

**DETERMINATION OF ORGANOPHOSPHORUS PESTICIDE  
RESIDUES IN EGGPLANT AND CAULIFLOWER COLLECTED  
FROM DIFFERENT MARKETS OF DHAKA**

**BY**

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*CERTIFICATE*

This is to certify that the thesis entitled “**DETERMINATION OF ORGANOPHOSPHORUS PESTICIDE RESIDUES IN EGGPLANT AND CAULIFLOWER COLLECTED FROM DIFFERENT MARKETS OF DHAKA**” 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 **MARZIA HABIB**, Registration No. **10-03792** 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.

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**June, 2016**



*Dedicated to  
My  
Beloved Parents*





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# DETERMINATION OF ORGANOPHOSPHORUS PESTICIDE RESIDUES IN EGGPLANT AND CAULIFLOWER COLLECTED FROM DIFFERENT MARKETS OF DHAKA

## ABSTRACT

The study was conducted to analyze pesticide residues in two common vegetables (eggplant and cauliflower) collected from five different areas (Kawran Bazaar, Mohammadpur Krishi Market, Mirpur-1 Bazar, Taltola Bazar and Jatrabari Bazar) of Dhaka city from September 2016 to March 2017. The collected samples were carried to the Pesticide Analytical Laboratory, Entomology Division of Bangladesh Agricultural Research Institute (BARI), Gazipur on the same sampling day. The collected samples were analyzed using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and Gas Chromatography (GC) coupled with Flame Thermionized Detector (FTD) for the determination of pesticide residues in 50 samples of eggplant and 50 samples of cauliflower. Among the 50 analyzed samples of eggplant, 9 samples (18% of the total number of samples) contained pesticide residues of Chlorpyrifos, Diazinon and Dimethoate. Among these 9 samples, 8 had residues above the Maximum Residue Limit (MRL) set by European Commission and one had the residue level exactly same as the Maximum Residue Limit (MRL) set by European Commission. Out of 50 samples of cauliflower, 8 samples (16% of the total no. of samples) contained pesticide residues of Chlorpyrifos, Diazinon and Quinalphos. All the contaminated samples of cauliflower contained residue above Maximum Residue Limit (MRL). Chlorpyrifos and Dimethoate were detected in most of the contaminated eggplant sample, while Chlorpyrifos and Diazinon were detected mostly in cauliflower samples. This study reflects the overall scenario of pesticide residue remain in eggplant and cauliflower in the local markets of Dhaka city, which will help the consumer to be aware of their health and safety.

**Key words:** Eggplant, Cauliflower, Gas Chromatography, Pesticide residues, QuEChERS Extraction

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## LIST OF ABBREVIATIONS

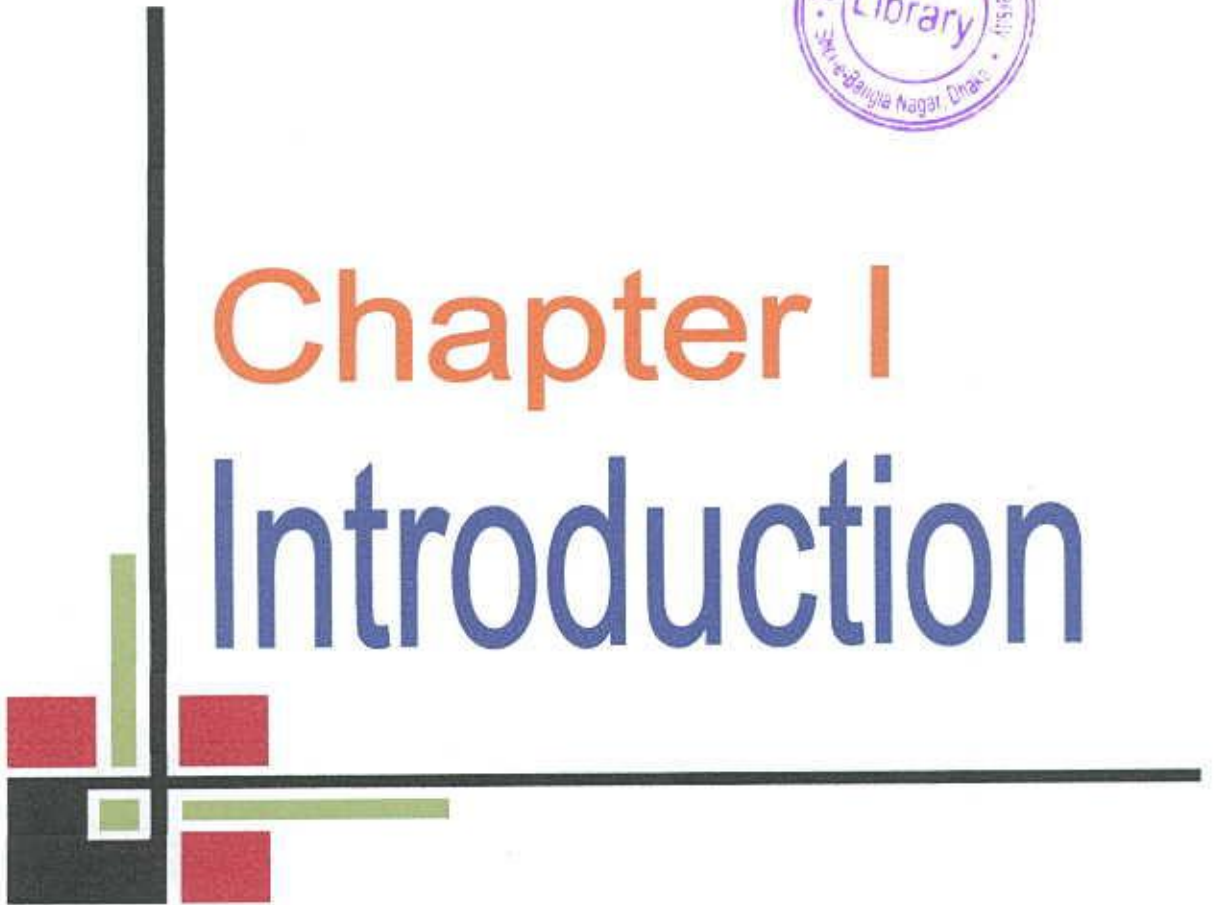
ADI	Acceptable Daily Intake
AOAC	Association of Analytical Chemist
BARI	Bangladesh Agricultural Research Institute
CSN	Committee for Standardization
DAS	Days After Spraying
DLLME	Dispersive Liquid-Liquid Microextraction
d-SPE	dispersive Solid Phase Extraction
ECD	Electron Capture Detector
<i>et all</i>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





# Chapter I

# Introduction





## CHAPTER I INTRODUCTION

Vegetable becomes the other name of healthy diet in today's life. Being the source of vitamins, minerals, dietary fiber, and phytochemicals, vegetables are essential for balanced diet. They are naturally low in fat and calories. None have cholesterol. They are important sources of many nutrients, including potassium, folate (folic acid), vitamin A, and vitamin C. In the daily diet vegetables have been closely associated with the improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some forms of cancer (Dias, 2012). Low vegetable intake, in unbalanced diets, has been estimated to cause about 31% of ischemic heart disease and 11% of stroke worldwide. According to the 2007 World Health Report, unbalanced diets with low vegetable consumption and low intake of complex carbohydrates and dietary fiber are estimated to cause 2.7 million deaths each year, and were among the top 10 risk factors contributing to mortality (Dias, 2011).

Like other vegetables eggplant and cauliflower also contain lots of vitamins and minerals. In addition to featuring a host of vitamins and minerals, eggplant also contains important phytochemicals which have antioxidant activity. Phytochemicals contained in eggplant include phenolic compounds, such as caffeic and chlorogenic acid, and flavonoids, such as nasunin (Dias, 2012).

Eggplant, (*Solanum melongena*) was originally domesticated from the wild nightshade species, the thorn or bitter apple, *S. incanum*, (Tsao and Lo, 2006; Doijode, 2001); probably with two independent domestications, one in the region of South Asia, and one in East Asia (USDA, 2014) is an important vegetable in Bangladesh. Eggplant is one of the healthiest, most nutritious and most consumed vegetable as it is available all the year round. It is cultivated in kitchen and commercial gardens during both Rabi and Kharif season in Bangladesh. Eggplant is a primary source of cash income for resource-poor farmers in Bangladesh. Currently, 7.8 percent of the land under vegetable crops, 64,208, hectares is dedicated to growing about 340,000 metric tons of eggplant per annum.

In the tropics, eggplant production is severely constrained by several insect and mite pests. The major pests include eggplant shoot and fruit borer, whitefly, thrips, aphid, leaf roller, red spider mite, and little leaf disease. Growers rely heavily on chemical pesticides to protect their eggplant fruit as well as the plant. For instance, farmers in certain areas of Philippines spray chemical insecticides up to 56 times during a cropping season; the total quantity of pesticide used per hectare of eggplant was about 41 liters of different brands belonging to the four major pesticide groups (Gapud and Canapi, 1994; Orden *et al.*, 1994). In Bangladesh, some farmers spray about 180 times during a cropping season (SUSVEG-Asia, 2007). Pesticide misuse has adverse effects on the environment and human health and on the other hand increases the cost of production. The share of the expense of pesticide to total material input cost was 55% for eggplant compared with cabbage (49%) and tomato (31%) in Philippines (Orden *et al.*, 1994), and accounted for 40–50 percent in Bangladesh (SUSVEG-Asia 2007). Many farmers avoid growing eggplant due to the cost of pesticides (Gapud and Canapi, 1994).

Eggplant suffers heavily at fruiting stage due to attack of shoot and fruit borers causing 70% damage to the crop and making it totally unfit for human consumption (Misra *et al.*, 1996). As the manual control is costly, time consuming and laborious, most of the farmer prefer chemical control and use different types of pesticides, many of them without knowing the correct application rate, application procedure, and precaution to be taken.

Cauliflower, (*Brassica oleracea botrytis*) is a vegetable of the family of Brassicaceae. Typically, only the head is eaten. The head is composed of a white inflorescence meristem. Cauliflower is an excellent source of vitamin C, vitamin K, folate, pantothenic acid, and vitamin B6. It is a high quality source of choline, dietary fiber, omega-3 fatty acids, manganese, phosphorus, and biotin. Furthermore, it is a good source of vitamin B1, B2, and B3, the minerals potassium and magnesium, and protein. Like other vegetables, cauliflower is also very susceptible to insect infestation. Diamond back moth, leaf webber, stem borer, tobacco caterpillar, aphids, painted bug etc. are main insects of cauliflower.

In case of severe infestation in both eggplant and cauliflower farmers are being bound to use chemical control methods as the manual control is costly, time consuming and laborious. So,





pesticides are being unavoidable for economic production as the market value of insect infested eggplant fruits and cauliflower heads are very much lower than the un-infested one. The warm and humid climatic condition of Bangladesh, increased use of modern high yielding varieties and disproportionate use of chemical fertilizers are highly favorable for development and multiplication of pests and diseases. As a sub-tropical country, in Bangladesh we face a wide range of temperature and humidity in different season of the year which provides a chance of multiplication and development to a wide range of insects. There are many genus/species of insects which attack multiple crops and already attained resistance because of using some common pesticides year after year. These things may sometimes make confusion and farmers are likely to use overdose more frequently.

Moreover, in Bangladesh, the food production has been more than doubled since 1971, but the food security still remains a major development issue for its huge population (Weinberger *et al.*, 2005). As the process of agricultural production is supported by the increasing use of agrochemicals and multiple cropping, the Government of Bangladesh has called for diversify the cropping pattern (Hoque, 2000). Diversification in vegetable crops and increasing commercialization can support the development of the agriculture sector in several ways. Although in Bangladesh the vegetable production is increasing day by day, various statistical data shows that it is mainly because of area expansion, not because of yield improvement. One potential drawback associated with a shift toward more intensive vegetable production is the reliance of most vegetable producers on heavy application of pesticide (Hossain *et al.*, 2000).

The use of pesticides plays an important role to eliminate the pests and for economic production however, it is causing threat to the human health, non-target organisms and the ecosystem when used indiscriminately without knowing their hazardous effects. Either these pesticides reach the human body directly or via food chain, causing various problems like hazardous health effect, different human and animal diseases, pesticide pollution, resistance and accumulation of pesticide residues in the body of animals and human beings. Indiscriminate use of pesticides has resulted in contamination of natural sources, air, water, and food, the risk to humans may be short term as well as long term. The intensity of the risk depends on the persistence of the pesticide, the exposure period, and other environmental conditions. We must ensure the safe agricultural product by judicious use of chemicals in agriculture. The use of pesticides should be



in such a way that there must not any residues in food product or if any, the amount must not exceed the ADI (Acceptable Daily Intake) or the MRL's (Maximum Residue Level).

Now a day's consumers are being concern for the presence of pesticide residues in daily food products because of its potential detrimental effects on non-target organisms than the target pest. The most destructive effects are interfering with the reproductive systems and fetal development as well as their capacity to cause cancer and asthma (Gilden *et al.*, 2010). Many pesticides are also responsible for adverse effect on natural ecosystem and reducing biodiversity. Pesticides in soil hindered nitrogen fixation and root nodule formation, which is required for the growth of higher plants (Peter, 2011). Pesticide contaminated water is harmful for fish and other aquatic biota. Pesticide surface runoff into rivers and streams can be highly lethal to aquatic life, sometimes killing all the fish in a particular stream. On this basis, the developed countries prefer the use of bio-pesticides and hormonal pesticides under IPM program (Integrated Pest Management) to avoid any damage to human health and to the very important environment.

Plant protection products (more commonly known as pesticides) are widely used in agriculture to increase the yield, improve the quality, and extend the storage life of food crops. Pesticide residues are the deposits of pesticide active ingredient, its metabolites or breakdown products present in some component in the environment after its application, spillage or dumping (Alba and Reyes, 2008). Residue analysis provides a measure of the nature and level of any chemical contamination within the environment and of its persistence. The pesticides must undergo extensive efficacy, environmental, and toxicological testing to be registered by governments for legal use in specific applications. The applied chemicals and/or their degradation products may remain as residues in the agricultural food products, which becomes a concern for human exposure. Selected sampling programs can be used to investigate residual levels of pesticide in the environment, their movement and their relative rates of degradation (Alba and Reyes, 2008).

Pesticide residue in food has become a consumer's safety issue and the consumers have the right to know how much pesticides are getting incorporated in the food they eat daily basis. The detection, identification, and quantification of pesticide in the food are becoming a issue of high public interest. To ensure the supply of safe food, we should use pesticides following Good Agricultural Practices (GAP). To ensure GAP, we need to know the present status of the residue level in the food products that are available in local markets. In this thesis, we wanted to monitor

the residue levels of different organophosphorus pesticides in eggplant and cauliflower available in different local markets of Dhaka city.

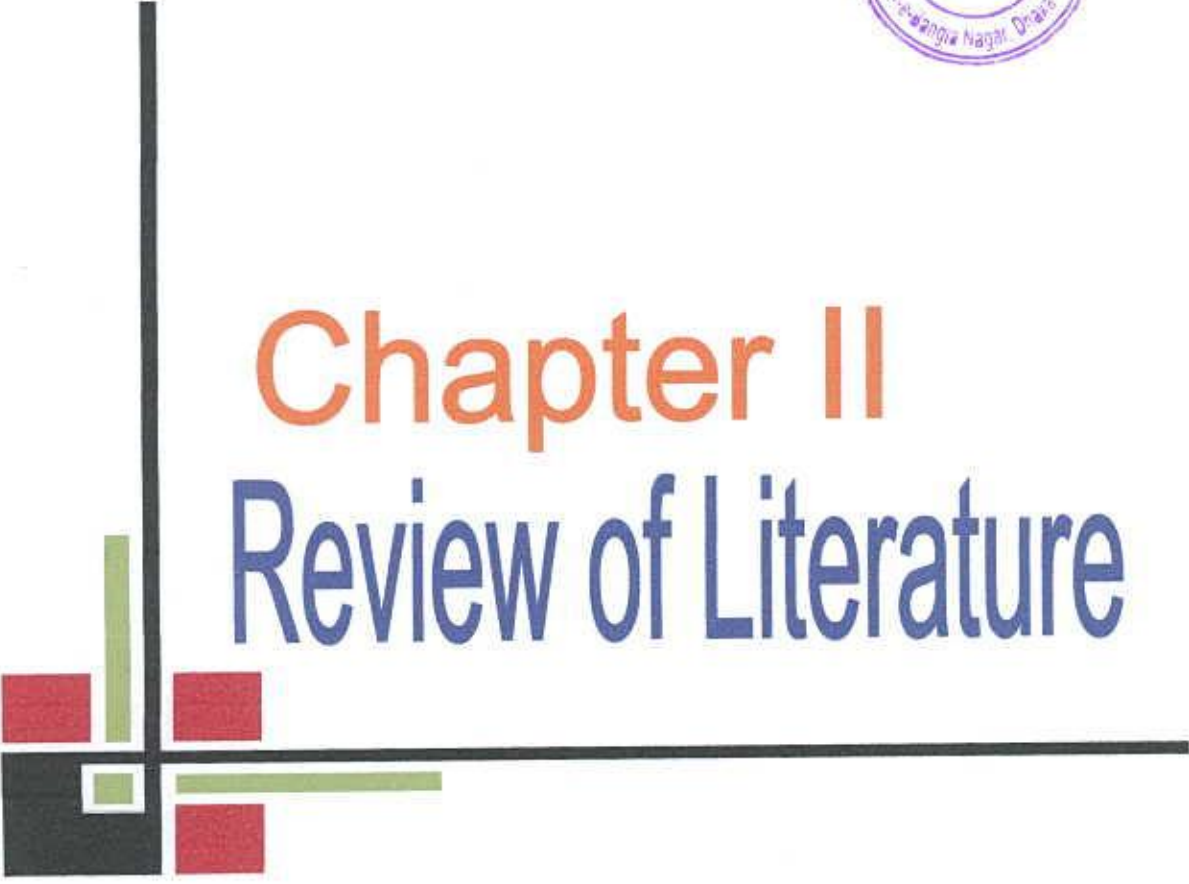
Gas Chromatography (GC) provides the most comprehensive and reliable screening method available for pesticides, metabolites, and suspected endocrine disrupters. Unlike most target compound methods in use today, this procedure has a good chance of finding and identifying unexpected or unknown pesticides, even in complex food extracts (Wylie and Quimby, 2000).

The quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for the extraction and clean up of 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 (Anastassiades *et al.*, 2003). 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 in 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.

Though several research works were conducted for the determination of pesticide residues in vegetables in Bangladesh however, a very little reference are available on the presence of pesticide residues in vegetables collected from Dhaka City. Therefore, the present research work was conducted with the following objectives:

- ⇒ To determine the level of different organophosphorus pesticide residues, present in the eggplant and cauliflower samples collected from several local markets of Dhaka city.
- ⇒ To compare the level of detected organophosphorus pesticide residues with the MRL's set by European Union.





# Chapter II

## Review of Literature



## CHAPTER II

# REVIEW OF LITERATURE

### 2. Pesticides

According to the Environmental Protection Agency (EPA), the government body that regulates pesticides in the U.S., a pesticide is any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Though often misunderstood to refer only to insecticides, the term pesticide also applies to herbicides, fungicides, and a variety of substances used to control pests. Pesticides also include plant regulators, defoliants and desiccants. We normally think of a pesticide as the product that can be purchased in the store, the insecticide, the weed killer or the fungicide. But, unfortunately, there is much more to it than it indicates. The product that one buys or is exposed to is actually a pesticide formulation that contains a number of different materials such as active and inert ingredients, as well as contaminants and impurities. In addition, pesticides, when subject to various environmental conditions, break down to other materials known as metabolites, which are sometimes more toxic than the parent material.

According to WHO Pesticides are chemical compounds that are used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). Pesticides are used in public health to kill vectors of disease, such as mosquitoes, and in agriculture, to kill pests that damage crops. By their nature, pesticides are potentially toxic to other organisms, including humans, and need to be used safely and disposed of properly (WHO, 1990).

Pesticides are substances that are meant to control pests or weeds (US EPA, 2007). The term pesticide includes all of the following: herbicide, insecticide, insect growth regulator, nematicide, termiticide, molluscicide, piscicide, avicide, rodenticide, predacide, bactericide, insect repellent, animal repellent, antimicrobial, fungicide, disinfectant (antimicrobial), and sanitizer (Randall *et al.*, 2013). In general, a pesticide is a chemical or biological agent such as a virus, bacterium, antimicrobial, or disinfectant) that deters, incapacitates, kills, or otherwise discourages pests. Target pests can include insects, plant pathogens, weeds, mollusks, birds, mammals, fish, nematodes (roundworms), and microbes that destroy property, cause nuisance, or spread disease, or are disease vectors. Although pesticides have benefits, some also have

drawbacks, such as potential toxicity to humans and other species. According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 most dangerous and persistent organic chemicals are organochlorine insecticide (Gilden *et al.*, 2010).

## 2.1. Classification of pesticides

### 2.1.1 Classification of pesticides based on chemical composition:

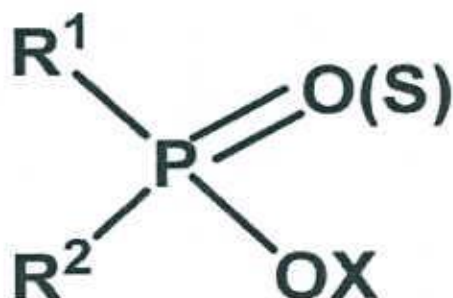
Under chemical classification, pesticides are categorized according to the chemical nature of the active ingredients. The chemical classification of pesticides is by far the most useful classification to researchers in the field of pesticides, agrochemicals, environment etc. and to those who search for details. Because this kind of classification gives the information about the efficacy as well as the knowledge of physical and chemical properties of the respective pesticides. This knowledge is very important for selection of the mode of application, precautions that need to be taken during preparation and application and the application rates. Based on chemical classification, pesticides are classified into four main groups namely; organochlorines, organophosphorus, carbamates and pyrethrin and pyrethroids (Buchel, 1983).

A little description of these four major groups of pesticides are given bellow (Zacharia and James, 2011)

(1) **Organochlorine pesticides** are organic compounds with five or more chlorine atoms. Organochlorines were the first synthetic organic pesticides to be used in agriculture and in public health. Most of them were widely used as insecticides for the control of a wide range of insects. They have a long-term residual effect in the environment because they are resistant to most chemical and microbial degradations. Organochlorine insecticides act as nervous system disruptors leading to convulsions and paralysis of the insect and its eventual death. Some of the commonly used representative examples of organochlorine pesticides are DDT, lindane, endosulfan, aldrin, dieldrin and chlordane and their chemical structures are presented hereunder (Zacharia and James, 2011).

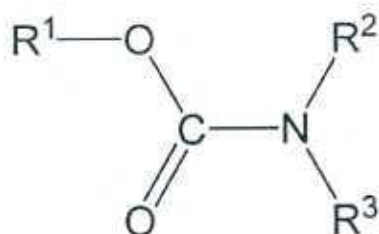


(2) **Organophosphorus insecticides** contain a phosphate group as their basic structural framework as defined by Schrader's formula:



Where,  $R^1$  and  $R^2$  are usually methyl or ethyl groups, the O in the OX group can be replaced with S in some compounds, whereas the X group can take a wide diversity of forms. Organophosphorus insecticides are generally more toxic to vertebrates and invertebrates as cholinesterase inhibitors leading to a permanent overlay of acetylcholine neurotransmitter across a synapse. As a result, nervous impulses fail to move across the synapse causing a rapid twitching of voluntary muscles and hence paralysis and death (Zacharia and James, 2011). Unlike organochlorines, organophosphorus insecticides are easily decomposed in the environment by various chemical and biological reactions, thus organophosphorus insecticides are not persistent in the environment (Martin, 1968). Some of the widely used organophosphorus insecticides include parathion, malathion, diazinon and glyphosate.

(3) **Carbamates** are organic pesticides derived from carbamic acid and the general formula is:



Where,  $R^1$  is an alcohol group,  $R^2$  is a methyl group and  $R^3$  is usually hydrogen. Both oxime and aryl carbamates have fairly high insect and mammalian toxicities as cholinesterase inhibitors (Zacharia and James, 2011). The cholinesterase inhibitions of carbamates differ from that of



organophosphorus in that, it is species specific and it is reversible (Drum, 1980). Some of the widely used insecticides under this group include carbaryl, carbofuran and aminocarb.

**(4) Pyrethroids** are synthetic analogues of the naturally occurring pyrethrins; a product of flowers from pyrethrum plant (*Chrysanthemum cinerariaefolium*). The insecticidal components of pyrethrum flowers are the optically active esters derived from (+)-trans-chrysanthemic acid and (+)-trans-pyrethroic acid. Pyrethroids are mainly acknowledged for their fast knocking down effect against insect pests, low mammalian toxicity and facile biodegradation. Although the naturally occurring pyrethrins are effective insecticides, their photochemical degradation is so rapid that their uses as agricultural insecticides become impractical. The synthetic analogues of the naturally occurring pyrethrins (pyrethroids) were developed by the modification of pyrethrin structure by introducing a biphenoxy moiety and substituting some hydrogen with halogens in order to confer stability at the same time retaining the basic properties of pyrethrins. The most widely used synthetic pyrethroids include permethrin, cypermethrin and deltamethrin (Zacharia and James, 2011).

Classification of pesticides based on their active ingredient according to EPA (U.S. EPA, 2007):

□ **Carbamate insecticides:** Carbamate insecticides are widely used in homes, gardens, and agriculture. They affect the functioning of the nervous system in ways similar to the organophosphates. The effects are usually reversible in humans.

□ **Neonicotinoids:** The neonicotinoids are insecticides that affect the central nervous system of insects. They have been associated with some bee kill incidents. Neonicotinoid pesticide products are applied to leaves and are used to treat seeds. They can accumulate in pollen and nectar of treated plants, which may be a source of exposure to pollinators. We are in the process of reevaluating this class of insecticides under the registration review program so we can understand whether there are effects to honeybees and other pollinators that need to be addressed.

□ **Organochlorine insecticides** were commonly used in the past. Many, like DDT, have been removed from the market due to their health and environmental effects and their persistence (e.g., DDT and chlordane). Others, like dicofol, lindane, and endosulfan, are now only available in the U.S. for limited uses.

□ **Organophosphate insecticides** are used in agriculture, homes, gardens, and on animals. They affect the insect's nervous system. Organophosphates range in toxicity for insects and humans (some are highly toxic), and they usually are not persistent in the environment. Our evaluation of risks from organophosphates resulted in the cancellation of several organophosphates and restrictions on the use of others. For example, parathion is no longer registered for any use, and chlorpyrifos is no longer registered for home use.

□ **Pyrethrin and pyrethroid insecticides** share a common origin. Pyrethroids are synthetic versions of the naturally occurring chemical pyrethrin, which is found in chrysanthemums. Pyrethrins are used mainly for indoor pest control. Synthetic pyrethroids are used in agriculture because they are designed to be more stable in the natural environment. Some synthetic pyrethroids are toxic to the nervous system.

### 2.1.2 Different types of organophosphates

OPs are nerve poisons which kill the target pest (usually insects). Most OP pesticides are insecticides, although there are also a number of related herbicide and fungicide compounds. Tetraethyl pyrophosphate (TEPP) was the first OP insecticide, which was developed in Germany during World War Two as a by-product of nerve gas development (Minton and Murray, 1988). OPs are generally acutely toxic. However active ingredients within the group show various degree of toxicity. Minton and Murray, have divided OPs into three groups. The first most and toxic group, e.g. chlorfenvinphos, has an LD<sub>50</sub> in the range 1-30 mg/kg. The LD<sub>50</sub> range for the second group, e.g. dichlorvos, is 30-50 mg/kg, and the least toxic group, e.g. malathion, has a range of 60-1,300 mg/kg (Minton and Murray, 1988). In humans, poisoning symptoms include: excessive sweating, salivation and lachrimation, nausea, vomiting, diarrhea, abdominal cramp, general weakness, headache, poor concentration and tremors. In serious cases, respiratory failure and death can occur (Eddleston *et al.*, 2008).

According to Pest control Technology or PCT OPs act by inhibiting the acetyl cholinesterase (AChE) enzyme in the nervous system. AChE performs a critical job in the nervous system by removing the neurotransmitter acetylcholine (ACh) from its receptor on the post-synapse nerve. Under natural conditions, AChE prevents overstimulation of the nervous system because it removes ACh. Without AChE, a stimulated nerve cannot return to its resting state. OPs tie-up (inhibit) to AChE, preventing it from removing ACh from its receptor site. The result is



overstimulation of the nerve cell, and death of the insect. Because the AchE enzyme is very similar between insects and mammals, the OPs are not very selective (Michael *et al.*, 2011).

At first OPs developed in the 1930s and '40s by Germany as a byproduct of their war efforts but now this class evolved into a number of newer members for the control of agricultural and daily life pests (Zacharia and James, 2011). At the height of use in the 1960s-1970s, there were >50 OP insecticides in use worldwide. Examples of commonly used Ops included chlorpyrifos (Dursban), dichlorvos (DDVP), malathion, diazinon, acephate (Orthene), propetamphos (Safrotin) and naled (Dibrom for mosquitoes) (Zacharia and James, 2011).

The World Health Organisation (WHO) estimates that there are in total three million acute severe cases of pesticide poisonings and 20,000 unintentional deaths each year, mostly in developing countries (WHO, 1990). Of these poisonings a large (but unknown) portion involves OPs. An assessment by the Pesticides Trust revealed azinphos methyl, chlorpyrifos, methamidophos, methomyl, monocrotophos, parathion and phosphamidon have caused a number of health concerns in a range of developing countries (Dinham, 1993). In 1995, there were 15,300 pesticide poisoning cases in China, 91% of which were caused by OPs (67% were caused by just three OPs, parathion, methamidophos and omethoate) (Shuyang and Peipei, 1996). Kyle Steenland at the US National Institute for Occupational Safety and Health maintains that acute poisoning from OPs remains a problem in industrialised countries. As estimated, 3,000-5,000 cases of accidental poisoning occur annually in the US, according to the Environmental Protection Agency (EPA) (Steenland, 1995). An FAO study in Indonesia found that most symptoms associated with pesticide toxicity were significantly greater in the time of year when spraying occurred. Farmers sprayed often using mixtures of hazardous pesticides, and over 50% were OPs. This study was typical of OP poisoning in developing countries where it is impossible to match specific pesticides with symptoms (Hirshhorn, 1993).



### 2.1.3. Description of seven selected organophosphates

#### 2.2.1 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).

IUPAC name of acephate is O,S-Dimethyl acetylphosphoramidothioate<sup>2</sup>, and the Chemical Abstracts Service (CAS) registry number is 30560-19-1(EPA, 2006).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 (EPA, 2006). Methamidophos inhibits acetylcholinesterase through phosphorylation (Farag *et al.*, 2000). Acetylcholine is the prominent insect stimulatory neurotransmitter for motor, sensory, and intermediate neurons (Chapman, 1998) and is broken down by acetylcholinesterase (Smith and Treherne, 1965). Organophosphates cause acetylcholine levels to increase and over-excite target nerves, muscles or tissues (Reigart and Robert, 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 (Farag *et al.*, 2000; Mahajna *et al.*, 1997).

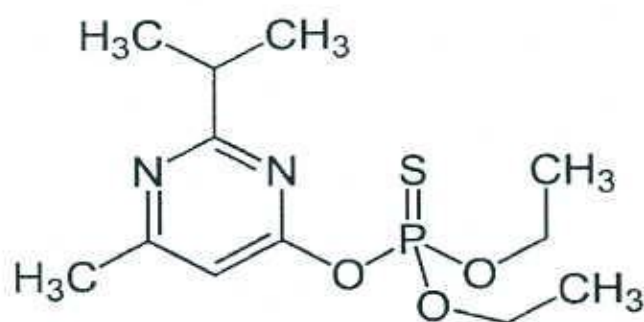


### 2.2.2. Diazinon

Diazinon is a non-systemic insecticide used in agriculture to control soil and foliage insects and pests on a variety of fruit, vegetable, nut and field crops. Diazinon is also used on non-lactating cattle in an insecticidal ear tag. Prior to the cancellation of all residential uses by 2004, diazinon was used outdoors on lawns and gardens, indoors fly control in household and commercial institutions and in pet collars designed to control fleas and ticks (US EPA, 2008). Uses for individual diazinon products vary widely.

The diazinon Chemical Abstracts Service (CAS) registry number is 333-41-5. The CAS name of diazinon is O, O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate and the International Union of Pure and Applied Chemistry (IUPAC) name is O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate (EPA, 2006; Tomlin, 2006). Pure diazinon is colorless oil. Technical grade diazinon ( $\geq 90\%$  active ingredient) is amber to brown liquid (EPA, 2006). Vapor pressure is  $1.40 \times 10^{-4}$ ,  $8.4 \times 10^{-5}$  mmHg at 20 °C (EPA, 2006; Howard, 1991) and molecular weight is 304.3 g/mol (EPA, 2006).

Solubility (water) of diazinon is about 0.04 g/L (40 mg/L) at 20°C and 30°C, though other values between 0.054 and 0.069 g/L (54 and 69 mg/L) have been reported in the 20-40 °C temperature range. Diazinon is absolutely miscible in acetone, benzene, ethanol, toluene, and xylene and is soluble in petroleum oils (EPA, 2006; Howard, 1991; Hornsby *et al.*, 1996; Ney, 1995).



**Diazinon**

Diazinon products are formulated as dusts, granules, liquids, concentrates, micro-encapsulations, wettable powders, seed coating and impregnated materials (USEPA, 2006). Diazinon is actually a contact insecticide which kills by altering normal neurotransmission within the nervous system

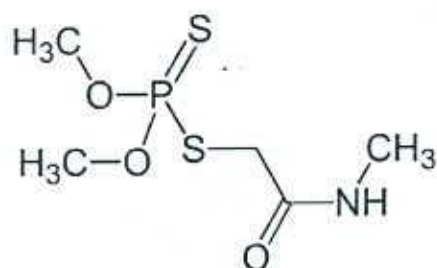


of target organisms (EPA, 2000). Diazinon inhibits the enzyme acetylcholinesterase (AChE), which hydrolyzes the neurotransmitter acetylcholine (ACh) in cholinergic synapses as well the neuromuscular junctions. This results in abnormal accumulation of ACh in the nervous system (Timchalk, 2001). Diazinon shares a common mechanism of toxicity with other organophosphate insecticides such as chlorpyrifos, malathion and parathion thus, diazinon would not be useful against organophosphate-resistant insect populations (EPA, 2000; 2006). Diazinon is metabolized within organisms to form diazoxon (sometimes referred to as "activation," see metabolism section below) and diazoxon is a more effective cholinesterase (ChE) inhibitor compared to diazinon itself (US.DHHS, 2006).

The U.S. EPA has classified diazinon as "Group D - not classifiable as to human carcinogenicity" based on the lack of evidence of carcinogenicity found in mice and rat studies (EPA, 2000; Daniels *et al.*, 1997). Several case-control studies have suggested possible links between exposure to diazinon and childhood cancers and non-Hodgkin's lymphoma (US.DHHS, 2006). In one study, families who used diazinon in their gardening were found to have an increased incidence of childhood cancers (Davis *et al.*, 1993). Another study found farmers using organophosphate insecticides, including diazinon, had higher rates of non-Hodgkin's lymphoma compared to non-farmers (Cantor *et al.*, 1992). In both studies however, it was not possible to conclusively attribute the increased cancer risk to diazinon exposure (US.DHHS, 2006). The acute reference dose (RfD) for diazinon is 0.0025 mg/kg/day (EPA, 2006). The chronic RfD is 0.0002 mg/kg/day (EPA, 2006).

### **2.2.3. 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).



**Dimethoate**

The CAS No. of dimethoate is 60-51-5 and the IUPAC name is *O,O*-dimethyl *S*-[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 degrees 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, nitrogen oxides (Meister, 1992). Dimethoate is possibly carcinogenic (Hayes, 1982; Hallenbeck and Cunningham, 1985). An increase in malignant tumors was reported in rats given oral doses of 5, 15 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).

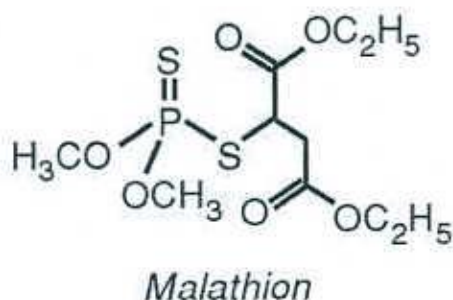
#### **2.2.4. Malathion**

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 US, 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 (EPA, 2006). Malathion is a colorless to amber liquid with a skunk- or garlic-like odor (US.DHHS, 2008). The vapor pressure of malathion is  $1.78 \times 10^{-4}$  mmHg at 25°C or 5.3 mPa at 30°C and also  $1.2 \times 10^{-4}$  to  $8 \times 10^{-6}$  mmHg at 20 °C (HSDB, US.DHHS, 2008; Tomlin,



2006; Hornsby *et al.*, 1006), the molecular weight is about 330.4 g/mol (Tomlin, 2006) and the solubility (water) is 145 mg/L(Tomlin, 2006).



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).

### 2.2.5. Fenitrothion

Fenitrothion is a contact insecticide and selective acaricide with low ovicidal properties (Spencer, 1981). It belongs to the organophosphate family of insecticides (Meister, 1994; Worthing, 1987). It is considered a cholinesterase inhibitor (Agrochemicals Handbook, 1983). Fenitrothion is effective against a wide range of pests, i.e. penetrating, chewing and sucking insect pests (coffee leaf miners, locusts, rice stem borers, wheat bugs, flour beetles, grain beetles, grain weevils) on cereals, cotton, orchard fruits, rice, vegetables, and forests. It may also be used as a fly, mosquito, and cockroach residual contact spray for commercial farms and community health programs (Worthing, 1983, Meister, 1994; Thomson, 1989). Fenitrothion is also effective against household insects and all of the nuisance insects listed by the World Health Organization. Its effectiveness as a vector control agent for malaria is confirmed by the World Health

Organization (Worthing, 1983, Worthing, 1987). Fenitrothion is non-systemic, and non-persistent (Hassall, 1990; Briggs, 1992; Spencer, 1981).

Fenitrothion was introduced in 1959 by both Sumitomo Chemical Company and Bayer Leverkusen and later by American Cyanamid Company (Worthing, 1987; Hayes and Laws, 1990; Hayes, 1982). Fenitrothion comes in dust, emulsifiable concentrate, flowable, fogging concentrate, granules, ULV, oil-based liquid spray, and wettable powder formulations (Anonymous, 1983; Worthing, 1983; Meister, 1994; Thomson, 1989;). It is available as a 95% concentrate, 50% emulsifiable concentrate, 40% and 50% wettable powder and 2%, 2.5%, 3% and 5% dusts (Hayes and Laws, 1990; Hayes, 1982). It is compatible with other neutral insecticides (Anonymous, 1983). Fenitrothion is an organophosphorus insecticide. The IUPAC name of fenitrothion is O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate and the CAS no. is 122-14-5. The vapor pressure is about  $7 \times 10^{-5}$  mbar at 20°C (Anonymous, 1983); 18 mPa at 20°C (Worthing, 1983) and the volatility is 0.09 mg/m<sup>3</sup>. The solubility in water at 20°C is 30 mg/l (Agrochemicals Handbook, 1983, Melnikov, 1971); at 30°C is 14 mg/l water (Worthing, 1983); nearly insoluble in water (Hassall, 1990; Meister, 1994); insoluble in water (Hayes and Laws, 1990).



Fenitrothion

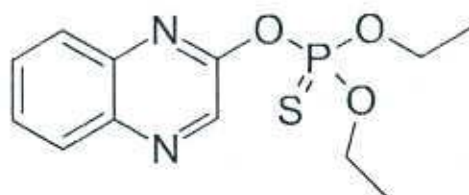
The active ingredient of fenitrothion is found in a variety of commercial insecticides and fungicides. Trade names for products containing fenitrothion include Accothion, Agrothion, Bay 41831, Cyfen, Cytel, Dicofen, Fenstan, Folithion, Kaleit, Mep, Metathion, Micromite, Novathion, Nuvanol, Pestroy, Sumanone, Sumithion, and Verthion (Meister, 1994; Thomson, 1989; Worthing, 1987; Fairchild, 1977). The common name methylnitrophos is used in Eastern Europe (Hayes and Laws, 1990).



The acute toxicity of fenitrothion to mammals is considered to be low (Spencer, 1981; Hayes and Laws, 1990; Hayes, 1982). Chronic symptoms in humans include: usual malaise, fatigue, headache, cramps, loss of memory and ability to concentrate, loss of weight, nausea, thirst, loss of weight, anorexia, muscular weakness and tremors. This pesticide produces typical cholinergic poisoning at sufficient dosage (Hayes and Laws, 1990; Hayes, 1982). Preliminary data shows fenitrothion degrades fairly rapidly in soil with a half-life of less than 7 days in non-sterile muck, sandy loam soils. Generally, fenitrothion is intermediately mobile in a variety of soils ranging from sandy loam to clay (Meister, 1994; EPA, 1987).

### 2.2.6. Quinalphos

Quinalphos is an organophosphorus insecticide. The IUPAC name of quinalphos is *O,O*-diethyl *O*-quinoxalin-2-yl phosphorothioate (Pramila *et al.*, 2004). World Health Organization has ranked it as a 'moderately hazardous' in WHO's acute hazard ranking and use of quinalphos is either banned or restricted in many countries. Solubility in water is 17.8 mg/L at 22 °C and melting point is 31 °C (88 °F; 304 K)



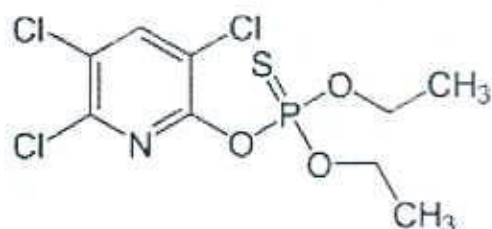
Quinalphos

It is a reddish-brown liquid 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 (Pramila *et al.*, 2004).

### 2.2.7. Chlorpyrifos

Chlorpyrifos is a broad-spectrum organophosphate (OP) pesticide, which is a non-systemic insecticide designed to be effective by direct contact, ingestion, and inhalation (Tomlin, 2006). The Chemical Abstracts Service (CAS) registry number is 2921-88-2 and the IUPAC name is *O,O*-dimethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate (US.EPA, 2006). It is a colorless to

white crystalline solid (US.EPA, 2006; Tomlin, 2006). Chlorpyrifos has a mild mercaptan (thiol) odor, similar to the smell of sulfur compounds found in rotten onions, garlic, eggs and skunks (Tomlin, 2006; Lewis, 1998). The vapor pressure is  $1.87 \times 10^{-5}$  mmHg at 25°C and the solubility in water is about 0.0014g/L (1.4mg/L) at 25°C (US.EPA, 1999).



Chlorpyrifos



Chlorpyrifos is used on agriculture sector such as on food and feed crops, cattle ear tags, golf course turf, industrial plants and vehicles, non-structural wood treatments including processed wood products, fence posts and utility poles, and to control municipal health pests such as mosquitoes and fire ants. It is registered for indoor residential use only in the form of containerized baits (US.EPA, 2006). Uses for individual products containing chlorpyrifos vary widely.

The United States Department of Agriculture (USDA) Pesticide Data Program collects data on pesticide residues in food products and compiles those data on an annual report of the findings. The 2007 annual summary reported 9734 samples of fruit and vegetable commodities tested for chlorpyrifos residues. Chlorpyrifos was detected in 339 (3.48%) of these samples (USDA, 2014). Chlorpyrifos residues were found in 18.0% of peaches tested (100 detections), in 15.8% of nectarines tested (89 detections), in 6.8% of broccoli tested (50 detections) and in 5.2% of kale greens (20 detections). Chlorpyrifos residues were also examined in almonds (46% of samples tested, 166 detections) and corn grain (30% of samples tested, 195 detections) (USDA, 2014). Chlorpyrifos was detected at levels exceeding the U.S. EPA tolerance in one sample each of collard greens (353 samples, 10 with detectable residues) and summer squash (742 samples, 5 with detectable residues). In collard greens, residues were detected in one sample at 6.3 ppm (tolerance of 2.0 ppm). In summer squash, residues were detected in one sample at 0.33 ppm (tolerance 0.10 ppm) (USDA, 2014).



In 1993, chlorpyrifos was categorized in Group E, evidence of non-carcinogenicity for humans, by the U.S. EPA (US EPA, 2005). No human data were found regarding carcinogenic effects of chlorpyrifos.

### **2.3. 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 (USEPA, 2006). 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 (Stephen *et al.*, 2011).

#### **2.3.1. 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).

#### **2.3.2. 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 (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.

#### **2.3.3. Determination of Pesticide Residues in Food**

A method was applied for the analysis of 48 crude pollen samples collected from 8 provinces of China. The main detection rates of each compound were 77.1% for carbendazim, 58.3% for fenpropathrin, 56.3% for chlorpyrifos, 50.0% for fluvalinate, 31.3% for chlorbenzuron, and

29.2% for triadimefon in crude pollen samples. The maximum values of each pesticide were 4516 ng/g for carbendazim, 162.8 ng/g for fenpropathrin, 176.6 ng/g for chlorpyrifos, 316.2 ng/g for fluvalinate, 437.2 ng/g for chlorbenzuron, 79.00 ng/g for triadimefon (Zhou *et al.*, 2016).

Pesticide residues are extracted from samples with a little amount of ethyl acetate and anhydrous sodium sulfate. Analyses are performed by large volume GC injection using the through oven transfer adsorption desorption (TOTAD) interface. The calculated limits of detection for each pesticide injecting 50 µl of extract which are 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 brinjal, lettuce, pepper, cucumber, and tomato (Corteaş *et al.*, 2013).

The concentration of organophosphorus pesticide residues such as dichlorvos, diazinon, chlorpyrifos and fenitrothion was monitored in some fresh vegetables (spinach, lettuce, cabbage, tomato and onion) and soil samples from different areas within Alau Dam and Gongu long agricultural areas in Borno State, Nigeria. Samples collection and preparation were carried out using standard procedures. The concentration of all the pesticides in the vegetables and soil samples were estimated using GC equipped with electron capture detector (ECD). The lowest concentrations of dichlorvos, diazinon, chlorpyrifos and fenitrothion in the Alau Dam and Gongu long agricultural areas were observed specially in the spinach root while the lowest concentrations were observed in the tomato leaf. The concentrations of all the pesticides in the soil samples were spotted to be higher at a depth of 21-30cm, while the lowest concentrations were observed at a depth of 0-10cm. The concentrations of all the organophosphorus pesticides in the vegetables and soil samples from the two agricultural areas were observed to be at worrying levels, much higher than the maximum residue limits (MRLs) and acceptable daily intake values (ADIs) set for vegetables and soil by the Codex 2009 (Akan *et al.*, 2013).

A research was conducted in Egyptian fruits and vegetables regarding the presence of pesticide residues. About 2381 samples of different types of fruits and vegetables were collected from eight Egyptian local markets in 6 different regions of the country. All samples were examined for residues of 54 pesticides, including organophosphorus, organonitrogen, organohalogen and certain pyrethroids. However, dithiocarbamates were analysed in only 318 samples. Overall, 81.50% of the samples had no detectable pesticide residues. Of the contaminated samples,



18.50% contained detectable residues and 1.90% exceeded their maximum residue limits (MRLs). Root and leafy vegetables showed the lowest contamination rates (1.90 and 4.70%, respectively), slightly exceeding the MRLs in leafy vegetables. However, fruit samples showed a slightly higher amount of contamination than vegetables (29 and 14.30%, respectively). Fruit also exhibited a higher level of violation than vegetables (2.30 and 1.90%, respectively). Dithiocarbamates residues were found in 21 of the 318 samples analyzed, which when expressed as a percentage contamination was 6.60%, and only one sample exceeded the MRL (Dogheim *et al.*, (1997).

An experiment was conducted to determine pesticide residues of five commonly used pesticides on vegetables, namely endosulfan, carbendazim, chlorpyrifos, cypermethrin and imidacloprid was done using GCECD and HPLC UV-VIS type analytical techniques. Out of the 5 pesticides monitored, 4 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 cypermethrin respectively. Amongst the different vegetable samples cauliflower head and tomato fruit had carbendazim residues higher than the recommended MRL's whereas cabbage head had endosulfan contamination higher than the recommended MRL values (Chauhan, 2012).

Pesticide residue detection methods from food matrices mainly involve two preparation steps. Extraction of intended analytes from the bulk of the matrices and partitioning of the residues in an immiscible solvent and or cleanup of the analytes from the matrix co-extractives is the 1st step and the 2nd one is identification and quantification of pesticides. Complex samples like meat and meat products need two step clean-up which combines different complex chromatographic techniques (Rimkuset *et al.*, 1996).

A method was developed and validated to detect and quantify 7 pesticide residues in the cabbage samples collected from different market places in Thessaloniki, Greece. They have been detected three insecticides (chlorpyrifos, cypermethrin and deltamethrin) and two fungicides (fluopicolide and propamocarb hydrochloride) in the collected cabbage samples. Among the 132

analyzed samples, 41 (31% of the total no. of samples) had pesticide residues, of which, 2 had multiple pesticide residues and 39 had single pesticide residues (Prodhan *et al.* 2016).

A method was also been developed and validated to detect and quantify 7 pesticide residues in the cauliflower samples collected from different market places in Thessaloniki, Greece. They have been detected four insecticides (chlorpyrifos, cypermethrin, deltamethrin and indoxacarb) in the collected cauliflower samples. Among the 120 analyzed samples, 48 (40% of the total no. of samples) were found to have pesticide residues (Prodhan *et al.* 2016a).

An easy and efficient sample preparation method for the determination of natamycin residues in wine samples by solvent extraction and ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) analysis was developed. Different amounts of C18 and primary secondary amine (PSA) sorbents in order to optimize the clean-up step with the extraction solvent acetonitrile was observed. Validation results were satisfactory. The method was applied in 10 Brazilian wine samples and no residues of natamycin were detected (Gabrieli *et al.*, 2016).

A total of 230 pesticide residues in 8496 samples of fresh leafy vegetables (e.g. Brassica lee ssp. namai, spinach, leafy lettuce, chinese chive, crown daisy, marshmallow, aster scaber and perilla leaves) was examined. The result showed that among 8469 samples, 61 different pesticides were detected in 890 samples, of which 118 samples exceeded the Korean maximum residue limits (KMRLs) (Park *et al.*, 2016).

An experiment was conducted to determine 10 pesticide residues containing seven insecticides (chlorpyrifos, dimethoate, deltamethrin, thiamethoxam, thiacloprid, pirimicarb and indoxacarb) and three fungicides (azoxystrobin, fluopicolide and propamocarb hydrochloride) in 122 fresh melon samples collected from different market places in Thessaloniki, Greece. Among the 122 analyzed samples, 32 (26% of the total no. of samples) were found to have pesticide residues (Prodhan *et al.*, 2015).

A research was conducted to analyze the effect of different traditional food processing methods on the reduction or minimization of pesticide residues in cauliflower head through GC- $\mu$ ECD and HPLC. The results indicate that the residual level of pesticides in unwashed and unprocessed cauliflower head 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 show that, the plain washing and detergent washing minimize 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) (Panhwar and Sheikh, 2013).

Soil extracts, water and fresh vegetable samples were examined using GC. The amounts of pesticides residues are less than 20µg/kg of dry material in soil sample. In 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, it is between 0.01 and 0.10 mg/kg of dry material (Kunda *et al.*, 2012).

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

A Gas Chromatography (GC) methodology has been validated for the determination of some halogenated insecticides. Complete separation of the pesticide prepared in ethyl acetate was achieved on Rtx - 1 column with dimension, 30m x 0.25mm x 0.25m. 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 injector and detector were respectively at temperatures of 225 and 300°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 (Afful *et al.*, 2010).

A multi-residue method has been developed to determine 5 groups of 85 pesticides - chlorinated, pyrethroid, carbamate, phosphorous and others - in fresh vegetables, fruits and green tea using stir bar sorptive extraction (SBSE) coupled with 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  $K_{o/w}$  values (e.g. pyrethroid). The method enables to determine pesticides at low  $\mu\text{g}/\text{kg}$  level in green soybeans, cucumber, spinach, grape, green tea and tomato (Yamagami *et al.*, 2009).

A study on the quantification of organophosphorus and organochlorine insecticide residues from different fish sample was conducted using simple GC technique to develop the simple procedure of quantification of organophosphorus and organochlorine insecticide residues from fish samples using Electron Capture Detector (ECD) and Flame Thermionic Detector (FTD) of Gas Chromatograph (GC). 68 samples of fish (Rui, Shrimp & Others) were collected from Chittagong, Dhaka and Khulna offices of Department of Fisheries as well as different fish export companies and supplied to Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur. They extracted and prepared all samples for injection using the standard procedures for residue analyses during August, 2008 to July, 2009. They also injected all samples in GC-FTD for the determination of organophosphorus insecticides and in GC-ECD for the determination of organochlorine insecticides. Their results revealed that among 68 samples, 13 had residues of different insecticides. 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 0.03-0.120ppm. 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 ADI (Acceptable Daily Intake) (Prodhan M.D.H. *et al.*, 2009).

Three supervised field trials (2 for eggplant and 1 for yard long bean) were carried out sprayed with the field dose (1.5 ml/L of water) of diazinon, carbosulfan, and quinalphos. All the samples were collected daily after spraying till residues were found. In case of Diazinon, left over residue



was found up to six days after spray (DAS), and up to 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 up to 3 DAS. Left over residue of quinalphos in yard long bean sample was detected up to 6 DAS and up to 4 DAS the level of residue was above the MRL (Kabir *et al.*, 2008).

A multi-residue method was developed and validated to determine 85 pesticides-chlorinated, phosphorous, pyrethroid and others - in vegetables, fruits and green tea. This procedure involved the use of stir bar sorptive extraction (SBSE) coupled with thermal desorption and retention time locked (RTL) GCMS. Pre-extraction with methanol and dilution with water prior to SBSE (60 min) were performed. Dilution of methanol extract for SBSE was examined to obtain high sensitivity and to compensate the effect of adsorption to the glass wall of extraction vessel and to sample matrix for the compounds with high log  $K_{ow}$  values (e.g. pyrethroid) (Ochiai *et al.*, 2008).

A multi-residue method was developed and validated using liquid chromatography–time-of-flight mass spectrometry (LC TOF-MS) for the quantitative (routine) analysis of 25 pesticides. The analytical performance of the method was evaluated and validated for different types of fresh fruits and fresh vegetables; melon, tomato, broccoli, orange, lemon, apple and pepper. 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. This methodology was applied to the analysis of two samples from an inter-laboratory exercise (Ferrer *et al.*, 2005).

An experiment was conducted to detect and quantify pesticide residues in fresh fruits and fresh vegetables by gas chromatography/tandem mass spectrometry (GC/MS/MS). Electron impact (EI)/MS/MS and chemical ionization (CI)/MS/MS were used 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 good linearity and excellent selectivity allowed identification and quantification of low levels of pesticide residues in the most difficult matrixes. The method has been used for routine analysis of many fruits and vegetables (Gamon *et al.*, 2001).





A research was carried out 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 insect-pest control, were monitored. 397 fresh fruits and vegetables samples were collected from 8 local markets and examined for 52 active ingredients. Of all analyzed samples, 42.8% contained detectable residues, of which 1.76% exceeded their maximum residue limits (MRL's). The range of contamination with different pesticides was 0-86%. The most commonly detected pesticide residues were dithiocarbamates 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 chlorpyrifos-methyl (1.5%). Among all samples, 62 tomato samples (15.01%) contained 13 pesticide residues, 65 grape samples (15.73%) contained 11 pesticides residues and 22 strawberry samples (5.32%) contained 10 pesticide residues. Cauliflower, onion and guava samples were 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 fruits and vegetables. Use of these pesticides in Egypt is completely prohibited by law since 1987 (Salwa *et al.*, 1999).

Gas chromatography–mass spectrometry detection (GC–MSD) technique was applied in the detection of 50 pesticides in baby food. QuEChERS (quick, easy, cheap, effective, rugged and safe) method was used as a sample preparation procedure. The recoveries were investigated at three levels (5, 10 and 50 µg/kg) and the results obtained showed compliance with the contemporary EU requirements with a few exceptions. LOQs for most of the tested pesticides were below the EU MRLs (10 µg/kg), except deltamethrin, cypermethrin, fenvalerate, phosalone and beta-cyfluthrin (LOQs were 10 µg/kg). The techniques were applied in the analysis of 50 samples of baby food manufactured in Serbia (Vukovic *et al.*, 2012).

To improve the efficiency of traditional methods a new sample preparation was introduced by Anastassiades *et al.* (2003). This procedure is known as “QuEChERS” an anagra for Quick, Easy, Cheap, Effective, Rugged and Safe. This procedure uses a single extraction in acetonitrile and required a very small amount (10-15g) of sample. This simple initial step simultaneously extracts the pesticides from the sample and prepares it for the next dispersive solid-phase extraction (d-SPE) step. The salts and SPE sorbents chosen for the d-SPE step serve to remove residual water and further remove matrix interferences from the sample. The resulting



acetonitrile extract is typically analyzed directly by GC/MS or by LC/MS/MS with proper dilution. This is a 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 (Anastassiades *et al.*, 2003). This is a sample preparation approach entailing solvent extraction of high-moisture samples with acetonitrile, ethyl acetate, or acetone and partitioning with magnesium sulphate alone or in combination with other salts followed by clean up using d-SPE. It is very flexible and since its inception, there have been several modifications of the technique depending on analytes, matrices, instrumentation and analyst preferences (Anastassiades *et al.*, 2003).

Nowadays the QuEChERS technique, is widely used for the extraction and clean-up of the extracts of fruit and vegetable matrices (Prodhan *et al.*, 2016; Prodhan *et al.*, 2016a; Prodhan *et al.*, 2015; Prodhan *et al.*, 2015a; Paramasivam and Chandrashekhar, 2012; Gilbert *et al.*, 2010; Lehotay *et al.*, 2005; Lehotay *et al.*, 2007; Lehotay *et al.*, 2010; Sinha and Sharma, 2007; Nguyen *et al.*, 2008; Dasika *et al.*, 2012; Mantzos *et al.*, 2013; Schenck and Hobbs, 2004; Paya *et al.*, 2007; Kirchner *et al.*, 2008; Krueve *et al.*, 2008; Cajka *et al.*, 2005; Wang *et al.*, 2007; Aysal *et al.*, 2007; Banerjee *et al.*, 2007; Schenck *et al.*, 2008; Kovalczuk *et al.*, 2008; Romero *et al.*, 2008; Kmellar *et al.*, 2008; Cunha *et al.*, 2007; Li *et al.*, 2009; Schenck *et al.*, 2009; Huskova *et al.*, 2009; Liu *et al.*, 2005; Bolanos *et al.*, 2007; Mol *et al.*, 2007; Ji *et al.*, 2008; Mezcuca *et al.*, 2008; Fernandez *et al.*, 2008; Banerjee *et al.*, 2008; Hayama and Takada, 2008; Waloreczyk, 2008; Zhang *et al.*, 2009). This technique was modified by several authors (AOAC Official methods, 2007; the European Committee for Standardization (CSN) Standard Method, CSN EN 15662, 2008). This technique is gaining popularity day by day compared to the other existing techniques as it has a lot of advantages. With the high analyte recoveries, the low organic solvent consumption, and the low cost per sample it becoming more popular. 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.



The QuEChERS approach appears to have a bright future in the analysis of pesticides in foods and other agricultural products. In addition to the references cited previously, the technique has proven successful for the extraction of pesticides from a variety of other fruits and vegetables (Schenck and Hobbs, 2004). Olives and olive oil (Cunha *et al.*, 2007), barley (Diez and Barcelo, 2006), green beans, peaches, peppers, snow peas (Schenck *et al.*, 2003) fatty matrices (such as milk, eggs and avocado) among others (Lehotay *et al.*, 2005a). The technique has been used to investigate non-pesticide food toxins such as acrylamide in various food matrices (Mastovska *et al.*, 2006) and drugs in blood (Plossl *et al.*, 2006).

A validation work of Quick Easy Cheap Effective Rugged and Safe (QuEChERS) multi-residue method was done for the extraction of 82 pesticides belonging to various chemical classes from grapes and pomegranate (commodities with high sugar and low lipid contents). Gas chromatography tandem mass spectrometry (GC-MS/MS) was used to recover 82 pesticides amenable to gas chromatography (GC) from spiked grapes and pomegranate. They use a water/ethyl acetate system for initial extraction, an extraction/partitioning step after the addition of salt, and a cleanup step utilizing dispersive solid-phase extraction (d-SPE); which indicates 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 (Satpathy *et al.*, 2014).

A study was conducted to determine multiple pesticide residues in eggplant with liquid chromatography-mass spectrometry by adopting (QuEChERS) extraction and liquid chromatography triple quadrupole-mass spectrometry. The method was developed and validated for the determination of 13 pesticides among which 10 were insecticides and 3 fungicides. The eggplant fruit samples were collected from different market places in Thessaloniki, Greece. Among the 72 analyzed samples, 34 (47 % of the total no. of samples) had pesticide residues, of



which, 5 had multiple pesticide residues and 29 had single pesticide residue. Only one sample contained residue above the EU-MRLs (Prodhan *et al.*, 2015).

A study was conducted on the detection and quantification of pesticide residues in some selected vegetables grown in Bangladesh. In intensive vegetable growing area, Narsingdi district of Bangladesh was chosen for this research on three major vegetables like eggplant, cauliflower, and country bean. They interviewed 23 farmers on the basis of a questionnaire and they found that 14 pesticides belonging to different groups are being commonly used on the selected vegetables by the respondent farmers to control the major pests. They record 8.33 to 45.00 percent farmers in two selected locations of Narsingdi to apply different pesticides every day and in some cases even twice in a day on vegetables. They collected 42 samples from fields and markets and operate multiple pesticide residue analysis by Gas Chromatography (GC) with Flame Thermionized Detector (FTD) and Electron Capture Detector (ECD). They found out of 42 samples, 27 had pesticide residue. Among these 27 samples, 14 samples had pesticide residues above the Maximum Residue Limit (MRL). The detected pesticides were Diazinon, Malathion, Quinalphos, Fenitrothion, Cypermethrin, Fenvalerate and Propiconazole (Islam *et al.*, 2014).

A total of 182 samples of six vegetables were collected for insecticide residue analysis from different agricultural fields of central Aravalli region, when they were ready to supply to the markets. The analysis of samples for different pesticide residues were carried out on 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% of total contaminated samples were exceeded the maximum residual limit (MRL) values (Charan *et al.*, 2010).

A study was conducted on the validation and uncertainty of the method for multi residue analysis of 35 pesticides in cantaloupe 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 insecticides and fungicides 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 (Milhome *et al.*, 2013).

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

The magnitude of contamination of DDT pesticides in vegetables, pulses and cereals was examined in a study. The samples 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 (Neetu, 2013).

A method was developed 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. Analyses are performed by large volume GC injection using the through oven transfer adsorption desorption (TOTAD) interface. The calculated limits of detection for each pesticide injecting 50 µL of extract which is much lower than the maximum residues levels (MRLs). Repeatability studies yielded a relative standard deviation lower than 10% in all cases. The method was applied to the analysis of eggplant, lettuce, pepper, cucumber, and tomato (Corteaş *et al.*, 2013).





# Chapter III

## Materials and Methods



## **CHAPTER III MATERIALS AND METHODS**

The vegetable (eggplant and cauliflower) samples were collected from 5 different markets of Dhaka City and carried to the Pesticide Analytical Laboratory, Entomology Division, BARI, Joydebpur, Gazipur for pesticide residue analysis during September 2016 to March 2017. From the collection of samples to the final analysis, all way required a number of processes which are described below.

### **3.1. Study area**

The study area included major five markets of Dhaka City. The area of Dhaka City is about 270 sq. km, located at 23.42° North latitude and 90.22° East longitude with an elevation of 4 meter from the sea level. In this study, vegetables were collected from Kawran Bazar, Mohammadpur Krishi Market, Mirpur-1 Bazar, Taltola Bazar and Jatrabari Bazar.





Figure 1: Map showing the places of sample collection.

### 3.2. Sample collection

A total of 100 samples (50 eggplants and 50 cauliflowers) were collected for this study. Ten samples of eggplant and ten samples of cauliflower were collected from each market.

**Table 1:** Sources and places of collection of eggplant samples

Area of collection	Sample ID	Source*
Jatrabari Bazar	DMBJ-1	Narshingdi
	DMBJ-2	Jessore
	DMBJ-3	Manikganj
	DMBJ-4	Chuadanga
	DMBJ-5	Narshingdi
	DMBJ-6	Manikganj
	DMBJ-7	Jessore
	DMBJ-8	Narshingdi
	DMBJ-9	Meherpur
	DMBJ-10	Savar
Kawran Bazar	DMBJ-11	Sripur
	DMBJ-12	Narshingdi
	DMBJ-13	Manikganj
	DMBJ-14	Sripur
	DMBJ-15	Chuadanga
	DMBJ-16	Narshingdi
	DMBJ-17	Sripur
	DMBJ-18	Sripur
	DMBJ-19	Chuadanga
	DMBJ-20	Chuadanga
Mirpur1 Bazar	DMBJ- 21	Narshingdi
	DMBJ- 22	Jessore
	DMBJ- 23	Meherpur
	DMBJ- 24	Meherpur
	DMBJ- 25	Jessore
	DMBJ- 26	Jessore
	DMBJ- 27	Manikganj
	DMBJ- 28	Chuadanga
	DMBJ- 29	Savar



	DMBJ- 30	Manikganj
Mohammadpur Krishi Market	DMBJ- 31	Narshingdi
	DMBJ- 32	Narshingdi
	DMBJ- 33	Jessore
	DMBJ- 34	Manikganj
	DMBJ- 35	Comilla
	DMBJ- 36	Comilla
	DMBJ- 37	Savar
	DMBJ- 38	Manikganj
	DMBJ- 39	Jessore
	DMBJ- 40	Sripur
Taltola bazar	DMBJ- 41	Savar
	DMBJ- 42	Jessore
	DMBJ- 43	Savar
	DMBJ- 44	Narshingdi
	DMBJ- 45	Comilla
	DMBJ- 46	Manikganj
	DMBJ- 47	Comilla
	DMBJ- 48	Narshingdi
	DMBJ- 49	Narshingdi
	DMBJ- 50	Jessore

Table 2: Sources and places of collection of Cauliflower samples

Area of collection	Sample ID	Source*
Jatrabari Bazar	DMCF 1	Manikganj
	DMCF 2	Tangail
	DMCF 3	Tangail
	DMCF 4	Manikganj
	DMCF 5	Savar
	DMCF 6	Savar
	DMCF 7	Tangail
	DMCF 8	Comilla

	DMCF 9	Sripur
	DMCF 10	Chuadanga
Kawran Bazar	DMCF 11	Tangail
	DMCF 12	Jessore
	DMCF 13	Manikganj
	DMCF 14	Jessore
	DMCF 15	Jessore
	DMCF 16	Sripur
	DMCF 17	Savar
	DMCF 18	Manikganj
	DMCF 19	Meherpur
	DMCF 20	Sripur
Mirpur1 Bazar	DMCF 21	Jessore
	DMCF 22	Gazipur
	DMCF 23	Gazipur
	DMCF 24	Chuadanga
	DMCF 25	Tangail
	DMCF 26	Jessore
	DMCF 27	Tangail
	DMCF 28	ManikganjManikganj
	DMCF 29	Sripur
	DMCF 30	Chuadanga
MohammadpurKrishi Market	DMCF 31	Jessore
	DMCF 32	Comilla
	DMCF 33	Sripur
	DMCF 34	Comilla
	DMCF 35	Jessore
	DMCF 36	Manikganj
	DMCF 37	Manikganj
	DMCF 38	Jessore
	DMCF 39	Tangail
	DMCF 40	Tangail



Taltola Bazar	DMCF 41	Savar
	DMCF 42	Meherpur
	DMCF 43	Gazipur
	DMCF 44	Chuadanga
	DMCF 45	Savar
	DMCF 46	Comilla
	DMCF 47	Tangail
	DMCF 48	Meherpur
	DMCF 49	Gazipur
	DMCF 50	Chuadanga

\* According to the retailer's opinion

Each sample was 1kg for both eggplant and cauliflower. Clean transparent airtight polyethylene bags were used to collect samples. Each bag was properly labeled with sample ID, sample number, source, location, collection date etc. A separate bag was used to collect each sample to avoid cross contamination.

### 3.3. Sample preparation for analysis

Just on the day of collection all samples were taken to the Pesticide Analytical Laboratory, Division of Entomology, Bangladesh Agricultural Research Institute (BARI), Gazipur. The whole unit of each sample was cut into small pieces and mixed properly. Clean air tight polythene bags were used to store chopped sample in refrigerator at -20°C until extraction and cleanup process started.

### 3.4. Chemicals and reagents

The standard of Chlorpyrifos, Acephate, Diazinon, Dimethoate, Quinalphos, Malathion and Fenitrothion were obtained from Sigma-Aldrich Laborchemikalien (St Louis, MO, USA) via SAF Scientific Ltd., Dhaka, Bangladesh.. All the pesticides standards contained >99.6% purity.

Methanol, acetone, gradient grade acetonitrile, sodium chloride (NaCl), anhydrous magnesium sulphate (MgSO<sub>4</sub>) and Primary Secondary Amine (PSA) were collected from Sigma-Aldrich Laborchemikalien (St Louis, MO, USA) via via SAF Scientific Ltd., Dhaka, Bangladesh.

### 3.5 Analytical Apparatus Required

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







**Plate 1: Centrifuge Machine**



**Plate 2: Electric Balance**



**Plate 3: Homogenizer**



**4: Vortex Mixer**



**Plate 5: Orbital Shaker**



**Plate 6: Gas Chromatograph (GC)**



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

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

### **3.6. Preparation of pesticide standard solution**

Pesticide standard stock solutions of Chlorpyrifos, Acephate, Diazinon, Dimethoate, Quinalphos, Malathion and Fenitrothion were prepared separately in acetone. The concentration was 1000 mg/L and they were stored at  $-20^{\circ}\text{C}$  until use. A mixed standard solution was prepared about 50 mg/L in acetone containing all the afore mentioned pesticides by adding the appropriate volume of each individual stock solution in a 50 ml volumetric flask and made to volume by addition of acetone. An intermediate mixed standard solution of 10 mg/L in acetone was prepared from the mixed standard solution of 50 mg/L. Then working standard solutions of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 mg/L in methanol were prepared by transferring the appropriate amount from 10 mg/L intermediate mixed standard solution into ten separate 10-mL volumetric flasks. All the standard solutions were kept in a freezer at  $-20^{\circ}\text{C}$  until use.

### 3.7 Extraction and clean up

QuEChERS extraction method is one of the latest and most efficient extraction and clean up techniques for pesticide residue analysis in food matrices which is an anagram for Quick, Easy, Cheap, Effective, Rugged and Safe. This techniques was first introduced by Anastassiades et al. (2003), which is gaining popularity day by day compared to the other existing techniques

In this study, the QuEChERS extraction technique was used for the extraction and clean-up of samples which was modified by Prodhan et al. 2015. The chopped samples were grounded thoroughly with the fruit blender. A representative 10-g portion of thoroughly homogenized sample was weighted in a 50 mL polypropylene centrifuge tube. Then 10 mL of acetonitrile (MeCN) was added into the centrifuge tube. The centrifuge tube was closed properly and shaken vigorously for 30 s by the use of a vortex mixer. Then, 4 g of anhydrous MgSO<sub>4</sub> and 1 g of NaCl were added into the centrifuge tube, and it was shaken immediately by the vortex mixer for 1 minute to prevent the formation of magnesium sulfate aggregates. Afterwards, the extract was centrifuged for 5 min at 5000 rpm. An aliquot of 3 mL of the MeCN layer was transferred into a 15 mL micro centrifuge tube containing 600 mg anhydrous MgSO<sub>4</sub> and 120 mg Primary Secondary Amine (PSA). Then it was thoroughly mixed by vortex for 30 s and centrifuged for 5 minutes at 4000 rpm. (Laboratory Centrifuges, Sigma-3K30, Germany). After centrifuge, a 1 mL supernatant was filtered by a 0.2 µm PTFE filter, and then it was taken in a clean HPLC vial for injection.

### 3.8 Detection and quantification of pesticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) with Flame Thermionized Detector (FTD) for the detection of Acephate, Dimethoate, Diazinon, Fenitrothion, Malathion, Chlorpyrifos and Quinalphos. The capillary column was AT-1, length was 30m, ID was 0.25mm and film thickness was 0.25µm. Helium was used as carrier and make up gas for FTD. The identification of suspected pesticide was performed by peak retention times in samples to those of peaks in the pure analytical standards (**Figure 2**). The instrument conditions are described in **Table 3** and **Table 4**.



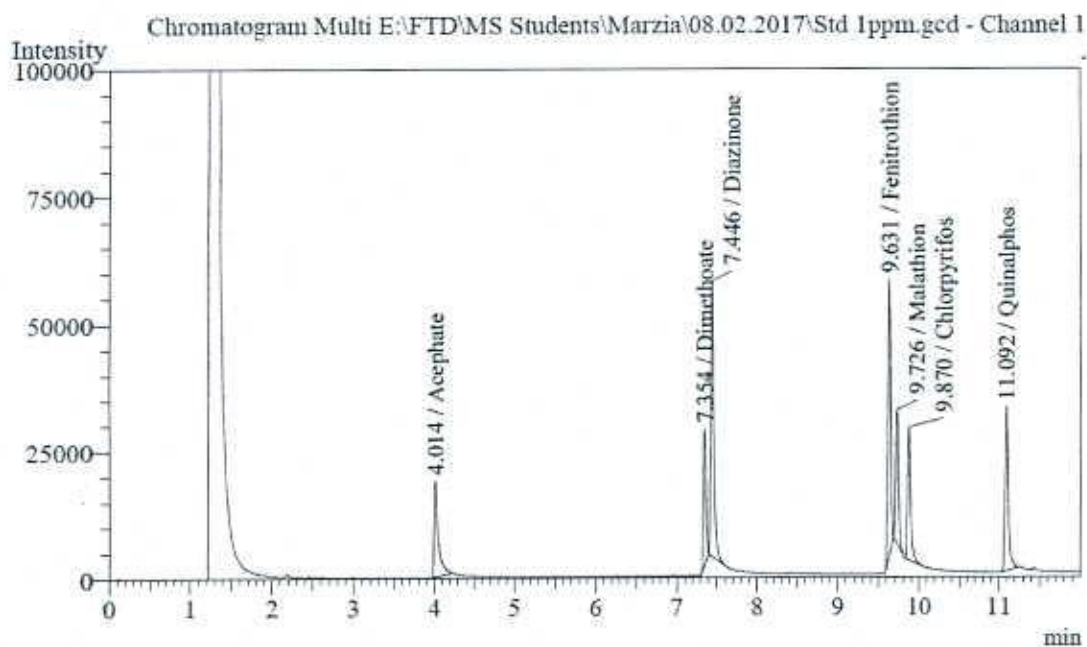


Figure 2. Typical Chromatograms of seven organophosphorus insecticide standards run by GC-FTD.

**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 <sub>2</sub> 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**

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

### 3.9 Calibration curve preparation

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





$f(x)=2.14476109468e-002*x+1.27409837292$   
 $R=0.999462912457$   $R^2=0.998926113376$   
 MeanRF:2.17625864931e-002 RFSD:1.36709257517e-003 RFRSD:6.28184786586  
 CurveType:Linear  
 ZeroThrough:Not through  
 WeightedRegression:None

External Standard

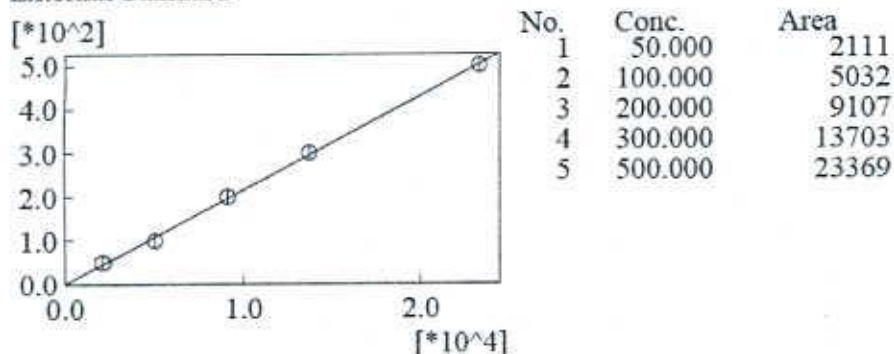


Figure 3. Calibration curve prepared for Dimethoate made with different concentrations ranging from 50µg/L to 500µg/L.

$f(x)=1.66718969691e-002*x-6.50897807754$   
 $R=0.999643866504$   $R^2=0.999287859839$   
 MeanRF:1.60231310913e-002 RFSD:4.02450756721e-004 RFRSD:2.51168610197  
 CurveType:Linear  
 ZeroThrough:Not through  
 WeightedRegression:None

External Standard

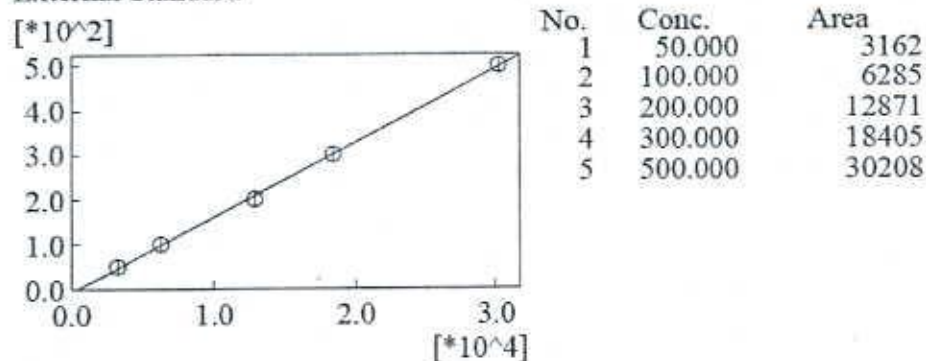


Figure 4. Calibration curve prepared for Chlorpyrifos made with different concentrations ranging from 50 µg/L to 500 µg/L.

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

External Standard

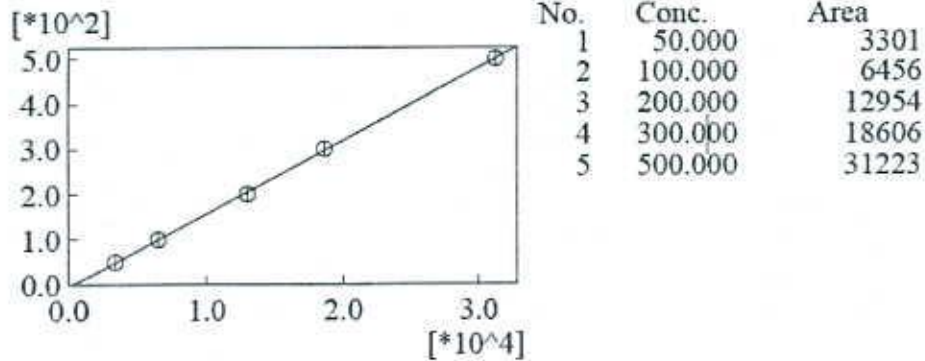


Figure 5. Calibration curve prepared for Acephate made with different concentrations ranging from 50 µg/L to 500 µg/L.

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

External Standard

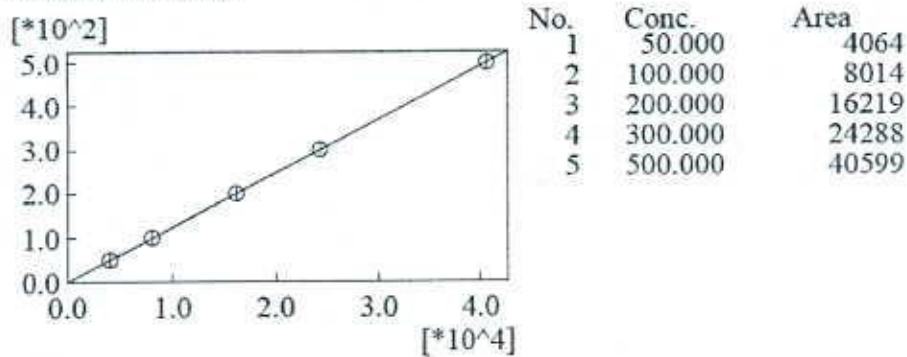
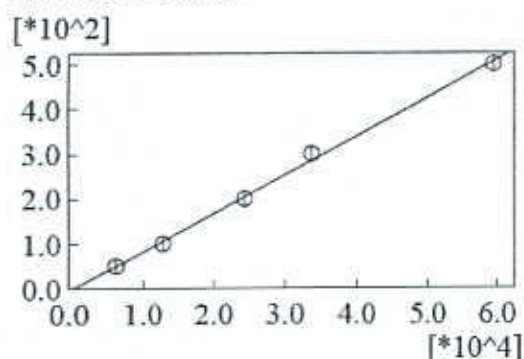


Figure 6. Calibration curve prepared for Quinalphos made with different concentrations ranging from 50 µg/L to 500 µg/L.



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

External Standard

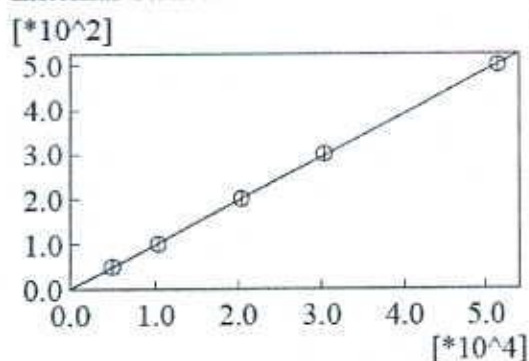


No.	Conc.	Area
1	50.000	6355
2	100.000	12850
3	200.000	24378
4	300.000	33704
5	500.000	59496

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

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

External Standard



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

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

$f(x)=2.5662119724e-002*x-13.8726059301$   
 $R=0.996563718496$   $R^2=0.993139245023$   
 MeanRF:2.28770289157e-002 RFSD:3.45826768136e-003 RFRSD:15.116769289  
 CurveType:Linear  
 ZeroThrough:Not through  
 WeightedRegression:None

External Standard

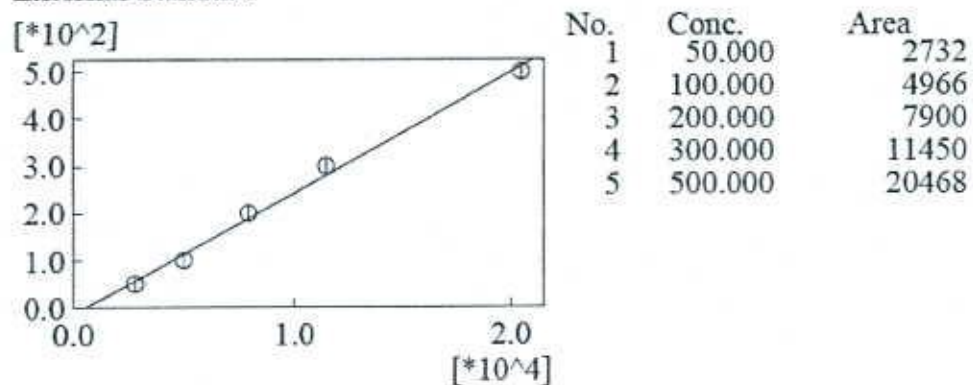


Figure 9. Calibration curve prepared for Malathion made with different concentrations ranging from 50 µg/L to 500 µg/L.





# Chapter IV

## Results and Discussion

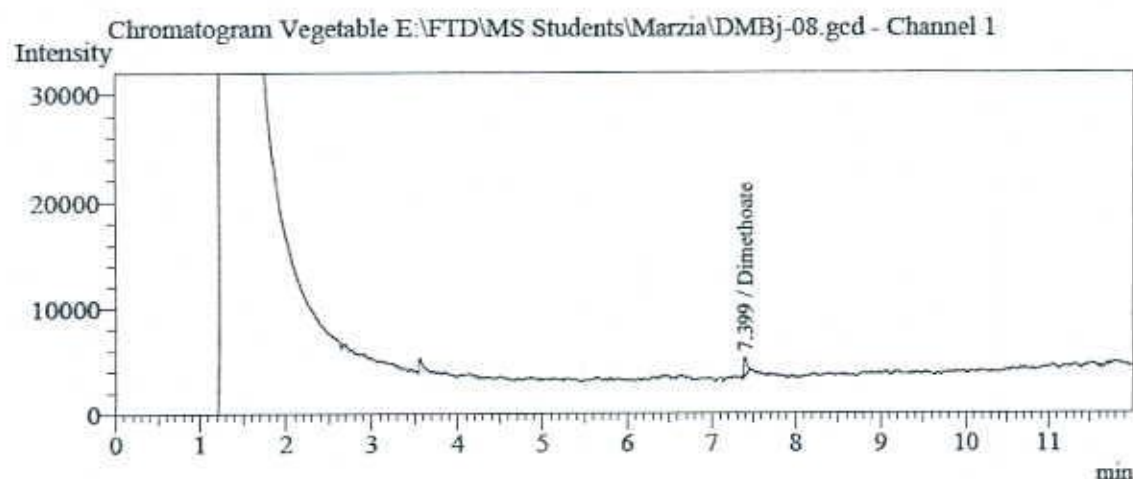
## CHAPTER IV

### RESULTS AND DISCUSSIONS

100 samples of vegetable (eggplant and cauliflower) were collected from 5 different markets (Kawran Bazar, Mohammadpur Krishi Market, Mirpur-1 Bazar, Taltola Bazar and Jatrabari Bazar) of Dhaka city to detect and quantify pesticide residues. The results obtained from this study are presented and described in this chapter using figures and tables.

#### 4.1 Pesticide residues in eggplant

The concentrated extracts of eggplant samples collected from different markets were analyzed by GC-2010 (Shimadzu) with Flame Thermionized Detector (FTD) with the pre-set parameters. **Figure 10-18** shows the chromatograms of the injected extracts of eggplant sample containing detected pesticides.



**Figure 10.** Chromatogram of Dimethoate found in one of the eggplant marketed sample (DMBj.08) showing retention time.



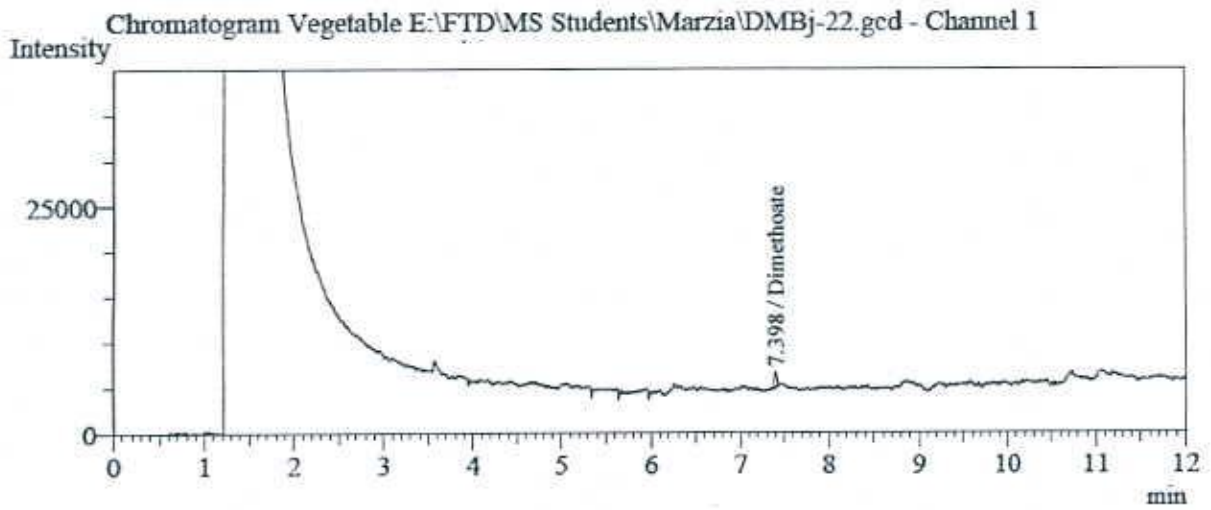


Figure 11. Chromatogram of Dimethoate found in one of the eggplant marketed sample (DMBj-22) showing retention time.

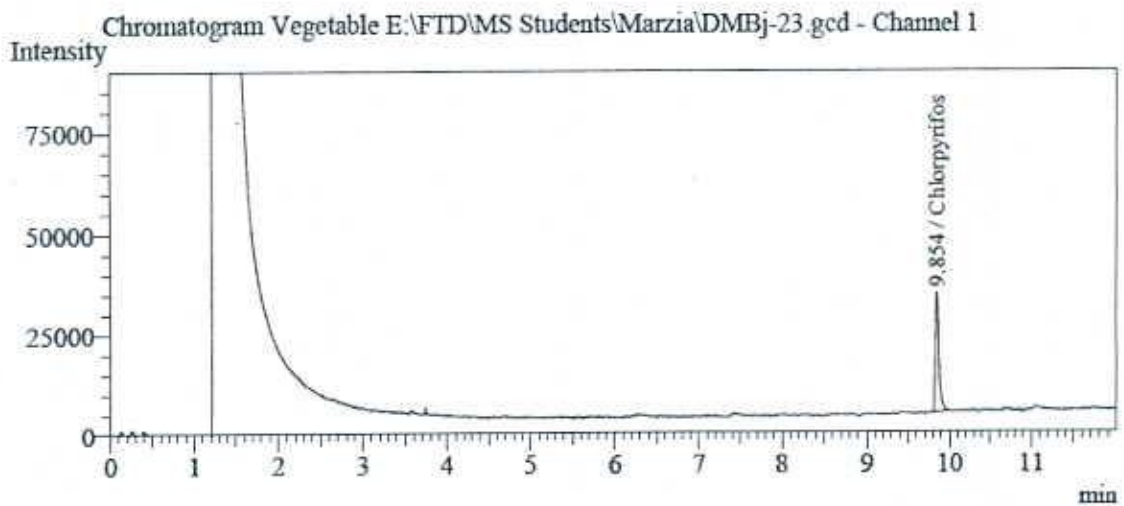


Figure 12. Chromatogram of Chlorpyrifos found in one of the eggplant marketed sample (DMBj-23) showing retention time.



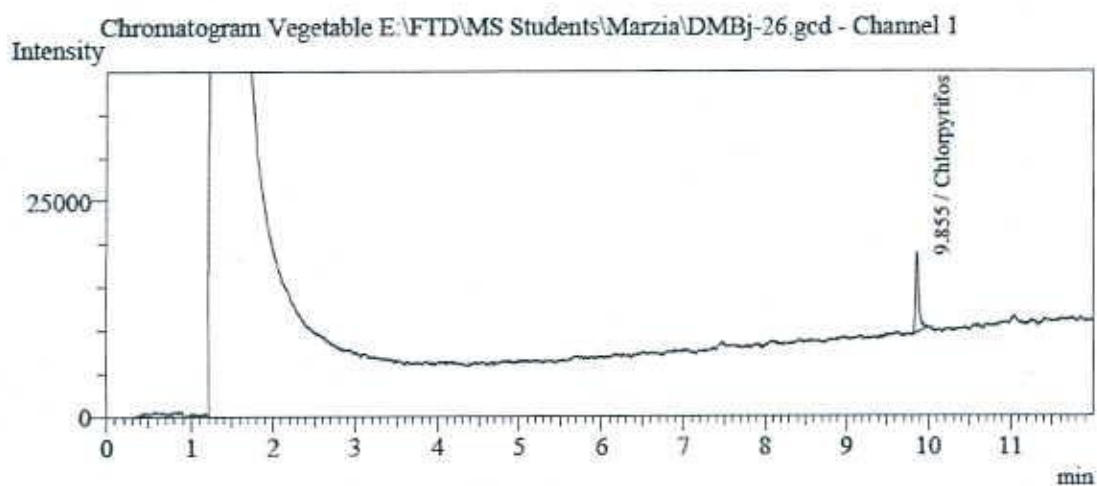


Figure 13. Chromatogram of Chlorpyrifos found in one of the eggplant marketed sample (DMBj-26) showing retention time.

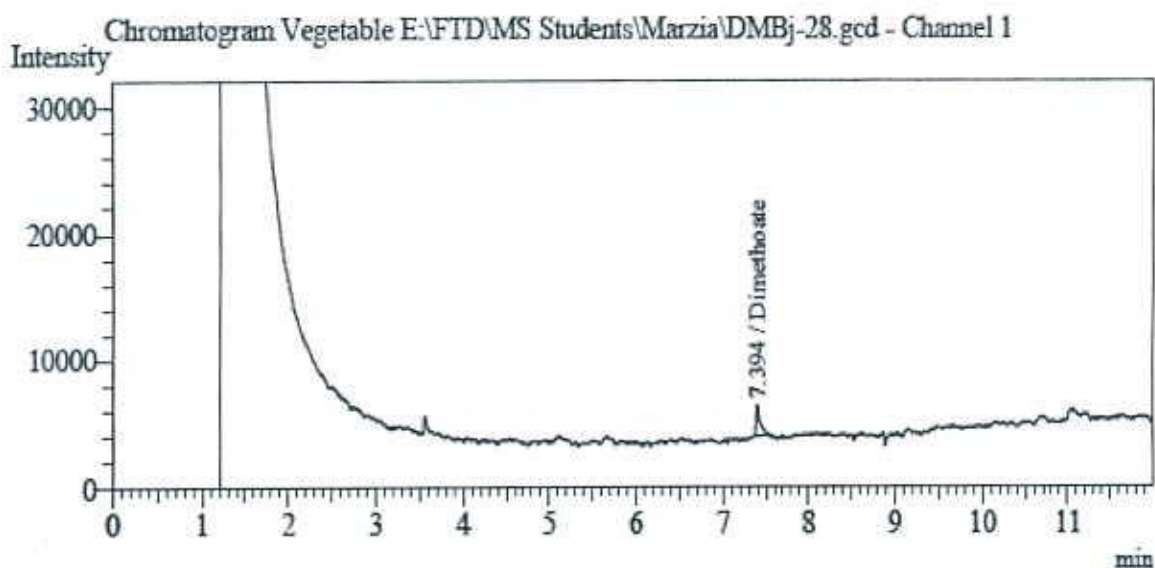


Figure 14. Chromatogram of Dimethoate found in one of the eggplant marketed sample (DMBj-28) showing retention time.



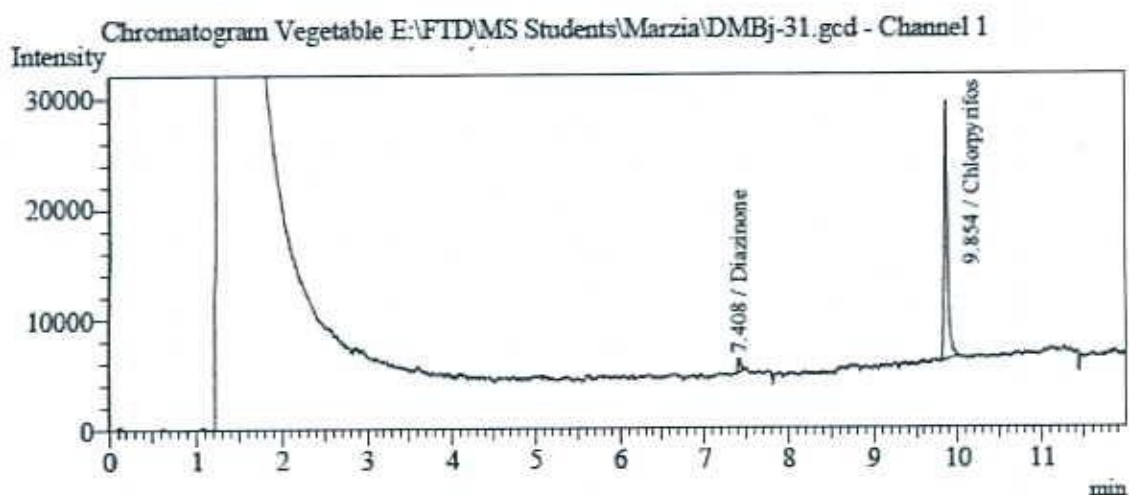


Figure 15. Chromatogram of Diazinon and Chlorpyrifos found in one of the eggplant marketed sample (DMBj-31) showing retention time.

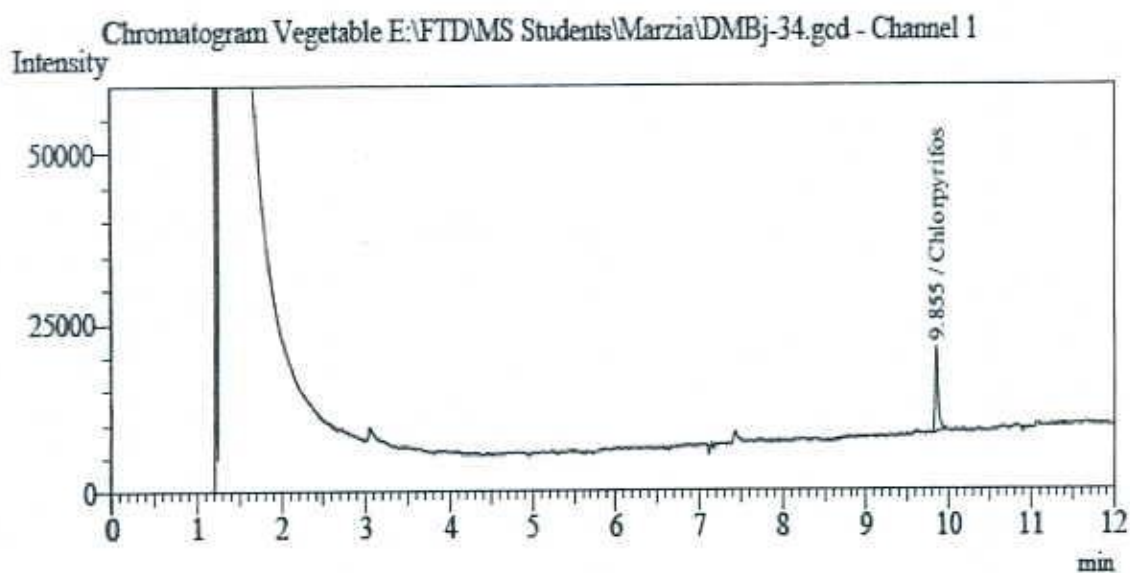


Figure 16. Chromatogram of Chlorpyrifos found in one of the eggplant marketed sample (DMBj-34) showing retention time.

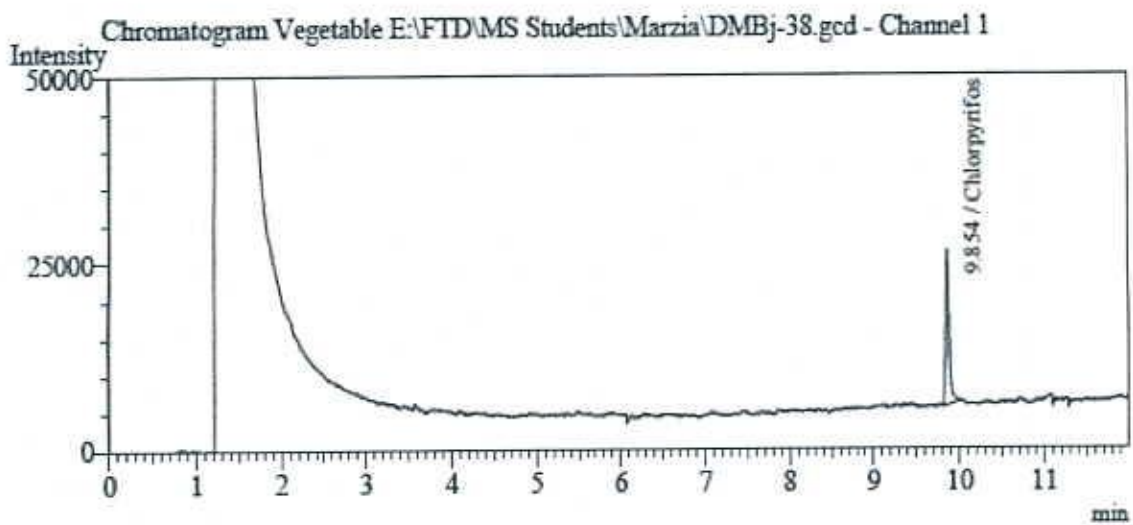


Figure 17. Chromatogram of Chlorpyrifos found in one of the eggplant marketed sample (DMBj-38) showing retention time.

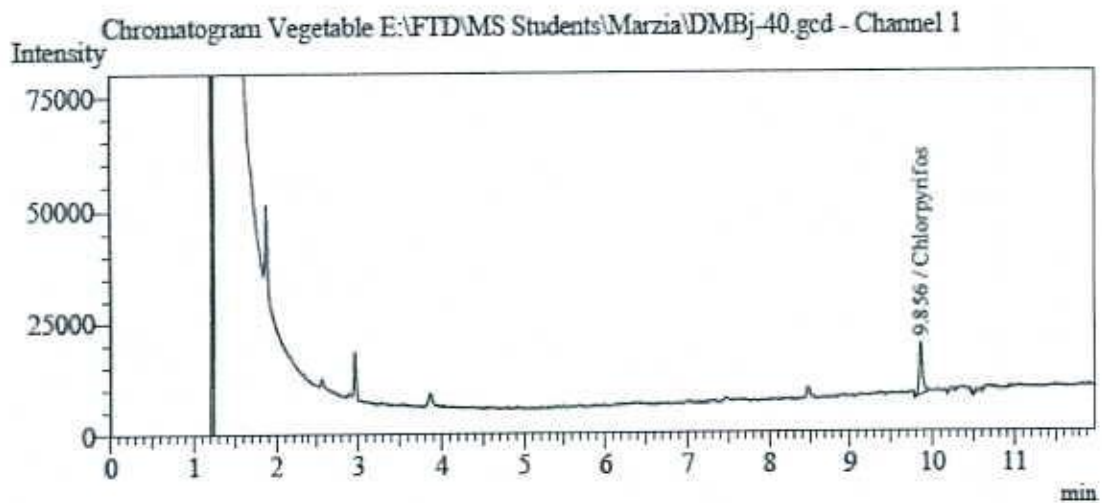


Figure 18. Chromatogram of Chlorpyrifos found in one of the eggplant marketed sample (DMBj-40) showing retention time.



The level of pesticide residues found in the analyzed eggplant samples and their maximum residue levels are outlined in **Table 5.**

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

Area of collection	Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)
Jatrabari Bazar	DMBj-1	ND	-	-
	DMBj-2	ND	-	-
	DMBj-3	ND	-	-
	DMBj-4	ND	-	-
	DMBj-5	ND	-	-
	DMBj-6	ND	-	-
	DMBj-7	ND	-	-
	DMBj-8	Dimethoate	0.08	0.02*
	DMBj-9	ND	-	-
	DMBj-10	ND	-	-
Kawran Bazar	DMBj-11	ND	-	-
	DMBj-12	ND	-	-
	DMBj-13	ND	-	-
	DMBj-14	ND	-	-
	DMBj-15	ND	-	-
	DMBj-16	ND	-	-
	DMBj-17	ND	-	-
	DMBj-18	ND	-	-
	DMBj-19	ND	-	-
	DMBj-20	ND	-	-
Mirpur1 Bazar	DMBj-21	ND	-	-
	DMBj-22	Dimethoate	0.052	0.02*
	DMBj-23	Chlorpyrifos	0.980	0.4*

	DMBj-24	ND	-	-
	DMBj-25	ND	-	-
	DMBj-26	Chlorpyrifos	0.470	0.4*
	DMBj-27	ND	-	-
	DMBj-28	Dimethoate	0.132	0.02*
	DMBj-29	ND	-	-
	DMBj-30	ND	-	-
Mohammadpur Krishi Market	DMBj-31	Diazinon	0.010	0.01*
		Chlorpyrifos	0.920	0.4*
	DMBj-32	ND	-	-
	DMBj-33	ND	-	-
	DMBj-34	Chlorpyrifos	0.500	0.4*
	DMBj-35	ND	-	-
	DMBj-36	ND	-	-
	DMBj-37	ND	-	-
	DMBj-38	Chlorpyrifos	0.762	0.4*
	DMBj-39	ND	-	-
DMBj-40	Chlorpyrifos	0.384	0.4*	
Taltola Bazar	DMBj-41	ND	-	-
	DMBj-42	ND	-	-
	DMBj-43	ND	-	-
	DMBj-44	ND	-	-
	DMBj-45	ND	-	-
	DMBj-46	ND	-	-
	DMBj-47	ND	-	-
	DMBj-48	ND	-	-
	DMBj-49	ND	-	-
	DMBj-50	ND	-	-

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





Fifty samples of eggplant collected from 5 different markets of Dhaka city (Kawran Bazar, Mohammadpur Krishi Market, Mirpur-1 Bazar, Taltola Bazar and Jatrabari Bazar) were analyzed to find out the presence of left over residue of seven organophosphorus pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos).

Out of 50 samples, 9 samples (18% of the total number of samples) contained pesticide residues and 41 samples (82% of the total number of samples) contained no detectable residues of the sought pesticides. The present results can be compared to a research conducted by Islam *et al.* (2014). They have collected 42 samples of eggplant, cauliflower and country bean from fields and local markets of Narsingdi district, Bangladesh, where they found 15 samples (above 68% of total samples) contained no residues of the sought pesticides.

#### **Pesticide residue status in the samples of eggplant collected from Jatrabari area:**

Only 1 sample (DMBj-08) among 10 eggplant samples collected from Jatrabari area contained Dimethoate at a level of 0.08 mg/kg, which is above the MRL (0.02mg/kg) set by the European Commission. The other 9 samples contain no detectable pesticide residues.

#### **Pesticide residue status in the samples of eggplant collected from Kawran Bazar and Taltola Bazar area:**

Among the 20 samples collected from Kawran Bazar and Taltola Bazar none of the sample contained any residues of the 7 tested pesticides.

#### **Pesticide residue status in the samples of eggplant collected from Mirpur-1 area:**

Among 10 samples collected from Mirpur-1 area, 2 samples (DMBj-22 and DMBj-28) contained Dimethoate at the level of 0.052 mg/kg and 0.132 mg/kg respectively, which were above the MRL (0.02mg/kg) set by the European Commission. And another 2 samples (DMBj-23 and DMBj-26) contained Chlorpyrifos at the level of 0.980 mg/kg and 0.470 mg/kg respectively which were above MRL (0.40mg/kg) set by the European Commission.

#### **Pesticide residue status in the samples of eggplant collected from mohammadpur Krishi Market:**

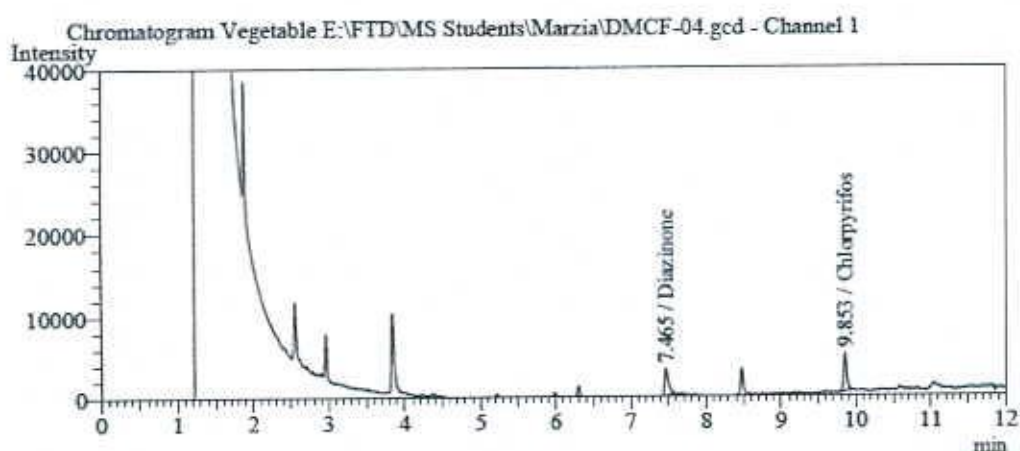
Among the ten samples of eggplant collected from Mohammadpur Krishi Market, 1 sample (DMBj-31) contained both Diazinon and Chlorpyrifos at the level of 0.01 mg/kg and 0.920 mg/kg respectively. In this sample the concentration of Diazinon is exactly same as the MRL (0.01mg/kg) set by the European Commission and the concentration of Chlorpyrifos is above the



MRL (0.40mg/kg) set by the European Commission. Another 3 samples (DMBj-34, DMBj-38 and DMBj-40) from the same market contained Chlorpyrifos at the level of 0.50 mg/kg, 0.762 mg/kg and 0.384 mg/kg respectively. Among which 2 samples (DMBj-34 and DMBj-38) had Chlorpyrifos concentration above MRL (0.40mg/kg) set by the European Commission and the other one (DMBj-40) had Chlorpyrifos concentration below the MRL (0.40mg/kg) set by the European Commission.

#### 4.2 Pesticide residues in cauliflower

The concentrated extracts of cauliflower samples collected from different markets of Dhaka city were analyzed by GC-2010 (Shimadzu) with Flame Thermionized Detector (FTD) with the pre-set parameters. **Figure 19-26** shows the chromatograms of the injected extracts of cauliflower sample containing detected pesticides.



**Figure 19.** Chromatogram of Diazinon and Chlorpyrifos found in one of the cauliflower marketed sample (DMCF-04) showing retention time.

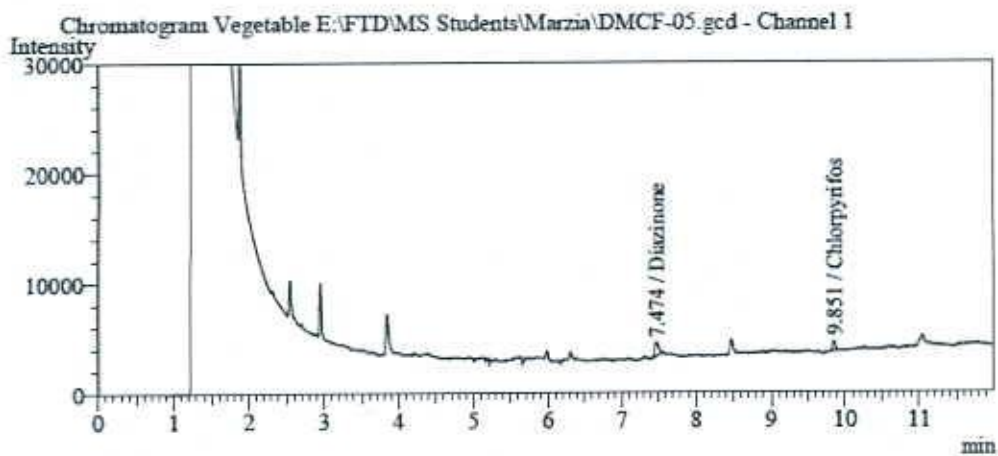


Figure 20. Chromatogram of Diazinon and Chlorpyrifos found in one of the cauliflower marketed sample (DMCF-05) showing retention time.

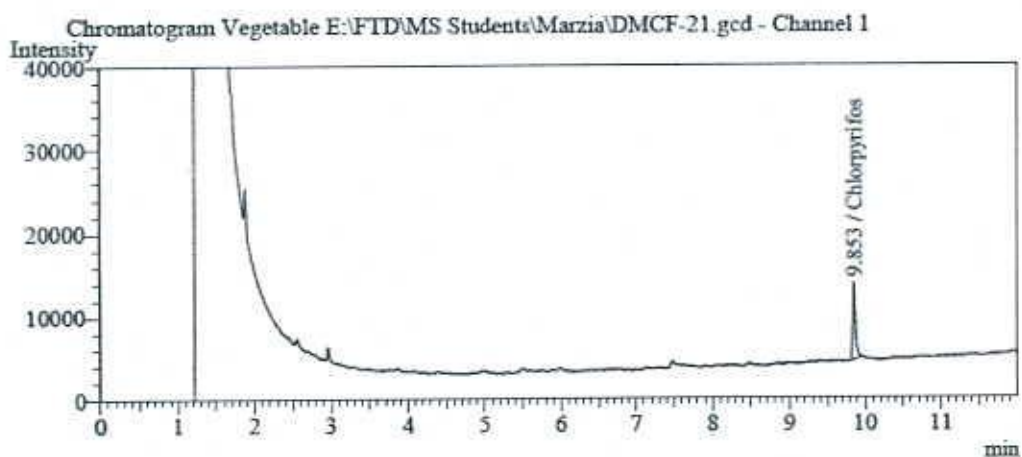


Figure 21. Chromatogram of Chlorpyrifos found in one of the cauliflower marketed sample (DMCF-21) showing retention time.



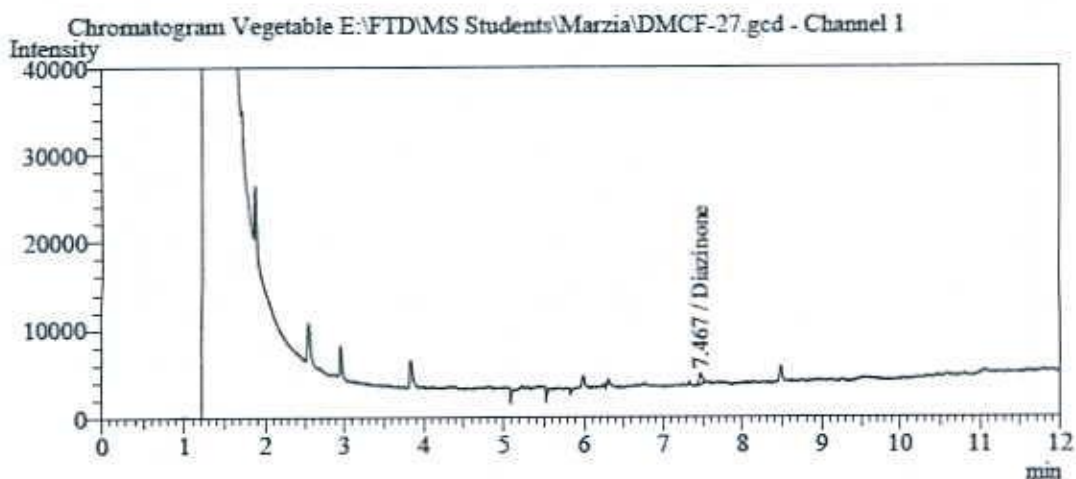


Figure 22. Chromatogram of Diazinon found in one of the cauliflower marketed sample (DMCF-27) showing retention time.

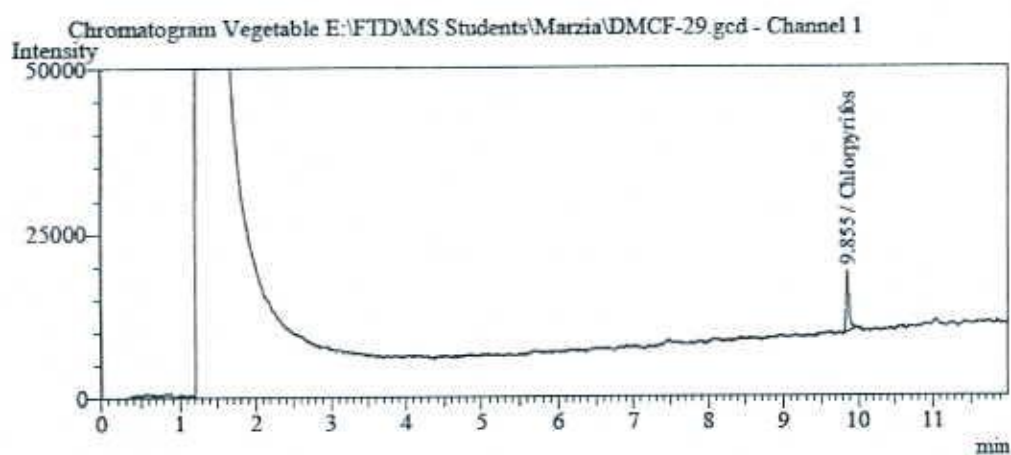


Figure 23. Chromatogram of Chlorpyrifos found in one of the cauliflower marketed sample (DMCF-29) showing retention time.



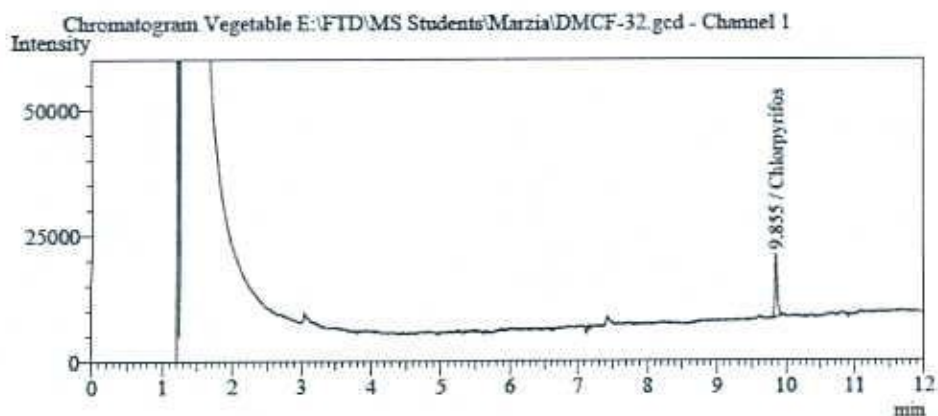


Figure 24. Chromatogram of Chlorpyrifos found in one of the cauliflower marketed sample (DMCF-32) showing retention time.

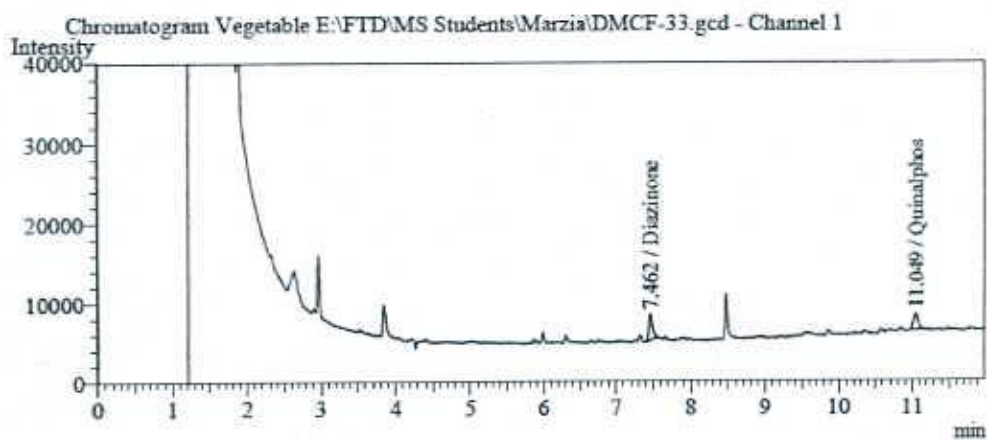


Figure 25. Chromatogram of Diazinon and Quinalphos found in one of the cauliflower marketed sample (DMCF-33) showing retention time.

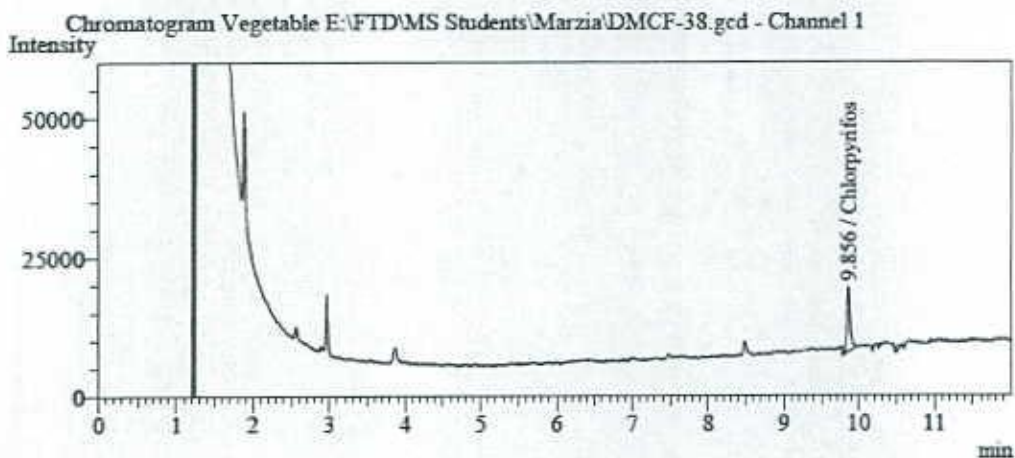


Figure 26. Chromatogram of Chlorpyrifos found in one of the cauliflower marketed sample (DMCF-38) showing retention time.

The levels of pesticide residues found in the analyzed cauliflower samples and their maximum residue levels are presented in Table 6.

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

Area of collection	Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)	
Jatrabari	DMCF-01	ND	-	-	
	DMCF-02	ND	-	-	
	DMCF-03	ND	-	-	
	DMCF-04		Diazinon	0.094	0.01*
			Chlorpyrifos	0.18	0.05*
	DMCF-05		Diazinon	0.42	0.01*
			Chlorpyrifos	0.03	0.05*
	DMCF-06	ND	-	-	
	DMCF-07	ND	-	-	
	DMCF-08	ND	-	-	
DMCF-09	ND	-	-		

Kawranbazar	DMCF-10	ND	-	-
	DMCF-11	ND	-	-
	DMCF-12	ND	-	-
	DMCF-13	ND	-	-
	DMCF-14	ND	-	-
	DMCF-15	ND	-	-
	DMCF-16	ND	-	-
	DMCF-17	ND	-	-
	DMCF-18	ND	-	-
	DMCF-19	ND	-	-
	DMCF-20	ND	-	-
Mirpur-1 Bazar	DMCF-21	Chlorpyrifos	0.373	0.05*
	DMCF-22	ND	-	-
	DMCF-23	ND	-	-
	DMCF-24	ND	-	-
	DMCF-25	ND	-	-
	DMCF-26	ND	-	-
	DMCF-27	Diazinon	0.033	0.01*
	DMCF-28	ND	-	-
	DMCF-29	Chlorpyrifos	0.42	0.05*
	DMCF-30	ND	-	-
	Mohammadpur krishi market	DMCF-31	ND	-
DMCF-32		Chlorpyrifos	0.54	0.05*
DMCF-33		Diazinon	0.08	0.01*
		Quinalphos	0.06	0.01*
DMCF-34		ND	-	-
DMCF-35		ND	-	-
DMCF-36		ND	-	-
DMCF-37		ND	-	-
DMCF-38		Chlorpyrifos	0.525	0.05*



	DMCF-39	ND	-	-
	DMCF-40	ND	-	-
Taltola Bazar	DMCF-41	ND	-	-
	DMCF-42	ND	-	-
	DMCF-43	ND	-	-
	DMCF-44	ND	-	-
	DMCF-45	ND	-	-
	DMCF-46	ND	-	-
	DMCF-47	ND	-	-
	DMCF-48	ND	-	-
	DMCF-49	ND	-	-
	DMCF-50	ND	-	-

\*According to the EU Pesticide Data base (European Commission, 2005)

Fifty samples of cauliflower collected from 5 different markets of Dhaka city (Kawran Bazar, Mohammadpur Krishi Market, Mirpur-1 Bazar, Taltola Bazar and Jatrabari Bazar) were analyzed to find out the presence of left over residue of seven organophosphorus pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos).

Out of 50 samples, 8 samples (16% of the total no. of samples) contained pesticide residues and 42 samples (84% of the total number of samples) contained no detectable residues of the sought pesticides. The present results can be compared to a research conducted by Islam *et al.* (2014). They were collected 42 samples of eggplant, 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.

#### **Pesticide residue status in the samples of cauliflower collected from Jatrabari area:**

10 samples of cauliflower were collected from Jatrabari area, among them 2 samples (DMCF-04 and DMCF-05) contained multi residue. Sample DMCF-04 contained residue of Diazinon at a level of 0.094 mg/kg which was above MRL (0.01mg/kg) set by the European Commission and Chlorpyrifos at a level of 0.18 mg/kg which was also above MRL (0.05mg/kg) set by the European Commission. And a sample DMCF-05 contained residue of Diazinon at a level of 0.042 mg/kg which was above MRL (0.01mg/kg) set by the European Commission and

Chlorpyrifos at a level of 0.03mg/kg which was also above MRL (0.05mg/kg) set by the European Commission.

**Pesticide residue status in the samples of cauliflower collected from Mirpur-1 area:**

Among the 10 samples of cauliflower collected from Mipur-1 area two samples (DMCF-21 and DMCF-29) contained residue of Chlorpyrifos. Where the residue of Chlorpyrifos was 0.373 mg/kg in sample DMCF-21 and was 0.42 mg/kg in sample DMCF-29. Both had Chlorpyrifos concentration above the MRL (0.05mg/kg) set by the European Commission. One sample from the same place contained residue of Diazinon which was 0.033 mg/kg and it was above the MRL (0.01mg/kg) set by the European Commission.

**Pesticide residue status in the samples of cauliflower collected from Kawran Bazar and Taltola Bazar area:**

In case of 20 samples of cauliflower collected from Kawran Bazar and Taltola Bazar, none of the sample contained any residues of the 7 tested pesticides.

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

Among the 10 samples of cauliflower collected from Mohammadpur Krishi Market area, two samples (DMCF-32 and DMCF38) contained residue of Chlorpyrifos, where the residue of Chlorpyrifos was 0.54 mg/kg in sample DMCF-32 and was 0.525 mg/kg in sample DMCF-38. These two concentrations of Chlorpyrifos in cauliflower sample were above MRL (0.05mg/kg) set by the European Commission. One sample (DMCF-33) from the same place contained residue of Diazinon and Quinalphos. Where Diazinon was at the level of 0.08 mg/kg which was above MRL (0.01mg/kg) and Quinalphos was at the level of 0.06 mg/kg which was also above MRL (0.01mg/kg) set by European Commission.





# Chapter V

## Summary and Conclusion



## CHAPTER V

### SUMMERY AND CONCLUSION

Most nutritious and vitamin rich component of our daily food menu is fresh vegetables. Vegetable helps in buildup our body immune system and protect us from different types of diseases. People becoming more health conscious day by day are being interested in fresh vegetable consumption. But the contribution of vegetable in our daily intake is very poor because of its low production. One of the major obstacles is insect-pests and diseases of vegetables. The farmers of our country mostly depend on chemical pesticides to control them. These chemical pesticides get incorporated in our food product, vegetables in the field and the pesticides with higher residual effect may remain in them when reach our dining table. Pesticide residues in food products may result in different types of chronic diseases e.g., cancer, heart attack etc. as a result of long term consumption of pesticide contaminated vegetables.

The purpose of this study is to detect and identify pesticide residues in eggplant and cauliflower collected from local markets of Dhaka city and to quantify them for the comparison with the international standards. Regarding this, 50 samples of eggplant and 50 samples of Cauliflower were collected from 5 different markets of Dhaka City 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 thermionized 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. diazinon, acephate, chlorpyrifos, malathion, fenitrothion, dimethoate and quinalphos were selected for the study.

Out of 50 samples of eggplant, 9 samples (18% of the total number of samples) contained residues of diazinon, chlorpyrifos and dimethoate. Among these 9 samples, 8 had residues above the MRL set by European Commission and one had the residue level exactly same as the MRL set by European Commission. Other 41 samples (82% of the total number of samples) contained no detectable residues of the sought pesticides.

Among the 50 samples of cauliflower, 8 samples (16% of the total number of samples) contained residues of diazinon, chlorpyrifos and quinalphos. All of these 8 samples had residues above the MRL set by European Commission. Other 42 samples (84% of the total number of samples) contained no detectable residues of the sought pesticides.

In present days, pesticide residue in food product has become a consumer safety issue and general people are becoming more concern about it. Media is also well aware about this topic. This study will help us to understand this matter. People may get an idea about the present conditions of the pesticide residue level in vegetables in local markets of Dhaka city and it will also may able to raise public awareness. This study also may help the exporters to gain the trust of foreign buyers.



# Chapter VI

# References





## CHAPTER VI

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Plate 7. Collected and labeled samples





Plate 8. Sample Chopping



Plate 9. Sample Grinding



Plate 10. Measuring different ingredient for sample preparation





Plate 11. Sample in vortex mixture



Plate 12. Samples in Orbital Shaker



Plate 13. Prepared Samples





Plate 14. Vial Preparation

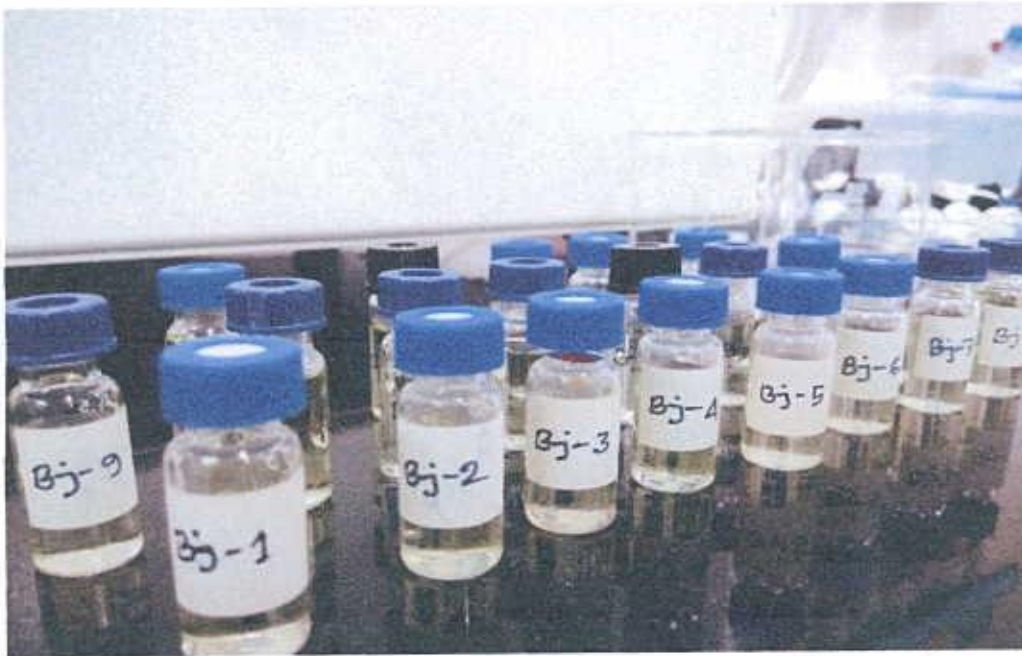


Plate 15. Prepared Vials



Plate 16. Vials in Gas-Chromatography (GC)

Sher-e-Bangla Agricultural University  
Library  
Accession No. 40347  
Sign. *Amay* Date 18/10/17