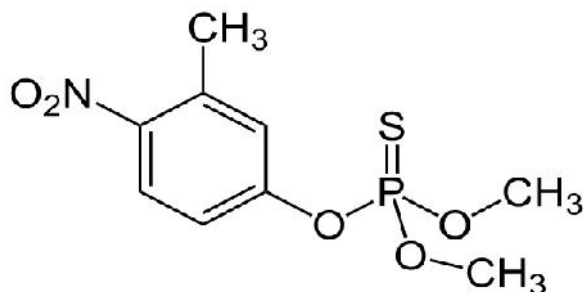


Fig: Structural formula of Fenitrothion

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).

2.3.4 Quinalphos

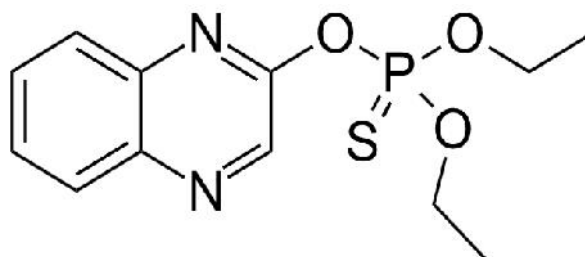


Fig: Structural formula of Quinalphos

Quinalphos (IUPAC name: O, O-Diethyl O-2-quinoxaliny phosphorothioate) 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.

2.3.5 Chlorpyrifos

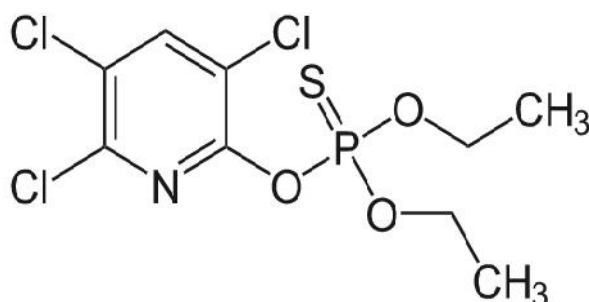


Fig: Structural formula of Chlorpyrifos

Chlorpyrifos (IUPAC name: O, O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) is a crystalline organophosphate insecticide, acaricide and miticide (EPA 2006). 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 cause overstimulation of the neuronal cells, which leads to neurotoxicity and eventually death. (Karanth and Pope, 2000). 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.

2.3.6 Acephate

Acephate is an organophosphate insecticide. It is a general-use insecticide registered for use on cereals, pulses, vegetables and other crops, agricultural seed and non-bearing plants, horticultural nursery plants, commercial infrastructures and institutions including municipal health facilities, golf course turf, ant mounds etc. (EPA 2006).

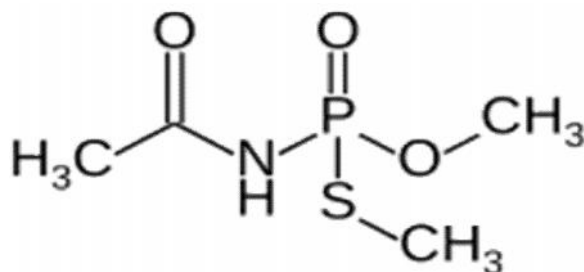


Fig: Structural formula of Acephate

IUPAC name of acephate is O, S-Dimethyl acetylphosphoramidothioate2, and the Chemical Abstracts Service (CAS) registry number is 30560-19-1. Molecular weight is 183.16 g/mol and the solubility (water) is 79 - 83.5 g/100 ml (Davy *et al.*, 2007). In soil, plants, and insects, acephate is converted to methamidophos. Methamidophos is another organophosphate insecticide that is registered by the U.S. EPA. Methamidophos inhibits acetylcholinesterase through phosphorylation (Farag *et al.*, 2000). Acetylcholine is the prominent insect stimulatory neurotransmitter for motor, sensory, and intermediate neurons (Chapman *et al.*, 1998) and is broken down by acetylcholinesterase (Smith and Treheme, 1965). Organophosphates cause acetylcholine levels to increase and over-excite target nerves, muscles or tissues (Reigart and Roberts, 1999).

Acephate is actually a systemic insecticide specially used to control sucking and biting insects by direct contact or ingestion (Tomlin, 2006; Thomson, 1989). Like other organophosphates acephate also bind to and inhibit the enzyme acetylcholinesterase (AChE) in nervous system tissues. Therefore, the

neurotransmitter acetylcholine accumulates and constantly activates cholinergic receptors (Klaassen, 2001; Reigart and Robert, 1999). Acephate itself is a weak acetylcholinesterase inhibitor (Chuck *et al.*, 1984). Methamidophos is a more potent organophosphate than acephate (Reigart and Robert, 1999). Insects metabolize acephate into methamidophos by hydrolysis, whereas mammals metabolize acephate more readily into des- O-methylacephate, accounting for acephate's comparatively high selectivity against insects (Farang *et al.*, 2000; Mahajna *et al.*, 1997).

2.3.7. Malathion

Malathion is a non-systemic, wide-spectrum organophosphorus insecticide. It is a widely used insecticide in agriculture, residential landscaping, community recreation areas, and in municipal health pest control programs such as mosquito eradication. In the U.S. it is the most commonly and widely used organophosphate insecticide (Bonner *et al.*, 2007).

The Chemical Abstracts Service (CAS) registry number is 121-75-5 and the International Union of Pure and Applied Chemistry (IUPAC) name for malathion is O, O-dimethyl dithiophosphate of diethyl mercaptosuccinate. Malathion is a colorless to amber liquid with a skunk- or garlic-like odor (U. S. DHHS, 2008). The vapor pressure of malathion is 1.78×10^{-4} mmHg at 25°C or 5.3 mPa at 30°C and also 1.2×10^{-4} to 8×10^{-6} mmHg at 20 °C (HSDB, US. DHHS, 2008; Tomlin, 2006; Hornsby *et al.*, 1996), the molecular weight is about 330.4 g/mol (Tomlin, 2006) and the solubility (water) is 145 mg/T (Tomlin, 2006).

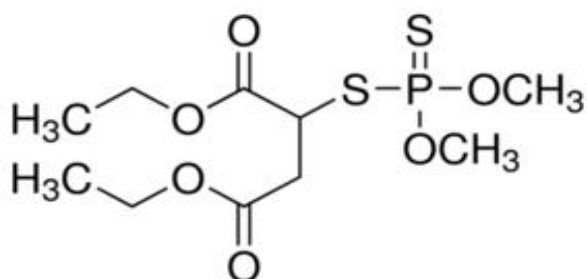


Fig:Structural formula of Malathion

Malathion is a broad-spectrum insecticide used to control a variety of outdoor insects in both agricultural and residential sectors. Malathion is registered for use on food, feed, and ornamental crops and for household use mosquito, boll weevil and fruit fly

eradication programs (EPA, 2006). Malathion is also an ingredient in shampoos regulated by the United States Food and Drug Administration (FDA) to control head lice (EPA, 2006). Malathion is toxic via skin contact, ingestion, and inhalation exposure (Tomlin, 2006). The International Agency for Research on Cancer (IARC) concluded in 1987 that the carcinogenic potential of malathion was not classifiable, and placed it in Group 3 (IARC, 1998).

2.4 Instrumental Techniques for Pesticide Residue Determination

For the determination of pesticide residues in foods different instrumental techniques are used. Gas Chromatography (GC), Gas Chromatography associated with Mass Spectrometry (GC-MS), 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 *et al.* 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 and Selim 2013; Chauhan *et al.*, 2012; Hadian *et al.*, 2008; Chandra *et al.* 2010; 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 (eg. during the production process of pharmaceutical and biological products), legal (eg. detecting performance enhancement drugs in urine), research (eg. separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (eg. 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 CO₂, as it has relatively low critical temperature (31⁰ C) and low critical pressure (73 kPa) (Atkins and 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 CO₂ 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 CO₂ will be able to extract analytes of low polarity at low pressure, and high polarity analytes at high pressure. SFE with CO₂ is usually performed at pressures that are not high enough to achieve efficient extraction of polar compounds. In such conditions, the supercritical CO₂ 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 CO₂ 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 CO₂, supercritical N₂O 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 (-NH₂) 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 (MgSiO₃), aluminum oxide (Al₂O₃) and silica (SiO₂) 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 200⁰ C) 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) Extraction 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 MgSO₄ 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 MgSO₄ 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 MgSO₄ 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; Drozdzy ski and Kowalska, 2009; Lehotay *et al.*, 2005, 2005a, 2005b; Paramasivam & chandrasekaran, 2012; 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 and 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 and Chan, 2010) and 14 OCPs in apricot, plum, cherry, nectarine, pear and apple (Cielik *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), azadirachtin, spinosad, rotenone (Drozdyski and Kowalska, 2009), oxadiazyl (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. Pesticide Residue

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. Acceptable Daily Intake or ADI

Acceptable daily intake or ADI is a measure of the amount of a specific substance (originally applied for a food additive, residue of a veterinary drug or pesticide) in food or drinking water that can be ingested (orally) on a daily basis over a lifetime without an appreciable health risk (WHO, 1987). ADIs are expressed usually in milligrams (of the substance) per kilograms of body weight per day (Frank and Kacew, 2002; Faustman and Omenn, 2001).

2.7.2. Maximum Residue Level (MRL)

A maximum residue level (MRL) is the highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly (Following Good Agricultural Practice) (European commission). Many countries have their own set of MRL's. MRL's set by European commission and Codex are recognized worldwide.

2.7.3 Determination of Pesticide Residues in Food

To up-date our knowledge regarding the current status of research and information on the insecticide residues remain in farm gate and markets samples of cauliflower and bitter gourd, the level of detected insecticides residues above the Maximum Residue Limit (MRL) or not etc., at home and abroad an effort has been made to review the

available literatures. Although the review could not be made so comprehensive due to limited scope and facility, it is hoped that most of the relevant information available in and around Bangladesh was collected and reviewed. It is revealed that most of the information on the aspects searched as mentioned above are mostly available from research station and information of farmers' field condition are scanty. However, a significant number of study-reports on insecticides residues in vegetable crops conducted under farmers' field conditions are available. The studies on the quantification of detected insecticides residues below or above the Maximum Residue Limit (MRL) of cauliflower and bitter melon in Bangladesh are rarely reported. With this background, the information collected from different sources have been reviewed and presented below:

Nahar *et al.* (2020) conducted a study to assess the health hazards associated with the residual effect of pesticides in two common vegetables (cauliflower and tomato) collected from five different markets of a northern city of Bangladesh. A total of 80 samples (i.e. 40 of each vegetable) were collected for the analysis of seven major organophosphorus insecticides namely acephate, chlorpyrifos, diazinon, dimethoate, fenitrothion, malathion and quinalphos used in that region. Modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction technique and GC-FTD (Gas Chromatography coupled with Flame Thermionic Detector) used for the analysis of the samples. Results indicate that 11 (6 cauliflower, 5 tomato) analyzed samples contained residues which is about 14% of the total number of samples. Most of the samples contaminated diazinon at a level above EU-MRLs. However, health risk assessment based on ADI, the contaminated samples were safe. Continuous monitoring together with a sample traceability system is suggested to protect consumers' health from the cumulative effects of other contaminated dietary products.

Collimore *et al.* (2020) conducted a study following newly modified QuEChERS method for the multiclass, multiresidue determination of organophosphate (OPP) and organochlorine pesticide (OCP) residues in fruits and vegetables. The method incorporated a solvent extraction with acetonitrile followed by partitioning with magnesium sulphate (MgSO₄) and sodium chloride (NaCl). The final step involved a dispersive solid phase extraction (d-SPE) clean-up prior to gas chromatography with electron capture detection (GC-ECD) analysis. Apples and lettuce were the selected matrices for fruits and vegetables, respectively in the method development stages.

Various combinations of sorbents were tested in the clean-up step. Florisil and MgSO₄ (FM) d-SPE proved to be the best combination for the clean-up step for both OCPs and OPPs in both the fruit and vegetable matrices. Recovery values fell within the acceptable range of 70 to 120% (RSD = 20%). The new method is unique as it employs the standard addition calibration technique for the quantification of OCP and OPP residues. The newly modified QuEChERS method provides a cheaper alternative for the analysis of pesticide residues in fruits and vegetables as samples can be analyzed at a fraction of the cost of the original QuEChERS method. The efficacy of the method was tested on several fruits and vegetables from the Central Division of Trinidad and Tobago. OCP and OPP residues were found in 61% of the samples, most of which were above the maximum residue limits.

Bempah et al. (2020) has been conducted a study to investigate the organochlorine, organophosphorus and synthetic pyrethroid pesticide residues in fruits and vegetables from different markets in Ghana. For this purpose, a total of 309 fruits and vegetable samples were collected and analyzed by gas chromatography with electron capture detector. The obtained results showed that the predominance of organochlorine followed by organophosphorus and synthetic pyrethroid pesticides in most of the analyzed samples. The detected concentrations of them were most significant in vegetable samples. The results obtained showed that 39.2 % of the fruits and vegetable samples analyzed contained no detectable level of the monitored pesticides, 51.0 % of the samples gave results with trace levels of pesticide residues below the maximum residue limit (MRL), while 9.8 % of the samples were above the MRL. The findings point to the urgent need to establish reliable monitoring programs for pesticides, so that any exceedance in concentration over environmental quality standards can be detected and appropriate actions taken.

Luo *et al.* (2020) initiated a research programme on the determination, residue analysis, risk assessment and processing factors of tebufenozide in okra fruits under field conditions. A simple LC-MS/MS method was established and validated for determining the tebufenozide residues in okra fruits. The recoveries of tebufenozide in okra fruits were > 72% with relative standard deviations of 0.6%-6.1%. The dissipation rates of tebufenozide were different in okra fruits cultivated under open land and greenhouse field conditions because of the discriminating humidity and temperature conditions. The dietary intake of the tebufenozide residues from okra

fruit consumption for Chinese consumers was fairly low, with approximately no potential health risk. The processing factor values of washing, blanching and soaking were all.

Ye Shao *et al.* (2020) conducted a study following modified QuEChERS protocol with a nitrogen-doped graphitized carbon derived from dicyandiamide sludge as a clean-up sorbent to determine 20 organochlorine pesticides in tomatoes using gas chromatography coupled to a triple quadruple mass analyzer. The modified protocol exhibited the advantages of environmental friendly and low-cost. The use of dicyandiamide sludge to produce NGB as an adsorbent for QuEChERS opened up a new field for the comprehensive utilization of dicyandiamide waste. Under the optimum conditions, the modified method provided excellent linearity with correlation coefficient higher than 0.9980 and low limits of detection ranging from 0.001 to 0.1 $\mu\text{g}/\text{kg}$. The mean recoveries of the majority of pesticides in tomatoes ranged from 71.2 to 95.3% with relative standard deviation lower than 20%.

Mao *et al.* (2020) carried out a study on the analysis of organophosphorus and pyrethroid pesticides in organic and conventional vegetables using QuEChERS combined with dispersive liquid-liquid micro extraction based on the solidification of floating organic droplet. The key parameters were optimized through orthogonal array experimental design and statistical analysis. The linearity of the calibration curves was satisfied in matrix-matched standard solution with $R^2 = 0.99$. The limits of detection and limits of quantification were 0.3–1.5 and 0.9–4.7 $\mu\text{g}/\text{kg}$, respectively. The average recoveries of pesticides were 61.6–119.4% with relative standard deviations $< 16.1\%$. Furthermore, the method was applied successfully to analyze the pesticides in 15 pairs of organic and conventional vegetables. These results reflect the efficiency, reliability and robustness of the developed method.

Loha *et al.* (2020) carried out a study to determine the level of residues of seven pesticides such as profenofos, metalaxyl, λ -cyhalothrin, 4,4'-DDT, 4,4'-DDE, and γ - and δ -endosulfan in vegetables (tomato, onion) from 20 locations and surface waters from 12 locations in the Central Rift Valley (CRV) of Ethiopia. The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) and solid phase extraction (SPE) methods were used for the vegetables and water, respectively. In 2.5% of the tomato samples, profenofos was detected above European maximum residue limits (MRLs),

in 12.5% of the samples metalaxyl, and in 2.5% - and -endosulfan. In 5% of the onion samples, profenofos was detected above European MRLs, in 7.5% of the onion samples metalaxyl, and in 5% -cyhalothrin. In surface water, profenofos was detected at the highest concentration of 2300 µg/L in the Bulbula River, 890 µg/L near the agricultural land north of Lake Ziway (ANLZ-1), 1700 µg/L in the floriculture effluent (FE-1), and 900 µg/L in tap water at the Batu Drinking Water (BDW) supply. These results show that the levels of pesticides are in several cases substantially elevated, and emphasize the need of regular pesticide monitoring programs for surface waters and vegetables in the Ethiopian CRV.

Alnedhary *et al.* (2020) undertook a research on the optimization and efficiency comparison of is dispersive and cartridge solid phase extraction cleanup techniques in the analysis of pesticide residues in some vegetables using Gas chromatography-Mass Spectrometry. The method applied for the analysis of four pesticides of different classes; dimethoate (Organophosphorus), fenvalerate (Pyrethroid), difenoconazole (Triazole) and deltamethrin (Pyrethroid) on four types of vegetables (i.e. tomato, potato, cucumber, and carrot). The procedures simply involve the use of acetonitrile containing 1% acetic acid for the extraction, and for cleanup; a manually prepared solid-phase extraction cartridge containing primary secondary amine (PSA) and normal charcoal were used. The validated GC-MS analysis method for the pesticide residues in the selected vegetables has high linearity with R² ranged from 0.9965 to 0.9999. The precision of the method estimated as relative standard deviation (%RSD) was 9.4% for all target pesticides which were indicative of the high repeatability of the optimized method. The accuracy calculated as average recoveries (%R) was between 80.52% and 99.63%. LODs for target pesticides in spiked cucumber, tomato, carrot, and potato samples ranged between 0.0950 and 0.5590 ng/g. The combined sample preparation method is cost-effective and has shown good simplification, recovery and cleanup capacity and proved to be efficient and suitable for the proposed application.

Regassa *et al.* (2020) developed a research programme on the determination of residue levels of DDT and its metabolites in khat and cabbage samples using QuEChERS sample preparation method combined with GC-MS detection. Parameters that primarily affect the extraction efficiency of the analytes were optimized. The significance of the use of cleanup was investigated and its optimum amount was

found to be 6 mg PSA and 12 mg C18. The optimum values for acetonitrile volume and pH of the sample was found to be 3 mL and 7, respectively. The linearity of the analytical response was acceptable with correlation coefficients of 0.992 or better. The precision associated with the analytical method, expressed as %RSD were lower than 8.6 and 9.1% for the intraday and interday precision, respectively. The limit of detection (LOD) and limit of quantification (LOQ) of the proposed method for cabbage sample were in the range of 2×10^{-5} – 4×10^{-5} mg/kg and 9×10^{-5} – 14×10^{-5} mg/kg, respectively. The LOD and LOQ of the proposed method for khat sample were in the range of 2×10^{-5} – 6×10^{-5} mg/kg and 7×10^{-5} – 19×10^{-5} mg/kg, respectively. The recoveries of the method were ranging from 97.16 to 107.99 for cabbage and 72.1 to 90.55 for khat sample. The analytical applications of this method indicated the presence of p, p'-DDE, p, p'-DDD and p, p'-DDT in both cabbage and khat samples. The amount of p, p'-DDE, p, p'-DDD and p, p'-DDT in cabbage were found to be 0.004, 0.01 and 0.01 mg/kg, respectively. The amount of p, p'-DDE, p, p'-DDD and p, p'-DDT in khat were found to be 0.01, 0.03 and 0.07 mg/kg, respectively. The results indicate that there should be continuous monitoring of DDT and its metabolites residues in cabbage and khat samples which in turn is helpful to assess the potential risk of the residues to consumers' health.

Ramadan *et al.* (2020) conducted a research on the evaluation of pesticide residues in vegetables from the Asir Region, Saudi Arabia. This study's aim was to determine pesticide residues in 10 different vegetable commodities from the Asir region, Saudi Arabia. They evaluated 211 vegetable samples, collected from supermarkets between March 2018 and September 2018, for a total of 80 different pesticides using ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) and gas chromatography–tandem mass spectrometry (GC-MS/MS) after extraction with a multi-residue method (the QuEChERS method). The results were assessed according to the maximum residue limit (MRL) provided by European regulations for each pesticide in each commodity. All lettuce, cauliflower, and carrot samples were found to be free from pesticide residues. A total of 145 samples (68.7%) contained detectable pesticide residues at or lower than MRLs, and 44 samples (20.9%) contained detectable pesticide residues above MRLs. MRL values were exceeded most often in chili pepper (14 samples) and cucumber (10 samples). Methyl, imidacloprid, metalaxyl, and cyproconazole were the most frequently detected

pesticides. Based on the results of this study, we recommend that a government-supported program for the monitoring of pesticide residues in vegetables be established to promote consumers' health and achieve sustainable farming systems.

Polat *et al.* (2019) evaluated a study on the analysis of the pesticide residues in bitter gourd using modified QuEChERS extraction coupled with Gas Chromatography. This study was undertaken to monitor the presence of seven organophosphorous pesticide residues like acephate, dimethoate, fenitrothion, chlorpyrifos, quinalphos, diazinon and malathion in bitter gourd. 65 samples were collected from retail markets located at the adjacent area of Jahangirnagar University, Savar, Dhaka, Bangladesh namely Genda bazaar, Savar bazaar, Nayarhat bazaar, Islampur bazaar, Pallibiddut bazaar, Baipayl bazaar and Sreepur bazaar. The samples were extracted by modified quick, easy, cheap, effective, rugged and safe (QuEChERS) method and analyzed by gas chromatography coupled with flame thermo ionic detector (GC-FTD). Among the 65 analyzed samples, eight (12.3% of the total number of samples) were contaminated with pesticide residues and all of them contained residues above Maximum Residue Limit (MRL) set by European Commission (EC). Another fifty-seven samples (87.7% of the total number of samples) contained no detectable pesticide residues of the sought pesticides. The findings from this current study showed the common scenario of pesticide residues in daily consumed vegetables of Savar, Dhaka, Bangladesh that pointed to the imminent health hazards.

Tankiewicz (2019) carried out a study on the determination of selected priority pesticides in high water fruits and vegetables by modified QuEChERS and GC-ECD with GC-MS/MS confirmation. Chosen compounds are commonly detected in fruit and vegetable crops, and some of their metabolites have even been found in human urine. In addition, some of them are known or suspected carcinogens according to the International Agency for Research of Cancer. Extraction and clean up parameters were optimized, thus the original QuEChERS method was modified to decrease solvent usage, in accordance with 'green chemistry' principles. The proposed methodology was validated in terms of selectivity, specificity, linearity, precision and accuracy. The obtained limits of detection (LODs) for all investigated pesticides ranged from 5.6 µg/kg to 15 µg/kg and limits of quantification (LOQs) from 17 µg/kg to 45 µg/kg. The obtained data demonstrated the good reproducibility and stability of the procedure in the tested concentration range up to 10 µg/kg, with relative standard

deviations (RSDs) lower than 10%. Recoveries for spiked pear samples at LOQ level for each pesticide were from 90% to 107% with RSDs lower than 9.6%.

Rani *et al.* (2019) developed a domestic method for the removal of pesticide residues in chilies. Normally available chilies in the market are treated with five pesticides which includes Bifenthrin, Deltamethrin, Hexaconazole, Lambda cyhalothrin and Profenophos and the treated sample was subjected to four different household methods for removal of residues like T1 (Running Tap water wash, T2 (Boiling for 10 min), T3 (Soaking in 2% salt solution for 10 min), T4 (Soaking in 2% salt solution for 10 min+ boiling for 10 min). The residues present in the sample were analysed by using QTOF GC/MS instrument. Among the four methods 2% salt solution + boiling method had greatest residue removal effect for Deltamethrin (72.90%) to Hexaconazole (37.23%).

Islam *et al.* (2019) carried out a study for the determination of major organophosphate insecticide residues in cabbage samples from different markets of Dhaka. The study was conducted to analyse 4 organophosphorus pesticide namely Chlorpyrifos, Diazinon, Fenitrothion and Quinalphos residues in cabbage. Between September 2016 and March 2017, 50 Cabbage samples were collected from 5 vegetables markets of Dhaka city, namely Rampura kacha bazar, Jatrabari krishi market, Kawran Bazar, Taltola Bazar and Mohammadpur Krishi Market. The collected samples were extracted and analyzed by QuEChERS based Gas Chromatography coupled with Flame Thermionic Detector (GC-FTD) method. Total 6 samples (12%) contained pesticide residues and interestingly all of them were above the MRLs set by EC. Among the four organophosphorus insecticides tested only Chlorpyrifos and Diazinon were detected above the MRLs. This research represents a snapshot situation of contamination of pesticides in one of the common winter vegetables available in Dhaka City's local markets linked to consumer safety.

Islam *et al.* (2019a) initiated a study for the analysis of pesticide residues in bitter gourd using modified QuEChERS extraction coupled with Gas Chromatography. This study was undertaken to monitor the presence of seven organophosphorous pesticide residues like acephate, dimethoate, fenitrothion, chlorpyrifos, quinalphos, diazinon and malathion in bitter gourd. 65 samples were collected from retail markets located at the adjacent area of Jahangirnagar University, Savar, Dhaka, Bangladesh namely

Genda bazaar, Savar bazaar, Nayarhat bazaar, Islampur bazaar, Pallibiddut bazaar, Baipayl bazaar and Sreepur bazaar. The samples were extracted by modified quick, easy, cheap, effective, rugged and safe (QuEChERS) method and analyzed by gas chromatography coupled with flame thermo ionic detector (GC-FTD). Among the 65 analyzed samples, eight (12.3% of the total number of samples) were contaminated with pesticide residues and all of them contained residues above Maximum Residue Limit (MRL) set by European Commission (EC). Another fifty-seven samples (87.7% of the total number of samples) contained no detectable pesticide residues of the sought pesticides. The findings from this current study showed the common scenario of pesticide residues in daily consumed vegetables of Savar, Dhaka, Bangladesh that pointed to the imminent health hazards. Therefore, it is suggested to control the overuse of pesticide in vegetable field strictly as well as to increase the awareness of the harmful effect of pesticide residues in vegetables for the growers and the consumers as well.

Islam *et al.*(2019b) established a method for the determination of major organophosphorus pesticide residues in eggplant using modified QuEChERS Extraction and Gas Chromatography. This investigation was undertaken to scrutinize the entity of seven Organophosphorus pesticide residues like acephate, dimethoate, fenitrothion, chlorpyrifos, quinalphos, diazinon and malathion in eggplant. Seventy eight eggplant samples were collected from retail markets located at the surrounding area of Jahangirnagar University, Savar, Dhaka, Bangladesh namely Genda bazaar, Savar bazaar, Nayarhat bazaar, Islampur bazaar, Pallibiddut bazar, Baipayl bazaar and Sreepur bazaar. The samples were extracted by modified quick, easy, cheap, effective, rugged and safe (QuEChERS) method and analyzed by Gas Chromatography coupled with Flame Thermionic Detector (GC-FTD). Among the seventy eight analyzed samples, nine (11.5%) were contaminated by pesticide residues. Two of them were exceeded the EU-MRL (EC, 2015). Another sixty nine samples (88.5%) were free from the contamination of the sought pesticides. The findings from this current study showed the subsistence of pesticide residues in daily consumed vegetables of Savar, Dhaka, Bangladesh that pointed to the imminent health hazards. So, public awareness about the pesticides and other related matter should be increased for practicing a pesticide free agriculture as well as gain contaminate free environment.

Tankiewicz et al. (2019) has been developed a modified quick, easy, cheap, efficient, rugged and safe (QuEChERS) method coupled to gas chromatography with electron capture detector (GC-ECD) for simultaneous determination of selected electronegative pesticides in fruits and vegetables with high water content. The chosen compounds are commonly detected in fruit and vegetable crops, and some of their metabolites have even been found in human urine. Extraction and clean up parameters were optimized, thus the original QuEChERS method was modified to decrease solvent usage, in accordance with 'green chemistry' principles. The proposed methodology was validated in terms of selectivity, specificity, linearity, precision and accuracy. The obtained limits of detection (LODs) for all investigated pesticides ranged from 5.6 $\mu\text{g kg}^{-1}$ to 15 $\mu\text{g kg}^{-1}$ and limits of quantification (LOQs) from 17 $\mu\text{g kg}^{-1}$ to 45 $\mu\text{g kg}^{-1}$. The obtained data demonstrated the good reproducibility and stability of the procedure in the tested concentration range up to 10 mg kg^{-1} , with relative standard deviations (RSDs) lower than 10%. Recoveries for spiked pear samples at LOQ level for each pesticide were from 90% to 107% with RSDs lower than 9.6%. The suitability of the developed procedure was tested on various fruit and vegetable samples available on the market at different seasons. The proposed methodology is applicable for detection and monitoring of selected pesticides not only in fruits and vegetables with high water content, but also in samples containing large amounts of pigments and dyes.

Hadiana *et al.* (2019) studied forty-eight pesticide residues from different chemical structures including organochlorine, organophosphorus, organonitrogen, dicarboximides, strobilurin, triazine, pyrethroids, and other chemical groups. Pesticide residues in 85 fruits and vegetables were determined and confirmed by GC-MS. The pesticide was extracted with ethyl-acetate, then, the extracts cleaned using high performance gel permeation column chromatography (GPC) and solid phase column (SPE). The mean recoveries of the pesticides were between 81 and 136%. The reproducibility of the relative standard deviation values was 2.1% and 14.8%. Pesticide residues were more frequently found in vegetables (65.5%) than in fruits (26.7%). The limits of detection and quantification of pesticide residues for the method were ranged from 0.003 to 0.06 $\mu\text{g/g}$ and between 0.01 to 0.1 $\mu\text{g/g}$ respectively. The analyzed samples did not contain residues from the monitored

pesticides that were higher than the accepted maximum residue limits (MRLs) as adapted by the FAO/WHO Codex alimentarius commission.

Kumari *et al.* (2019) initiated a study to assess the health hazards associated with the presence of pesticide residues in fruits and vegetables sampled from farms and markets of Kinnaur district of Himachal Pradesh (India). Residues of predominant pesticides used in the region, belonging to the group of organophosphates, pyrethroid and phthalimide, were analysed using gas chromatograph quadrupole mass spectrometer (GC-MS/MS). The pesticide extraction from the matrix was done following the modified QuEChERS method. Results indicated varying concentrations of pesticide residue in market and farm samples with farm samples more contaminated than market samples. Chronic health hazards prediction indicated that organophosphorus groups (methyl parathion and triazophos) posed health risk to children in the study area.

Prodhan *et al.* (2018) conducted a research on the variability of pesticide residues in eggplant units collected from a field trial and marketplaces in Greece. In total, 120 samples from a trial field and 142 samples from different marketplaces in Thessaloniki, Greece, were collected to estimate the variability of pesticide residues in eggplant units. They were extracted by the QuEChERS method and the residues were determined by LC-MS/MS. For the field samples, the level of estimated cypermethrin and deltamethrin residues were 0.01-0.349 mg/Kg and 0.01-0.097 mg/Kg, respectively; and the unit-to-unit variability factors (VFs) obtained for cypermethrin and deltamethrin residues were 2.54 and 2.51, respectively. The mean residue levels of both pesticides were higher in the composite samples than in the individual samples. The average VFs for the marketplace samples was 3.89. The eggplant units exposed to pesticides were higher in residues than the non-exposed units.

Prodhan *et al.* (2018a) conducted a study to determine the pre harvest interval (PHI) for quinalphos in Eggplant, Cabbage and Yard long bean; malathion in Eggplant, Yard Long bean and Cauliflower; cypermethrin in Tomato and Yard long bean; and diazinon in Eggplant and Yard long bean depending on Maximum Residue Limit (MRL) set by FAO/WHO. Five supervised field trials were conducted and sprayed with the field dose (2 ml/L of water) of each pesticide except cypermethrin (1 ml/L of

water). Samples were collected at 0, 1, 3, 5, 7, 10, 12, 15 and 18 days after spray. The collected samples were analyzed using Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD) and Electron Capture Detector (ECD) for the determination of pesticide residues. The level of residues were above MRL up to 10 DAS for quinalphos in Cabbage, 7 DAS in Eggplant, 5 DAS in Yard long bean; for malathion 5 DAS in Yard long bean and Eggplant, 7 DAS in cauliflower; for diazinon 5 DAS in Yard long bean and Eggplant; and 3 DAS for cypermethrin in Yard long bean and Tomato. The determined PHI for quinalphos were 12 DAS in Cabbage and 10 DAS in Eggplant and 7 DAS in Yard long bean; For malathion 7 DAS in Yard long bean and Eggplant and 10 DAS in cauliflower; For diazinon 7 DAS in Yard long bean and Eggplant; For cypermethrin 5 DAS in Yard long bean and Tomato.

Prodhan *et al.* (2018b) conducted a study to quantify the residue loss of Quinalphos, Diazinon and Fenitrothion from eggplant and Malathion from Yard long bean through washing and cooking procedures. Samples were collected from the Research field of Entomology Division of Bangladesh Agricultural Research Institute (BARI). The samples were analyzed using a simple Gas Chromatographic technique. Washing with water reduced 34% Quinalphos, 28% Diazinon and 41% Fenitrothion and heating with water at 100 °C reduced 95% Quinalphos, 84% Diazinon and 100% Fenitrothion from eggplant. Washing with water reduced 45% Malathion and heating with water at 100 °C reduced 100% Malathion from Yard long bean. Effect of O₃ sterilizer in reducing pesticide residues from eggplant was also investigated in this study and found that O₃ sterilizer reduced 79.00% Diazinon and 62.50% Quinalphos while washing with only water reduced 60.50% Diazinon and 40.00% Quinalphos from eggplant.

Prodhan *et al.* (2018c) has been developed and validated a simple and efficient multiple organochlorine pesticide residues analytical method using quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction technique and Gas Chromatography coupled with Electron Capture Detector (ECD) for the determination of 19 organochlorine pesticides (Alpha BHC, Delta BHC, Beta BHC, Gama BHC, Heptachlor, Aldrin, Heptachlor Epoxide, Gama Chlordane, Alpha Chlordane, Alpha Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, Beta Endosulfan, 4,4 DDT, Endrin Aldehyde, Endosulfan sulphate, Methoxychlor, and Endrin Ketone) in shrimp. The method was validated by evaluating the accuracy, precision and linearity limit of

detection (LOD) and limit of quantification (LOQ). The average recoveries of the selected pesticides ranged from 84% to 106% with RSDr = 14% in four fortification levels of 0.05, 0.1, 0.2 and 0.3 mg/Kg. The linearity was 0.996 for all of the selected pesticides with matrix matched calibration standards. The LOD ranged from 0.003 to 0.009 mg/Kg and the LOQ was 0.05 mg/Kg. This method was applied successfully for the residue analysis of 40 shrimp samples collected from different market places in Bangladesh.

Lawal *et al.* (2018) have been used modified QuEChERS dispersive solid phase extraction technique coupled with ionic liquid-based dispersive liquid-liquid microextraction for the determination of multi-pesticide residues in fruit and vegetable samples. The analysed samples were jackfruit, strawberries, cucumber, pears, and carrots. The resulting linearity range (5–400 µg/kg) and regression coefficient (>0.99) results were satisfactory. The 94.2 and 95.8% accuracy (89–138%) and precision (0–25%) results were satisfactory and within the recommended ranges (20%) and (70–120%), respectively. The limits of detection (0.01–0.54 µg/kg) and quantitation (0.03–1.79 µg/kg) were excellent. The matrix effects (≤–87%) for all analysed samples were not significant. The estimated measurement uncertainties (27%) were within the acceptable range (50%). Justifiably, the response surface methodology optimized instrument and sample treatment techniques were reliable and convenient for multi-pesticide residue determination in various fruits and vegetables.

Nimsha *et al.* (2018) have been determined pesticide residues in locally grown vegetables of tomato, cabbage and capsicum in Puttalam, Dambulla and Nuwara Eliya district in Sri Lanka by Gas Chromatography with Mass Spectrometry after multi residue extraction procedure (QuEChERS method). The QuEChERS method was validated using five pesticides named Diazinon, Chlopyrifos, Fipronil, Prothiofos and Tebuconazole and their retention times in minutes were 15.948, 19.566, 20.342, 22.308, and 26.201 respectively. Coefficient of detection was obtained near 0.99 for all tested standard pesticides confirming the accuracy of the test method. Out of 45 vegetables samples, 15 samples were detected with pesticide residues, either Chlopyrifos, Prothiofos or Tebuconazole. However, pesticide residual values were less than Maximum Residual Levels for all the tested pesticides. Tebuconazole was the mostly detected pesticide residue with 0.128 ppm and 0.052 ppm in tomato and cabbage collected from Matale and Puttalam districts respectively.

Akter *et al.* (2017) conducted a study for the determination of pesticide residues in eggplant collected from different local markets of Mymensingh Sadar, Mymensingh. The collected samples were extracted using modified QuEChERS Extraction and analyzed with Gas chromatography. This study reflects the overall scenario of pesticide residue contamination in eggplant available in the local markets of Mymensingh Sadar, Mymensingh. In this study, a simple and efficient multiple pesticide residue analytical method based on QuEChERS extraction and gas chromatography-flame thermionic detector (GC-FTD) was used for the determination of pesticide residues. Among the 50 analyzed samples, 11 (22% of the total number of the samples) contained residues of diazinon, dimethoate, quinalfos, and chlorpyrifos, of which, 2 had multiple pesticide residues and 5 samples contained residue above the European Union maximum residue limit (EU-MRLs). Chlorpyrifos was detected as the most used pesticide in eggplant in the studied area.

Hasan *et al.* (2017) initiated a study to quantify pesticide residues in country bean collected from different markets of Dhaka city. The collected samples were analyzed using modified QuEChERS Extraction and Gas Chromatography. They have been detected two organophosphorus insecticides (Dimethoate and Quinalphos) in the analyzed country bean samples. Among the 50 analyzed samples of country bean, 10 samples (20%) contained residues of Dimethoate and Quinalphos, of which 5 samples were above the maximum residue limits (MRLs). Most of the contaminated samples (8 samples) contained residue of Dimethoate.

Jallow *et al.* (2017) has been monitored the presence of pesticide residues in primary and derived agricultural products raises serious health concerns for consumers. The aim of this study was to assess the level of pesticide residues in commonly consumed fruits and vegetables in Kuwait. A total of 150 samples of different fresh vegetables and fruits were analyzed for the presence of 34 pesticides using the quick easy cheap effective rugged and safe (QuEChERS) multi-residue extraction, followed by gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS). Pesticide residues above the maximum residue limits (MRL) were detected in 21% of the samples and 79% of the samples had no residues of the pesticides surveyed or contained residues below the MRL. Multiple residues were present in 40% of the samples with two to four pesticides, and four samples were contaminated with more than four pesticide residues.

Mebdoua *et al.* (2017) were analyzed a total of 160 samples of 13 types of fresh fruits and vegetables from domestic production and import to detect the presence of pesticide residues. Analysis was performed by multi-residual extraction followed by gas chromatography–mass spectrometry. In 42.5% of the tested samples, no residues were found and 12.5% of samples contained pesticide residues above maximum residue limits.

Stachniuk *et al.* (2017) analyzed 144 samples (of black currants, red currants, raspberries, cherries, strawberries, blackberries, cauliflowers and broccoli) using LC-MS/MS method for the determination of 60 pesticides. The QuEChERS extraction, matrix-matched calibration and dynamic multiple reaction monitoring method were used. Residues of 15 compounds, mainly fungicides and insecticides, were detected in 46 samples. The percentage of samples with residues above the maximum residue levels (MRL) was 15%, whereas samples with residues below MRL were 17%. A total of 13 samples contained more than one pesticide residue. Pesticide residues were detected most often in samples of black currants (50%), broccoli (36.4%), raspberries (29%) and red currants (21.8%). A most frequently detected pesticide were carbendazim and acetamiprid.

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

Prodhan *et al.* (2016) has been developed and validated a multiresidue analytical method for the determination of pesticide residues in cauliflower collected from different market places in Thessaloniki, Greece. In this study, the liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for the quantification of pesticide residues at trace levels. Among the 120 analyzed samples, 48 (40% of the total no. of samples) were found to have pesticide residues. The detected pesticides were chlorpyrifos, cypermethrin, deltamethrin and indoxacarb.

Prodhan *et al.* (2016a) has been developed and validated a multiresidue analytical method to determine pesticide residues in cabbage collected from different market

places in Thessaloniki, Greece. In this study, the modified QuEChERS extraction in combination to liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for the quantification of pesticide residues at trace levels. 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. Of the detected pesticides, three were insecticides (chlorpyrifos, cypermethrin and deltamethrin) and two were fungicides (fluopicolide and propamocarb hydrochloride).

Prodhan *et al.* (2015) undertook an experiment to determine pesticide residues in 72 fresh eggplant samples collected from different market in Thessaloniki, Greece with Liquid Chromatography-Mass Spectrometry by adopting QuEChERS extraction method. Among the 72 analyzed samples, 34 (47 % of the total number of samples) had pesticide residues, of which, 5 had multiple pesticide residues and 29 had single pesticide residue. Only one sample contained residue above the EU-MRLs (European Union- Maximum Residue Limits).

Prodhan *et al.* (2015a) has been developed and validated a precise and an effective analytical method to determine pesticide residues in melon collected from different market places in Thessaloniki, Greece. In this study, the modified QuEChERS extraction in combination to liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for the quantification of pesticide residues at trace levels. In this study, both insecticides and fungicides have been detected in melon samples. Among the 122 analyzed samples, 32 (26% of the total number of samples) were found to have pesticide residues.

Satpathy *et al.* (2014) conducted a research on the 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 GCMS/MS with Compound Based Screening. They validated the Quick Easy Cheap Effective Rugged and Safe (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 use a mixture of 82 pesticides amenable to gas chromatography (GC) was quantitatively recovered from spiked grapes and pomegranate and determined using gas chromatography tandem mass spectrometry (GC-MS/MS). The method they employed involved initial extraction in a water/ethyl acetate system, an extraction/partitioning step after the

addition of salt, and a cleanup step utilizing dispersive solid-phase extraction (d-SPE); which ensured that it was a rapid, simple and cost-effective procedure. Their method setup was streamlined with the new software approach of Compound Based Scanning (CBS). They found that matrix-matched calibration results have demonstrated good reproducibility, robustness and linearity and spiking levels for the recovery experiments as 0.005, 0.01 and 0.1 mg/kg for GC-MS/MS analyses. They attained adequate pesticide quantification and identity confirmation, even at the lowest concentration levels, considering the high signal-to-noise ratios, the very good accuracies and precisions, as well as the good matches between the observed ion ratios. They found the mean recoveries mostly ranged between 70 and 110 % (91% on average), and RSD were generally below 12% (7.3% on average). The use of analyte standards during GC analysis was demonstrated to provide a good alternative to the use of matrix-matched standards to minimize matrix-effect related errors. For all compounds LODs were 0.001 to 0.005 mg/kg and LOQs were 0.005 to 0.020 mg/kg.

Islam *et al.* (2014) conducted a survey in intensive vegetable growing area in the Narsingdi district of Bangladesh regarding pesticides used by farmers on three major vegetables like eggplant, cauliflower, and country bean. On the basis of questionnaires, 23 farmers were interviewed and it was noted that fourteen pesticides belonging to different groups were found to be commonly used on the selected vegetables by the respondent farmers to control the major pests. In two selected locations of Narsingdi 8.33 to 45.00 percent farmers were recorded to apply different pesticides everyday, in some cases even twice in a day on vegetables. A total of 42 samples were collected from fields and markets and multiple pesticide residue analysis was done by Gas Chromatography (GC) with Flame Thermionized Detector (FTD) and Electron Capture Detector (ECD). Out of 42 samples, 27 had pesticide residue. Among these 27 samples, 14 samples had pesticide residues above the Maximum Residue Limit (MRL). The detected pesticides were Diazinon, Malathion, Quinalphos, Fenitrothion, Cypermethrin, Fenvalerate and Propiconazole.s

Hossain *et al.* (2014) conducted a study to determine the pre harvest interval (PHI) for cypermethrin and acephate in Yard long bean depending on Maximum Residue Limit (MRL) set by FAO/ WHO. Two supervised field trials were conducted and sprayed with the field dose (2 ml/L of water for acephate) and for cypermethrin, it was 1 ml/L of water. Samples were collected at 0, 1, 3, 5, 7, 10, 12, and 15 days after spray. The

collected samples were analyzed using Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD) and Electron Capture Detector (ECD). The level of residues are detected up to 10 DAS for cypermethrin (0.096 mg/kg), and 7 DAS for acephate (0.435 mg/kg), however, the level of detected residues for both of the pesticides were above MRLs up to 5 DAS. Therefore, The PHI was determined for both of the pesticides were 7 DAS.

Akan *et al.* (2013) carried out a research entitled “Organophosphorus pesticide residues in vegetables and soil samples from alau dam and gongulong agricultural areas, Borno State, Nigeria”. They observed concentrations of organophosphorus pesticide residues (dichlorvos, diazinon, chlorpyrifos, and fenitrothion) in some vegetables (spinach, lettuce, cabbage, tomato and onion) in Borno State, Nigeria. They determined concentrations of all the pesticides in the vegetables using GC equipped with electron capture detector (ECD). The highest concentrations of diclorvos, diazinon, chlorpiryfos and fenitrothion 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 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 set acceptable daily intake values (ADIs) for vegetables Codex 2009.

Milhome *et al.* (2013) conducted a research 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 were from 63-117% with RSD lower than 15% in the concentration range of 0.05 0.20mg/kg. They also found the LOQ for most compounds were below the MRLs established in Brazil.

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

Cortea *et al.* (2013) developed a method for the determination of organophosphorus pesticides in vegetables. Pesticide residues were extracted from samples with a small amount of ethyl acetate and anhydrous sodium sulfate. Analysis were 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 μ L 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.

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

Cho *et al.* (2013) carried out a research on the “Evaluation of QuEChERS Method for 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 pesticide 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 0.1 μ g/g and about 90% of the compound had LODs of less than 0.05 μ g/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 work was conducted by Kanda *et al.* (2012) with GC analysis 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) conducted a research work on “Pesticide residue analysis of fruits and vegetables” where they described an efficient and effective analytical method to screen pesticides in fruits and vegetable samples using liquid chromatography tandem mass spectrometry (LC-MS/MS). They used QuEChERS method with acetate buffering (AOAC Official Method 2007.01) for sample preparation, which has been previously shown to yield high-quality results for hundreds of pesticide residues in foods.

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

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

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

Farag *et al.* (2011) conducted an experiment on the ‘‘Monitoring of pesticide residues in some Egyptian herbs, fruits and vegetables’’. They have collected 132 samples offruits, vegetables, herbs and spices from Egyptian local markets and analyzed forpesticide residues. They found that contamination with pesticide residues reached 54.55% while samples free from contamination reached 45.45%. He observed onlyone sample from 132 analyzed samples violated the Maximum Residue Limits (MRLs) of the Codex Committee. From the 132 analyzed samples, 72 samples (54.55%) were contaminated, from which 43.18% contaminated with residues from one pesticide residue, 6.06% with 2 residues and 5.3% with more than 2 residues.

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

Prodhan *et al.* (2010) has been developed an analytical method for the determination of pesticide residues in fish using Gas Chromatogarchy-Mass Spectrometry (GC-MS). A total of 125 samples of fish covering Rui, Carp, Shrimp, White fish, and Fin fish were collected from Dhaka, Khulna and Chittagong region of Bangladesh and carried to the Pesticide Analytical Laboratory (PAL), Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur. All samples were extracted and prepared for injection using the standard protocols for multi-residue analyses during September, 2007 to April, 2008. The elutes were injected into either GCMS-EI or GCMS-NCI depending on the nature of target insecticides. Results revealed that among 125 samples, 49 had insecticide residues. Out of seven samples from Dhaka, 2

had multiple insecticide residues and 1 had single insecticide residue. The detected carbofuran residues ranged from 0.29 to 1.13 ppm, the residues of diazinon, carbaryl and fenvalerate were 1.38, 0.35 and 0.009 ppm, respectively. Out of 60 samples from Chittagong, 8 had multiple insecticide residues and 15 had single insecticide residue. The detected residues of carbofuran, diazinon, fenvalerate, chlorpyrifos, heptachlor and DDT ranged from 0.17-0.89 ppm, 0.03-2.75 ppm, 0.01-0.03 ppm, 0.005 ppm, 0.19-1.15 ppm and 0.06-0.52 ppm, respectively. Out of 58 samples from Khulna, 9 had multiple insecticide residues and 14 had single insecticide residue. The detected residues of carbofuran, diazinon and DDT ranged from 0.03-1.25 ppm, 0.02-1.03 ppm and 0.04-0.12 ppm respectively. Thus 22 samples contained insecticide residue above ADI (Acceptable Daily Intake) referring to average body weight of 50 kg/person, of which 02 samples collected from Dhaka, 10 from Chittagong and 10 from Khulna region.

Afful *et al.* (2010) carried out a study 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, 30m x 0.25mm x 0.25: m. 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.

Charan and Sharma (2010) monitored pesticide residues in a total of 182 samples of six vegetables collected from different agricultural fields of central Aravalli region, India to find out the severity of synthetic agrochemicals on human being. They analyzed pesticide residues using GC-ECD and GC-NPD systems equipped with

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

Gilden *et al.* (2010) found the presence of pesticide residues, which is a concern for consumers because pesticides are known to have potential harmful effects to other non-targeted organisms than pests and diseases. The major concerns are their toxic effects such as interfering with the reproductive systems and fetal development as well as their capacity to cause cancer and asthma.

Rahman (2010) conducted a study on the multi insecticide residue analysis in environmental sample collected from different regions of Bangladesh. A total of 94 samples of vegetables (brinjal, hyacinth bean, cauliflower and yard long bean) were collected from farmers field and market of different regions like Barisal, Bogra, Chittagong, Comilla, Dhaka, Dinajpur, Gazipur, Jessore, Khagrachuri, Narsingdi, Rajshahi and Rangpur and carried out the Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur. Results revealed that among 94 samples, 46 had insecticide residues, of them 18 had above MRL. Among 38 analyzed brinjal samples, 18 samples contained residue of Cypermethrin, Diazinon and Quinalphos; and only 8 had above MRL. Cypermethrin is the frequent one which was found in 14 samples. Of 24 analyzed hyacinth bean samples, 15 had the residue of Cypermethrin, Diazinon, Quinalphos, Fenotrothion and Malathion; and 7 had above MRL. Among the 16 analyzed cauliflower samples, 5 samples contained residue of Cypermethrin, Diazinon, Quinalphos and Malathion; and only 2 had above MRL. Of 16 yard long bean samples, 8 samples contained residue of Cypermethrin, Diazinon, Quinalphos and Malathion; and only 2 had above MRL.

Schreiber and Wittrig (2010) conducted a research for the identification and quantitation of pesticide residues in apple, banana, carrot, cucumber, curry powder grapes, grapefruit, hazelnut, lemon, nectarine, orange, pear, raspberry, red pepper, raisin, salad, spinach and tomato from a supermarket by QuEChERS extraction method and liquid chromatography mass spectrometry (LC/MS). They injected the extracted sample into a liquid chromatography tandem mass spectrometry system where a total number of 12 pesticides was detected where the amount of

methamidophos, omithoate, thiamethoxam, dimethoate, clothianidin, imadacloprid, promamocarb, carbyl, metalaxyl, myclobutanil, aspinosyn and dspinosyn were 130 µg/kg, 42 µg/kg, 48 µg/kg, 54µg/kg, 14µg/kg, 2.4µg/kg, 98µg/kg, 499µg/kg, 5.1µg/kg, 3.4µg/kg, 6.1µg/kg and 6.8 µg/kg, respectively.

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

Ochiai *et al.* (2009) conducted a study for the 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 K_{ow} 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 a simple technique for the quantification of organophosphorus and organochlorine insecticide residues from fish samples using

Gas Chromatograph (GC) couple to Electron Capture Detector (ECD) and Flame Thermionic Detector (FTD). They collected sixty eight samples of fish (Rui, Shrimp & Others) from Dhaka, Khulna and Chittagong and carried to the 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.

Yamagami *et al.* (2009) undertook a research to determine five groups of 85 pesticides- chlorinated, carbamate, phosphorous, pyrethroid and others - in vegetables, fruits and green tea using stir bar sorptive extraction (SBSE) coupled to thermal desorption and retention time locked (RTL) GC-MS. They found the residual limit between 4-100 µg/kg for 66 pesticides.

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 was 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.

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

Kabir *et al.* (2008) developed an analytical method for the determination of residue of diazinon and carbosulfan in brinjal and quinalphos in yard long bean under supervised field trial. The present study was undertaken to detect and quantify the left over residue of Diazinon and Carbosulfan in brinjal and Quinalphos in yard long bean and comparison between the detected residue level with maximum residue level (MRL) set by FAO (1970). Three supervised field trials (two for brinjal and one for yard long bean) were carried out sprayed with the field dose (1.5 ml/L of water) of Diazinon, Carbosulfan, and Quinaiphos. Samples were collected daily after spraying till residue were found. In case of Diazinon, left over residue was found upto 6 days after spray (DAS), and upto 3 DAS, the level of residue was above the MRL. Carbosulfan residue was detected till 7 DAS and the detected quantity of residue was above MRL upto 3 DAS. Left over residue of Quinalphos in yard long bean sample was detected upto 6 DAS and upto 4 DAS the level of residue was above the MRL.

Kabir *et al.* (2008a) conducted an experiment to quantify the purity in respect of active ingredient (AI) of some common insecticides used against vegetable insect pests. A series of analyses were made at Pesticide Research Laboratory, Pesticide Research & Environmental Toxicology Section, Entomology Division, BARI, Gazipur using GC-2010 (with FID and ECD detectors) and HPLC- 20A Prominence (with PDA detector). Tested insecticides were 9 brands of Carbofuran 5G and 3G, 2 brands of Carbaryl 85SP, 3 brands of Carbosulfan 20EC, 8 brands of Malathion 57EC, 9 brands of Diazinon 10G and 60EC, 3 brands of Quinalphos 25EC, 3 brands of Dimethoate 40EC, 4 brands of Cypermethrin 10EC and 7 brands of Chloropyrifos 20EC). These collected insecticides were collected from pesticide traders of Jessore and Tongi region of Bangladesh. Results of the present investigation clearly indicated

that most of the marketed insecticides were found to have lower AI ($\leq 60\%$) than that stated on the label. In some cases AI of unknown chemical was being used. Of 9 tested brands of Carbofuran, 3 were found to have 100% purity, 5 had 75-78% AI and 1 had nothing except carrier. Among 2 tested brands of Carbaryl none had $>50\%$ purity while all brands of Carbosulfan were found to have 78-98% purity. Eight brands of Malathion were tested, of which only 2 were found as pure, 5 had 80-90% AI and 1 had 60% AI. Of 9 tested brands of Diazinon, only 1 had $>90\%$ AI, while 3 had 80%, 4 had 40% and the other 1 had 10%. All 3 tested brands of Quinalphos provided 68-76% AI, while all Dimethoate had only 15-20% AI. Among 4 brands of Cypermethrin, 3 consisted of 100% AI, only 1 had 65%. Seven brands of Chlorpyrifos were analyzed, 2 were found to have 100% purity, 3 had 91-97% and 2 had 85-87% AI.

Nguyen *et al.* (2008) conducted a research for the simultaneous determination of 156 pesticides in watermelon collected from various markets of Korea using gas chromatography with electron impact mass spectrometric detection in the selected ion monitoring mode. They found the limit of quantifications (LOQs) for most compounds was below 0.005 mg/kg.

Kabir *et al.* (2007) conducted an experiment at the Regional Sugarcane Research Station, Gazipur in which carbofuran (2 kg AI/ha) was applied in sugarcane field to document the level of carbofuran residue left in soil and plant samples after different days of application (DAA). Plant and soil samples were analyzed by using GCMS-EI. Carbofuran residues were found even at 90 DAA both in soil and plant. In case of soil, the amount of carbofuran residues were 24.84, 3.32, 2.12, 0.59, 0.035, 0.02 and 0.005 ppm at 0, 3, 7, 15, 30, 60 and 90 DAA, respectively. In case of plant samples, the lower residue (0.0035 ppm) was observed at 0 DAA compared to those at 3 DAA (0.075 ppm), 7 DAA (0.035 ppm) and 15 DAA (0.015 ppm). At 60 and 90 DAA, residues were the same (0.002 ppm) while at 30 DAA it was 0.0025 ppm. The highest level of carbofuran residue (0.075 ppm) in plant samples was found at 3 DAA which is lower than FAO/WHO recommended MRL (0.1mg/kg crop).

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.

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.

Khan (2005) examined the residues of commonly used insecticides on fruits and vegetables grown in NWFP-Pakistan during crop season 2000, by using HPTLC. The initial residues of cypermethrin were 0.67 mg/kg. After 10 days, it was dissipated to 0.10 mg/kg, thus representing a loss of 85%. The samples did not contain any detectable residues 15 days after application. The year 2001, the initial residues of cypermethrin on tomato fruits were found to be 0.87 mg/kg which were reduced to 0.10 mg/kg after 15 days. The initial residue of chlorpyrifos 2.61 mg/kg degraded to

1.02 mg/kg 14 days after application. No residues were detected in the fruits 21 days after application.

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

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.

Colume *et al.* (2001) observed 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.

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

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

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.

Lehotay (2000) investigated 22 diverse pesticide residues in green bean and carrot extracts by bench top gas chromatography. The targeted pesticides which were incurred in the samples, included chlorpyrifos, azinphos-methyl, parathion-methyl, diazinon, terbufos, DDE, endosulfan sulfate, carbofuran, carbaryl, propargite, bifenthrin, dacthal, trifluralin, metalaxyl, pendimethalin, atrazine, piperonyl butoxide,

diphenylamine, vinclozolin, chlorothalonil, quintozene, and tetrahydrophthelimide (the breakdown product of captan). Average recoveries of the pesticides were 103% with relative standard deviations of 14 to 5% on 16 average.

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 organochlorines, 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.

Dogheim *et al.* (1999) has been monitored the residues of organophosphorous, dithiocarbamates and few synthetic pyrethroid pesticides commonly used in Egyptian fruits and vegetables, in addition to those organochlorines pesticides which had been termed as persistent and prohibited to use on foodstuffs several years ago. From 8 local markets, total of 397 fruit and vegetable samples were collected and analyzed for 52 pesticides. Out of total 397 samples, there were 42.8% were found positive and contained residues at detectable levels, out of which 1.76% found above to the MRLs. Residues of organochlorine pesticides were not found in most of the samples. Cauliflower, guava and 16 onion samples were found free from any pesticide residues otherwise among all samples, 65 grape samples contained 11 pesticide residues, 22 samples of strawberry contained 10 pesticide residues and 62 samples of tomato

contained 13 different pesticide residues. The most frequently detected pesticide was dithiocarbamates. Out of 98 samples residues of dithiocarbamates were found in 70.4% and only one sample of grape contained residues above to the MRLs. Samples of eggplant and carrot were found contaminated by the trace amounts of residues of pp'-DDT and pp'-DDE pesticides.

Ahuja *et al.* (1998) carried a research to monitor insecticide residues in cauliflowers, cabbages, tomatoes, brinjal, okras, field beans and cucumbers for GCH and its isomers, Endosulfan, Dimethoate, Monocrotophos, Quinalphos, Fenvalerate, and 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 (MRLs).

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" in India. Detectable levels or residues were observed in 33.3% of tomatoes (diazinon, 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.

CHAPTER III

MATERIALS AND METHODS

The vegetable (cauliflower and bitter gourd) samples were collected from different markets of Patuakhali district and carried to the Pesticide Analytical Laboratory, Entomology Division, BARI, Joydebpur, Gazipur for pesticide residue analysis during November 2019 to February 2020. All way required a number of processes from the collection of samples to the final analysis which are described below.

3.1 Study area

The study area included major five markets of Patuakhali district. The area of Patuakhali district is about 3220.15 sq km, located at 22.33° North latitude and 90.31° East longitude with an elevation of 6 meters (19.69 ft) from the sea level. In this study, vegetables were collected from 5 markets such as Baga Bazar, Pirtola Bazar, Patuakhali New Market, Kalapara Bazar and Galachipa Bazar of Patuakhali district.

3.2 Sample collection

A total of 60 samples (30 cauliflower and 30 bitter gourd) were collected for this study. Six samples of cauliflower and six samples of bitter gourd were collected from each market. Each sample was 1kg for both cauliflower and bitter gourd. Transparent airtight clean polyethylene bags were used to collect samples and each bag was properly labeled with source, sample number, sample ID, collection date, location etc. To avoid cross contamination, each sample was collected in a separate polyethylene bag.

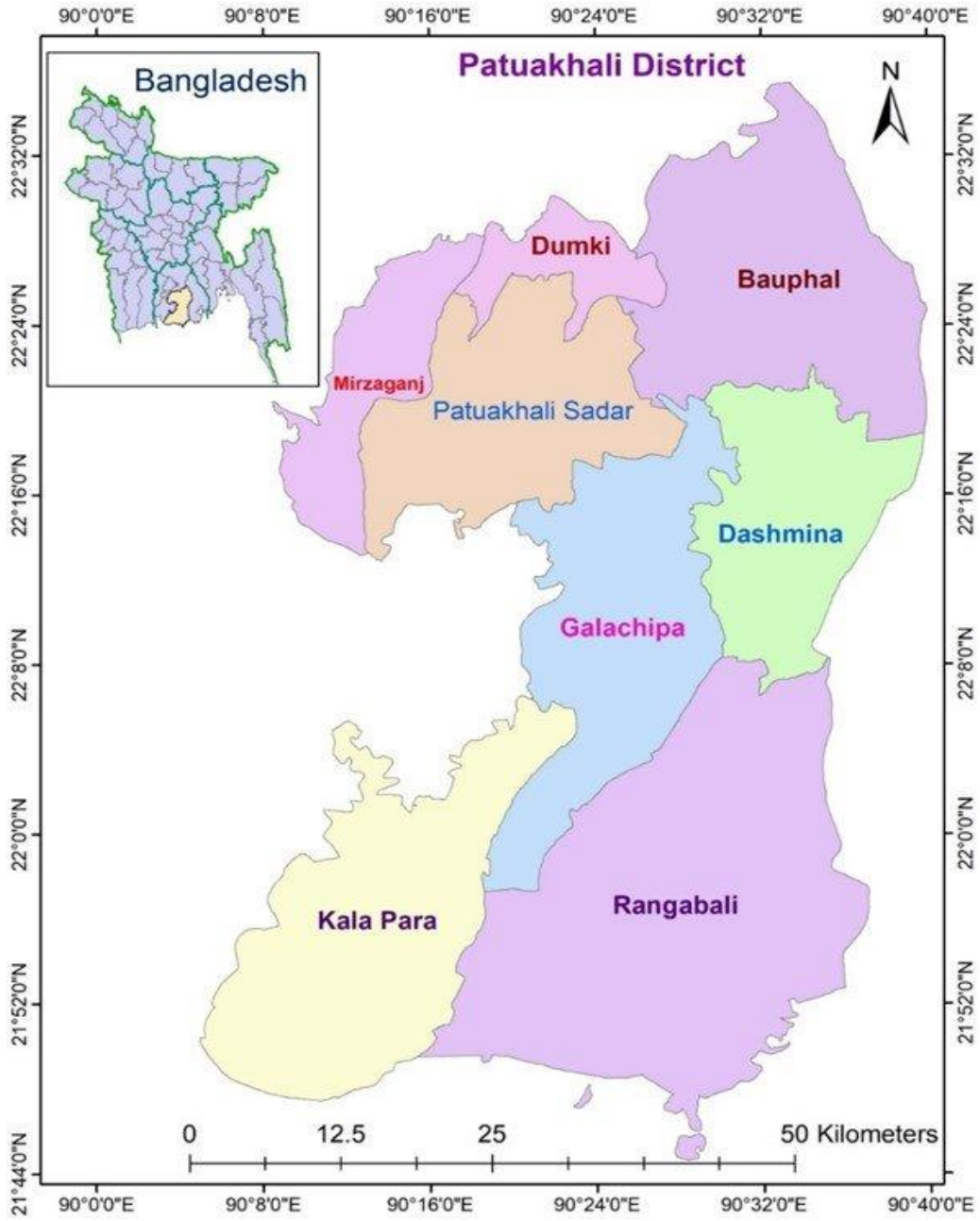


Figure 1. District map of Patuakhali

Table 1: Sources and places of collection of cauliflower samples

Area of collection	Sample ID	Source
Baga Bazar	PaCf-01 PaCf-02 PaCf-03 PaCf-04 PaCf-05 PaCf-06	Bauphal Kanakdia Chor Borhan Adabaria Kanchipara Sabupura
Pirtola Bazar	PaCf-07 PaCf-08 PaCf-09 PaCf-10 PaCf-11 PaCf-12	Dumki Shrirampur Pangasia Muradia Lebukhali Angaria
Patuakhali New Market	PaCf-13 PaCf-14 PaCf-15 PaCf-16 PaCf-17 PaCf-18	Patuakhali Morichbunia Jainkathi Bodorpur Vuria Borobigha
Kalapara Bazar	PaCf-19 PaCf-20 PaCf-21 PaCf-22 PaCf-23 PaCf-24	Kalapara Tiakhali Nilgong Lalua Champapur Dhankhali
Galachipa Bazar	PaCf-25 PaCf-26 PaCf-27 PaCf-28 PaCf-29 PaCf-30	Galachipa Amkhola Dakua Panpotti Chor kajol Kalagachia

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

Area of collection	Sample ID	Source
Baga Bazar	PaBg-01 PaBg-02 PaBg-03 PaBg-04 PaBg-05 PaBg-06	Bauphal Kanakdia Chor Borhan Adabaria Kanchipara Sabupura
Pirtola Bazar	PaBg-07 PaBg-08 PaBg-09 PaBg-10 PaBg-11 PaBg-12	Dumki Shrirampur Pangasia Muradia Lebukhali Angaria
Patuakhali New Market	PaBg-13 PaBg-14 PaBg-15 PaBg-16 PaBg-17 PaBg-18	Patuakhali Morichbunia Jainkathi Bodorpur Vuria Borobigha
Kalapara Bazar	PaBg-19 PaBg-20 PaBg-21 PaBg-22 PaBg-23 PaBg-24	Kalapara Tiakhali Nilgong Lalua Champapur Dhankhali
Galachipa Bazar	PaBg-25 PaBg-26 PaBg-27 PaBg-28 PaBg-29 PaBg-30	Galachipa Amkhola Dakua Panpotti Chor kajol Kalagachia

3.3 Sample preparation for analysis

The collected samples were carried to the Pesticide Analytical Laboratory, Division of Entomology, Bangladesh Agricultural Research Institute (BARI) just after 1 day of collection. 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 then cleanup process started.

3.4 Chemicals and reagents

The standard of malathion, diazinon, fenitrothion, quinalphos, chlorpyrifos, acephate, and dimethoate 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, HPLC 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. GC-2010, Shimadzu corporation, Japan (Plate 4)



Plate 1. Centrifuge Machine

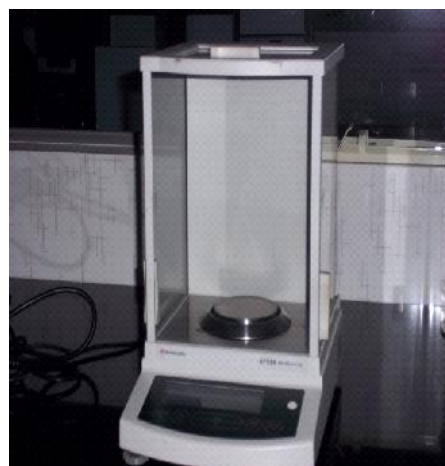


Plate 2: Electric Balance



Plate 3: Vortex Mixer



Plate 4: Gas Chromatograph (GC)

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

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

Some pictorial view related to sample preparation



Plate 5: Chopping of Sample



Plate 6. Weighing of homogenized sample



Plate 7: Adding Acetonitrile



Plate 8: Extract Vortexing

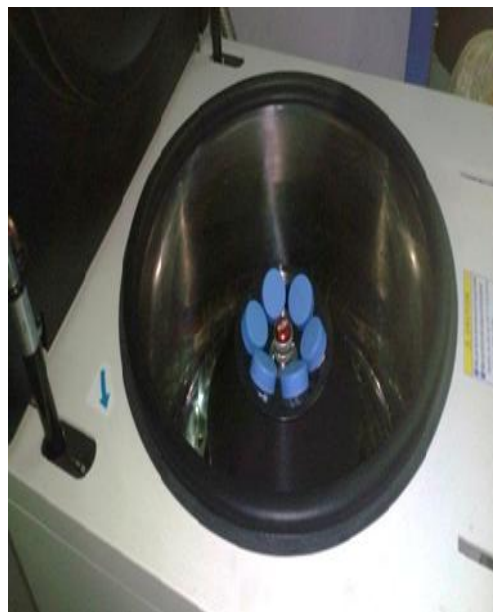


Plate 9: Centrifuging the Extract



Plate 10. Filtration through PTFE filter



Plate 11. Sample extract ready for injection

3.6 Preparation of pesticide standard solution

Pesticide standard stock solutions of malathion, diazinon, fenitrothion, quinalphos, chlorpyrifos, acephate, and dimethoate were prepared separately in MeCN at a concentration of 1000 mgL^{-1} and they were stored at -20°C until use. A mixed standard solution of 50 mgL^{-1} in MeCN containing all the aforementioned pesticides was prepared by adding the appropriate volume of each individual stock solution in a 50 mL volumetric flask and then made to volume by addition of MeCN. An intermediate mixed standard solution of 10 mgL^{-1} in MeCN was prepared from the mixed standard solution of 50 mgL^{-1} . Then working standard solutions of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 mgL^{-1} in MeCN were prepared by transferring the appropriate amount from 10 mgL^{-1} of intermediate mixed standard solution into 10 separate 10mL volumetric flasks. All of 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 stands for Quick, Easy, Cheap, Effective, Rugged and Safe. This technique was first introduced in 2003 by Anastassiades *et al.* and gaining popularity day by day compared to the other existing techniques such as Solid phase extraction (SPE), Liquid-liquid extraction (LLE), Supercritical Fluid Extraction (SFE), 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.* in 2015, where the chopped samples were grounded thoroughly with the fruit blender. In a 50 mL polypropylene centrifuge tube, a representative 10g portion of thoroughly homogenized sample was weighted and 10 mL of acetonitrile (MeCN) was added into the centrifuge tube. Then the centrifuge tube was closed properly and shaken vigorously for 30 s by the use of a vortex mixer and 1 g of NaCl and 4 g of anhydrous MgSO_4 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 at 5000 rpm for 5 min. An aliquot of 3 mL of MeCN layer was transferred into a 15 mL micro centrifuge tube containing 120 mg

Primary Secondary Amine (PSA) and 600 mg anhydrous MgSO₄ and then it was thoroughly mixed by vortex for 30 s and centrifuged at 4000 rpm for 5 minutes. (Laboratory Centrifuges, Sigma-3K30, Germany). After centrifugation, a 1 mL supernatant was filtered by a 0.2 µm PTFE filter. 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 Thermionic Detector (FTD) for the detection of acephate, chlorpyrifos, dimethoate, diazinon, fenitrothion, malathion and quinalphos. The capillary column was AT-1 length was 30m, ID was 0.25mm and film thickness was 0.25µm. Helium was used as carrier and make up gas for FTD. The identification of suspected pesticide was performed by peak retention times in samples to those of peaks in the pure analytical standards. A typical chromatogram containing seven selected organophosphorus insecticides is presented in Figure 2. The instrument conditions are described in Table 3 and Table 4.

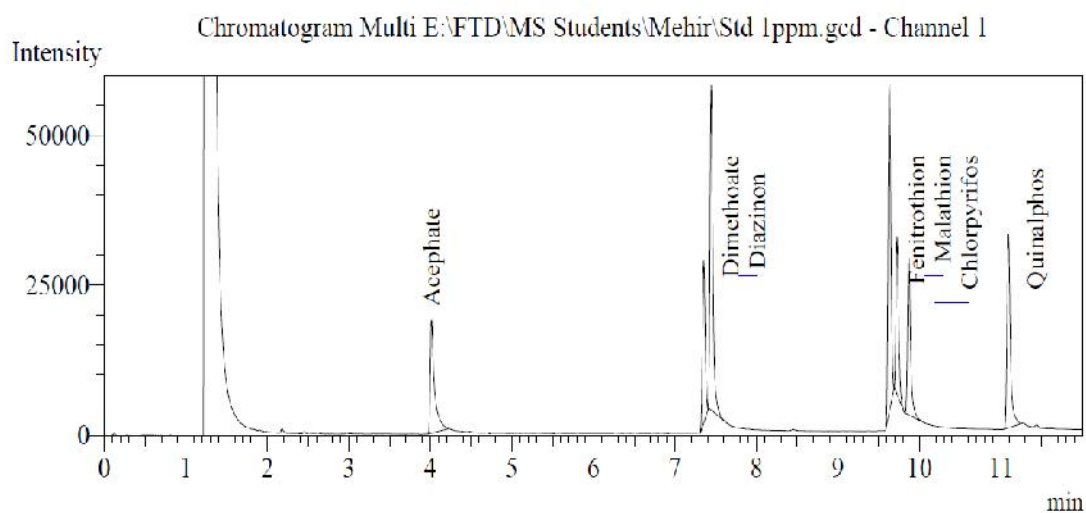


Figure 2: Typical chromatograms of seven organophosphorus insecticide standards run by GC-FTD.

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

3.9 Preparation of Calibration Curve

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

Calibration Curve - Analytical Line 1 - Channel 1

ID#:1 Name:Acephate

$$f(x) = 9.10326225705e-003 * x + 5.93037080704$$

$$R = 0.999804593187 \quad R^2 = 0.999609224558$$

MeanRF:9.48346399648e-003 RFSD:2.47060312499e-004 RFRSD:2.6051695097

CurveType:Linear

ZeroThrough:Not through

WeightedRegression:None

External Standard

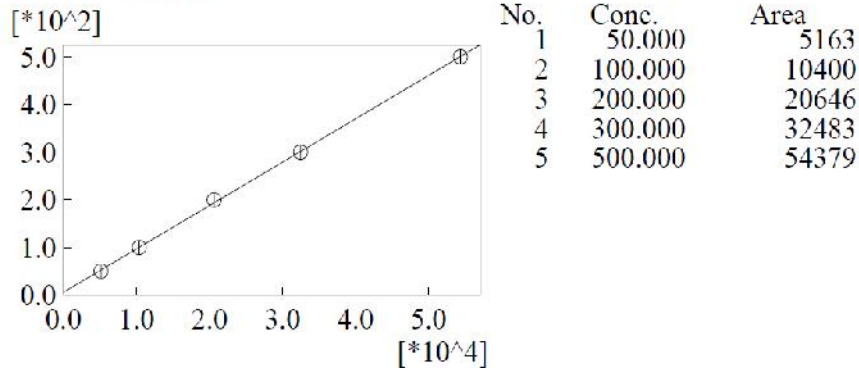


Figure 3. Calibration curve prepared for acephate made with different concentrations ranging from 50 µg/L to 500 µg/L.

ID#:2 Name:Dimethoate

$$f(x) = 1.11366545268e-002 * x + 3.92198354599$$

$$R = 0.999907208959 \quad R^2 = 0.999814426528$$

MeanRF:1.1426908424e-002 RFSD:1.67213835175e-004 RFRSD:1.46333399176

CurveType:Linear

ZeroThrough:Not through

WeightedRegression:None

External Standard

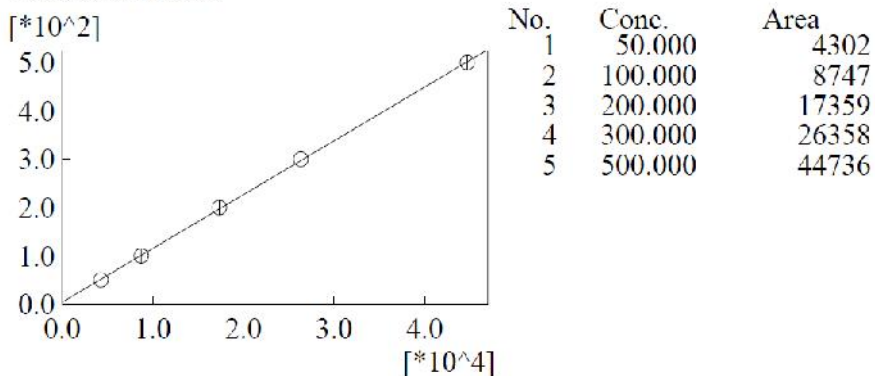


Figure 4. Calibration curve prepared for dimethoate made with different concentrations ranging from 50 µg/L to 500 µg/L.

ID#:3 Name:Diazinone

$$f(x)=8.58182478378e-003*x-4.76864750449$$

$$R=0.9987293676 \quad R^2=0.997460349706$$

MeanRF:8.23177212761e-003 RFSID:4.50915039717e-004 RFRSD:5.47773957694

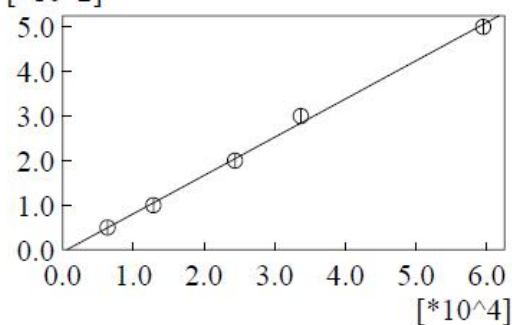
CurveType:Linear

ZeroThrough:Not through

WeightedRegression:None

External Standard

[*10²]



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

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

ID#:4 Name:Fenitrothion

$$f(x)-6.38668665212e-003*x+0.368481716024$$

$$R=0.999989187395 \quad R^2=0.999978374907$$

MeanRF:6.4079315158e-003 RFSID:3.43245591173e-005 RFRSD:0.535657396348

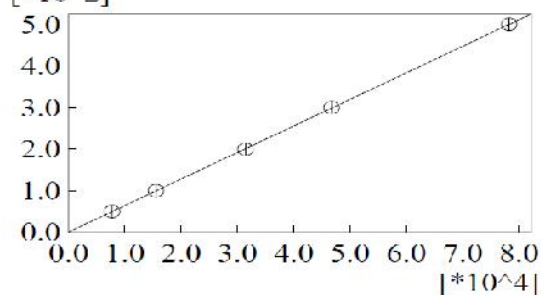
CurveType:Linear

ZeroThrough:Not through

WeightedRegression:None

External Standard

[*10²]



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

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

ID#:5 Name:Malathion

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

External Standard

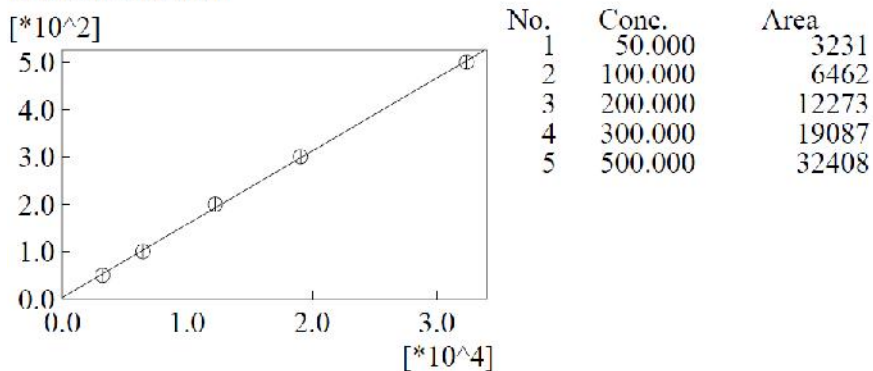


Figure 7. Calibration curve prepared for malathion made with different concentrations ranging from 50 µg/L to 500 µg/L.

ID#:6 Name:Chlorpyrifos

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

External Standard

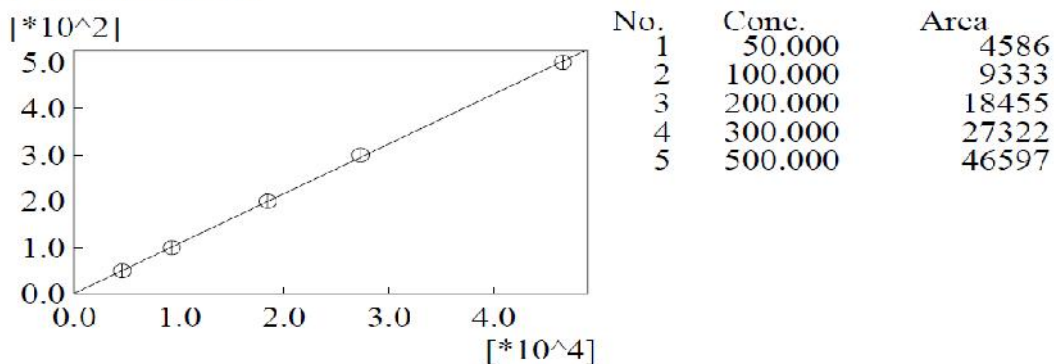


Figure 8. Calibration curve prepared for chlorpyrifos made with different concentrations ranging from 50 µg/L to 500 µg/L.

ID#:7 Name:Quinalphos

$$f(x)=1.2305211624e-002*x+0.670992116044$$

R=0.999994904248 R²=0.999989808522

MeanRF:1.23562145806e-002 RFSD:7.05710649982e-005 RFRSD:0.571138227959

CurveType:Linear

ZeroThrough:Not through

WeightedRegression:None

External Standard

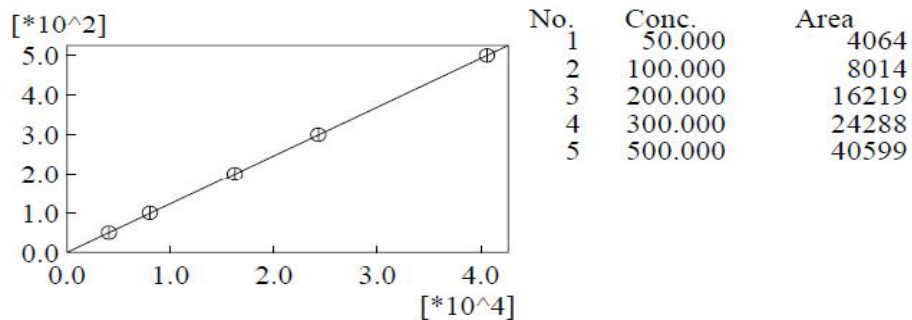


Figure 9. Calibration curve prepared for quinalphos made with different concentrations ranging from 50 µg/L to 500 µg/L.

CHAPTER IV

RESULTS AND DISCUSSIONS

Sixty (60) samples of vegetable (cauliflower and bitter gourd) were collected from 5 different markets of Patuakhali district (Baga Bazar, Pirtola Bazar, Patuakhali New Market, Kalapara Bazar, Galachipa Bazar) to detect and quantify pesticide residues. The results obtained from this study are presented and described in this chapter using figures and tables.

4.1 Pesticide Residues in cauliflower

The concentrated extracts of cauliflower samples collected from different markets of Patuakhali were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. Figure 10-15 shows the chromatograms of the injected extracts of cauliflower sample containing detected pesticides.

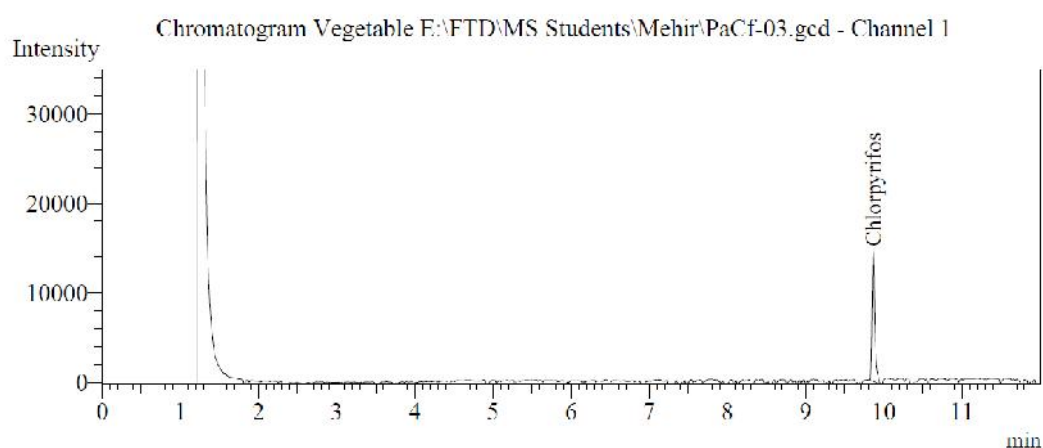


Figure 10. Chromatogram of chlorpyrifos found in one of the cauliflower sample (PaCf-03) collected from Baga Bazar.

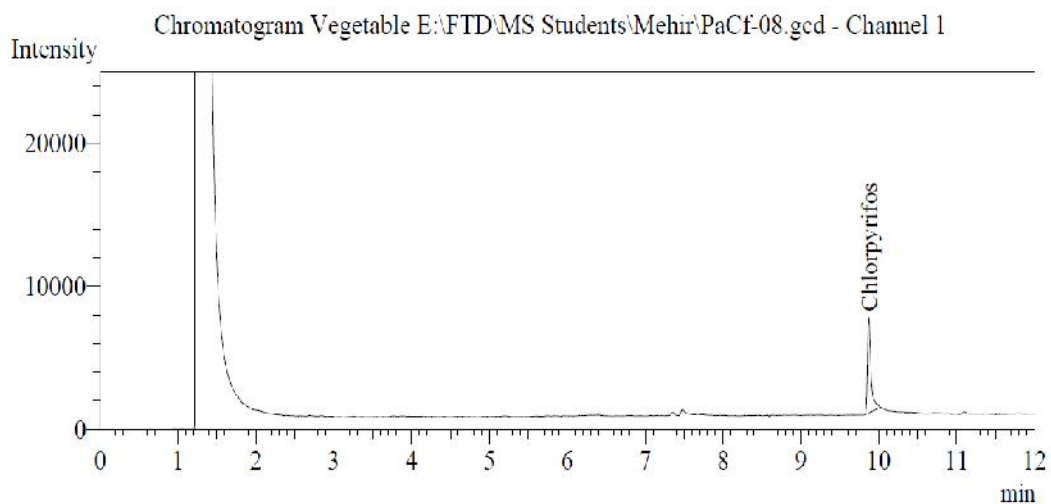


Figure 11. Chromatogram of chlorpyrifos found in one of the cauliflower sample (PaCf-08) collected from Pirtola Bazar.

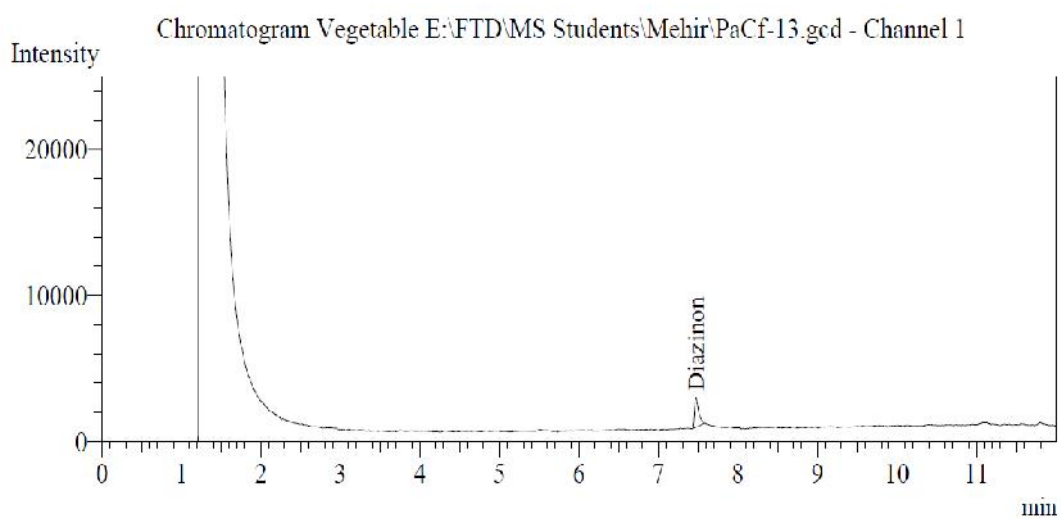


Figure 12. Chromatogram of diazinon found in one of the cauliflower sample (PaCf-13) collected from Patuakhali New Market.

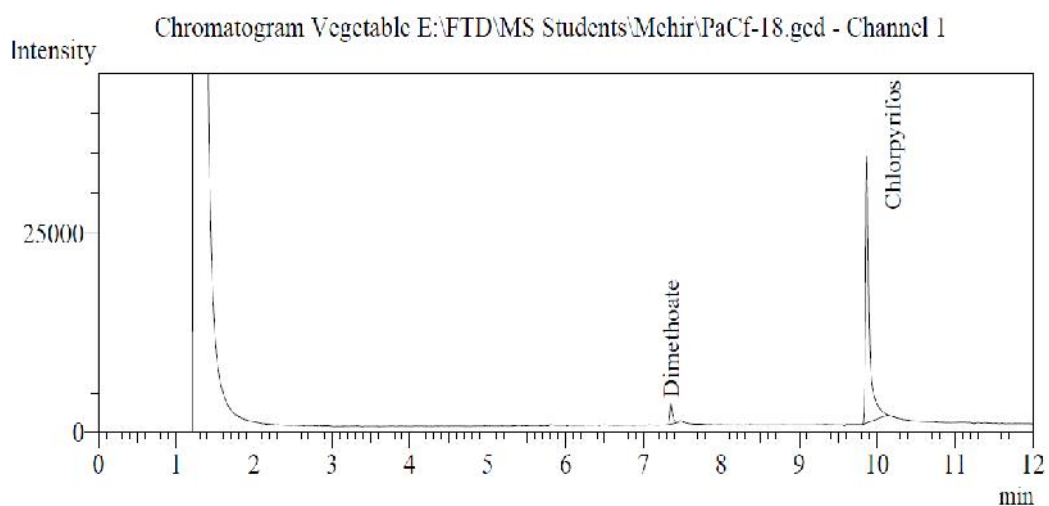


Figure 13. Chromatogram of dimethoate and chlorpyrifos found in one of the cauliflower sample (PaCf-18) collected from Patuakhali New Market.

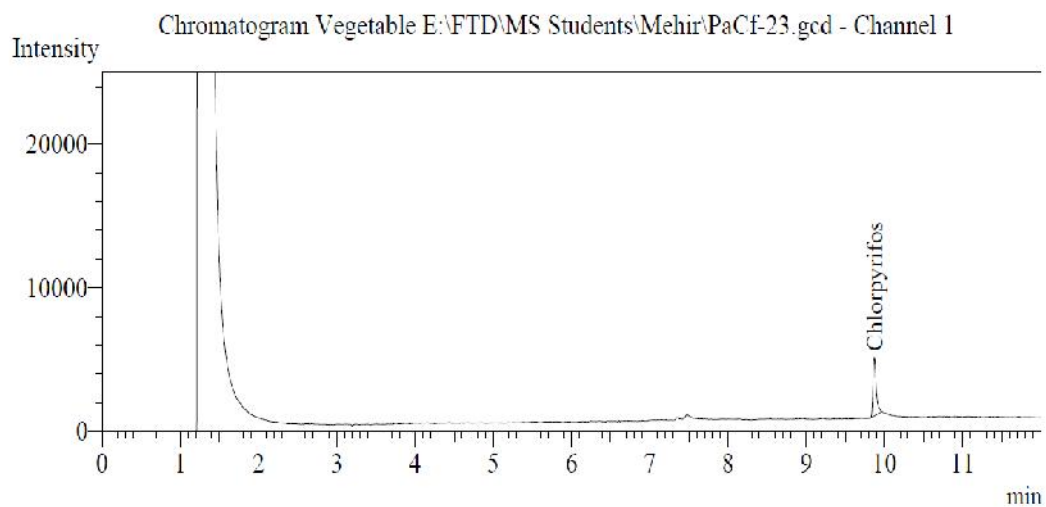


Figure 14. Chromatogram of chlorpyrifos found in one of the cauliflower sample (PaCf-23) collected from Kalapara Bazar.

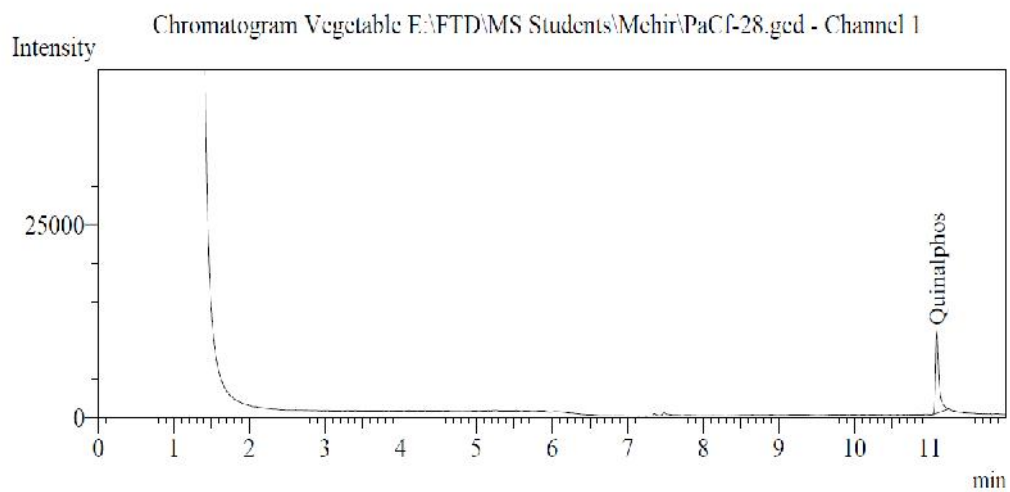


Figure 15. Chromatogram of quinalphos found in one of the cauliflower sample (PaCf-28) collected from Galachipa Bazar.

Table 5. The level of residues (mg/kg) of different pesticides found in the analyzed cauliflower samples.

Area of collection	Sample ID	Name of detected pesticides	Level of residue (mg/kg)	MRLs (mg/kg)
Baga Bazar	PaCf-01	ND	-	
	PaCf-02	ND	-	
	PaCf-03	Chlorpyrifos	0.521	0.01*
	PaCf-04	ND	-	
	PaCf-05	ND	-	
	PaCf-06	ND	-	
Pirtola Bazar	PaCf-07	ND	-	
	PaCf-08	Chlorpyrifos	0.299	0.01*
	PaCf-09	ND	-	
	PaCf-10	ND	-	
	PaCf-11	ND	-	
	PaCf-12	ND	-	
Patuakhali New Market	PaCf-13	Diazinon	0.052	0.01*
	PaCf-14	ND	-	
	PaCf-15	ND	-	
	PaCf-16	ND	-	
	PaCf-17	ND	-	
	PaCf-18	Dimethoate Chlorpyrifos	0.109 1.617	0.02 0.01*
Kalapara Bazar	PaCf-19	ND	-	
	PaCf-20	ND	-	
	PaCf-21	ND	-	
	PaCf-22	ND	-	
	PaCf-23	Chlorpyrifos	0.163	0.01*
	PaCf-24	ND	-	
Galachipa Bazar	PaCf-25	ND	-	
	PaCf-26	ND	-	
	PaCf-27	ND	-	
	PaCf-28	Quinalphos	0.362	0.01*
	PaCf-29	ND	-	
	PaCf-30	ND	-	

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

Thirty samples of cauliflower collected from 5 different markets of Patuakhali (Baga Bazar, Pirtola Bazar, Patuakhali New Market, Kalapara Bazar and Galachipa Bazar) and were analyzed to find out the presence of left over residue of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos).

Out of 30 analyzed samples of cauliflower, 6 samples (20% of the total number of samples) contained pesticide residues and 24 samples (80% of the total number of samples) contained no detectable residues of the sought pesticides. The present results can be compared to Islam *et al.* (2014). They have collected 42 samples of brinjal, cauliflower and country bean from fields and markets of Narsingdi district, Bangladesh, where they found 15 samples (above 68% of total samples) contained no residues of the sought pesticides. The findings of the present study can also be compared with Akter *et al.* (2017). They have been monitored pesticide residues in eggplant collected from Mymensingh district and found that among the 50 analyzed samples, 11 (22% of the total number of the samples) contained pesticide residues of diazinon, dimethoate, quinalfos, and chlorpyrifos, of which, 2 had multiple pesticide residues and 5 samples contained residue above the European Union maximum residue limit (EU-MRLs). Chlorpyrifos was detected as the most used pesticide in eggplant in the studied area. The results of this study are in a good agreement with Hasan *et al.* (2017). They have been detected two types of insecticides (dimethoate and quinalphos) in country bean samples collected from different market places of Dhaka. Among the 50 analyzed samples of country bean, 10 samples (20%) contained residues of Dimethoate and Quinalphos, of which 5 samples were above the maximum residue limits (MRLs). Most of the contaminated samples (8 samples) contained residue of dimethoate.

Six cauliflower samples were collected from Baga Bazar area, among them, one samples (PaCf-03) contained chlorpyrifos at a level of (0.521 mg/kg), which was above the EU-MRL (European Commission 2015). The other 5 samples contain no detectable pesticide residues. From Pirtola Bazar, six samples were collected and analyzed, of which one sample (PaCf-08) contained chlorpyrifos residue (0.299 mg/kg). But other 5 samples contained no detectable pesticide residues. The level of detected Chlorpyrifos residue (0.299 mg/kg) was above EU-MRL (0.01 mg/kg).

Six cauliflower samples were collected from Patuakhali New Market. Of them, two were contaminated: one sample (PaCf-13) contained residue of diazinon (0.052 mg/kg), which was above EU-MRL (0.01 mg/kg); the another sample (PaCf-18) contained residue of dimethoate residue (0.109 mg/kg) and chlorpyrifos (1.617 mg/kg), which was above EU- MRL (0.02 mg/kg) and (0.01 mg/kg), respectively. The other 4 samples contain no detectable pesticide residues.

From Kalapara Bazar, six samples were collected and analyzed, of which one sample (PaCf-23) contained chlorpyrifos residue (0.163 mg/kg). But other 5 samples contained no detectable pesticide residues. The level of detected chlorpyrifos residue (0.163 mg/kg) was above EU-MRL (0.01 mg/kg). In case of Galachipa Bazar, one sample (PaCf-28) of cauliflower contained residue of quinalphos at a level of 0.362 mg/kg, which was above EU-MRL (0.01 mg/kg). However, the other 5 samples contained no detectable pesticide residues.

4.2 Pesticide residues in bitter gourd

The concentrated extracts of bitter gourd samples were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. Figure 17-20 shows the chromatograms of the injected extracts of bitter gourd sample containing detected pesticides.

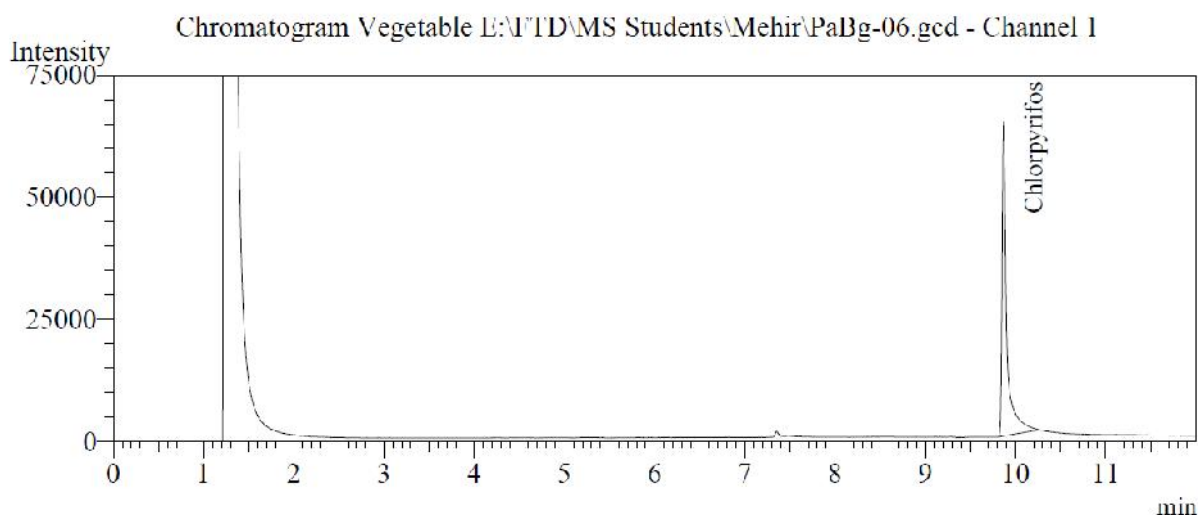


Figure 16. Chromatogram of chlorpyrifos found in one of the bitter gourd sample (PaBg-06) collected from Baga Bazar.

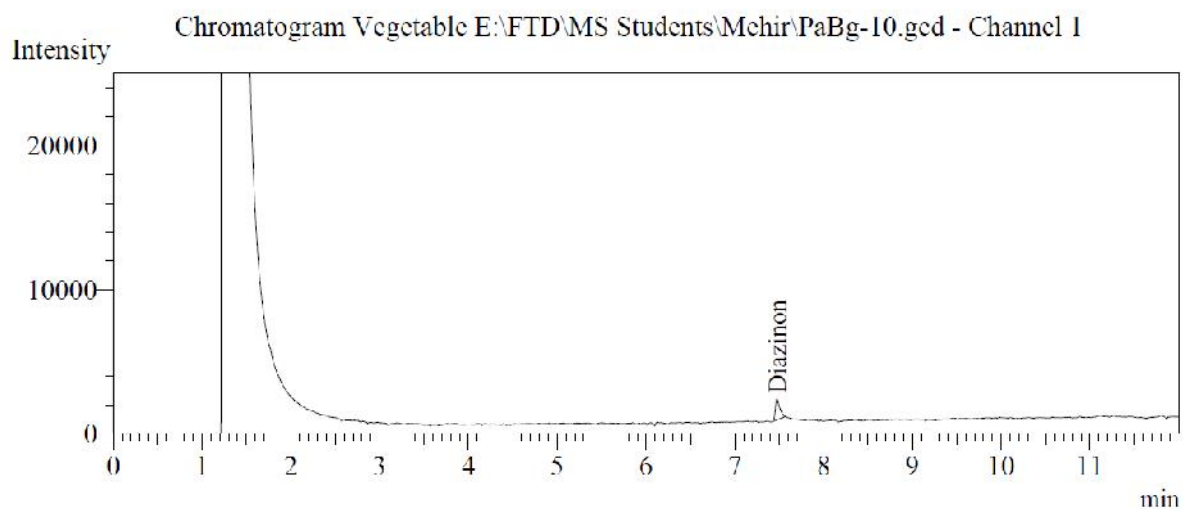


Figure 17. Chromatogram of diazinon found in one of the bitter gourd sample (PaBg-10) collected from Pirtola Bazar.

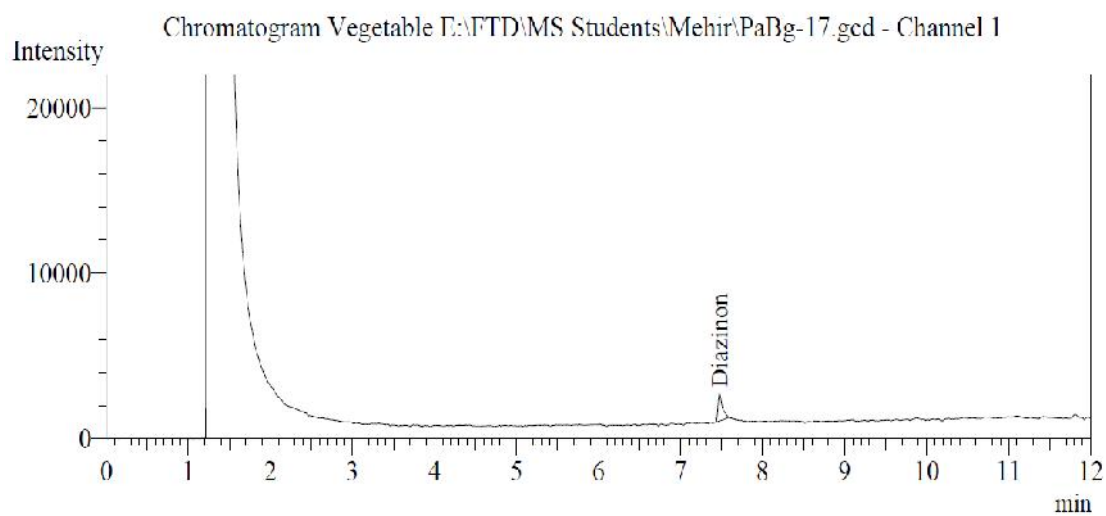


Figure 18. Chromatogram of diazinon found in one of the bitter gourd sample (PaBg-17) collected from Patuakhali New Market.

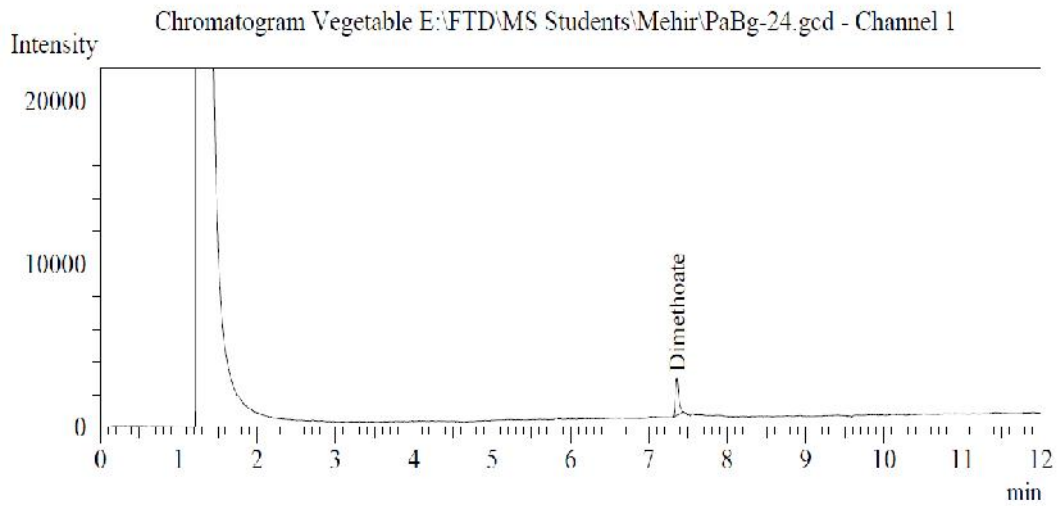


Figure 19. Chromatogram of dimethoate found in one of the bitter melon sample (PaBg-24) collected from Kalapara Bazar.

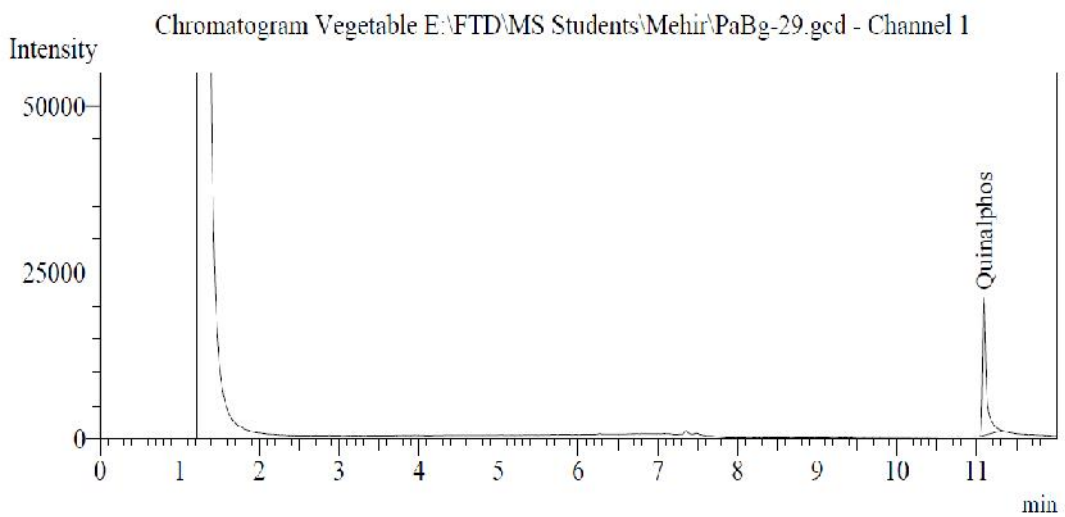


Figure 20. Chromatogram of quinalphos found in one of the bitter melon sample (PaBg-29) collected from Galachipa Bazar.

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

Area of collection	Sample ID	Name of detected pesticides	Level of residue (mg/kg)	MRLs (mg/kg)
Baga Bazar	PaBg-01	ND	-	
	PaBg-02	ND	-	
	PaBg-03	ND	-	
	PaBg-04	ND	-	
	PaBg-05	ND	-	
	PaBg-06	Chlorpyrifos	2.301	0.04*
Pirtola Bazar	PaBg-07	ND	-	
	PaBg-08	ND	-	
	PaBg-09	ND	-	
	PaBg-10	Diazinon	0.037	0.04*
	PaBg-11	ND	-	
	PaBg-12	ND	-	
Patuakhali New Market	PaBg-13	ND	-	
	PaBg-14	ND	-	
	PaBg-15	ND	-	
	PaBg-16	ND	-	
	PaBg-17	Diazinon	0.045	0.04*
	PaBg-18	ND	-	
Kalapara Bazar	PaBg-19	ND	-	
	PaBg-20	ND	-	
	PaBg-21	ND	-	
	PaBg-22	ND	-	
	PaBg-23	ND	-	
	PaBg-24	Dimethoate	0.092	0.02*
Galachipa Bazar	PaBg-25	ND	-	
	PaBg-26	ND	-	
	PaBg-27	ND	-	
	PaBg-28	ND	-	
	PaBg-29	Quinalphos	0.754	0.01
	PaBg-30	ND	-	

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

Thirty samples of bitter gourd were collected from 5 different markets of Patuakhali district (Baga Bazar, Pirtola Bazar, Patuakhali New Market, Kalapara Bazar and Galachipa Bazar) and were analyzed to find out the presence of left over residue of seven selected pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos).

Out of 30 samples of bitter gourd, 5 samples (16.66% of the total number of samples) contained pesticide residues and 25 samples (83.33% of the total number of samples) contained no detectable residues of the sought pesticides. The present results can be compared to Islam *et al.* (2019a), they found that among the 65 analyzed bitter gourd samples, 8 (12.3% of the total number of samples) were contaminated with pesticide residues and all of the contaminated samples contained residues above Maximum Residue Limit (MRL) set by European Commission (EC) collected from different markets of Savar, Dhaka of Bangladesh. The results of the present study are also supported by the findings of Nahar *et al.* (2020), who reported that among the 80 analyzed samples of cauliflower and tomato, 11 (6 cauliflower, 5 tomato) contained pesticide residues, which was about 14% of the total number of samples. Most of the samples contaminated with diazinon at a level above EU-MRLs.

Six bitter gourd samples were collected from Baga Bazar, of which one sample (PaBg-06) contained chlorpyrifos residue (2.301 mg/kg). The level of detected chlorpyrifos residue (2.301 mg/kg) was above EU-MRL (0.04 mg/kg). But other 5 samples contained no detectable pesticide residues. From Pirtola Bazar, six samples of bitter gourd were collected, of which one sample (PaBg-10) contained diazinon residue (0.037 mg/kg), which was below EU-MRL (0.04 mg/kg). But other 5 samples contained no detectable pesticide residues.

Among the six samples of bitter gourd collected from Patuakhali New Market, one sample (PaBg-17) contained residue of diazinon (0.045 mg/kg). The other 5 samples contain no detectable pesticide residues. The level of detected diazinon residue (0.045 mg/kg) was above EU-MRL (0.04 mg/Kg). While, in case of Kalapara Bazar, out of six analyzed samples, one sample (PaBg-24) contained residues of dimethoate (0.092 mg/Kg). The level of detected dimethoate residue was above EU-MRL (0.02 mg/Kg). But other 5 samples contained no detectable pesticide residues.

Among the six samples of bitter gourd collected from Galachipa Bazar, one sample (PaBg-29) contained residue of quinalphos (0.754 mg/kg). The other 5 samples contain no detectable pesticide residues. The level of detected quinalphos residue (0.754 mg/kg) was above EU-MRL (0.01 mg/Kg).

CHAPTER V

SUMMARY AND CONCLUSION

The farmers of Bangladesh are using different types of organophosphorus pesticides for the control of insect pests of fruits and vegetables in order to increase the production and to reduce the economic loss. However, it is well known that the application of pesticides may leave residues, which is a global concern for the consumers due to their detrimental effects on human health and in the environment. Especially, the exposure towards organophosphate group for long duration and high relative amount will inhibit function of acetylcholine esterase, this can cause salivation, dizziness etc. Therefore, the present study was initiated to identify organophosphorus pesticide residues in cauliflower and bitter gourd collected from local markets of Patuakhali district and to quantify them for the comparison with the maximum residue limits (MRLs) set by European Union.

Regarding this, 30 samples of cauliflower and 30 samples of bitter gourd were collected from 5 different markets of Patuakhali district and carried to the Pesticide Analytical Laboratory, Entomology Division, BARI, Joydebpur, Gazipur, Bangladesh. The QuEChERS extraction technique was applied for the extraction and cleanup of these collected samples. Gas chromatography coupled with flame thermionic detector (FTD) was used to identify and quantify the level of pesticide residues present in the extracted samples. Seven most commonly used organophosphorus pesticides i.e. acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos were considered for this study.

Out of 30 samples of cauliflower, 6 samples (20% of the total number of samples) contained residues of diazinon, chlorpyrifos, dimethoate and quinalphos. Among these 6 samples, 5 had residues above the Maximum Residue Limit (MRL) and 1 sample contained two different types of pesticide residues such as dimethoate and chlorpyrifos. The level of detected dimethoate and chlorpyrifos residues was above MRL and the other 24 samples (70% of the total number of samples) contained no detectable residues of the sought pesticide. Among the 30 analyzed samples of bitter gourd, 5 samples (16.66% of the total number of samples) contained residues of diazinon, dimethoate, chlorpyrifos and quinalphos. Among these 5 samples, 4 had residues above the MRL and 1 had residues below the MRL set by European

Commission. On the other hand, 25 samples (83.33% of the total number of samples) contained no detectable residues of the sought pesticides. At present, pesticide residues in vegetables are becoming a major food safety concern for the consumers and the governments as well. Therefore, the generated knowledge on pesticide residue levels in vegetables at local markets of Patuakhali district will help to increase public awareness and the policy planners to take necessary action in order to minimize the pesticide residues level in vegetables at Patuakhali district in Bangladesh.

Recommendations for further research:

The research has done only five different markets of Patuakhali district of Bangladesh. More research work should be done in other areas of Patuakhali district of Bangladesh in order to find out the actual scenario of pesticide residue load in different vegetables grown in this district. This research work has been carried out for two vegetables (Cauliflower and Bitter gourd) only, more research work containing different vegetables should be done to find out the harmful effect of pesticide residues in order to save the peoples of Bangladesh.

CHAPTER VI

REFERENCES

- Afful, S., Enimil, E., Blewu, B., Mantey, G.A. and Ewusie, E.A. (2010). Gas chromatographic methodology for the determination of some halogenated pesticides. *Res. J. Appl. Sci. Eng. Tech.* **2**(6): 592-595.
- Afify, A.E.M.M.R., Mohamed, M.A., El-Gammal, H.A. and Attallah, E.R. (2010). Multiresidue Method of Analysis for Determination of 150 Pesticides in Grapes Using Quick and Easy Method (QuEChERS) and LC-MS/MS Determination. *J. Food Agri. Environ.* **8**(2): 602-606.
- Agüera, A., Piedra, L., Hernando, M.D., Fernandez-Alba, A.R. and Contreras, M. (2000). Splitless large-volume GC-MS injection for the analysis of organophosphorus and organochlorine pesticides in vegetables using a miniaturised ethyl acetate extraction. *Analyst.* **125**: 1397-1402.
- Ahuja, A.K., Soudamini, M., Debi, S., Awasthi, M.D., Mohapatra, S., Sharma, D., Reddy, P.P., Kumar, N.K.K. and Verghese, A. (1998). Monitoring of vegetables of insecticide residue contamination at harvest, Advances in IPM for horticultural crops. Proc. of the 1st national symposium on pest management in horticultural crops: Environmental implications and thrusts, Bangalore, India, 15-17 Oct. 1997, pp. 243-246.
- Akan, J.C., Jafiya, L., Mohammed, Z. and Abdulrahman, F.I. (2013). Organophosphorus pesticide residues in vegetables and soil samples from alau dam and gongulong agricultural areas, Borno State, Nigeria. *Int. J. Environ. Monitoring and Analysis.* **1**(2): 58-64.
- Akhlaghi, H., Motavalizadehkakhky, A. and Emamiyan, R. (2013). Determination of diazinon in fruits from northeast of Iran using the QuEChERS sample Preparation method and GC/MS. *Asian J. Chem.* **25**(3): 1727-1729.
- Aktar, M.A., Khatun, R. and Prodhan, M.D.H. (2017). Determination of pesticide residues in eggplant using modified QuEChERS Extraction and Gas chromatography. *Int. J. Agron. Agri. Res.* **11**(2): 22-31.

- Alnedhary, A.A., AL-Hammadi, M.M., Numan, A.A. and Murshed, F.A. (2020). Optimization and Efficiency Comparison of Dispersive and Cartridge Solid Phase Extraction Cleanup Techniques in the Analysis of Pesticide Residues in Some Vegetables Using Gas Chromatography-Mass Spectrometry. *PSM Biological Research*. **5**(1): 40-54.
- Anastassiades, M., Lehotay, S.J., Stajnbaher, D. and Schenck, F.J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solidphase extraction” for the determination of pesticide residues in produce. *J. AOAC Int.* **86**: 412–431.
- Anastassiades, M., Scherbaum, E., Ta delen, B. and Štajnbaher, D. (2007). In: Crop protection, public health, environmental safety, H. Ohkawa, H. Miyagawa and P. W. Lee (Eds.), 439, Wiley-VCH, Weinheim, Germany.
- Anonymous (2000). Annual report 1999-2000, Entomology division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh.
- Asensio-Ramos, M., Hernández-Borges, J., Ravelo-Pérez, L.M. and Rodríguez-Delgado, M. A. (2010). Evaluation of a Modified QuEChERS Method for the Extraction of Pesticides from Agricultural, Ornamental and Forestal Soils. *Anal. Bioanal. Chem.* **396**(6): 2307-2319.
- Atkins, P. and De Paula, J. (2002). Atkins` physical chemistry. The 7th Edition, Oxford University Press Inc., New York, p.18
- Bempah, C.K., Asomaning, J. and Boateng, J. (2020). Market basket survey for some pesticides residue in fruits and vegetables from Ghana. *Journal of Microbiology, Biotechnology and Food Sciences*, **9**(4), 850-871.
- Bempah, C.K., Buah-Kwofie, A., Denutsui D, Asomaning, J. and Tutu, A.O. (2011). Monitoring of pesticide residues in fruits and vegetables and related health risk assessment in Kumasi Metropolis, Ghana. *Res. J. Environ. Earth Sci.* **3**(6): 761-771.

- Blodgett, D.J. (2006). Organophosphate and Carbamate Insecticides. Small Animal Toxicology, 2nd ed.; Peterson, M.E.; Talcott, P.A., Eds.; Elsevier Saunders: St. Louis. pp. 941-947.
- Bonner, M.R., Cobie, J. and Blair, A. (2007). 'Malathion Uxposure and the Incidence of Cancer in the Agricultural Health Study. *American J. of Epidemiology*. **166**(9): 1023-1034.
- Butler, J., Steiniger, D. and Phillips, E. (2008). Analysis of pesticide residues in lettuce using a modified quechers extraction technique and single quadrupole GC/MS, Retrieved from www.thermo.com. (Date: 12 Nov., 2013).
- Caboni, P., Sarais, G., Angioni, A., Vargiu, S., Pagnozzi, D., Cabras P. and Casida J.E. (2008). Liquid chromatography tandem mass spectrometric ion switching determination of chlorantraniliprole and flubendiamide in fruits and vegetables. *J. Agric. Food Chem.* **56**: 7696-7699.
- Camino-Sánchez, A., Zafra-Gómez, J., Ruiz-García, R., Bermúdez-Peinado, O., Ballesteros, A., Navalón, A. and Vílchez J.L. (2011). UNE-EN ISO/IEC 17025:2005 accredited method for the determination of 121 pesticide residues in fruits and vegetables by gas chromatography–tandem mass spectrometry, *J. Food Composit. Anal.* **24**: 427-440.
- Camino-Sancheza, F.J., Zafra-Gomez, A., Oliver-Rodriguezb, B., Ballesteros, O., Navalón, A., Crovetto, G. and Vilchez, J.L. (2010). UNE-EN ISO/IEC 17025:2005-accredited method for the determination of pesticide residues in fruit and vegetable samples by LC-MS/MS. *Food Addit. Contam.* **27**(11): 1532-1544.
- Chandra, S., Mahindrakar, A.N. and Shinde, L.P. (2010). Determination of cypermethrin and chlorpyrifos in vegetables by GC-ECD. *Int. J. Chem. Tech. Res.* **2**(2): 908-911.
- Chapman, R.F. (1998). The Insects Structure and Function, 4th ed.; Cambridge University Press: Cambridge. UK. p. 542.

- Charan, P.D. and Sharma, K.C. (2010). Monitoring of pesticide residues in farmgate vegetables of Central Aravalli region of Western India. *American-Eurasian J. Agril. Env. Sci.* **7**(3): 255-258.
- Chauhan, S.S., Negi, S., Singh, N., Bhatia, G. and Srivastava, A. (2012). Monitoring of pesticides residues in farmgate vegetables of Uttarakhand, India. *Wudpecker J. Agri. Res.* **1**(7): 250 – 256.
- Chauhan, S.S., Negi, S., Singh, N., Bhatia, G. and Srivastava, A. (2012). Monitoring of pesticides residues in farmgate vegetables of Uttarakhand, India. *Wudpecker J. Agri. Res.* **1**(7): 250 – 256.
- Cho, T.H., Park, Y.H., Park, H.W., Hwang, L.H., Cho, I.S., Kim, H.J., Kim, M.J., Kim, M.S. and Chae, Y.Z. (2013). Evaluation of QuEChERS method for determination of pesticide residues using GC/NPD and GC/ECD. *The Korean J. Pesticide Sci.* **17**(1): 65-71.
- Chuck W.A.C., Hussain. M.A. and Oloffs, P.C. (1984). Hydrolytic and Metabolic Products of Acephate. *J. Environ. Sci. Health. B* **19**(6): 501-522.
- Chung, S.W.C. and Chan, B.T.P. (2010). Validation and use of a fast sample preparation method and liquid chromatography-tandem mass spectrometry in analysis of ultra-trace levels of 98 organophosphorus pesticide and carbamate residues in a total diet study involving diversified food types. *J. Chromatogr. A.* **17**(29): 4815-4824.
- Chung, S.W.C. and Chen, B.L.S. (2011). Determination of organochlorine pesticide residues in fatty foods: A critical review on the analytical methods and their testing capabilities. *J. Chromatogr. A.* **1218**(33): 5555–5567.
- Cielik, E., Sadowska-Rociek, A., Ruiz, J.M.M. and Surma-Zadora, M. (2011). Evaluation of QuEChERS Method for the Determination of Organochlorine Pesticide Residues in Selected Groups of Fruits. *Food Chem.* **125**(2): 773-778.
- Collimore, W. A. and Bent, G. A. (2020). A newly modified QuEChERS method for the analysis of organochlorine and organophosphate pesticide residues in fruits and vegetables. *Environ. Monit. Assess.* **192**(2): 128.

- Colume, A., Cardenas, S., Gallego, M. and Valcarcel, M. (2001). Semiautomatic multiresidue gas chromatographic method for the screening of vegetables for 25 organochlorine and pyrethroid pesticides. *Anal. Chim. Acta.* **436**: 153-162.
- Corteaş, J. M., Sanchez, R., Plaza, E.M.D., Villean, J. and Vaazquez A. (2013). Large volume GC injection for the analysis of organophosphorus pesticides in vegetables using the through oven transfer adsorption desorption (TOTAD) interface. *Int. J. Env. Monit. Anal.* **1**(2): 58-64.
- Craig, W. and Beck, L. (1999). Phytochemicals: health protective effects. *Can. J. Dietetic Prac. Res.* **60**(2): 78-84.
- Cressey, D. (2013). Europe debates risk to bees. *Nature.* **496**(7446): 408.
- Dasika, R., Tangirala, S. and Naishadham, P. (2012). Pesticide residue analysis of fruits and vegetables. *J. Env. Chem. Ecotoxicol.* **4**(2):19-28.
- Dauterman, W.C., Viado, G.B., Casida, J.E. and O'Brien, R.D. (1960). "Insecticide Residues, Persistence of Dimethoate and Metabolites Following Foliar Application to Plants". *J. of Agric. and Food Chem.* **8**(2): 115.
- Dethe, M.D., Kale, V.D and Rane, S.D. (1995). Insecticide residues in/on farm gate samples of vegetables Pest Management. *Hort. Ecosystem.* **1**(1): 49-53.
- Dias, J. S. and Ryder, E. (2011). World vegetable industry: production, breeding, trends. *Hort. Review.* **38**: 299-356.
- Dogheim, S.M., Gad Alla, S.A., El-Marsafy, A.M. and Fahmy, S.M. (1999). Monitoring pesticide residues in Egyptian fruits and vegetables in 1995. *J. Assoc. Off. Anal. Chem.* **82**: 948-955.
- Dong, F., Liu, X., Cheng, L., Chen, W., Li, L., Qin, D. and Zheng, Y. (2009). Determination of Metaflumizone Residues in cabbage and Soil Using Ultra-Performance Liquid Chromatography/ESI-MS/MS. *J.Sep.Sci.* **32**(21):3692-3697.

- Drozdzy ski, D. and Kowalska, J. (2009). Rapid Analysis of Organic Farming Insecticides in Soil and Produce Using Ultra-Performance Liquid Chromatography/Tandem Mass Spectrometry. *Anal. Bioanal. Chem.* **394**(8): 2241-2247.
- EL-Saeid, M.H. and Selim M.T. (2013). Multiresidue analysis of 86 pesticides using gas chromatography mass spectrometry: ii-nonleafy vegetables. *J. Chem.* **2013**: 1-10.
- EPA (Environmental Protection Agency). (2006). Climate Change Indicators in the United States. <https://www3.epa.gov/climatechange/pdfs/climateindicators>.
- European Commission. (2015). EU Pesticide Residue MRLs. Regulation no. 396/2005. <http://ec.europa.eu/sanco-pesticides/public/index.cfm>.
- Fan, S., Zhao, P., Yu, C., Pan, C. and Li, X. (2014). Simultaneous determination of 36 pesticide residues in spinach and cauliflower by LC-MS/MS using multiwalled carbon nanotubes-based dispersive solid-phase clean-up. *Food Addit. Contam.* **31**(1): 73–82.
- FAO. (2012) Global pact against plant pest marks 60 years in action. FAO celebrates anniversary of creation of the international plant protection convention in 3 April 2012, Rome. <http://www.fao.org/news/story/en/item/131114/icode/>
- FAOSTAT (Food and Agricultural Organization Statistics). (2002). www.faostat.fao.org.
- FAOSTAT. (2012). Global pact against plant pests marks 60 years in action. FAO celebrates anniversary of creation of the International Plant Protection Convention in 3 April 2012, Rome. www.faostat.fao.org.
- Farag, A.T., Eweidalt. M.H. and El-Okazy. A.M. (2000). Reproductive toxicology of acephate in male mice. *Reprod. Thxicol.* **14**: 457-462.
- Farag, R.S., Latif, M.S., El-Gawad, A.E. and Dogheim, S.M. (2011). Monitoring of pesticide residues in some Egyptian herbs, fruits and vegetables. Biochemistry Department, Faculty of Agriculture, Cairo University, Egypt. Agricultural Research Center, Ministry of Agricultural, Dokki, Egypt.

- Faustman, E.M. and Omenn, G.S. (2001). "Risk assessment". In Klaassen, Curtis D. Casarett & Doull's Toxicology: The Basic Science of Poisons (6th ed.). New York: McGraw-Hill. pp. 92-4.
- Fenik, J., Tankiewicz, M., & Biziuk, M. (2011). Properties and determination of pesticides in fruits and vegetables. *TrAC Trends in Anal. Chem.* **30**(6), 814-826.
- Fenoll, J., Hellin, P., Martinez, C.M., Miguel, M. and Flores, P. (2007). Multiresidue method for analysis of pesticides in pepper and tomato by gas chromatography with nitrogen–phosphorus detection. *Food Chem.* **105**: 711-719.
- Fernández, M.J.L., Garrido, F.A., Plaza, B.P. and Martínez V.J.L. (2008). Multiresidue method for the analysis of more than 140 pesticide residues in fruits and vegetables by gas chromatography coupled to triple quadrupole mass spectrometry. *J. Mass Spectrom.* **43**(9): 1235-1254.
- Fernández-Cruz, M.L., Grimalt, S., Villarroya, M., López, F.J., Llanos, S. and García-Baudín, J.M. (2006). Residue levels of captan and trichlorfon in field-treated kaki fruits, individual versus composite samples, and after household processing. *Food Addit. Contam.* **23**(6): 591-600.
- Ferrer, I., Garcia-Reyes, J.F., Mezcua, M., Thurman, E.M. and Fernandez-Alba, A.R. (2005). Multi-residue pesticide analysis in fruits and vegetables by liquid chromatography–time-of-flight mass spectrometry. *J. Chromatogr. A.* **1082**(1): 81-90.
- Ferrer, I., Garcia-Reyes, J.F., Mezcua, M., Thurman, E.M. and Fernandez-Alba, A.R. (2005). Multi-residue pesticide analysis in fruits and vegetables by liquid chromatography–time-of-flight mass spectrometry. *J. Chromatogr. A.* **1082**(1): 81-90.
- Frank, L.C. and Kacew, S. (2002). Lu's Basic toxicology: Fundamentals, Target Organs and Risk Assessment. Taylor & Francis. pp. 364.

- Frenich, A.G., Vidal, J.M., Pastor-Montoro, E. and Romero-González, R. (2008). High throughput determination of pesticide residues in food commodities by use of ultra-performance liquid chromatography–tandem mass spectrometry. *Anal. Bioanal. Chem.* **390**(3): 947-959.
- Gamon, A., Leo, C. and Ten, A. (2001). Multiresidue determination of pesticides in fruit and vegetables by gas chromatography. *J. AOAC Int.* **84**(4): 342-354.
- Garrido Frenich, A., Martinez Vidal, J. L., Cruz Sicilia, A.D., Gonzalez Rodriguez, M.J. and Plaza, B. (2006). Multiresidue analysis of organochlorine and organophosphorus pesticides in muscle of chicken, pork and lamb by gas chromatography-triple quadrupole mass spectrometry. *Anal. Chim. Acta.* **558**: 42-52.
- Garrido Frenich, A., Martinez Vidal, J.L., Lopez Lopez, T., Aquado, S.C. and Martinez Salvador, I. (2004). Monitoring multiclass pesticide residues in fresh fruits and vegetables by liquid chromatography with tandem mass spectrometry. *J. Chromatogr. A.* **1048**: 199-206.
- Garrido Frenich, A., Martinez Vidal, J.L., Moreno Frias, M., Olea-Serrano, F., Olea, N. and Cuadros-Rodreguez, L. (2003). Determination of organochlorine pesticides by GC-ECD and GC-MS-MS techniques including an evaluation of the uncertainty associated with the results. *Chromatographia.* **5**: 213-220.
- Garrido Frenich, A., Martinez Vidal, J.L., Pastor-Montoro, E. and Romero Gonzalez, R. (2008). High-throughput determination of pesticide residues in food commodities by use of ultra-performance liquid chromatography- tandem mass spectrometry. *Anal. Bioanal. Chem.* **390**: 947-959.
- Gilden, R.C., Huffling, K. and Sattler, B. (2010). Pesticides and health risks. *J. Obst. Gynecol. Neon. Nur.* **39**: 103-110.
- Hadian, Z., Azizi, M.H., Hosseiny, H. and Khosravi-darani, K. (2008). Determination of Pesticides in Fruits by Gas Chromatography/Mass Spectrometry after High Performance Gel Permeation Clean up. *Asian J. Chem.* **20**: 2643-2650.

- Hadiana, Z., Eslamizad, S. and Yazdanpanah, H. (2019). Pesticide residues analysis in Iranian fruits and vegetables by gas chromatography-mass spectrometry. *Iranian J. Pharma. Res.* **18**(1): 275-285.
- Hajslova, J., Holadova, K., Kocourek, V., Poustka, J., Godula, M., Cuhra, P. and Kempny, M. (1998). Matrix-induced effects: a critical point in the gas chromatographic analysis of pesticide residues. *J. Chromatogr. A.* **800**: 283-295.
- Hajšlová, J., & Zrostlikova, J. (2003). Matrix effects in (ultra) trace analysis of pesticide residues in food and biotic matrices. *J. Chromatogr. A.* **1000**(1-2), 181-197.
- Hallenbeck, W.H. and Cunningham, B.K.M. (1985). Pesticides and human health. New York: Springer-Verlag. pp. 28-29.
- Hans, G., Mol, J. Ruud, C.J., Dam, V. and Odile, M.S. (2003). Determination of polar organophosphorus pesticides in vegetables and fruits using liquid chromatography with tandem mass spectrometry: selection of extraction solvent. *J. Chromatogr. A.* **1015**: 119–127.
- Hasan, R., Prodhan, M.D.H., Rahman, S.M.M., Khanom, R. and Ullah, A. (2017) Determination of Organophosphorus Insecticide Residues in Country Bean Collected from Different Markets of Dhaka. *J. Env., Anal. Toxicol.* **7**: 489.
- Hayes, W.J. (1982). Pesticides studied in man. Baltimore. MD: Williams & Wilkins. Academic Press, Inc., NY. Vol. 1.
- Hayes, W.J. and Laws E.R. (1990). Handbook of Pesticide Toxicology. Classes of Pesticides. Academic Press, Inc., NY. Vol. 3.
- Heimstra, M. and Kok Andre de. (2007). Comprehensive multi-residue method for the target analysis of pesticides in crops using liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A.* **1154**: 3-25.
- Hercegová, A., Dömötöröová, M. and Matisová, E. (2007). Sample preparation methods in the analysis of pesticide residues in baby food with subsequent chromatographic determination. *J. Chromatogr. A.* **1153**(1-2): 54-73.

- Hornsby, A.G., Wauchope, R.D. and Herner, A.E. (1996). Pesticide Properties in the Environment; Springer-Verlag: New York. pp. 83-84.
- Hossain, M.S., Rahman, M.M., Kabir K.H., Miah, M.R.U. and Prodhan, M.D.H. (2014). Determination of Pre Harvest Interval (PHI) for cypermethrin and acephate in yard-long bean under supervised field trial. *Bangladesh J. Entomol.* **24**(1): 101-115.
- Hrouzkova, S. and Matisova, E. (2011). Fast gas chromatography and its use in pesticide residues analysis. Retrieved from www.intechopen.com. (Date: 12 Nov. 2013).
- IARC. (1998). Monographs on the Evaluation of Carcinogenic Risks to Humans; International Agency for Research on Cancer. Miscellaneous Pesticides. Lyon, France. **Vol.** 30, pp. 103.
- Islam, M.A., Ullah, A., Habib, M., Chowdhury, M.T.I., Khan, M.S.I., Kaium, A. and Prodhan, M.D.H. (2019). Determination of major organophosphate pesticide residues in cabbage collected from different markets of Dhaka. *Asia Pac.j.public Health.* **5**(2).
- Islam, M.S., Prodhan, M.D.H. and Uddin, M.K. (2019a). Analysis of the pesticide residues in bitter melon using modified QuEChERS extraction coupled with Gas Chromatography. *Asia Pac. J. public health* **5**(3).
- Islam, M.S., Prodhan, M.D.H. and Uddin, M.K. (2019b). Determination of Major Organophosphorus Pesticide Residues in Eggplant using QuEChERS Extraction and Gas Chromatography. **4**(8): 2165.
- Islam, M.W., Dastogeer, K.G., Hamim, I., Prodhan, M.D.H. and Ashrafuzzaman, M. (2014). Detection and quantification of pesticide residues in selected vegetables of Bangladesh. *J. of Phytopathol. and Pest Mgmt.***1**(2): 17-30.
- IUPAC (International Union of Pure and Applied Chemistry). (1997). Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Online corrected version: pesticide residue.
- Jallow, M.F., Awadh, D.G., Albaho, M.S., Devi, V.Y. and Ahmad, N. (2017). Monitoring of pesticide residues in commonly used fruits and vegetables in Kuwait. *Int. J. Environ. Res. and Public Health.* **14**(8), 833.

- Jansson, C., Pihlström T., Österdahl, B.G. and Markides, K.E. (2004). A new multi-residue method for analysis of pesticide residues in fruit and vegetables using liquid chromatography with tandem mass spectrometric detection. *J. Chromatogr. A*. **1023**: 93–104.
- Kabir, K.H., Abdullah, M., Prodhan, M.D.H., Ahmed, M.S. and Alam, M.N. (2007) Determination of carbofuran residue in the samples of sugarcane and soil of sugarcane field. *The Agriculturist*. **5**(1& 2):61-66.
- Kabir, K.H., Rahman, M.A., Ahmed, M.S., Prodhan, M.D.H. and Akon, M.W. (2008). Determination of residue of diazinon and carbosulfan in brinjal and quinalphos in yard long bean under supervised field trial. *Bangladesh J. Agril. Res.* **33**(3): 503-513.
- Kabir, K.H., Rahman, M.A., Ahmed, M.S., Prodhan, M.D.H. and Akon, M.W. (2008a). Quantitative analysis of some commonly used insecticides in vegetables. *Bangladesh J. Agriculturist*. **1**(2):259-264.
- Kaihara, A., Yoshii, K., Tsumura, Y., Ishimitsu, S. and Tonogai, Y. (2002). Multi-Residue Analysis of 18 Pesticides in Fresh Fruits, Vegetables and Rice by Supercritical Fluid Extraction and Liquid Chromatography-Electrospray Ionization Mass Spectrometry. *J. Health Sci.* **48**(2): 173-178.
- Kale, A.A., Cadakh, S.R. and Adsule, R.N. (1991). Physico-chemical characteristics of improved varieties of bittergourd (*Mom ordica chorantio* L.). *Maharashtra J. Hort.* **5**:56-59.
- Karant, S. and Pope, C. (2000). Carboxylesterase and A-Esterase Activities during Maturation and Aging: Relationship to the Toxicity of Chlorpyrifos and Parathion in Rats. *Toxicol. Sci.* **58**: 282-289.
- Khan, B.A. (2005). *Studies on the residues of commonly used Insecticides on fruits and vegetables grown in NWFP-Pakistan*. NWFP AGRICULTURAL UNIVERSITY, PESHAWAR-PAKISTAN.

- Kikuchi, Y., Hayatsu, M, Hosokawa, T., Nagayama. A., Tago, K. and Fukatsu, T. (2012). Symbiont-mediated insecticide resistance. *Proc. Natl Acad. Sci.* **109**(22): 861-885.
- Klaassen, C.D. (2001). Casarett and Doull's Toxicology. The Basic Science of Poisons. 6th ed.; McGraw-hill: New York. pp. 1236.
- Kmellár, B., Abrankó, L., Fodora, P. and Lehotay, S.J. (2010). Routine Approach to Qualitatively Screening 300 Pesticides and Quantification of Those Frequently Detected in Fruit and Vegetables Using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). *Food Addit. Contam.* **27**(10): 1415-1430.
- Kmellár, B., Fodor, P., Pareja, L., Ferrer, C., Martínez-Uroz, M.A., Valverde, A. and Fernandez-Alba, A.R. (2008). Validation and Uncertainty Study of a Comprehensive List of 160 Pesticide Residues in Multi-Class Vegetables by Liquid Chromatography–Tandem Mass Spectrometry. *J. Chromatogr. A.* **1215**(1-2): 37-50.
- Koesukwiwat, U., Lehotay, S.J., Miao, S. and Leepipatpiboon, N. (2010). High Throughput Analysis of 150 Pesticides in Fruits and Vegetables Using QuEChERS and Low Pressure Gas Chromatography-Time-of-Flight Mass Spectrometry. *J. Chromatogr. A.* **1217**(43): 6692-6703.
- Kumar, Y. and Hossmanni, R. S. (2001). Magnitude of the residue of carbofuran and 3-hydroxy carbofuran in/on rice in India following furadan 3G insecticide treatment. p. 309.
- Kumari, D. and John, S. (2019). Health risk assessment of pesticide residues in fruits and vegetables from farms and markets of Western Indian Himalayan region. *Chemosphere*, **224**: 162-167.
- Latif, Y., Sherazi, S.T.H. and Bhangar, M.I. (2011). Assessment of pesticide residues in commonly used vegetables in Hyderabad, Pakistan. *Ecotoxicol. Environ. Saf.* **74**: 2299-2303.

- Lawal, A., Wong, R.C.S., Tan, G.H. and Abdula'uf, L.B. (2018). Determination of Pesticide Residues in Fruit and Vegetables by High-Performance Liquid Chromatography–Tandem Mass Spectrometry with Multivariate Response Surface Methodology. *Analytical Letters*. pp. 1-18.
- Lehotay, S.J. (2000). Analysis of pesticide residues in mixed fruit vegetable extracts by direct sample introduction gas chromatography. *J. AOAC Int.* **83**(3): 312-314.
- Lehotay, S.J. (2007). Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning With Magnesium Sulfate: Collaborative Study. *J. AOAC Int.* **90**(2): 485-520.
- Lehotay, S.J., de Kok, A., Hiemstra, M. and van Bodegraven, P. (2005). Validation of a Fast and Easy Method for the Determination of Residues from 229 Pesticides in Fruits and Vegetables Using Gas and Liquid Chromatography and Mass Spectrometric Detection. *J. AOAC Int.* **88**(2): 595-614.
- Lehotay, S.J., Mastovská, K. and Light field, A.R. (2005a). Use of buffering to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. *J. AOAC Int.* **88**: 615-629.
- Lehotay, S.J., Mastovska, K. and Yun, S.J. (2005b). Evaluation of Two Fast and Easy Methods for Pesticide Residue Analysis in Fatty Food Matrixes. *J. AOAC Int.* **88**(2): 630-638.
- Liu, H., Bai, X. and Pang, X. (2020). Intercity variability and local factors influencing the level of pesticide residues in marketed fruits and vegetables of China. *Science of The Total Environment*, 700, 134481.
- Loha, K. M., Lamoree, M. and De Boer, J. (2020). Pesticide residue levels in vegetables and surface waters at the Central Rift Valley (CRV) of Ethiopia. *Environ. Monit. and Assess.* **192**(8):1-14.
- Lotti, M. and Moretto, A. (2005). Organophosphate-Induced Delayed Polyneuropathy. *Toxicol.* **24**(1): 37-49.

- Lucini, L. and Molinari, G.P. (2011). Performance and matrix effect observed in QuEChERS extraction and tandem mass spectrometry analyses of pesticide residues in different target crops. *J. Chromatogr. Sci.* **49**:709-714.
- Luo, X., Qin, X., Chen, D., Liu, Z., Zhang, K. and Hu, D. (2020). Determination, residue analysis, risk assessment and processing factors of tebufenozide in okra fruits under field conditions. *Int. J. Food Sci. Tech.* **100**(3): 1230-1237.
- Mahajna, M., Quistad, G.B. and Casida, J.E. (1997). Acephale insecticide Toxicity: Safety Conferred by Inhibition of the Bioactivating Carboxamidase by the Metabolite Methamidophos. *Chem. Res. Toxicol.* **10**: 64-69.
- Malsha, K., John, I., William, B.A., Michael, H.J. and Lee, A. B. (2011). Fipronil toxicity in northern bobwhite quail *Colinus virginianus*: Reduced feeding behaviour and sulfone metabolite formation. *Chemosphere.* **83**(4): 524–530.
- Mao, X., Wan, Y., Li, Z., Chen, L., Lew, H. and Yang, H. (2020). Analysis of organophosphorus and pyrethroid pesticides in organic and conventional vegetables using QuEChERS combined with dispersive liquid-liquid micro extraction based on the solidification of floating organic droplet. *Food chemistry.* **309**: 125755.
- McIntyre, A.N., Allison, N. and Penman, D.R. (1989). Pesticides issues and options for New Zealand. Ministry for the Environment, Wellington, New Zealand. **7**:29.
- Mebdoua, S., Lazali, M., Ounane, S.M., Tellah, S., Nabi, F. and Ounane, G. (2017). Evaluation of pesticide residues in fruits and vegetables from Algeria. *Food Additives & Contaminants: Part B*, **10**(2): 91-98.
- Meister, R.T. (1992). Farm Chemicals Handbook. Meister Publishing Co. Willoughby, OH. pp. 54.
- Milhome, M.A.L., Sousa, J.S., Andrade, G.A., Castro, R.C.D., Lima, L.K., Lima, C.G. and Nascimento, R.F. (2013). Validation and uncertainty of the method for multiresidue analysis of 35 pesticides in melon using gas chromatography coupled to quadrupole mass spectrometry (GC-QP/MS). *J. Physics: conf. ser.* **575**: 1-5.

- Mol, H.G.J., Plaza- Bolanos, P., Zomer, P., De Rijk, T.C., Stolker, A.A.M. and Mulder, P.P.J. (2008). Toward a generic extraction method for simultaneous determination of pesticides, mycotoxins, plant toxins and veterinary drugs in feed and food matrixes. *Anal. Chem.* **80**: 9450-9459.
- Nahar, K.M., Khan, M.S.I., Habib, M., Hossain, S.M., Prodhan, M.D.H. and Islam, M.A. (2020) Health risk assessment of pesticide residues in vegetables collected from northern part of Bangladesh. *Food Research*, **4**(6): 2281 – 2288.
- Narendaran, S. T., Meyyanathan, S. N. and Babu, B. (2020). Review of pesticides residue analysis in fruits and vegetables. Pre-treatment, extraction and detection techniques. *Food Research International*, 109141.
- Neetu, T. (2013). Determination of chlorinated pesticide in vegetables, cereals and pulses by gas chromatography in east national capital region, *Delhi, India. Res. J. Agri. Forestry Sci.* **1**(1): 27-28.
- Nguyen, T.D., Lee, M.H. and Lee, G.H. (2008). Multiresidue Determination of 156 Pesticides in Watermelon by Dispersive Solid Phase Extraction and Gas Chromatography/Mass Spectrometry. *Bull. Korean Chem. Soc.* **29**: 12.
- Nguyen, T.D.; Yun, M.Y. and Lee, G.H. (2009). A Multiresidue Method for the Determination of 118 Pesticides in Vegetable Juice by Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Tandem Mass Spectrometry. *J. Agric. Food Chem.* **57**(21): 10095-10101.
- Nimsha, M.K.L.K.R., Weerakkody, S. and Yoshida, P.W. (2018). Quantification of Pesticide Residues in Selected Vegetables using the QuEChERS Method. *OUSL Journa.* **13**(01), 29-42.
- Obana, H., Okihashi, M., Akutsu, K., Kitagawa, Y. and Hori, S. (2003). Determination of neonicotinoid pesticide residues in vegetables and fruits with Solid Phase Extraction and Liquid Chromatography Mass Spectrometry. *J. Agric. Food Chem.* **51**: 2501-2505.
- Ochiai, N., Sasamoto, K., Kanda, H., Yamagami, T. and David, F. (2009). Multi-residue method for determination of 85 pesticides in vegetables, fruits and

green tea by stir bar sorptive extraction and thermal desorption GC-MS. Retrieved from www.gerstel.de. (Date: 16th Nov., 2013).

- Oellig, C. and Schmid, S. (2019). Polyethyleneimine as weak anionic exchanger adsorbent for clean-up in pesticide residue analysis of fruits and vegetables. *J. Chromatograph. A.* **1597**: 9-17.
- Ono, Y., Yamagami, T., Nishina, T. and Tobino, T. (2006). Pesticide Multiresidue Analysis of 303 Compounds Using Supercritical Fluid Extraction., *Anal.Sci.* **22**(11): 1473-1476.
- Ortelli, D., Edder, P. and Corvi, C. (2004). Multi-residue analysis of 74 pesticides in fruits and vegetables by liquid chromatography-electrospray-tandem mass spectrometry. *Analytic. Chemical. Acta.* **520**: 33-45.
- Pang, G.F., Fan, C.L., Liu, Y.M., Cao, Y.Z., Zhang, J.J., Li, X.M., Li, Z.Y., Wu, Y.P. and Guo, T.T. (2006). Determination of residues of 446 pesticides in fruits and vegetables by three-cartridge solid-phase extraction-gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. *J. AOAC Int.* **89**(3): 740-771.
- Panhwar, A.A. and Sheikh, A.S. (2013). Assessment of pesticide residues in cauliflower through gas chromatography- μ ECD and High Performance Liquid Chromatography (HPLC) analysis. *Int. J. Agric. Sci. Res.* **3**(1): 7-16.
- Paramasivam, M. and Chandrasekaran, S. (2012). Determination of fipronil and its major metabolites in vegetables, fruit and soil using QuEChERS and gas chromatography-mass spectrometry. *Intern. J. Environ. Anal. Chem.* **93**(11): 1203–1211.
- Paranthaman, R., Sudha, A. and Kumaravel, S. (2012). Determination of Pesticide Residues in Banana by Using High Performance Liquid Chromatography and Gas Chromatography-Mass Spectrometry. *American J. Biochem. Biotechnol.* **8**(1): 1-6.
- Park, D.W., Kim, K.G., Choi, E.A., Kang, G.R., Kim, T.S., Yang, Y.S., Moon, S.J., Ha, D.R., Kim, E.S. and Cho, B.S. (2016). Pesticide residues in leafy

- vegetables stalk and stem vegetables from South Korea: a long term study on safety and health risk assessment. *Food Addit. Contam.* **33**(1): 105-118.
- Parveen, Z., Riazuddin, Iqbal, S., Khuhro, M.I., Bhutto, M.A. and Ahmed, M. (2011). Monitoring of multi-residue pesticide residues in some fruits in Karachi, Pakistan. *Pak. J. Bot.* **43**(4): 1915-1918.
- Patel, K., Fussel, R.J., Hetmanski, M., Goodall, D.M. and Keely, B.J. (2005). Evaluation of gas chromatography–tandem quadrupole mass spectrometry for the determination of organochlorine pesticides in fats and oils. *J. Chromatogr. A.* **1068**: 289- 296.
- Pico, Y., Font, G., Ruiz, M.J. and Fernandez, M. (2006). Control of pesticide residues by liquid chromatography-mass spectrometry to ensure food safety. *Mass Spectrom. Rev.* **25**: 917-960.
- Polat, B. and Tiryaki, O. (2019). Determination of some pesticide residues in conventional-grown and IPM-grown tomato by using QuEChERS method. *Journal of Environmental Science and Health.* **54**(2): 112-117.
- Prodhan, M.D.H., Papadakis, E.N. and Mourkidou, E.P. (2015). Determination of multiple pesticide residues in eggplant with liquid chromatography-mass spectrometry. *Food Anal. Methods.* **8**: 229–235.
- Prodhan, M.D.H., Papadakis, E.N. and Mourkidou, E.P. (2015a). Analysis of pesticide residues in melon using QuEChERS extraction and liquid chromatography triple quadrupole mass spectrometry. *Int. J. Env. Anal. Chem.* **95**(13): 1219-1229.
- Prodhan, M.D.H., Papadakis, E.N. and Mourkidou, E.P. (2016). Variability of pesticide residues in cauliflower units collected from a field trial, and market places in Greece. *J. Env.Sci. Health.* **51**(9): 644-653.
- Prodhan, M.D.H. and Alam, S.N. (2018c). Determination of multiple organochlorine pesticide residues in shrimp using modified QuEChERS extraction and gas chromatography. *SAARC J. Agri.* **16**(1): 81-93.
- Prodhan, M.D.H. Emmanouil-N. Papadakis, Euphemia Papadopoulou Mourkidou (2018). Variability of pesticide residues in eggplant units collected from a

- field trial and marketplaces in Greece. *J. Sci. Food. Agric.* 2018; **98**: 2277–2284.
- Prodhan, M.D.H., Akon, M.W. and Alam, S.N. (2018a). Determination of pre-harvest interval for quinalphos, malathion, diazinon and cypermethrin in major vegetables. *J Environ. Anal. Toxicol.* **8**(553): 2161-0525.
- Prodhan, M.D.H., Akon, M.W. and Alam, S.N. (2018b). Decontamination of organophosphorus insecticide residues from eggplant and yard long bean. *Int. J. Expt. Agric.* **8**(1): 6-9.
- Prodhan, M.D.H., Papadakis, E.N. and Papadopoulou-Mourkidou, E. (2016a). Analysis of Pesticide Residues and Their Variability in Cabbage Using QuEChERS Extraction in Combination with LC-MS/MS. *Food Anal. Methods.* **9**(12): 3470–3478.
- Prodhan, M.D.H., Rahman, M.A., Ahmed, M.S. and Kabir, K.H. (2009). Quantification of organophosphorus and organochlorine insecticide residues from fish samples using simple GC technique. *Bangladesh J. Agriculturist.* **2**(2): 197-204.
- Prodhan, M.D.H., Rahman, M.A., Ahmed, M.S. and Kabir, K.H. (2010). Pesticide residues in fish samples collected from different fish cultivation regions of Bangladesh. *SAARC J. Agri.* **8**(2): 53-64.
- Rahman, A.M. (1999). Effect and residues of two selected insecticides applied for control of brinjal shoot and fruit borer in eggplant. An MS Thesis submitted to BSMRAU, Gazipur, Bangladesh, pp.1-4.
- Rahman, M.M. (2010). Crop and commodity pest management. In: Radcliffe's IPM World Textbook, E.B. Radcliffe, W.D. hutchison and R.E. Cancelado (eds.). URL:<http://ipmworld.umn.edu>, University of Minnesota, St Paul, MN.
- Ramadan, M.F., Abdel-Hamid, M., Altorgoman, M.M., Al Garamah, H.A., Alawi, M.A., Shati, A.A. and Awwad, N.S. (2020). Evaluation of Pesticide Residues in Vegetables from the Asir Region, Saudi Arabia. *Molecules.* **25**(1): 205.

- Randall, C., Arnold, J., Patterson, F., Silvester, J., Robertson, I., Cooper, C.L., Burnes, B., Harris, D. and Axtell, C. (2013). National Pesticide Applicator Certification Core Manual. National Association of State Departments of Agriculture Research Foundation, Washington, DC, Ch.1.
- Rani, G.B., Sri, C.N.S., Rishita, Y., Saikia, N. and Rao, C.S. (2019). Domestic methods for the removal of pesticide residues in chillies. *J. Pharmacogn. Phytochem.* **8**(4): 2690-2693.
- Rashid, A., Nawaz, S., Barker, H., Ahmad, I. and Ashraf, M. (2010). Development of a Simple Extraction and Clean-Up Procedure for Determination of Organochlorine Pesticides in Soil Using Gas Chromatography-Tandem Mass Spectrometry. *J. Chromatogr. A.* **1217**(17): 2933-2939.
- Rasul, G. and Thapa, G.B. (2004). Sustainability of ecological and conventional agricultural systems in Bangladesh: An assesment based on environmental, economic and social perspectives. *Agricultural system.* **79**(3): 327-351.
- Regassa, C., Tolcha, T., Gomoro, K. and Megersa, N. (2020). Determination of Residue Levels of DDT and Its Metabolites in Khat and Cabbage Samples using QuEChERS Sample Preparation Method Combined with GC-MS Detection. *Ethiop. J.Sci. Sust. Develop.* **7**(1): 44-53.
- Reigart, J.R. and Roberts, J.R. (1999). Organophosphate Insecticides, Recognition and Management of Pesticide Poisonings, 5th ed.: U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticides Programs; U.S. Government Printing Office: Washington, DC. pp. 34-47.
- Rimkus, G.G., Rummler, M. and Nausch, I. (1996). Gel permeation chromatography-high performance liquid chromatography combination as an automated clean-up technique for the multiresidue analysis of fats. *J. Chromatogr. A.* **737**(1): 9-14.

- Rissato, S.R., Galhiane, M.S., Apon, B. and Arruda, M. (2005). Multiresidue Analysis of Pesticides in Soil by Supercritical Fluid Extraction/Gas Chromatography with Electron-Capture Detection and Confirmation by Gas Chromatography-Mass Spectrometry. *J. Agric. Food Chem.* **53**(1): 62-69.
- Rissato, S.R., Galhiane, M.S., De Souza, A.G. and Apon, B.M. (2005a). Development of a Supercritical Fluid Extraction Method for Simultaneous Determination of Organophosphorus, Organohalogen, Organonitrogen and Pyrethroids Pesticides in Fruit and Vegetables and Its Comparison with a Conventional Method by GC-ECD and GC-MS. *J. Braz. Chem. Soc.* **16** (5): 1038-1047.
- Robert, G.J., Gaylord, L.P., Stephen, C., Diana, L., George, B.F. and Susan, G.E. (2003). Atropine availability as an antidote for nerve agent casualties: Validated rapid reformulation of high-concentration atropine from bulk powder. *Annal. Emerg. Med.* **41**(4): 453-456.
- Sahoo, S.K., Battu, R.S. and Singh, B. (2011). Development and validation of quechers method for estimation of propamocarb residues in tomato (*Lycopersicon esculentum* Mill) and soil. *American J. Anal. Chem.* **2**: 26-31.
- Salwa, M., Dogheim, A., Gad A.S. and Ashraf, M. E. (1999). Monitoring pesticide residues in Egyptian fruit and vegetables in 1995. *J. Offic. Associ. Analyt. Chem.* **82**(4): 948-955.
- Satoshi, T., Yamamoto, H., Fukui, N., Yamaguchi, S., Kitagawa, Y., Kakimoto, Y., Osakada, M., Okihashi, M., Kajimura, K. and Obana, H. (2013). Validation Study on a Rapid Multi-Residue Method for Determination of Pesticide Residues in Vegetables and Fruits by LC-MS/MS. *Food Hyg. Saf. Sci.* **54**(3): 237-249.
- Satpathy, G., Tyagi, Y.K. and Gupta, R.K. (2014). Development and validation of multi-residue analysis of 82 pesticides in grapes and pomegranate as per the requirements of the European Union (EU) and codex alimentations using GC-MS/MS with compound based screening. *American J. Food Sci. and Tech.* **2**(2): 53-61.
- Schreiber, A. and Wittrig, R. (2010). Enhanced LC/MS for the quantitation and identification of pesticide in food sample. *Agro Food Ind. Hi Tech.* **21**: 18-22.

- Shi, C., Gui, W., Chen, J. and Zhu, G. (2010). Determination of Oxadiargyl Residues in Environmental Samples and Rice Samples. *Bull. Environ. Contam. Toxicol.* **84** (2): 236-239.
- Smegal, D.C. (2000). Human Health Risk Assessment Chlorpyrifos; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, Health Effects Division, U.S. Government Printing Office: Washington, DC. pp. 1-131.
- Smith, D.S. and Treheme, J.E. (1965). The Electron Microscopic Localizations of Cholinesterase Activity in the Central Nervous System of an Insect, *Periplaneta Americana* I. *J. Cell Biol.* **26**: 445-465.
- Srivastava, A.K., Trivedi, P., Srivastava, M.K., Lohani, M. and Srivastava, L.P. (2011). Monitoring of pesticide residues in market basket samples of vegetable from Lucknow City, India: QuEChERS method. *Environ. Monit. Assess.* **176**: 465–472.
- Stachniuk, A., Szmagara, A., Czaczo, R. and Fornal, E. (2017). LC-MS/MS determination of pesticide residues in fruits and vegetables. *J. Environ. Sci. Health C, Part B.* **52**(7): 446-457.
- Tankiewicz, M. (2019). Determination of selected priority pesticides in high water fruits and vegetables by modified QuEChERS and GC-ECD with GC-MS/MS confirmation. *Molecules.* **24**(3): 417.
- Tankiewicz, M. (2019). Determination of selected priority pesticides in high water fruits and vegetables by modified QuEChERS and GC-ECD with GCMS/MS confirmation. *Molecules.* **24**(3): 417:1-17.
- Thanh, D.N., Byung, S.L., Bo, R.L., Dae, M.L. and Lee, G.H. (2007). A Multiresidue Method for the Determination of 109 Pesticides in Rice Using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) Sample Preparation Method and Gas Chromatography/Mass Spectrometry with Temperature Control and Vacuum Concentration. *Rapid Commun. Mass Spectrom.* **21**(18): 3115-3122.
- Thomson, W.T. (1989). Acephate. Agricultural Chemicals Book I - Insecticides, Acaricides, and Ovicides; Thomson Publications: Fresno. CA. pp. 1.

- Tomlin, C.D.S. (2006). *The Pesticide Manual. A World Compendium*. 14th ed.; British Crop Protection Council: Alton. Hampshire, UK. pp. 5-6.
- Toxicological Profile for Chlorpyrifos; U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Public Health Service: Atlanta, 1997.
- U.S. DHHS. (2008). Hazardous Substances Databank (HSDB). Malathion; U.S. Department of Health and Human Services. National Institutes of Health. National Library of Medicine. (<http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB> (accessed Jan 2008), updated June 2005)
- Valverde-García, A., Fernandez-Alba, A., Contreras, M. and Agüera, A. (1996). Supercritical Fluid Extraction of Pesticides from Vegetables Using Anhydrous Magnesium Sulfate for Sample Preparation. *J. Agric. Food Chem.* **44**(7): 1780-1784.
- Vidal, J.L.M., Arrebola, F.J., Garrido Frenich, A., Fernandez, J.M. and Mateu-Sanchez, M. (2004). Validation of a gas chromatographic-tandem mass spectrometric method for analysis of pesticide residues in six food commodities.selection of a reference matrix for calibration. *Chromatographia.* **59**: 321-327.
- Walter, J.C. (2009). Chlorinated Pesticides: Threats to Health and Importance of Detection. *Environ. Med.* **14**(4): 347–59.
- Wang, D., Atkinson, S., Hoover-Miller, A. and Li, Q.X. (2005). Analysis of organochlorines in harbor seal (*Phoca vitulina*) tissue samples from Alaska using gas chromatography/ion trap mass spectrometry by an isotopic dilution technique. *Rapid Commun.Mass Spectrom.* **19**: 1815-1821.
- Wang, J., Leung, D. and Chow, W. (2010). Applications of LC/ESI-MS/MS and UHPLCQqTOF MS for the Determination of 148 Pesticides in Berries. *J. Agric. Food Chem.* **58**(10): 5904-5925.
- Wargovich, M. J. (2000). Anticancer properties of fruits and vegetables. *Hort. Sci.* **35**:573-575.

- Wesselink, A.K., Hatch, E.E., Rothman, K.J., Willis, S.K., Orta, O.R. and Wise, L.A. (2020). Pesticide residue intake from fruits and vegetables and fecundability in a North American preconception cohort study. *Environment International*, 139, 105693.
- WHO. (1987). "Principles for the safety assessment of food additives and contaminants in food". *Environmental Health Criteria* 70.
- William, B.A., Paul, S.G., Karen, F.J., Russell, B.V. and Lee, A.B. (2008). Fenitrothion, an organophosphate, affects running endurance but not aerobic capacity in fat-tailed dunnarts (*Sminthopsis crassicaudata*). *Chemosphere*. **72**(9): 1315–1320.
- Worthing, C.R. (1987). *The pesticide manual: A world compendium*. 8th Ed. The British Crop Protection Council. Croydon, England.
- Yamagami, T., David, M. and Sandra, P. (2009). Multi-Residue Method for Determination of 85 Pesticides in Vegetables, Fruits and Green Tea by Stir Bar Sorptive Extraction and Thermal Desorption GC-MS. Retrieved from www.gerstel.de. (Date: 16th Nov., 2013).
- Yamamoto and Izuru (1999). *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*. Tokyo: Springer-Verlag. pp. 3-27.
- Yang, X.B., Ying, G.G. and Kookana, R.S. (2010). Rapid Multiresidue Determination for Currently Used Pesticides in Agricultural Drainage Waters and Soils Using Gas Chromatography-Mass Spectrometry. *J. Environ. Sci. Health*. **45**(2): 152-161.
- Ye, X., Shao, H., Zhou, T., Xu, J., Cao, X. and Mo, W. (2020). Analysis of Organochlorine Pesticides in Tomatoes Using a Modified QuEChERS Method Based on N-Doped Graphitized Carbon Coupled with GC-MS/MS. *Food Analytical Methods*. 1-10.