DETERMINATION OF PESTICIDE RESIDUES IN VEGETABLES COLLECTED FROM BOGURA DISTRICT IN BANGLADESH

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DETERMINATION OF PESTICIDE RESIDUES IN VEGETABLES COLLECTED FROM BOGURA DISTRICT IN BANGLADESH

BY

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CERTIFICATE

This is to certify that the thesis entitled **"DETERMINATION OF PESTICIDE RESIDUES IN VEGETABLES COLLECTED FROM BOGURA DISTRICT IN BANGLADESH"** submitted to the Department of Agricultural Chemistry, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.)** in **AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bona fide research work carried out by **ANEY PARVEN**, Registration No. **12-05023** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

Dated: December, 2017
Dhaka, Bangladesh

(Dr. Md. Sirajul Islam Khan)
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Dedicated to

My

Beloved Parents

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ABSTRACT

The study was conducted to analyze pesticide residues in two common vegetables (country bean and yard long bean) collected from five different areas (Raja Bazaar, Gabtali Bazar, Sonatola Bazar, Mohasthangor Bazar and Shibganj Bazar) of Bogura District from November, 2017 to February, 2018. The collected samples were carried to the Pesticide Analytical Laboratory, Entomology Division of Bangladesh Agricultural Research Institute (BARI), Gazipur. The collected samples were analyzed using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD) for the determination of pesticide residues in 35 samples of country bean and 35 samples of yard long bean. Among the 35 analyzed samples of country bean, 4 samples (11% of the total number of samples) contained pesticide residues of Quinalphos. Among these 4 samples, 2 had residues above the Maximum Residue Limit (MRL) and 2 had residues below the Maximum Residue Limit (MRL) set by European Commission. Similarly out of 35 samples of yard long bean, 5 samples (14% of the total number of samples) contained pesticide residues of Chlorpyrifos, Dimethoate and Quinalphos. Among this 5 samples, 2 had residues above the Maximum Residue Limit (MRL) and 2 had residues below the Maximum Residue Limit (MRL) set by European Commission and 1 sample contained two pesticide residues such as Dimethoate and Chlorpyrifos. The level of detected Dimethoate residue was above the Maximum Residue Limit (MRL), while the level of detected Chlorpyrifos residue was below the Maximum Residue Limit (MRL) in this contaminated sample. Quinalphos and Dimethoate were detected in most of the contaminated country bean and yard long bean samples, while Chlorpyrifos was also detected. This study reflects the overall scenario of pesticide contamination in vegetables especially in country bean and yard long bean available in the local markets of Bogura District, which will help the consumer to be aware of their health and safety.

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LIST OF ABBREVIATIONS

ADI	Acceptable Daily Intake
ACh	Acetylcholine
AChE	Acetycholinesterase
APCI	Atmospheric Pressure Chemical Ionization
ASE	Accelerated solvent extraction
AOAC	Association of Official Analytical Chemists
BARI	Bangladesh Agricultural Research Institute
CAS	The Chemical Abstracts Service
CCD	Colony collapse disorder
CBS	Compound Based Scanning
CSN	Committee for Standardization
DAS	Days After Spray
DDT	Dichloro Diphenyle Trichloroethen
DLLME	Dispersive Liquid–Liquid Micro Extraction
DV	Daily Value
d-SPE	dispersive solid phase extraction
ECD	Electron Capture Detector
EPA	Environmental Protection Agency
ESI	electrospray ionization
et al.	*et alibi* (and others)
etc.	*et cetra* (and so on)
ECD	Electron capture Detector
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FID	Flame Ionization Detector
FPD	Flame photometric Detector
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GC-MS	Gas Chromatograph-Mass Spectrometry
HPLC	High Performance Liquid Chromatography

HRI	Hazard Risk Index
IARC	The International Agency for Research on Cancer
IUPAC	Intrenational Union of Pure and Applied Chemistry
KMRL	Korean Maximum Residue Limits
LC-MS	Liquid Chromatography-Mass Spectrometry
LLE	Liquid-liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
MAE	Microwave Assisted Extraction
MCS	Multiple Chemical Sensitivity Syndrome
MDQ	Minimum Detectable Quantity
MRL	Maximum Residue Limit
MRM	multiple reaction monitoring
NPD	Nitrogen-phosphorus Detector
NPTN	National Pesticides Telecommunications Network
NTE	Neuropathy Target Esterase
OCPs	Organochlorin Pesticides
OPPs	organophosphorus Pesticides
PDA	Photodiode Array detection
PDI	Potential Daily Intake
PSA	Primary Secondary Amine
PHI	Pre-Harvest Interval
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RSM	Response Surface Methodology
RTL	Retention Time Locked
SAU	Sher-e-Bangla Agricultural University
SBSE	Stir Bar Sorptive Extraction
SE	Solvent Extraction
SFE	Supercritical fluid extraction
SF	Supercritical Fluid
SIM	Selected Ion Monitoring
SRM	Selected Reaction Monitoring
SPME	Solid Phase Micro Extraction (SPME)

SPE	Solid phase extraction
TOTAD	Through Oven Transfer Adsorption Desorption
TCD	Thermal conductivity Detector
UHPLC-MS/MS	Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry
VFs	Variability Factors
WHO	World Health Organization

CHAPTER I

INTRODUCTION

Bangladesh is an agricultural based country and its economy highly depends on agriculture. It has an area of 1, 47,570 sq. kilometer, only 0.31% of the total agricultural land in the world but 2.0 percent of total population in the world (Rasul and Thapa, 2004). Vegetables, rice, wheat, jute, sugarcane, and tea are major crops grown in Bangladesh (Islam *et al.* 2009). Pesticides and synthetic fertilizers have contributed significantly to improve yields of crops. High yielding variety is highly susceptible to pests and diseases. So the use of pesticide is now an inherent part of agriculture for pest control (Suvagata Bagchi *et. al.*, 2008). It has been found that many countries of the world including Bangladesh have been extensively using chemical pesticides to cultivate agricultural crops. About 900 chemical pesticides are used worldwide in various food products and for the treatment of crops and soil (Thurman *et. al.*, 2008).

Vegetables are considered essential for well balanced diets since they supply vitamins, minerals, dietary fiber, and phytochemicals. In the daily diet, vegetables have been strongly associated with improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some forms of cancer. Vegetables make up a major portion of the diet of humans in many parts of the world and play a significant role in human nutrition, especially as sources of phytonutriceuticals: vitamins (A, B_1, B_6, B_9, C, E), minerals, dietary fiber and phytochemicals (Quebedeaux and Eisa, 1990; Craig and Beck, 1999; Wargovich, 2000; Dias and Ryder, 2011). Vegetables are the fresh and edible portions of herbaceous plants. They contain valuable food ingredients which can be successfully utilized to build up and repair the body. Vegetables are valuable in maintaining alkaline reserve of the body. They are valued mainly for their high carbohydrate, vitamin and mineral contents. There are different kinds of vegetables. They may be edible roots, stems, leaves, fruits or seeds. Each group contributes to diet in its own way (Robinson, 1990).

The country bean, *Dolichos lablab* L. is an important winter vegetable crop in Bangladesh. It belongs to the family Fabaceae (Thompson, 1951). Though it is commonly known as country bean in Bangladesh, but it has different names at different regions of the country like Sheem, Uri, Ushi, Deshi Sheem etc. Internationally the crop has also different names e.g. Hyacinth bean, Bonavist bean, Dolichos bean, Indian bean, Egyotian kidney bean, Lima bean, Faba bean etc. (Jadhav *et al*., 1987). It is remarkable that bean crops show a broad range of adaptation to the most varied climatic conditions within a wide range of geographic area that extends from around 50^o north latitude to 32^o south latitude. Country bean is a big source of essential vitamins and generally cultivated in Rabi seasons usually around the homestead by trailing its vine either on trees or by providing different kinds of supports. Although beans are assessed as the major group of vegetables grown intensively in Rabi seasons, some varieties of country bean can be grown year round including kharif seasons. The importance of country bean has thus highly significance from growing season point of view (Hossain and Awrangzeb, 1992). The major vegetables grown in Rabi season are various kinds of beans, which appear as one of the most important group of vegetables in our daily food. Country bean plays a significant role in the agro-economy and national health of Bangladesh. The crop has multipurpose functions. Consumers use its young pods and tender beans as vegetables (Purseglove, 1968). As a split pulse (dhal) and in various curry preparation, the ripe and dried seeds are used. It is also used as a popular fodder crop. To produce “hay and silage’ as animal feed, its foliar portion is used. Country bean has a unique ability to fix atmospheric nitrogen to the soil through rhizobial symbiosis at root zone which enhance soil fertility as a green manure crop. Its mature dried stems are used as fuel and provide more or less an opportunity to combat fuel crisis. It has high digestibility and free from flatulent effects. Its green pods and mature seeds are good source (25%) of protein (on dry basis), vitamins (e.g. vitamin A, vitamin C, riboflavin etc.) and mineral such as magnesium, calcium, phosphorous, potassium, iron, sulfur and sodium (Deka and Sarker, 1990; Newaz, 1992). So it is an important source of cash income as well as alleviating malnutrition and sickness caused as by dietary deficiencies.

In spite of being a prospective crop, high rate of insect pest’s infestation is responsible to its low yield and poor quality. Farmers face significant yield loss of beans every year due to severe attack of various insect pests in our country. Generally, insect pests cause enormous quantity of yield losses in every season. Though, regular statistical records are not kept, as per conservative estimate the yield loss in country bean due to insect pests is reported to be about 12-30% (Hossain, 1990). Country bean is usually attacked by nine different insect species and one species of mite (Alam, 1969).

Yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Walp) is an important leguminous vegetable grown very profitably all over Bangladesh. It is also known as asparagus bean, string bean, snake bean or vegetable cowpea (Purseglove, 1977). It is extensively grown in kharif season when there is shortage of vegetables supply in the market. A serving of 100 g of yard long bean contains 50 calories, 9.0 g of total carbohydrates, 3.0 g of proteins, 0.2 g total fat and 0.8 g of minerals (Anon., 2013). Yard long bean is one of the economically important vegetable crops in Bangladesh. The area occupied by this crop was 5857.49 ha and the production was 21348 t during the year 2008- 2009 (Anon., 2010). It is one of the vegetable shaving exporting potential in Bangladesh. The cultivation of this crop faces various problems including the pest management (Rashid, 1993). Yard long bean is especially attractive to aphids (*Aphis craccivora, Myzus persicae* and *Aphis gossypii*), green stink bug (*Nezara viridula*) and red spider mite (*Tetranychus* spp.). Greasy cutworms (*Agrotisipsilon*) often cause damage just after emergence (Grubben, 1993). The insect pests have been reported as one of the serious problems to yard long bean cultivation in the country (Rashid, 1999).

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

All upazila under Bogura district are famous for vegetables production. Pesticides have been used legally or illegally in extensive quantities at these areas for various purposes. At present, detailed information is not available about the level of pesticide residues remain in different vegetable samples of these areas. But now a day's application of pesticides for the control of insect pests and diseases in Bogura district is very high for increasing crops yield. Moreover, there are no substantial works have yet been done for the determination of pesticide residues levels at this areas in order to investigate their harmful effects as a result of the irresponsible and indiscriminate use of pesticides in farmer's fields. The farmers of Bogura district are generally used Malathion, Diazinon, Chlorpyriphos and some other pesticides also (Hossain *et al.*, 2015).

Pesticide residue is becoming a major food safety concern for the consumers and governments. In order to remove residual effect of pesticides, accurate dose and the harvest time after spray of each pesticide which should be recommended to the farmer, so that the amount of residual pesticides in vegetables might be lower than the acceptable range (Tashkent, 1998).

Gas Chromatography (GC) and Gas Chromatography-Mass spectrometry (GC-MS) is the most sophisticated method for determination of pesticide residue in vegetable samples rather than High Performance Liquid Chromatography (HPLC) method and others. GC-MS has been widely heralded as a "Gold Standard" for forensic substance identification because it is used to perform a specific test. A specific test positively identifies the actual presence of a particular substance in a given sample. A non-specific test merely indicates that a substance falls into a category of substances. Although a non-specific test could statistically suggest the identity of the substance, this could lead to false positive identification (Sattar, 1993).

Bangladesh has been using pesticides since 1950's in agriculture. About 80 registered pesticides having 170 different trade names are commonly used in agriculture and public sectors in Bangladesh (Sattar, 1993). The indiscriminate use of agricultural pesticides has created very serious health and environmental problems in many developing countries. Worldwide, one to five million farm workers are estimated to suffer pesticide poisoning every year. Most of the poisonings take place in rural areas of developing countries, where safeguards typically are inadequate or lacking altogether. Although developing countries use 25% of the world's production of pesticides, they experience 99% of the deaths due to pesticide poisoning (WHO, 2004). So, the levels of pesticide residues in various samples should be monitored routinely, and effective measures must be adopted to control the use of pesticides to minimize human health hazards. Various agencies of United Nations and their established networks are extending co-operation, collaboration expert guidance in devising practical steps for the controlled use of pesticides to minimizing the undesirable levels of residues in food chains and untoward effects on target organisms in the environment (Ambrush, 1997). Therefore, the purpose of pesticide residues monitoring program is to know the pesticide residue level remain in the harvested crops and in the environment. A number of analytical methods are used to determine multiple pesticide residues for fruits and vegetables (Anastassiades *et al*., 2003; Prodhan *et al*., 2016; Prodhan *et al*., 2016a; Prodhan *et al*., 2015; Prodhan *et al*., 2015a; Schenck *et al*., 2008; Singh *et al.* 2012; Dasika *et al.*, 2012; Lehotay, 2010). Different extraction and clean-up methods are used for different food matrices; among them QuEChERS extraction techniques are widely used in the food testing laboratories. In 2003, the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method for pesticide residue analysis was introduced (Anastassiades *et al.*, 2003); it provides high-quality results in a fast, easy, inexpensive approach. Follow-up studies have further validated the method for more than 200 pesticides (Lehotay *et al.*, 2005), improved results for the remaining few problematic analytes (Lehotay *et al.*, 2005a), and tested it in fat-containing matrices (Lehotay *et al.*, 2005b). Therefore QuEChERS extraction techniques along with Gas Chromatography were used in this study to determine selected pesticides in country bean and yard long bean. Considering above factors, the present study was undertaken with the following objectives:

Objectives of Research Work

- To identify pesticide residues in country bean and yard long bean collected from different local markets of Bogura district.
- To quantify the level of pesticide residues in country bean and yard long bean collected from different local markets of Bogura district, and
- To compare the level of pesticide residues in the selected vegetables with the MRL of respective pesticide recommended by the EU-MRL.

CHAPTER II

REVIEW OF LITERATURE

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

2.1 Pesticides

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (EPA, 2006).The term pesticide includes herbicide, insecticide, insect growth regulator, nematicide, termiticide, molluscicide, piscicide, avicide, rodenticide, predacide, bactericide, insect repellent, animal repellent, antimicrobial, fungicide, disinfectant (antimicrobial), and sanitizer (Randall *et al*., 2013).

According to the Food and Agriculture Organization, a pesticide is any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal diseases, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies. The term includes substances intended for use as plant growth regulators, defoliants, desiccants, or agents for thinning fruit or preventing the premature fall of fruits. It is also used for all substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport (FAOSTAT, 2002). Due to their wide use in agriculture, pesticides are the most investigated priority pollutants in agricultural products (Garrido Frenich *et al*., 2008).

2.2 Classification of Pesticides

2.2.1 Organophosphate Pesticides

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

2.2.2 Organocarbamate Pesticides

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

2.2.3 Organochlorine Pesticides

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

2.2.4 Pyrethroid Pesticides

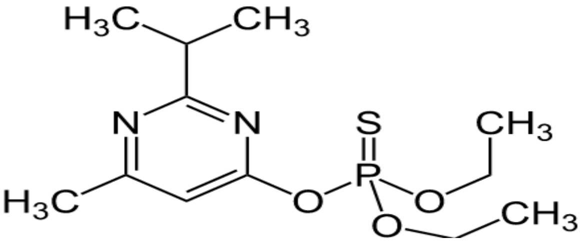
They were developed as a synthetic version of the naturally occurring pesticide pyrethrin, which is found in *chrysanthemums*. They have been modified to increase their stability in the environment. Some synthetic pyrethroids are toxic to the nervous system.

2.2.5 Neonicotinoid Pesticides

Neonicotinoids area class of neuroactive insecticides chemically similar to nicotine. In the late 1990s neonicotinoids came under increasing scrutiny over their environmental impact and were linked in a range of studies to adverse ecological effects, including honey-bee colony collapse disorder (CCD) and loss of birds due to a reduction in insect populations. In 2013, the European Union and a few non EU countries restricted the use of certain neonicotinoids (Cressey, 2013).Imidacloprid, of the neonicotanoid family, is the most widely used insecticide in the world (Yamamoto and Izuru, 1999).

2.3 Description of the Selected Pesticides

2.3.1 Diazinon



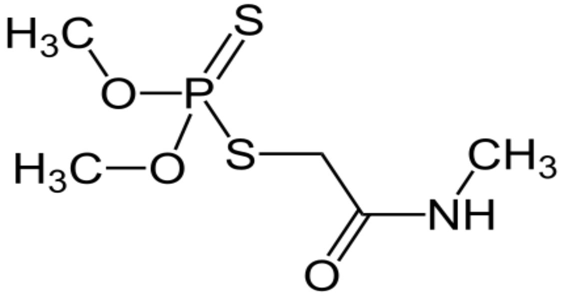
Structural formula of Diazinon

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

Diazinon is a contact insecticide which kills insects by altering normal neurotransmission within the nervous system of the insect. As mentioned above, diazinon inhibits the enzyme acetylcholinesterase (AChE), which hydrolyzes the neurotransmitter acetylcholine (ACh) in cholinergic synapses and neuromuscular junctions. This results in abnormal accumulation of ACh within the nervous system. Diazinon, although a thiophosphoric ester, shares a common mechanism of toxicity with other organophosphate insecticides such as chlorpyrifos, malathion and parathion, and is not very effective against the organophosphate-resistant insect populations. Symptoms of acute diazinon exposure develop in minutes to hours following exposure, depending of the exposure pathway. The initial symptoms of humans are nausea, dizziness, salivation, headache, sweating, lacrimation, and rhinorrhea. The symptoms can progress to vomiting, abdominal cramps, diarrhea, muscle twitching, weakness, tremor, a lack of coordination and miosis. Intermediate syndrome generally occurs within 24–96 hours after exposure. Intermediate syndrome in humans is characterized by difficulty breathing and muscular weakness, often in the face, neck and proximal limb muscles. Cranial nerve palsies and depressed tendon reflexes have also been reported.

2.3.2. Dimethoate

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

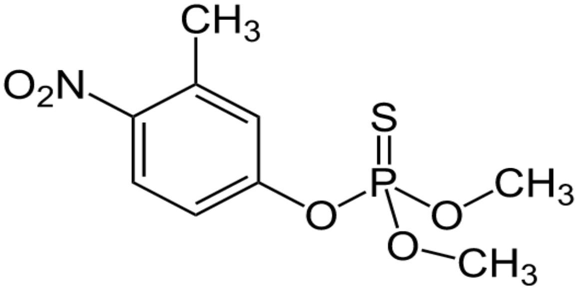


Structural formula of Dimethoate

The CAS No. of dimethoatc is 60-51-5 and the IUPAC name is O, O-dimethyl. 9-[2-(rnethvlamino)-2-oxoethyl] dithiophosphate (EPA 2006). Dimethoate is a colorless crystalline solid with a camphor-like (mercapian) odor (Worihing, 1987). It will breakdown rapidly when heated to temperatures above 80° C. creating the possibility of explosion. It should never he heated above 35 degrees C. Thermal decomposition may release hazardous ancitoxic fumes of dimethylsullide, methyl mercaptane, carbon monoxide, carbon dioxide, phosphorus penroxide and nitrogeiioxides (Meister, 1992). Dimethoate is possibly carcinogenic (Hayes, 1982;

Hallenbeck and Cunningham, 1985). An increase in malkznani tumors was reported in rats given oral closes of 'S. IS or 30 mg/kg dimethoate for 511 to 627 clays (Hayes and Laws, 1990). Dimethoate is available in aerosol spray, dust, emulsifiable concentrate and ULV concentrate formulations (Hayes and Laws, 1990: Meister, 1992).

2.3.3 Fenitrothion

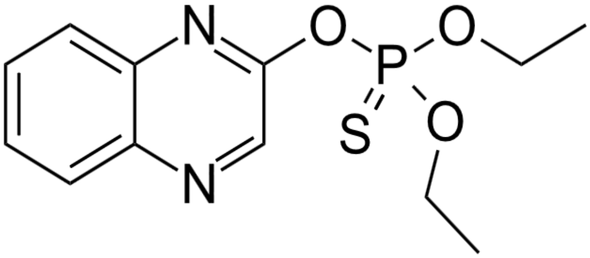


Structural formula of Fenitrothion

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

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

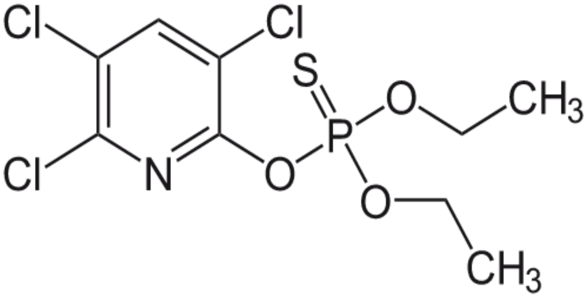
2.3.4 Quinalphos



Structural formula of Quinalphos

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

2.3.5 Chlorpyrifos



Structural formula of Chlorpyrifos

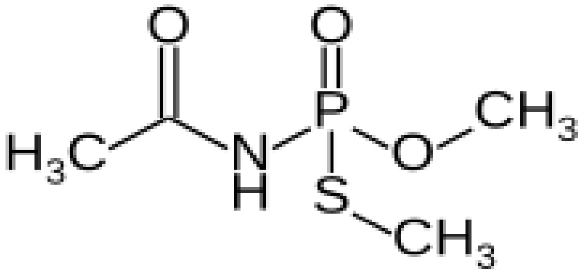
Chlorpyrifos (IUPAC name: O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) is a crystalline organophosphate insecticide, acaricide and miticide (EPA 2006). It was introduced in 1965 by Dow Chemical Company and is known by many trade names, including Dursban. It acts on the nervous system of insects by inhibiting acetyl cholinesterase. Chlorpyrifos is moderately toxic to humans, and exposure has been linked to neurological effects, persistent developmental disorders and autoimmune disorders. Exposure during pregnancy retards the mental development of children, and most home use was banned in 2001 in the U.S. In agriculture, it is "one of the most widely used organophosphate insecticides" in the United States, according to the United States Environmental Protection Agency (EPA), and before being phased out for residential use was one of the most used residential insecticides.

In case of target organisms, chlorpyrifos is a broad-spectrum insecticide which kills insects upon contact by affecting the normal function of the nervous system. Chlorpyrifos affects the nervous system by inhibiting the breakdown of acetylcholine (ACh), a neurotransmitter (Smegal, 2000). When insects are exposed, chlorpyrifos binds to the active site of the cholinesterase (ChE) enzyme, which prevents breakdown of ACh in the synaptic cleft. The resulting accumulation of ACh in the synaptic cleft causes overstimulation of the neuronal cells, which leads to neurotoxicity and eventually death. (Karanth and Pope, 2000; Toxicological Profile for Chlorpyrifos, 1997) Chlorpyrifos shares a common mechanism of toxicity with other organophosphate insecticides such as malathion and parathion, thus, chlorpyrifos would not be effective against organophosphate-resistant insect populations. In case of non-target organisms, the mode of action of chlorpyrifos is similar for target and non-target organisms (Reigart and Roberts, 1999). Acetylcholine is found throughout the mammalian nervous system, including at cholinergic synapses in the central nervous system, the junction of post-ganglionic parasympathetic neurons in exocrine glands and smooth and cardiac muscles, at pre- and post-ganglionic neurons in the autonomic nervous system, at neuromuscular junctions of the somatic nervous system, and on the surface of red blood cells (Reigart and Roberts, 1999; Blodgett, 2006). Chlorpyrifos affects ChE levels differently in various systems throughout the body. Scientists have observed plasma and red blood cell ChE inhibition in experimental animals at doses lower than those required to cause ChE inhibition in the brain (Smegal, 2000). The physiological functions of the neuropathy target esterase (NTE) enzyme were studied in genetically altered mice, which lacked the NTE enzyme. The results demonstrated that NTE plays an essential role in placental development, blood vessel development and protein synthesis in the central nervous system. (Lotti and Moretto, 2005) Chlorpyrifos can inhibit NTE by binding to the active site of the enzyme. Inhibition of the NTE enzyme results in loss of myelin and degeneration of axon fibers of the peripheral and central nerves (Reigart and Roberts, 1999; Blodgett, 2006). Chlorpyrifos can cause permanent inhibition of the ChE or NTE enzymes, a process known as aging. Cleavage of an alkyl group from the chlorpyrifos residue produces a negative charge at the active site of the enzyme.

This causes an unbreakable bond to form between the phosphorous atom on chlorpyrifos and the active site of the ChE or NTE enzyme (Blodgett, 2006; Lotti and Moretto, 2005). Chlorpyrifos also interacts with other enzymes, such as carboxylesterases and A-esterases. The functional role of these enzymes is not well understood, although they occur in many mammalian systems.

2.3.6 Acephate

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



Structural formula of Acephate

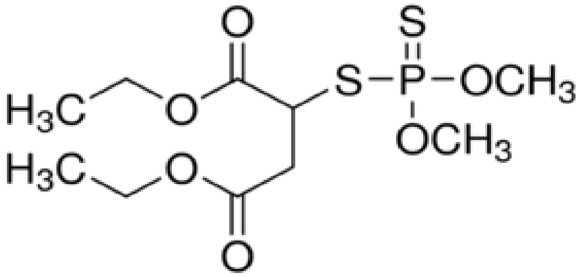
IUPAC name of acephate is O, S-Dimethyl acetylphosphoramidothioate2, and the Chemical Abstracts Service (CAS) registry number is 30560-19-1. Molecular weightis 183.16 g/mol and the solubility (water) is 79 - 83.5 g/100 ml (Davy *et al.*, 2007). In soil, plants, and insects, acephate is converted to methamidophos.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

Acephate is actually a systemic insecticide specially used to control sucking and biting insects by direct contact or ingestion (Tomlin, 2006; Thomson, 1989). Like other organophosphates acephate also bind to and inhibit the enzyme acetylcholinesterase (AChE) in nervous system tissues. Therefore, the neurotransmitter acetylcholine accumulates and constantly activates cholinergic receptors (Klaassen, 2001; Reigart and Robert, 1999). Acephate itself is a weak acetylcholinesterase inhibitor (Chuck *et al.*, 1984). Methamidophos is a more potent organophosphate than acephate (Reigart and Robert, 1999). Insects metabolize acephate into methamidophos by hydrolysis, whereas mammals metabolize acephate more readily into des-O-methylacephate, accounting for acephate's comparatively high selectivity against insects (Farag *et al.*, 2000; Mahajna *et al.*, 1997).

2.3.7. Malathion

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

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



Structural formula of Malathion

Malathion is a broad-spectrum insecticide used to control a variety of outdoor insects in both agricultural and residential sectors. Malathion is registered for use on food, feed, and ornamental crops and for household use mosquito, boll weevil and fruit fly eradication programs (EPA, 2006). Malathion is also an ingredient in shampoos regulated by the United States Food and Drug Administration (FDA) to control head lice (EPA, 2006). Malathion is toxic via skin contact, ingestion, and inhalation exposure (Tomlin, 2006). The International Agency for Research on Cancer (IARC) concluded in 1987 that the carcinogenic potential of malathion was not classifiable, and placed it in Group 3 (IARC, 1998).

2.4 Instrument Used for Pesticide Residues Determination

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

2.4.1 Liquid Chromatography-Mass Spectrometry

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

2.4.2 Gas Chromatography-Mass Spectrometry

In GC-MS, pesticides were identified by retention time and specific ions determined by selected ion monitoring (SIM) mode using the target and qualified ions. SIM mode provides adequate quantification at low levels as required for monitoring purposes but confidence in confirmation of identity is reduced if the selected ions are affected by matrix effect. Besides using the MS/MS it is possible to decrease the matrix effects, may achieve a higher selectivity levels and lower detection limit (Hercegová *et al.*, 2007**;** Patel *et al.*, 2005). GC-MS/MS with triple quadrupole (Patel *et al.*, 2005, Garrido Frenich *et al.*, 2006) and ion trap mass spectrometers (Wang *et al.*, 2005) has been used for pesticide residue analysis on fatty food. Both acquisition mode, multiple reaction monitoring (MRM) (Patel *et al.*, 2005) and the selected reaction monitoring (SRM) (Garrido Frenich *et al.*, 2006) mode have been used to analyze multiple pesticide residues from food matrices. Using the MS/MS may overcome the problems arising from the chromatographic interference that occurred with GC-ECD (Garrido Frenich *et al.*, 2003). Several single and multiresidue methods using GC-MS have been developed for the analysis of pesticides from different classes (Akhlaghi *et al.*, 2013; Latif *et al.*, 2011; EL-Saeid and Selim 2013; Chauhan *et al.*, 2012; Hadian *et al.*, 2008; Chandra *et al.* 2010; Paramasivam and Chandrasekaran, 2012; Vidal *et al.*, 2004; Kabir *et al.*, 2007).

2.4.3 Gas Chromatography

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

2.4.4 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) has been used for manufacturing (eg. during the production process of pharmaceutical and biological products), legal (eg. detecting performance enhancement drugs in urine), research (eg. separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (eg. detecting vitamin D levels in blood serum) purposes. Now a day, HPLC are mostly used for the purity analysis of pesticides. But still it is also used for single pesticide residue analysis of different food matrices (Panhwar & Sheikh, 2013; Paranthaman *et al.,* 2012).

2.5 Extraction and Clean-up

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

2.5.1 Supercritical Fluid Extraction (SFE)

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

2.5.2 Solid Phase Extraction (SPE)

SPE is one of the most commonly used sorbent techniques in analyzing pesticide residues. This method is based on the omission of extracts containing target analytes through a column filled with the appropriate sorbent (which was previously conditioned by an appropriate solvent or solvent mixture), or passing of an appropriate solvent through the SPE column to which a suitable amount of sample was previously added. It is easy to operate, costs less, it has been automated and uses small amounts of solvent. SPE is the multifunctional techniques, since the purification and the concentration occur in the same step. Unfortunately, SPE has certain limitations, primarily related to lower yields (recovery), i.e. slightly lower sensitivity, in situations where there is "clogging" of the SPE column (blocking of the sorption centers by solid and oily components originating from the sample). The most commonly used SPE sorbents in pesticide residues determination are: reversephase octadecyl (C_{18}), normal-phase aminopropyl ($-NH_2$) and primary-secondary amine (PSA), anion-exchanger three-methyl ammonium (SAX) and adsorbents such as graphitized carbon black (GCB). Normal-phase sorbents such as florisil ($MgSiO_3$), aluminum oxide (Al_2O_3) and silica (SiO_2) are usually used in combination with the previously mentioned sorbents. The SPE cartridge should be chosen depending on the physicochemical properties of pesticides that are searched for in a particular sample, and the nature of the sample matrix.

2.5.3 Accelerated Solvent Extraction (ASE)

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

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

Despite mentioned disadvantages related to conventional solvent extraction (SE) methods, they are still the most popular methods for routine analysis. To overcome the SE drawbacks, new trends in pesticide residues analysis have appeared. A good example of this is the QuEChERS method (Anastassiades *et al*., 2003). The authors questioned the conditions previously used for pesticide residues analysis, and through extensive experiments and novel use of $MgSO_4$ for salting out extraction/partitioning and dispersive solid-phase extraction (d-SPE) for cleanup, they devised a highly streamlined sample preparation method with excellent results for a wide range of pesticides in many types of samples. The original procedure consists in the sample extraction by hand-shaking or vortex mixing with the 10 mL of acetonitrile (MeCN). Gram quantities of salts (4 g of $MgSO_4$ and 1 g of NaCl) are then added to the sample by mixing, to drive analytes partitioning between the aqueous residue and the solvent. After vortex mixing and centrifugation, clean-up and removal of residual water is performed using a d-SPE procedure (PSA adsorbent and anhydrous $MgSO_4$ are mixed with the sample extract), that requires less time than the traditional SPE and simultaneously removes residual water and many polar matrix components, such as organic acids, some polar pigments, and sugars. As a polar solvent, miscible with water, with sufficient dispersive (hydrophobic) properties to extract effectively both polar and non-polar pesticides, MeCN is chosen as the QuEChERS solvent. Use of this solvent in the QuEChERS method proved to be successful for extraction of several pesticides classes from different matrices (Anastassiades *et al*., 2003; Asensio-Ramos *et al.,* 2010; Drozdzyński and Kowalska, 2009; Lehotay *et al.,* 2005, 2005a, 2005b; Paramasivam & chandrasekaran, 2012; Rashid *et al.*, 2010; Shi *et al.*, 2010; Yang *et al*., 2010, Prodhan *et al*., 2016; Prodhan *et al*., 2016a; Prodhan *et al*., 2015; Prodhan *et al*., 2015a;). Studies showed that some pesticides gave lower recoveries depending on pH of the matrix (Anastassiades *et al.*, 2007; Lehotay *et al.*, 2005, 2005a). Anastassiades *et al*. (2007) realized that buffering at pH=5 during extraction gave the optimum balance to achieve acceptably recoveries (>70%) for pH-dependent pesticides, independent of the matrix. On the other hand, Lehotay (2007) modified the method to use even stronger acetate buffering conditions. Both versions of methods went through extensive laboratory trials and successfully met statistical criteria for acceptability by independent scientific standards organizations. So the acetate-buffering version becomes AOAC Official Method 2007.01 (Lehotay, 2007) and the citratebuffering version being named as Standard EN 15662 Method (www.cen.eu). There is an abundance of the QuEChERS applications for pesticides determination in different plant samples. Thus, for example, QuEChERS provides satisfactory results for determination of 229 pesticides in lettuce and orange (Lehotay *et al.*, 2005), 109 in rice (Thanh *et al.*, 2007), 160 in tomato, pear and orange (Kmellár *et al.*, 2008), 140 in cucumber and orange (Fernández Moreno *et al.*, 2008), 118 in vegetables juice (Nguyen *et al.*,2009), 138 in apples, bananas, pears, apple juice, peas, creamed corn, squash and carrots (Wang and Leung, 2009), 150 in tomato, strawberry, potato, orange, and lettuce (Koesukwiwat *et al.*, 2010), 300 in tomato, apple, lettuce, cucumber, carrot, mushroom, grapes, lemon, pepper, pear, potato and cabbage (Kmellár *et al.*, 2010), 69 in zucchini, melon, cucumber, tomato, garlic, lettuce and pepper (Camino-Sánchez et al., 2010), 46 in onion, spinach, potato, carrot, cucumber, cabbage and tomato, 150 in grapes (Afify *et al.*, 2010), 148 in onion, spinach, potato, carrot, peas and tomato (Wang *et al.*, 2010), 73 OPPs and carbamates in rice, tree nuts and citric fruits (Chung and Chan, 2010) and 14 OCPs in apricot, plum, cherry, nectarine, pear and apple (Cieślik *et al.*, 2011), 13 in eggplant (Prodhan *et al.*, 2015), 10 in melon (Prodhan *et al.*, 2015a), 7 in cabbage (Prodhan *et al.*, 2016) and 7 in cauliflower (Prodhan *et al.*, 2016a). Besides, QuEChERS has been successfully used for determination of metaflumizone (Dong *et al.*, 2009), azadyrachtin, spinosad, rotenone (Drozdzyński and Kowalska, 2009), oxadiargyl (Shi *et al.*, 2010) and 38 pesticides (Yang *et al.*, 2010) in soil samples. As a modified version, it was applied for OCPs (Rashid *et al.*, 2010) and OPPs determination in soil samples (Asensio-Ramos *et al.*, 2010). The QuEChERS advantages are the high recovery, accurate results, low solvent and glassware usage, less labor and bench space, lower reagent costs, and ruggedness. The main QuEChERS disadvantage is that the final extract must be concentrated to furnish the necessary sensitivity i.e. to achieve the desired limits of quantification (LOQ).

2.7. Pesticide Residue

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

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

2.7.1. Acceptable Daily Intake or ADI

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

2.7.2. Maximum Residue Level (MRL)

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

2.7.3 Determination of Pesticide Residues in Food

To up-date our knowledge regarding the current status of research and information on the insecticide residues remain in farm gate and market samples of country bean, the level of detected insecticides residues above the Maximum Residue Limit (MRL) or not etc., at home and abroad an effort has been made to review the available literatures. Although the review could not be made so comprehensive due to limited scope and facility, it is hoped that most of the relevant information available in and around Bangladesh was collected and reviewed. It is revealed that most of the information on the aspects searched as mentioned above are mostly available fromresearch station and information of farmers’ field condition are scanty. However, asignificant number of study-reports on insecticides residues in vegetable cropsconducted under farmers’ field conditions are available. The studies on thequantification of detected insecticides residues below or above the Maximum Residue Limit (MRL) of country bean in

Bangladesh are rarely reported. With this background, the information collected from different sources have been reviewed and presented below:

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

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

Prodhan *et al.* (2018b) conducted a study to quantify the residue loss of Quinalphos, Diazinon and Fenitrothion from eggplant and Malathion from Yard long bean through washing and cooking procedures. Samples were collected from the Research field of Entomology Division of Bangladesh Agricultural Research Institute (BARI). The samples were analyzed using a simple Gas Chromatographic technique. Washing with water reduced 34% Quinalphos, 28% Diazinon and 41% Fenitrothion and heating with water at 100 ^{0}C reduced 95% Quinalphos, 84% Diazinon and 100% Fenitrothion from eggplant. Washing with water reduced 45% Malathion and heating with water at 100 ^{0}C reduced 100% Malathion from Yard long bean. Effect of O_3 sterilizer in reducing pesticide residues from eggplant was also investigated in this study and found that O_3 sterilizer reduced 79.00% Diazinon and 62.50% Quinalphos while washing with only water reduced 60.50% Diazinon and 40.00% Quinalphos from eggplant.

Prodhan *et al.* (2018c) has been developed and validated a simple and efficient multiple organochlorine pesticide residues analytical method using quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction technique and Gas Chromatography coupled with Electron Capture Detector (ECD) for the determination of 19 organochlorine pesticides (Alpha BHC, Delta BHC, Beta BHC, Gama BHC, Heptachlor, Aldrin, Heptachlor Epoxide, Gama Chlordane, Alpha Chlordane, Alpha Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, Beta Endosulfan, 4,4 DDT, Endrin Aldehyde, Endosulfan sulphate, Methoxychlor, and Endrin Ketone) in shrimp. The method was validated by evaluating the accuracy, precision and linearity limit of detection (LOD) and limit of quantification (LOQ). The average recoveries of the selected pesticides ranged from 84% to 106% with RSDr $\leq$ 14% in four fortification levels of 0.05, 0.1, 0.2 and 0.3 mg/Kg. The linearity was $\geq$0.996 for all of the selected pesticides with matrix matched calibration standards. The LOD ranged from 0.003 to 0.009 mg/Kg and the LOQ was 0.05 mg/Kg. This method was applied successfully for the residue analysis of 40 shrimp samples collected from different market places in Bangladesh.

Lawal *et al.* (2018) were used modified QuEChERS dispersive solid phase extraction coupled with ionic liquid-based dispersive liquid–liquid microextraction for the determination of multi-pesticide residues in fruit and vegetable samples. The analysed samples were jackfruit, strawberries, cucumber, pears, and carrots. The resulting linearity range (5–400 μg/kg) and regression coefficient (>0.99) results were satisfactory. The 94.2 and 95.8% accuracy (89–

138%) and precision (0–25%) results were satisfactory and within the recommended ranges (≤20%) and (70–120%), respectively. The limits of detection (0.01–0.54 µg/kg) and quantitation (0.03–1.79 µg/kg) were excellent. The matrix effects (≤−87%) for all analysed samples were not significant. The estimated measurement uncertainties (≤27%) were within the acceptable range (≤50%). Justifiably, the response surface methodology optimized instrument and sample treatment techniques were reliable and convenient for multi-pesticide residue determination in various fruits and vegetables.

Nimsha *et al*. (2018) were determined Pesticide residues in locally grown vegetables tomato, cabbage and capsicum in Puttalum, Dambulla and Nuwara Eliya districts in Sri Lanka by Gas Chromatography with Mass Spectrometry after multi residue extraction procedure (QuEChERS method). The QuEChERS method was validated using five pesticides named Diazinon, Chlopyrifos, Fipronil, Prothiofos and Tebuconazole and their retention times in minutes were15.948, 19.566, 20.342, 22.308, and 26.201 respectively. Coefficient of detection was obtained near 0.99 for all tested standard pesticides confirming the accuracy of the test method. Out of 45 vegetables samples, 15 samples were detected with pesticide residues, either Chlopyrifos, Prothiofos or Tebuconazole. However, pesticide residual values were less than Maximum Residual Levels for all the tested pesticides. Tebuconazole was the mostly detected pesticide residue with 0.128 ppm and 0.052 ppm in tomato and cabbage collected from Matale and Puttlum districts respectively.

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

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

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

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

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

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

Hossain *et al.* (2015) collected and analyzed 10 Brinjal samples from Bogura district and found that one Brinjal sample was contaminated with Diazinon and the level of detected Diazinon was 0.32 mg/Kg. Diazinon was also detected in one Cucumber sample CS-5 (0.18 ppm) among the ten Cucumber samples and one Tomato sample (TS-3 at 0.57 ppm) among five Tomato samples. Chlorpyriphos was being found in one Bringal sample (BS-7 at 0.4 ppm). It was also detected in three Cucumber samples among ten Cucumber samples. The IDs of Chlorpyriphos positive samples were CS-2 (0.05 ppm), CS-7 (0.02 ppm).

Prodhan *et al.* (2015) conducted a research on ‘‘Determination of Multiple Pesticide Residue in Eggplant with Liquid Chromatography-Mass Spectrometry’’ where a simple and efficient multiple pesticide residue analytical method using quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction and liquid chromatography triple quadrupole-mass spectrometry was developed and validated for the determination of ten insecticides and three fungicides in eggplant. The method was validated by evaluating the accuracy, precision, linearity, limit of detection, and limit of quantification. They evaluated matrix effect and it was found that thiamethoxam, cypermethrin, and deltamethrin had pronounced matrix effect (-69, +57, and +93 %, respectively). They applied this method for the residue analysis of 72 fresh eggplant fruit samples 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.* (2015a), where they use a rapid, precise and efficient method for the determination of seven insecticides (chlorpyrifos, dimethoate, deltamethrin, thiamethoxam, thiacloprid, pirimicarb and indoxacarb) and three fungicides (azoxystrobin, fluopicolide and propamocarb hydrochloride) in melon by employing quick, easy, cheap, effective, rugged and safe extraction method coupled with liquid chromatography triple quadrupole mass spectrometry which was developed and validated by evaluating the accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ). They evaluated matrix effect and it was found considerable for thiamethoxam and deltamethrin (-53% and +135%, respectively). Finally, this method was applied for the residue analysis of 122 fresh melon samples collected from different market places in Thessaloniki, Greece. Among the 122 analysed samples, 32 (26% of the total no. of samples) were found to have pesticide residues. None of the samples contained residues above the European Union Maximum Residue Levels. The most frequently detected pesticides were fluopicolide and thiamethoxam.

Satpathy *et al.* (2014) conducted a research on the ‘‘Development and Validation of Multi-residue Analysis of 82 Pesticides in Grapes and Pomegranate as per the Requirements of the European Union (EU) and Codex Alimentarius Using GCMS/MS with Compound Based Screening’’. They validated the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) multi-residue method for the extraction of 82 pesticides belonging to various chemical classes from grapes and pomegranate (commodities with high sugar and low lipid contents). They use a mixture of 82 pesticides amenable to gas chromatography (GC) was quantitatively recovered from spiked grapes and pomegranate and determined using gas chromatography tandem mass spectrometry (GC-MS/MS). The method they employed involved initial extraction in a water/ethyl acetate system, an extraction/partitioning step after the addition of salt, and a cleanup step utilizing dispersive solid-phase extraction (d-SPE); which ensured that it was a rapid, simple and cost-effective procedure. Their method setup was streamlined with the new software approach of Compound Based Scanning (CBS). They found that matrix-matched calibration results have demonstrated good reproducibility, robustness and linearity and spiking levels for the recovery experiments as 0.005, 0.01 and 0.1 mg/kg for GC-MS/MS analyses. They attained adequate pesticide quantification and identity confirmation, even at the lowest concentration levels, considering the high signal-to-noise ratios, the very good accuracies and precisions, as well as the good matches between the observed ion ratios. They found the mean recoveries mostly ranged between 70 and 110 % (91% on average), and RSD were generally below 12% (7.3% on average). The use of analyte Protestants during GC analysis was demonstrated to provide a good alternative to the use of matrix-matched standards to minimize matrix-effect related errors. For all compounds LODs were 0.001 to 0.005 mg/kg and LOQs were 0.005 to 0.020 mg/kg.

Islam *et al.* (2014) conducted a survey on the ‘‘Detection and quantification of pesticide residues in selected vegetables of Bangladesh’’ in intensive vegetable growing area in the Narsingdi district of Bangladesh regarding pesticides used by farmers on three major vegetables like eggplant, cauliflower, and country bean. On the basis of questionnaires, under their supervision 23 farmers were interviewed and they noted that fourteen pesticides belonging to different groups were found to be commonly used on the selected vegetables by the respondent farmers to control the major pests. They record 8.33 to 45.00 percent farmers in two selected locations of Narsinghdi to apply different pesticides every day and in some cases even twice in a day on vegetables. They collected 42 samples from fields and markets and operate multiple pesticide residue analysis by Gas Chromatography (GC) with Flame Thermionic 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.

Akan *et al*. (2013) carried out a research entitled ‘‘Organophosphorus pesticide residues in vegetables and soil samples from alau dam and gongulong agricultural areas, Borno State, Nigeria’’. They observed concentrations of organophosphorus pesticide residues (dichlorvos, diazinon, chlorpyrifos, and fenitrothion) in some vegetables (spinach, lettuce, cabbage, tomato and onion) in Borno State, Nigeria. They determined concentrations of all the pesticides in the vegetables using GC equipped with electron capture detector (ECD). The highest concentrations of diclorvos, diazinon, chlorpiryfos and fenithrothion in the Alau Dam and Gongulong agricultural areas were observed in the leaf of tomato, while the lowest concentrations were observed in the root of spinach. The concentrations of all the organophosphorus pesticides in the vegetables and soil samples from the two agricultural areas were observed to be at alarming levels, much higher than the maximum residue limits (MRLs) and set acceptable daily intake values (ADIs) for vegetables Cordex 2009.

Islam *et al.* (2013) carried out a research work entitled “Analysis of Pesticide Residue in Vegetables Collected from Local Market by Using GC Technique” where they detect and quantify the presence of pesticide residues in Cucumber, Spinach, and Brinjal available in local market of Mymensingh sadar upazila. They found that among the studied 9 samples, only 3 samples responded to two remarkable elusions. Mancozeb 64% + Symoxanil 8% residues occurred in only one Cucumber sample which was collected from seshmore BAU, the quantity of the Mancozeb 64% + Symoxanil 8% residue was about 50 ppm. On the other hand, out of 3 spinach samples, 1 of them showed presence of imidachloprid residues. But sample from BAU

Sesh More eluted a small area contained peak which was very minute level (less than 0.1 ppm). Cucumber sample from Seshmore, BAU showed a remarkable peak which was approximately 50 ppm level of Mancozeb 64% + Symoxanil 8% residue.

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

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

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

Corteas *et al.* (2013) developed a method for the determination in vegetables of organophosphorus pesticides. Pesticide residues are extracted from samples with asmall amount of ethyl acetate and anhydrous sodium sulfate. Analyses are performedby large volume

GC injection using the through oven transfer adsorption desorption (TOTAD) interface. The calculated limits of detection for each pesticide injecting 50µL 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, andtomato.

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

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

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

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

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

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

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

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

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

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

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

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

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

Rahman (2010) conducted a study on the multi insecticide residue analysis in environmental sample collected from different regions of Bangladesh. A total of 94 samples of vegetables (brinjal, hyacinth bean, cauliflower and yard long bean) were collected from farmers field and market of different regions like Barisal, Bogra, Chittagong, Comilla, Dhaka, Dinajpur, Gazipur, Jessore, Khagrachuri, Narsingdi, Rajshahi and Rangpur and carried out the Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur. Results revealed that among 94 samples, 46 had insecticide residues, of them 18 had above MRL. Among 38 analyzed brinjal samples, 18 samples contained residue of

Cypermethrin, Diazinon and Quinalphos; and only 8 had above MRL. Cypermethrin is the frequent one which was found in 14 samples. Of 24 analyzed hyacinth bean samples, 15 had the residue of Cypermethrin, Diazinon, Quinalphos, Fenotrothion and Malathion; and 7 had above MRL. Among the 16 analyzed cauliflower samples, 5 samples contained residue of Cypermethrin, Diazinon, Quinalphos and Malathion; and only 2 had above MRL. Of 16 yard long bean samples, 8 samples contained residue of Cypermethrin, Diazinon, Quinalphos and Malathion; and only 2 had above MRL.

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

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

Islam *et al.* (2009) analyzed pesticide residues in cauliflower by high performance liquid chromatography. They sprayed in cauliflower with 4 different pesticides (diazinon, malathion, chlorpyrifos and cypermethrin) at recommended dose and double of recommended dose. Calibration curves that constructed for the analytes spiked into samples followed linear relationships with good correlation coefficients (R^2>0.990). In the analysis, residual amounts of diazinone and chlorpyrifos were above respective maximum residue limit (MRL) values.

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

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

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

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

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

Kabir *et al.* (2008) studied the left over residue of Diazinon and Carbosulfan in brinjal and Quinalphos in yard long bean and made a comparison between the detected residue levels with maximum residue level (MRL) set by FAO 1970. Three supervised field trials (two for brinjal and one for yard long bean) were carried out sprayed with the field dose (1.5 ml/L of water) of Diazinon, Carbosulfan, and Quinaiphos. They collected sample daily after spraying. They found that in case of Diazinon, left over residue was up to 6 days after spray (DAS), and upto 3

DAS, the level of residue was above the MRL. They also detected Carbosulfan residue remainded till 7 DAS and the detected quantity of residue was above MRL up to 3 DAS. Left over residue of Quinalphos in yard long bean sample was detected upto 6 DAS and up to 4 DAS the level of residue was above the MRL.

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

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

Fernández-Cruz *et al.* (2006) carried out a research on the **"**Residue levels of captan and trichlorfon in field-treated kaki fruits, individual versus composite samples, and after household processing" where the dissipation of residue levels of captan and trichlorfon in field-treated kaki crops was studied according to good laboratory practices to propose maximum residue limits (MRLs). Residue levels of captan and trichlorfon were analyzed by GC/MS and LC-MS/MS, respectively. Residue levels of captan and trichlorfon permitted one to propose MRLs in kaki of 3 and 5 mg kg^{-1}, respectively. The behavior of these residues was also studied after peeling and cooking, and in individual fruits versus composite samples. Residue levels of these compounds for individual fruits suggested that a variability factor up to three could be set for the acute risk assessment. Levels of captan decreased by more than 90% after peeling and completely after cooking. Trichlorfon penetrates into the flesh in a proportion of 70% of the residue at the pre-harvest interval. Cooking resulted in a decrease of 27% of residue levels of trichlorfon.

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

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

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

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

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

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

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

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

Lehotay (2000) investigated 22 diverse pesticide residues in green bean and carrot extracts by bench top gas chromatography. The targeted pesticides which were incurred in the samples, included chlorpyrifos, azinphos-methyl, parathion-methyl, diazinon, terbufos, DDE, endosulfan sulfate, carbofuran, carbaryl, propargite, bifenthrin, dacthal, trifluralin, metalaxyl, pendimethalin, atrazine, piperonyl butoxide, diphenylamine, vinclozolin, chlorothalonil, quintozene, and tetrahydrophthelimide (the breakdown product of captan). Average recoveries of the pesticides were 103s 7% with relative standard deviations of 14 to 5% on 16 average.

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

Dogheim *et al.* (1999) monitored the residues of organophosphorous, dithiocarbamtes and few synthetic pyrethroid pesticides commonly used in Egyptian fruits and vegetables, in addition to those organochlorines pesticides which had been termed as persistent and prohibited to use on foodstuffs several years ago. From 8 local markets, total of 397 fruit and vegetable samples were collected and analyzed for 52 pesticides. Out of total 397 samples, there were 42.8% were found positive and contained residues at detectable levels, out of which 1.76% found above to the MRLs. Residues of organochlorine pesticides were not found in most of the samples. Cauliflower, guava and 16 onion samples were found free from any pesticide residues otherwise among all samples, 65 grape samples contained 11 pesticide residues, 22 samples of strawberry contained 10 pesticide residues and 62 samples of tomato contained 13 different pesticide residues. The most frequently detected pesticide was dithiocarbamtes. Out of 98 samples residues of dithiocarbamtes were found in 70.4% and only one sample of grape contained residues above to the MRLs. Samples ofeggplant and carrot were found contaminated by the trace amounts of residues of pp'-DDT and pp'-DDE pesticides.

Ahuja *et al.* (1998) carried a research to monitor insecticide residues in cauliflowers, cabbages, tomatoes, brinjal, okras, field beans and cucumbers for GCH and its isomers, Endosulfan, Dimethoate, Monocrotophos, Quinalphos, Fenvalerate, and Cypermethrin. The residues of alpha, beta, tau isomers of HCH, Endosulfan, monocrotophos, Quinalphos, Dimethoate were detected in most of the samples. However, the residues of Monocrotophos on tomatoes, brinjal and okras and those of Carbendazim on French beans were found to persist over the prescribed maximum residue limit values (MRLs).

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

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

However, the levels of pesticide residues were lower than the maximum residue limits (MRL) prescribed.

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

FAO/WHO (1993) reported that seventeen supervised trials from Switzerland and the USA using EC, WP and GR formulations. In the US trials a GR or EC formulations was used pre-planting at 4.4 kg a.i/ha followed by three or four foliar sprays at weekly intervals with WP or EC formulations at 0.56 kg a.i/ha. The Swiss trial was with granules at 8.7 kg a.1/ha at the 4-6 leaf stage. Residue of Diazinon in Radish root ranged from <0.01 to 0.08 mg/kg 7.33 days after the last application.

According to Singh and Kalra (1992) eggplant fruits, leaves and soil samples were collected at 0, 1, 2, 5 and 10 days after treatment to determine the Cypermethrin residues by Gas Chromatography. The analytical methodology used involved acetone and hexane extraction, silica gel column cleanup and 63 Ni gas liquid chromatographic estimation. The minimum limits of cis- Cypermethrin and trans-Cypermethrin were 0.008 and 0.006 mg/kg. Initial deposit of Cypermethrin on fruit in 1982 was observed 0.73 mg/kg after eighty sprays at 50g a.i./ha application rate, which declined to 0.61 mg/kg one day after treatment and then became 0.08 mg/kg after 10 days. At double the application rate of insecticides resulted in correspondingly increased levels of residues. During 1983 also almost simila rate of dissipation was observed. Half-life value on eggplant fruits varied between 2.2 and 2.9 days for Cypermethrin. Eggplant leaves when sprayed at low dosage received about 2 to 6 time’s higher level of insecticides than the fruits. Cypermethrin resulted in mean initial deposit of 1.69 and 1.16 mg/kg in 1982 and after eighth spray at 50g a.i./ha application rate, which dissipated by 68% after 10 days. At 100g a.i./ha dosage, the mean initial deposit was observed to be 3.18 and 2.98 mg/kg in 1982 and 1983 after eighth spray which declined to 0.68 mg/kg after 10 days of treatment. In soil, at the minimum effective dosage, the range of mean initial deposit was observed to be 0.28- 0.35 mg/kg in 1982 and 1983. Doubling the application rate resulted in higher recovery of insecticides. Half- life value of Cypermethrin in soil was 5.2 days. The dissipation of transisomer was found to be slightly faster than the cis- isomer in eggplant fruits and leaves. These isomers were lost almost at the same rate from soil.

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

Singh and Kalra (1989) stated that a study was carried out in Ludhiana, India to detemine the residues of Permethrin aplied for the control of brinjal shoot and fruit borer on fruit and leaves of eggplant and in the soil and to determine the waiting period. The compound was sprayed at a rate of 50g a.i/ha at fortnightly intervals. Initial deposits on fruits ranged from 1.3 to 0.7 mg/kg and MRL 1, 2, 3 and 10 days after spraying was 0.34, 0.2, 0.11 and 0.07 mg/kg respectively. The half-life of the insecticides on fruits ranged from 2.1 to 3.0 days. The mean initial deposit on eggplant leaves was 2.24 mg/kg; residues had dissipated by 70-80% after 10 days. Permethrin residue in soil after 8 sprays was 0.27- 0.28 mg/kg. The half- life value of Permethrin in the soil was about 3.2 days, the trans- isomers of Permethrin degradedslightly faster than the cis-isomers in leaves and fruits. A 1-day waiting period is recommended for consumption of fruits.

Devi and Manjunath (1985) found that Quinaiphos is a less persistent insecticide and dissipated on the fruits more quickly than Fenvalerate and Carbaryl. In the current study, the left over residue of Quinaiphos in the YLB sample had been detected upto 6 DAS, of which upto 4 DAS, the quantity of residue were above MRL. At the 0 DAS, the residue was 0.678 ppm and they were 0.552 ppm, 0.419 ppm, 0.306 ppm, and 0.214 ppm at 1 DAS, 2 DAS, 3 DAS, and 4

DAS, respectively. All these quantities were above MRL. The quantity decreased down to 0.164 ppm at 5 DAS and 0.087 ppm at 6 DAS. But Devi and Manjunath (1985) found the residue of Quinalphos (0.56 ppm) in brinjal above the tolerance level upto 10 DAS.

Awasthi *et al*. (1984) reported that residue levels of Phorate and Quinalphos were initially higher at soil depths of 0- 7.5cm and 15.0- 22.5cm than at 7.5- 15.0cm, but 60 days after application residue levels increased with soil depth as the insecticides move down the profile. Aldicarb and Carbofuran levels were highest at 0- 7.5cm depth throughout the 60 day experiment while Aldicarb was not detected below 15cm depth. Insecticides were taken up into foliage and fruits of okra, but were not detected after 45 (carbamates) or 50 (organophosphates) days.

Talekar *et al*. (1977) reported that the residues of DDT and Dieldrin in soil declined 20 and 25% in the fall and winter months and there was no accumulation of residue with further application of these chemicals during spring and summer. Two organophosphates (Fonofos and Phorate) and one carbamate (Carbofuran) degraded rapidly resulting in only 8, 0.4 and 32% recoveries respectively at the end of the fall and winter seasons. Their breakdown was further accelerated during the hot, rainy, spring and summer months when their recoveries were lower. When grown in treated silt loam soil, sweet potatoes and white potatoes absorbed the residues of these chemicals and their metabolites at different levels.

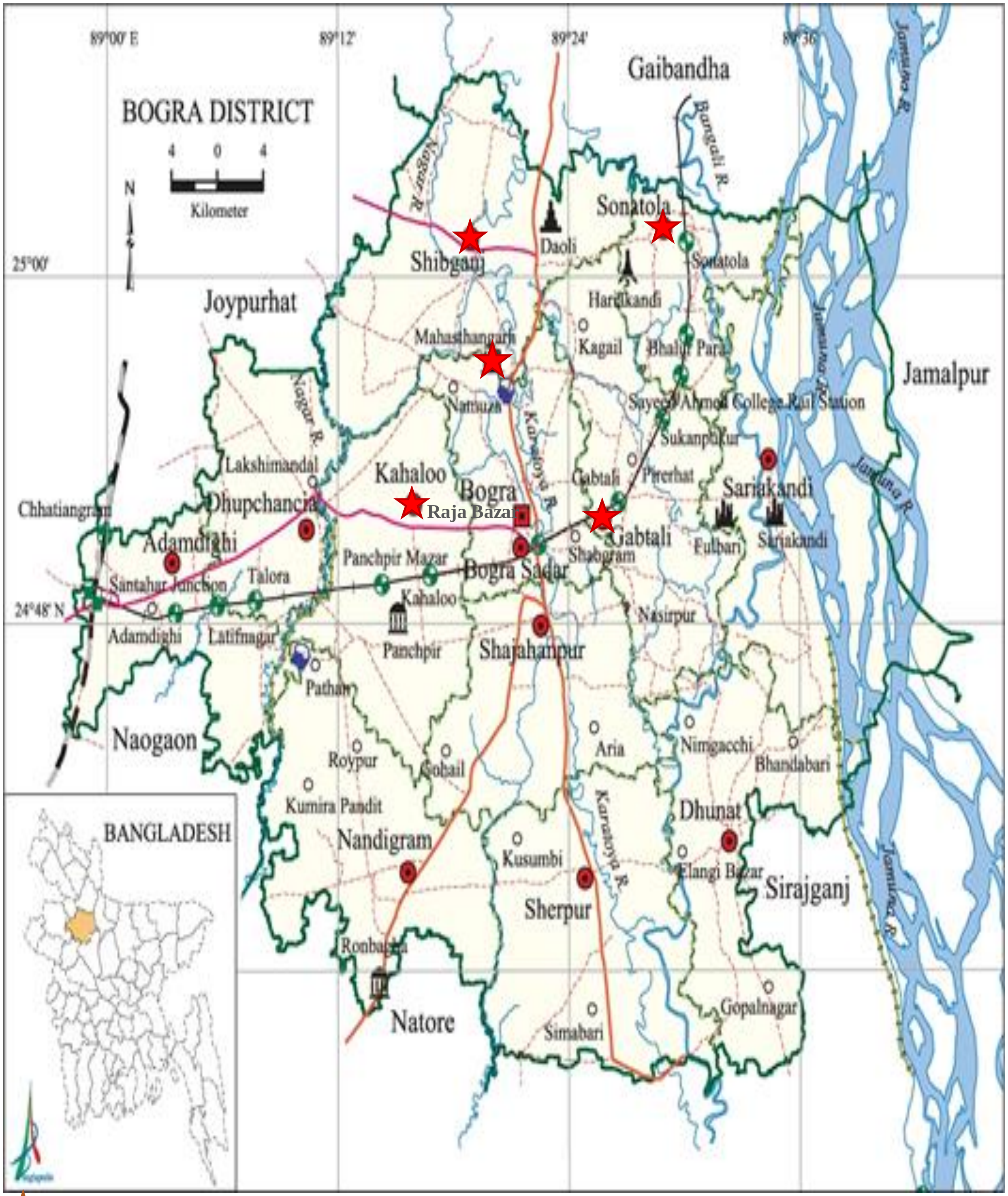
CHAPTER III

MATERIALS AND METHODS

The vegetable (country bean and yard long bean) samples were collected from 5 different markets of Bogura district and carried to the Pesticide Analytical Laboratory, Entomology Division, BARI, Joydebpur, Gazipur for pesticide residue analysis during November 2017 to February 2018. 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 Bogura district. The area of Bogura district is about 2898.68 km^2, located at 24.78° North latitude and 89.35° East longitude with an elevation of 4 meter from the sea level. In this study, vegetables were collected from Raja Bazaar, Gabtali Bazar, Sonatola Bazar, Mohasthangor Bazar and Shibganj Bazar in Bogura district.



★ Area of Sample Collection

Figure 1. Map showing the places of sample collection

3.2 Sample Collection

A total of 70 samples (35 country bean and 35 yard long bean) were collected for this study. Seven samples of country bean and seven samples of yard long bean were collected from each market. The amount of each sample was 1 Kg for all the selected vegetables. The samples were collected in clean transparent air tight polyethylene bag and each bag was properly labeled with sample number and sources. Sample was collected in individual polyethylene bag to avoid cross contamination.

Table 1: Sources and places of collection of country bean samples

Area of collection	Sample ID	Source
Raja Bazar	BORBe-1	Shibganj
	BORBe-2	Gabtali
	BORBe-3	Nandigram
	BORBe-4	Gabtali
	BORBe-5	Nandigram
	BORBe-6	Mohasthangor
	BORBe-7	Gabtali
Gabtali Bazar	BOGBe-1	Sukhanpukur
	BOGBe-2	Rameshwarpur
	BOGBe-3	Nepaltali
	BOGBe-4	Sukhanpukur
	BOGBe-5	Naruamala
	BOGBe-6	Gabtali
	BOGBe-7	Naruamala
Sonatola Bazar	BOSOBe-1	Mokamtola
	BOSOBe-2	Sukhanpukur
	BOSOBe-3	Mohasthangor
	BOSOBe-4	Sonatola
	BOSOBe-5	Baiguni
	BOSOBe-6	Mokamtola
	BOSOBe-7	Shibganj
Mohasthangor Bazar	BOMBe-1	Mohasthangor
	BOMBe-2	Shibganj
	BOMBe-3	Mohasthangor
	BOMBe-4	Mohasthangor
	BOMBe-5	Shibganj
	BOMBe-6	Mohasthangor
	BOMBe-7	Shibganj
Shibganj Bazar	BOSGBe-1	Shibganj
	BOSGBe-2	Mohasthangor
	BOSGBe-3	Mokamtola

	BOSGBe-4	Shibganj
	BOSGBe-5	Roynagar
	BOSGBe-6	Buriganj
	BOSGBe-7	Mohasthangor

Table 2: Sources and places of collection of yard long bean samples

Area of collection	Sample ID	Source
Raja Bazar	BORYLB-1	Shibganj
	BORYLB-2	Nandigram
	BORYLB-3	Sonatola
	BORYLB-4	Nandigram
	BORYLB-5	Gabtali
	BORYLB-6	Mohasthangor
	BORYLB-7	Subgram
Gabtali Bazar	BOGYLB-1	Sukhanpukur
	BOGYLB-2	Rameshwarpur
	BOGYLB-3	Naruamala
	BOGYLB-4	Gabtali
	BOGYLB-5	Sukhanpukur
	BOGYLB-6	Rameshwarpur
	BOGYLB-7	Gabtali
Sonatola Bazar	BOSOYLB-1	Shibganj
	BOSOYLB-2	Nandigram
	BOSOYLB-3	Sonatola
	BOSOYLB-4	Shibganj
	BOSOYLB-5	Sonatala
	BOSOYLB-6	Mohasthangor
	BOSOYLB-7	Shibganj
Mohasthangor Bazar	BOMYLB-1	Shibganj
	BOMYLB-2	Mohasthangor
	BOMYLB-3	Mohasthangor
	BOMYLB-4	Shibganj
	BOMYLB-5	Mohasthangor
	BOMYLB-6	Sonatola
	BOMYLB-7	Mohasthangor
Shibganj Bazar	BOSGYLB-1	Mokamtola
	BOSGYLB-2	Shibganj
	BOSGYLB-3	Mohasthangor
	BOSGYLB-4	Shibganj
	BOSGYLB-5	Sonatola
	BOSGYLB-6	Shibganj
	BOSGYLB-7	Mohasthangor

3.3 Sample Preparation for Analysis

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

3.4 Chemicals and Reagents

The standard of Chlorpyrifos, Acephate, Diazinon, Dimethoate, Quinalphos, Malathion and Fenitrothion were obtained from Sigma-Aldrich (St Louis, MO, USA) via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. Standards of all pesticides contained >99.6% purity. Methanol, acetone, gradient grade acetonitrile, sodium chloride (NaCl), anhydrous magnesium sulphate ($MgSO_4$) and Primary Secondary Amine (PSA) were purchased from Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh.

3.5 Analytical Apparatus Used

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

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

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

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

Plate 1. Electric Balance

Plate 2. Vortex Mixer

Plate 3. Centrifuge Machine

Plate 4. 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

Some pictorial view related to sample preparation

Plate 5: Chopping of Sample

Plate 6: Adding Acetonitrile

Plate 7. Weighing of Salt (NaCl and $MgSO_4$)

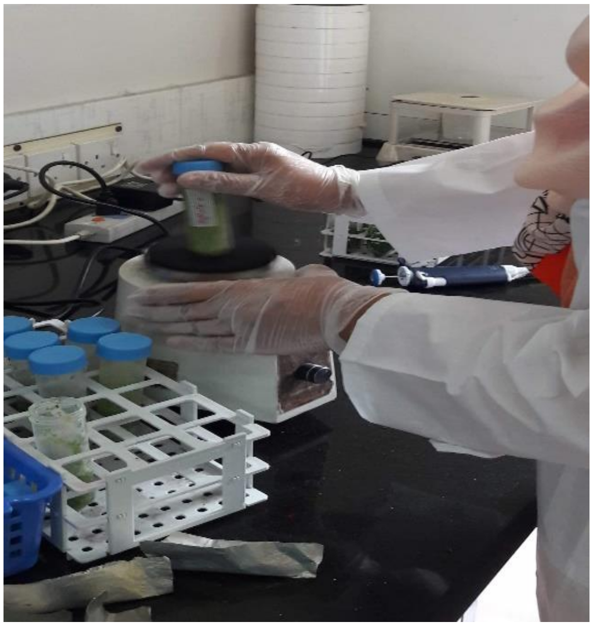
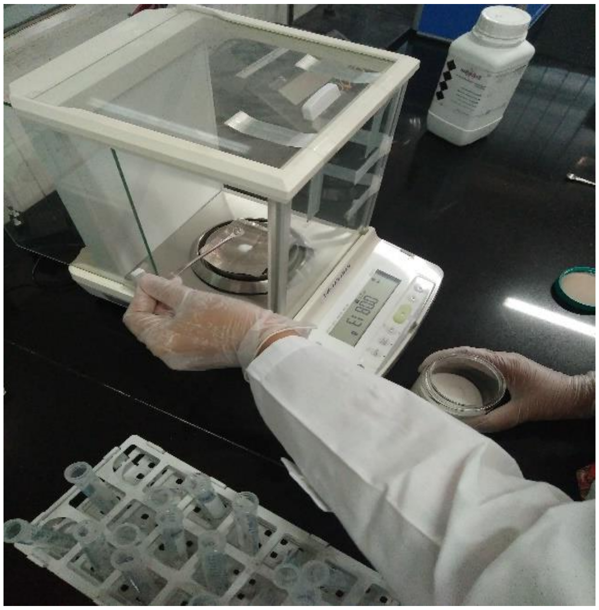


Plate 8: Shaking of Sample

Plate 9. Centrifuging the Sample



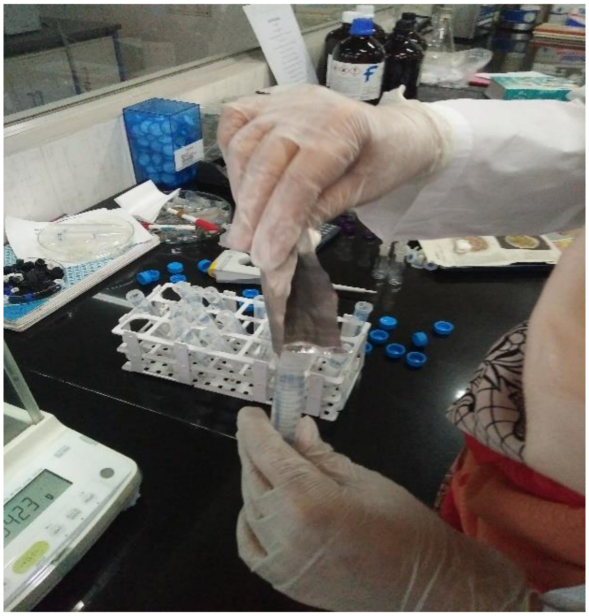


Plate 10. Weighing of PSA

Plate 11. Adding PSA in the Sample Extract

Plate 12: Filtration through PTFE Filter

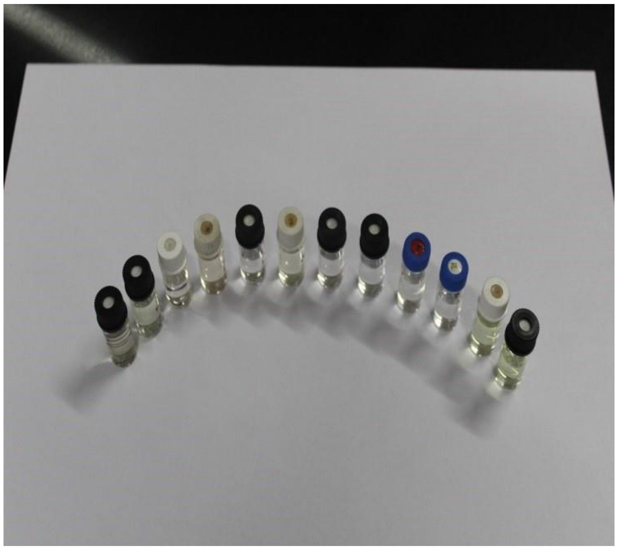


Plate 13. Sample Extract Ready for Injection

3.6 Preparation of Pesticide Standard Solution

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

3.7 Extraction and Clean up

QuEChERS extraction method is one of the latest extraction and clean up techniques for pesticide residue analysis in food matrices which is an anagram for Quick, Easy, Cheap, Effective, Rugged and Safe. This techniques was first introduced by Anastassiades *et al.* (2003), which is gaining popularity day by day compared to the other existing techniques such as Supercritical Fluid Extraction (SFE), Liquid-liquid extraction (LLE), Solid phase extraction (SPE), Solid phase micro extraction (SPME), Stir bar sorptive extraction (SBSE), and

Microwave assisted extraction (MAE). The technique uses a single extraction in acetonitrile and requires a very small amount of (10-15 gm) sample. A large excess of salts or buffers are added to extract to aid in the extraction of both polar and non-polar pesticide. This sample initial step simultaneously extract the pesticide from the samples and prepares it for the next dispersive solid phase extraction (d-SPE), the salts and SPE sorbents chosen for the d- SPE step serve to remove residual water and further remove matrix interference from the sample. The resulting acetonitrile extract is typically analyzed directly by gas chromatography (GC), gas chromatography-mass spectrometry (GC/MS) or liquid chromatography tandem mass spectrometry (LC/MS/MS) with proper dilution.

In this study, the QuEChERS extraction technique was used for the extraction and clean-up of samples which was modified by Prodhan *et al.* (2015). The chopped samples were grounded thoroughly with the fruit blender. A representative 10-g portion of thoroughly homogenized sample was weighted in a 50 mL polypropylene centrifuge tube. Then 10 mL of acetonitrile (MeCN) was added into the centrifuge tube. The centrifuge tube was closed properly and shaken vigorously for 30 s by the use of a vortex mixer. Then, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added into the centrifuge tube, and it was shaken immediately by the vortex mixer for 1 minute to prevent the formation of magnesium sulfate aggregates. Afterwards, the extract was centrifuged for 5 min at 5000 rpm. An aliquot of 3 mL of the MeCN layer was transferred into a 15 mL micro centrifuge tube containing 600 mg anhydrous $MgSO_4$ and 120 mg Primary Secondary Amine (PSA). Then it was thoroughly mixed by vortex for 30 s and centrifuged for 5 minutes at 4000 rpm. (Laboratory Centrifuges, Sigma-3K30, Germany). After centrifuge, a 1 mL supernatant was filtered by a 0.2 μm PTFE filter, and then it was taken in a clean GC vial for injection.

3.8 Detection and quantification of pesticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) for the detection of acephate, dimethoate, diazinon, fenitrothion, malathion, chlorpyrifos and quinalphos. The capillary column was AT-1 length was 30m, ID was 0.25mm and film thickness was 0.25μm. Helium was used as carrier and make up gas for FTD. The identification of suspected pesticide was performed by peak retention times in samples to those of peaks in the pure analytical standards.

The instrument conditions are described in Table 3 and Table 4.

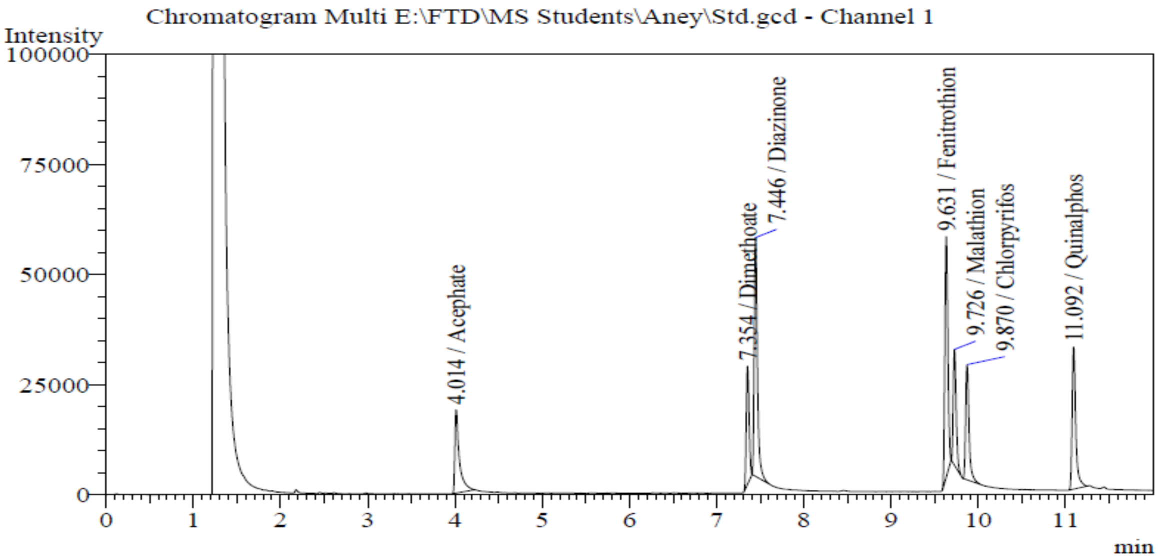


Figure 2: Typical chromatograms of seven organophosphorus insecticide standards run by GC-FTD.

Table 3: The instrument parameters for GC-FTD

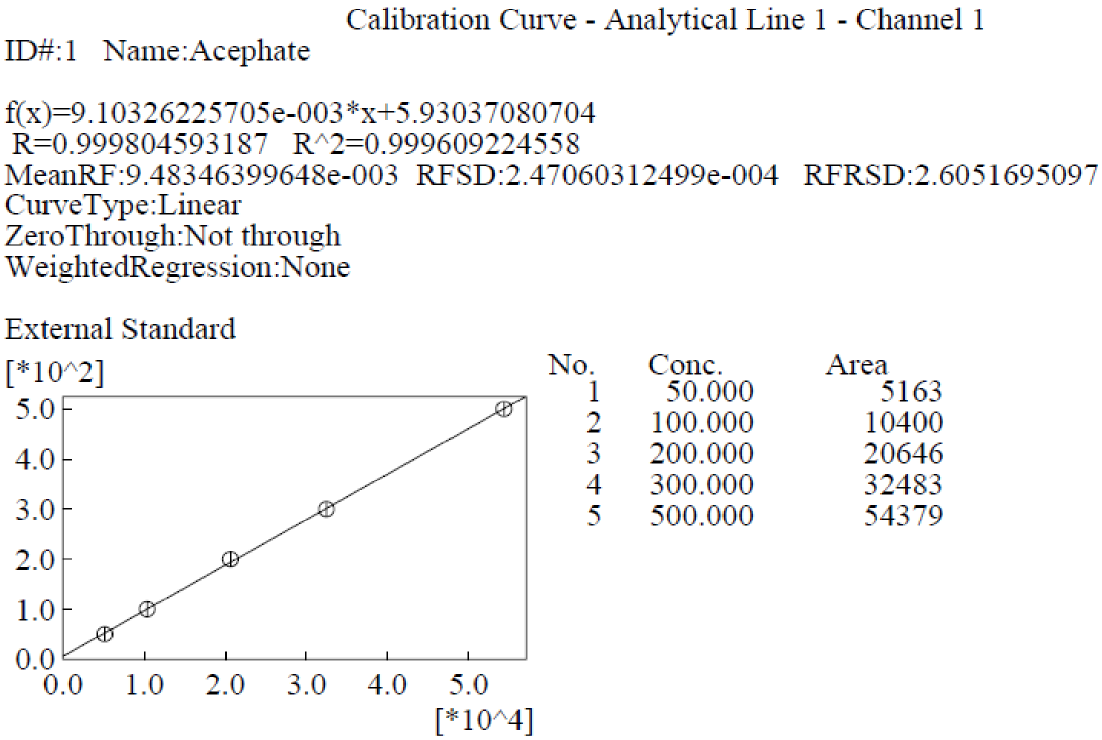
Instruments	Conditions
Injection port SPL	Injection mode: split; temperature: 250°C; flow control mode: linear velocity; split ratio: 30:0
Detector channel 1 FTD	Temperature: 280°C; current: 1.00 Pa; H_2 flow: 1.5 mL/min; stop time: 10 min; make up flow: 30 mL/min; air flow: 145 mL/min

Table 4: Conditions for column oven temperature for FTD

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

3.9 Preparation of Calibration Curve

Prior to the injection of the sample extract, standard solutions of different concentrations of each pesticide group were prepared and injected with suitable instrument parameters. The samples were calibrated (retention time, peak area etc.) against five 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.

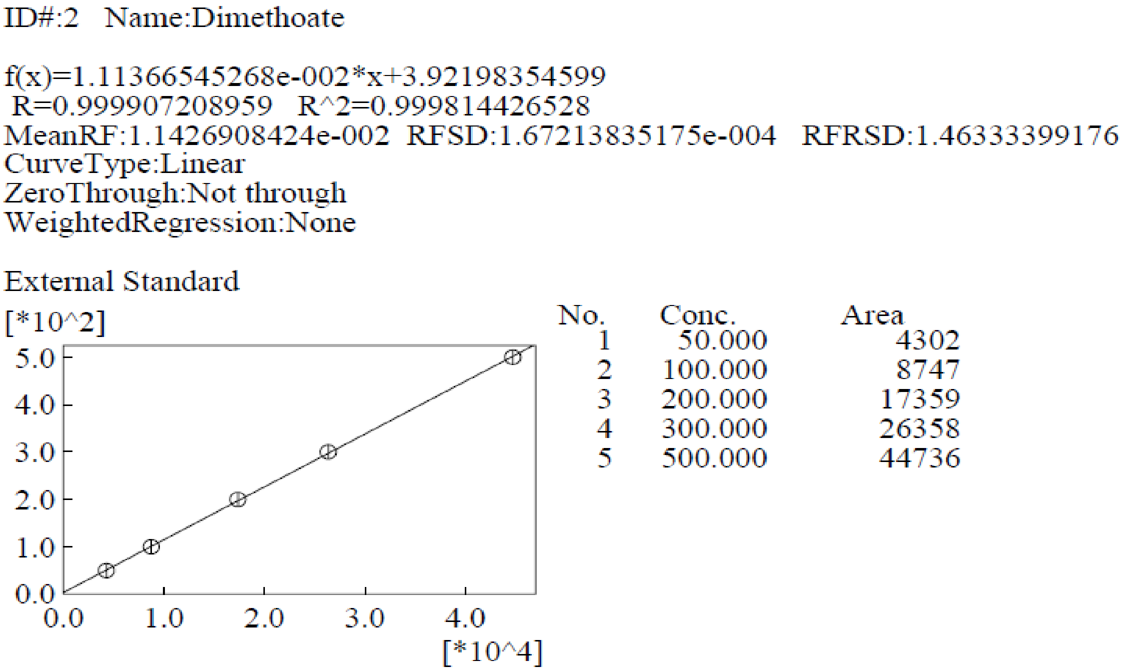


```
                         Calibration Curve - Analytical Line 1 - Channel 1
ID#:1   Name:Acephate

f(x)=9.10326225705e-003*x+5.93037080704
 R=0.999804593187   R^2=0.999609224558
MeanRF:9.48346399648e-003  RFSD:2.47060312499e-004   RFRSD:2.6051695097
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard
                              No.    Conc.        Area
                               1      50.000       5163
                               2     100.000      10400
                               3     200.000      20646
                               4     300.000      32483
                               5     500.000      54379
```

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

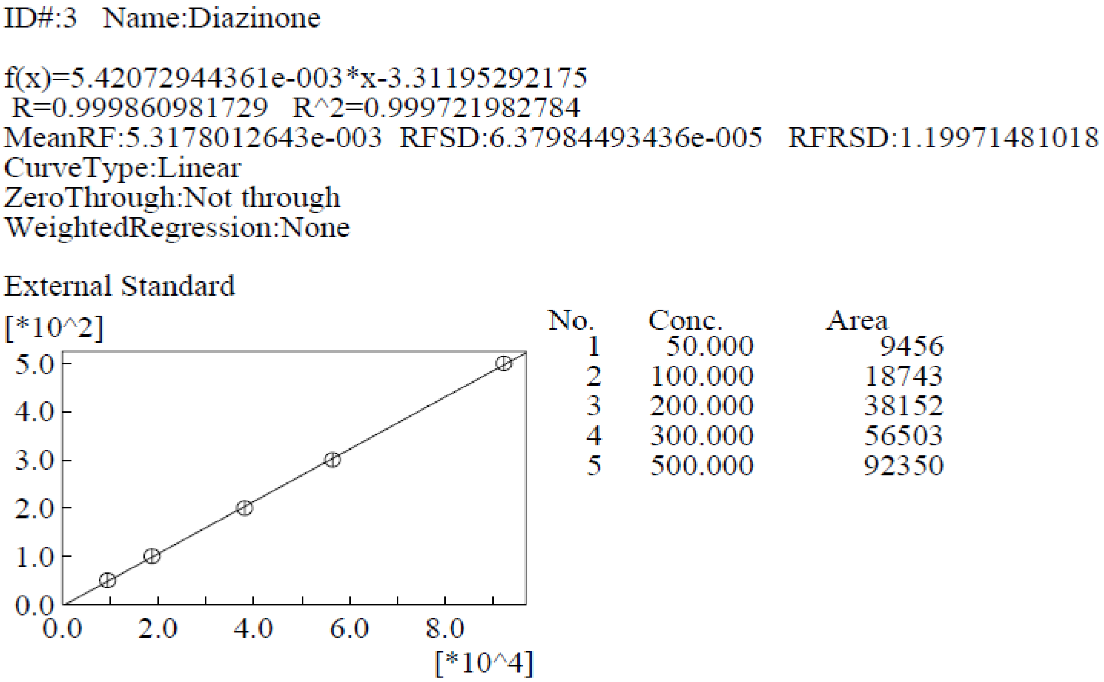


```
ID#:2   Name:Dimethoate

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

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

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

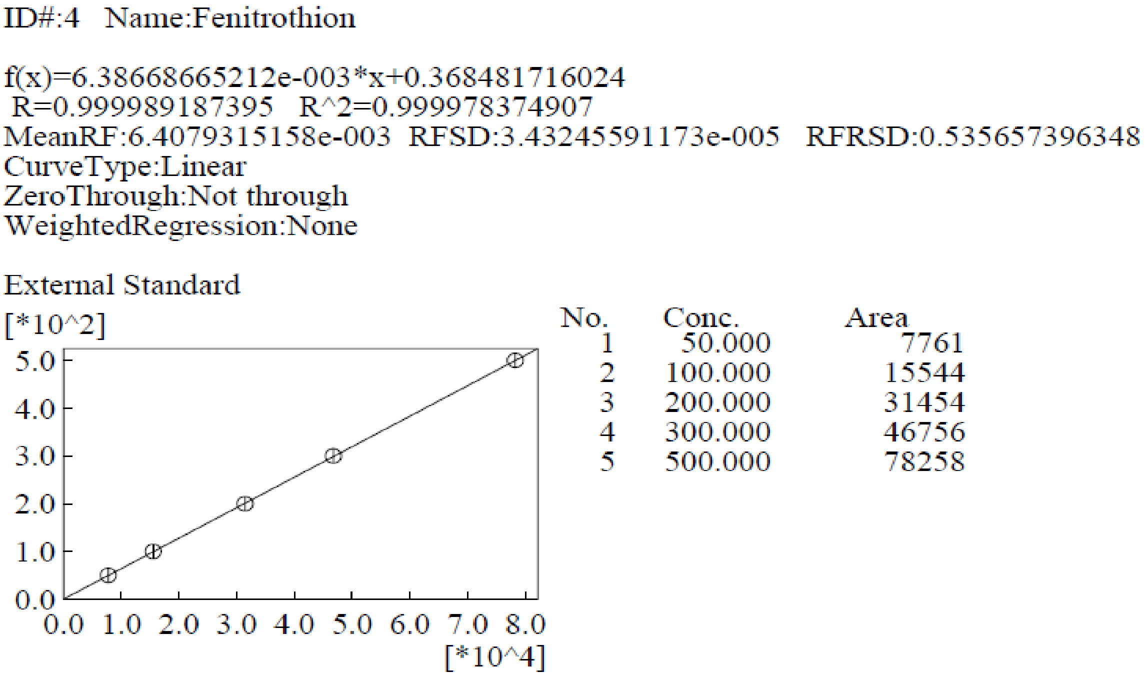


```
ID#:3   Name:Diazinone

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

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

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

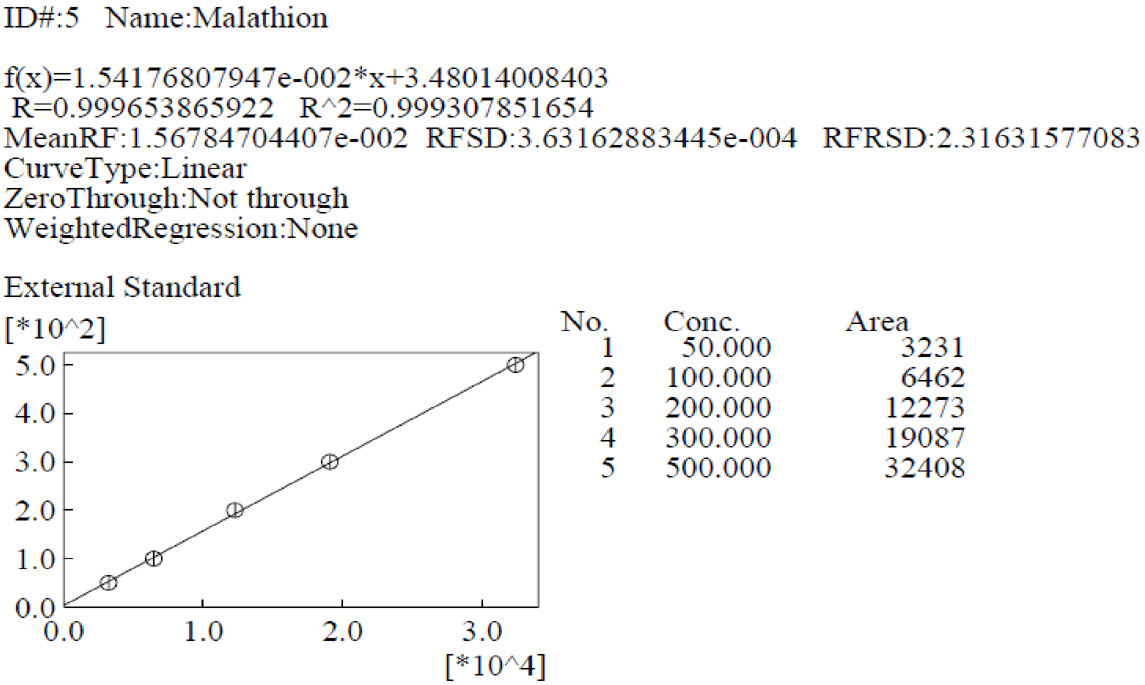


```
ID#:4   Name:Fenitrothion

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

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

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

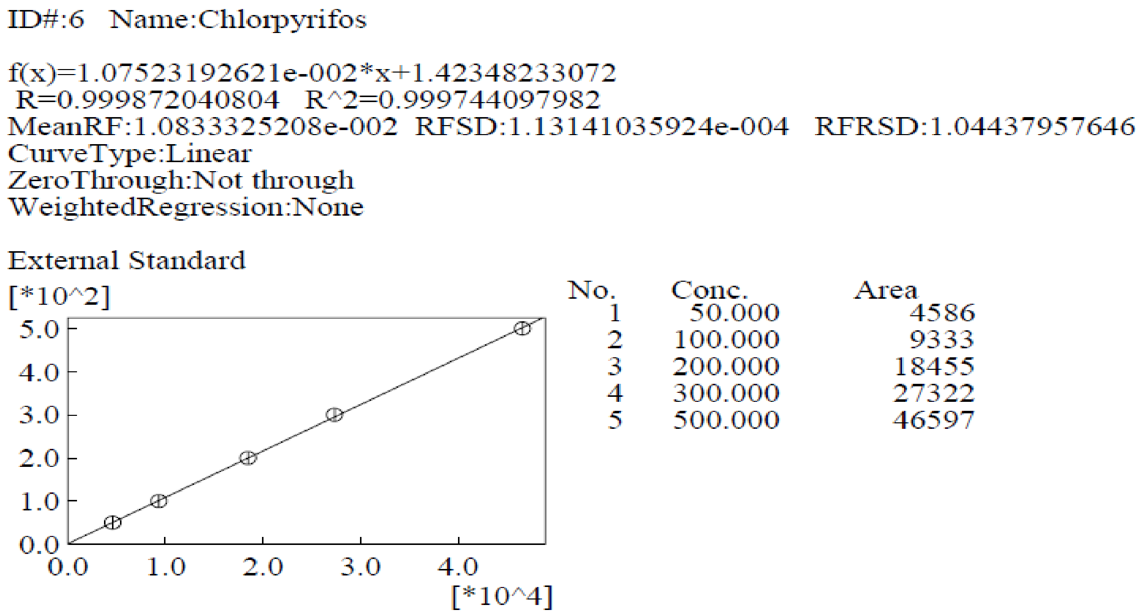


```
ID#:5   Name:Malathion

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

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

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

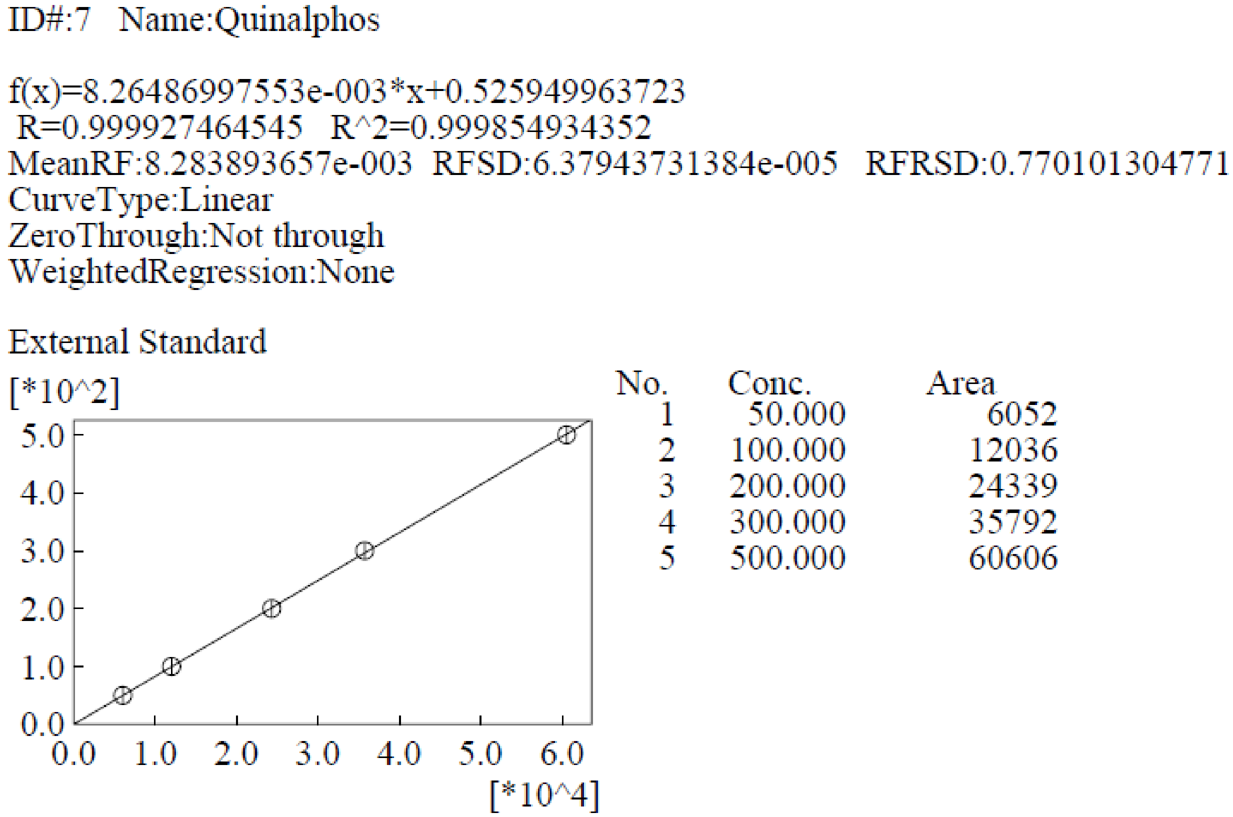


```
ID#:6   Name:Chlorpyrifos

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

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

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



```
ID#:7   Name:Quinalphos

f(x)=8.26486997553e-003*x+0.525949963723
 R=0.999927464545   R^2=0.999854934352
MeanRF:8.283893657e-003  RFSD:6.37943731384e-005   RFRSD:0.770101304771
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard
                                         No.     Conc.        Area
                                          1       50.000       6052
                                          2      100.000      12036
                                          3      200.000      24339
                                          4      300.000      35792
                                          5      500.000      60606
```

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

CHAPTER IV

RESULTS AND DISCUSSIONS

Seventy (70) samples of vegetable (country bean and yard long bean) were collected from 5 different markets of Bogura district (Raja Bazar, Gabtali Bazar, Sonatola Bazar, Mohasthangor Bazar and Shibganj Bazar) to detect and quantify pesticide residues. The results obtained from this study are presented and described in this chapter using figures and tables.

4.1 Pesticide Residues in Country Bean

The concentrated extracts of country bean samples collected from different markets of Bogura were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. Figure 10-13 shows the chromatograms of the injected extracts of country bean sample containing detected pesticides.

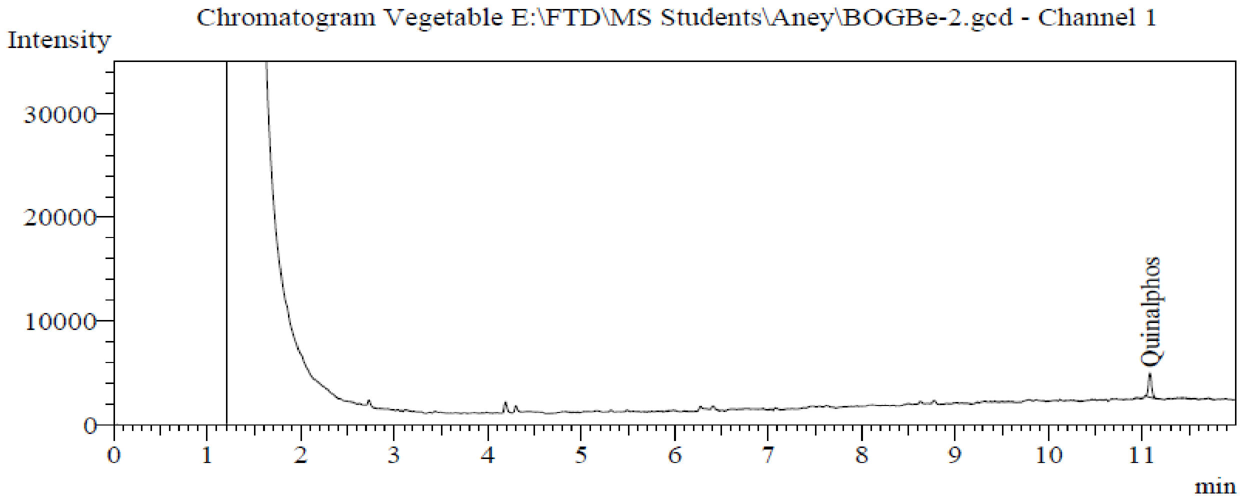


Figure 10. Chromatogram of Quinalphos found in one of the country bean sample (BOGBe-2) showing retention time

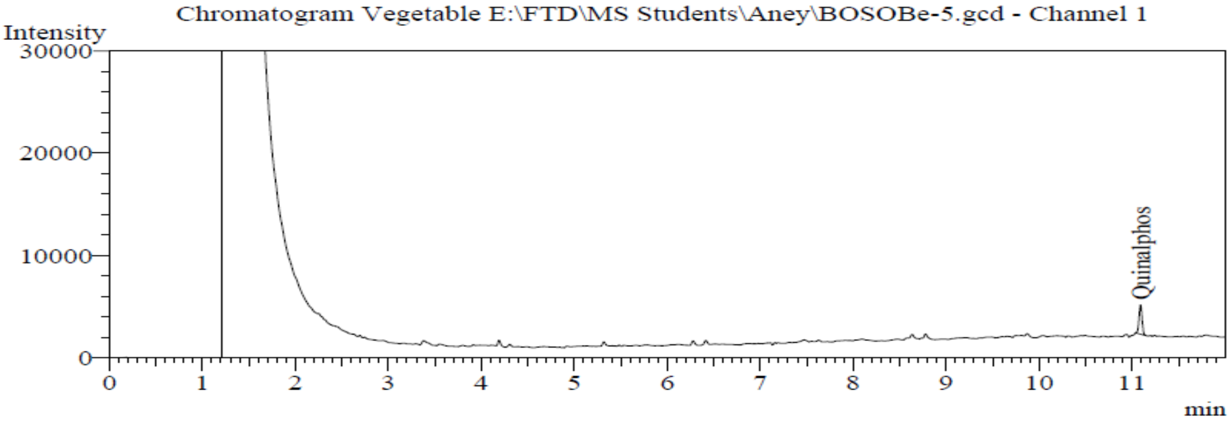


Figure 11. Chromatogram of Quinalphos found in one of the country bean sample (BOSOBe-5) showing retention time

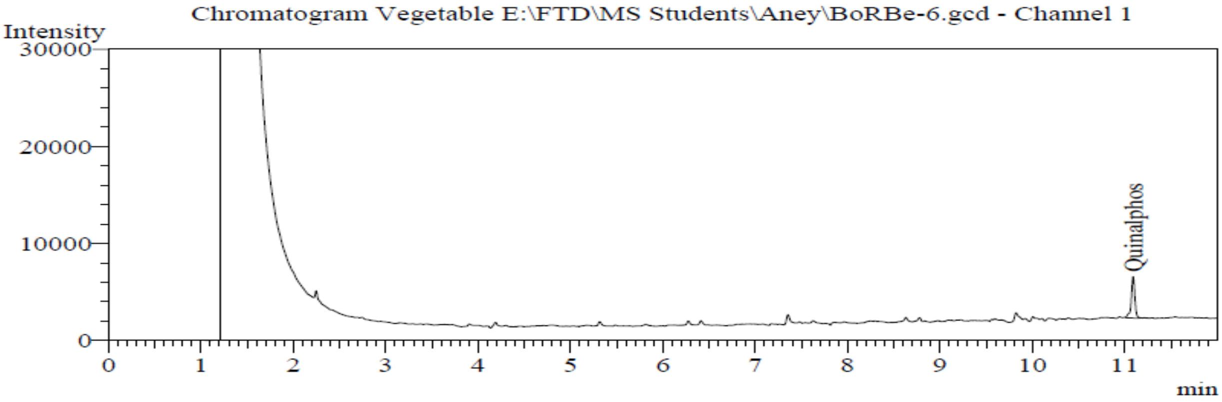


Figure 12. Chromatogram of Quinalphos found in one of the country country bean sample (BORBe-6) showing retention time.

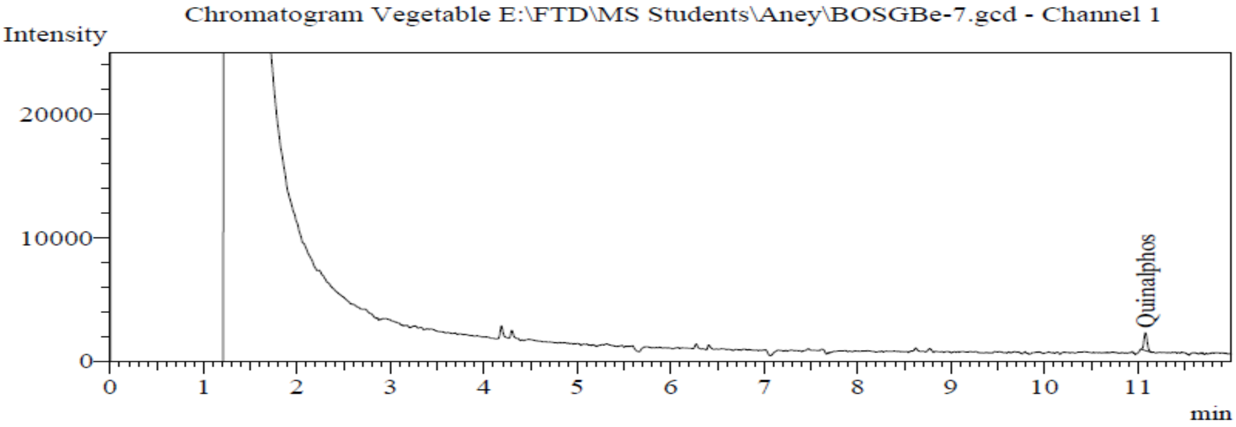


Figure 13. Chromatogram of Quinalphos found in one of the country bean sample (BOSGBe-7) showing retention time.

The level of pesticide residues found in the analyzed country bean 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 country bean samples.

Area of collection	Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)
Raja Bazar	BORBe-1	ND	-	
	BORBe-2	ND	-	
	BORBe-3	ND	-	
	BORBe-4	ND	-	
	BORBe-5	ND		
	BORBe-6	Quinalphos	0.107	0.01*
	BORBe-7	ND		
Gabtali Bazar	BOGBe-1	ND	-	
	BOGBe-2	Quinalphos	0.009	0.01*
	BOGBe-3	ND	-	-
	BOGBe-4	ND	-	
	BOGBe-5	ND	-	
	BOGBe-6	ND	-	
	BOGBe-7	ND		
Sonatola Bazar	BOSOBe-1	ND	-	
	BOSOBe-2	ND	-	
	BOSOBe-3	ND	-	
	BOSOBe-4	ND	-	
	BOSOBe-5	Quinalphos	0.101	0.01*
	BOSOBe-6	ND		
	BOSOBe-7	ND		
Mohasthangor Bazar	BOMBe-1	ND	-	
	BOMBe-2	ND		
	BOMBe-3	ND	-	
	BOMBe-4	ND	-	
	BOMBe-5	ND	-	
	BOMBe-6	ND	-	
	BOMBe-7	ND		
Shibganj Bazar	BOSGBe-1	ND		
	BOSGBe-2	ND	-	
	BOSGBe-3	ND	-	
	BOSGBe-4	ND	-	
	BOSGBe-5	ND	-	
	BOSGBe-6	ND	-	
	BOSGBe-7	Quinalphos	0.007	0.01*

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

Thirty five samples of country bean collected from 5 different markets of Bogura (Raja Bazar, Gabtali Bazar, Sonatola Bazar, Mohasthangor Bazar and Shibganj Bazar) and were analyzed to find out the presence of left over residue of seven pesticides (Acephate, Diazinon, Dimethoate, Malathion, Fenitrothion, Chlorpyrifos and Quinalphos).

Out of 35 samples of country bean, 4 samples (11% of the total number of samples) contained pesticide residues and 31 samples (89% of the total number of samples) contained no detectable residues of the sought pesticides. The present results can be compared to Islam *et al.* (2014). They have collected 42 samples of brinjal, cauliflower and country bean from fields and markets of Narsingdi district, Bandgladesh, where they found 15 samples (above 68% of total samples) contained no residues of the sought pesticides.

Seven country bean samples were collected from Raja Bazar area, among them one samples (BORBe-6) contained Quinalphos at a level of 0.107 mg/kg, which was above the EU-MRL (European Commission 2015). The other 6 samples contain no detectable pesticide residues.

From Gabtoli Bazar, seven samples were collected. Of which one sample (BOGBe-2) contained Quinalphos residue (0.009 mg/kg). But other six samples contained no detectable pesticide residues. The level of detected Quinalphos residue (0.009 mg/kg) was below EU-MRL (0.01 mg/kg).

One sample (BOSOBe-5) of country bean contained residue of Quinalphos (0.101 mg/kg) among the seven samples collected from Sonatola Bazar, which was above EU-MRL (0.01 mg/kg). The other 6 samples contain no detectable pesticide residues.

One sample (BOSGBe-7) of country bean contained residue of quinalphos (0.007 mg/kg) among the seven samples collected from Shibgonj Bazar, which was below EU- MRL (0.01 mg/kg). The other 6 samples contain no detectable pesticide residues.

Among the seven samples collected from Mohasthan Bazar, none of the samples contained no detectable residues of the sought pesticides.

4.2 Pesticide Residues in Yard Long Bean

The concentrated extracts of yard long bean samples collected from different markets of Bogura were analyzed by GC-2010 (Shimadzu) with Flame Thermonic Detector (FTD) with the pre-set parameters. Figure 14-18 shows the chromatograms of the injected extracts of yard long bean sample containing detected pesticides.

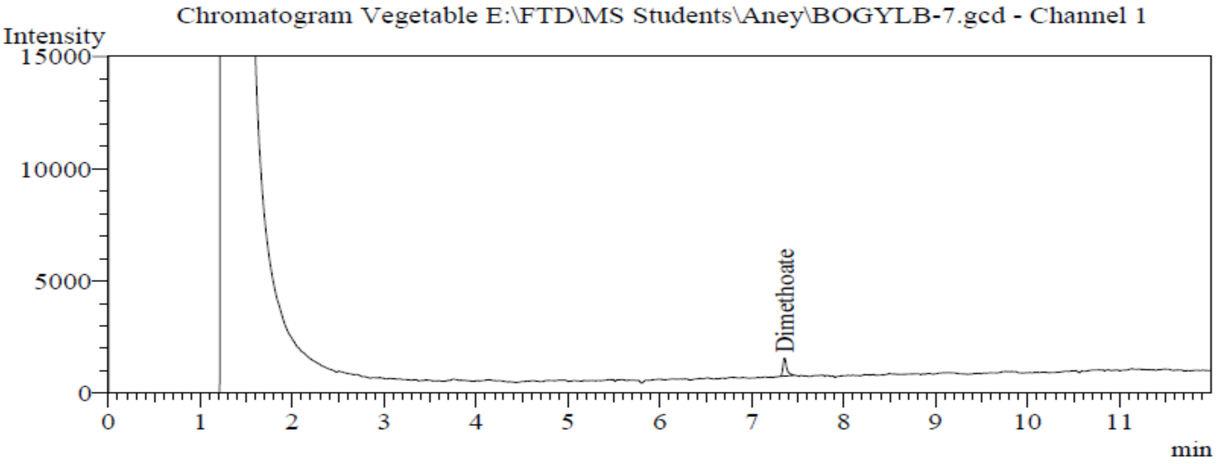


Figure 14. Chromatogram of Dimethoate found in one of the yard long bean sample (BOGYLB-7) showing retention time.

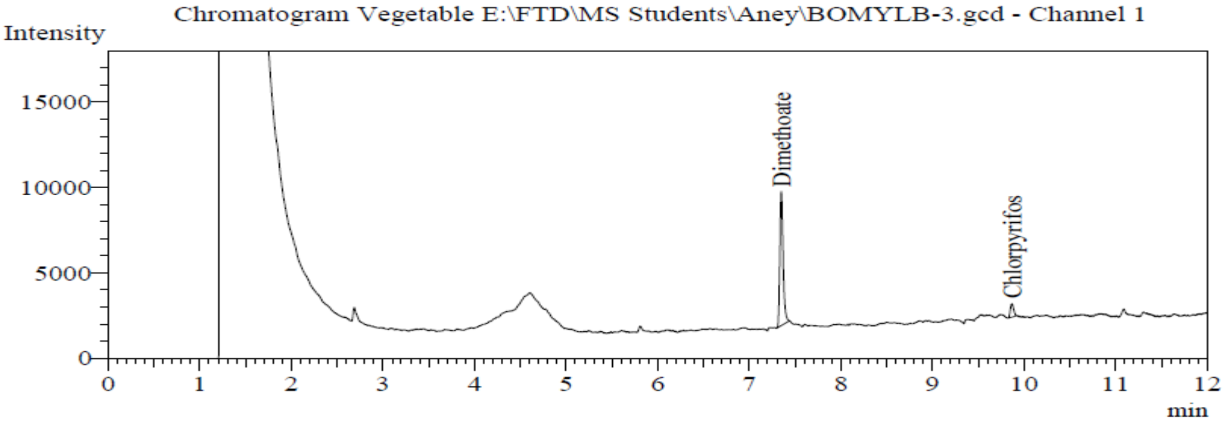


Figure 15. Chromatogram of Dimethoate and Chlorpyrifos found in one of the yard Long bean sample (BOMYLB-3) showing retention time.

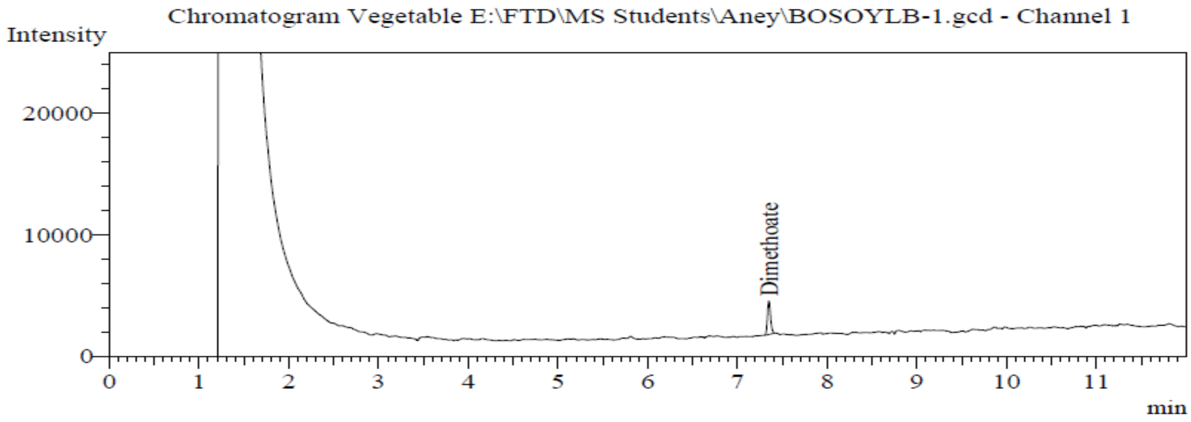


Figure 16. Chromatogram of Dimethoate found in one of the yard long bean sample (BOSOYLB-1) showing retention time.

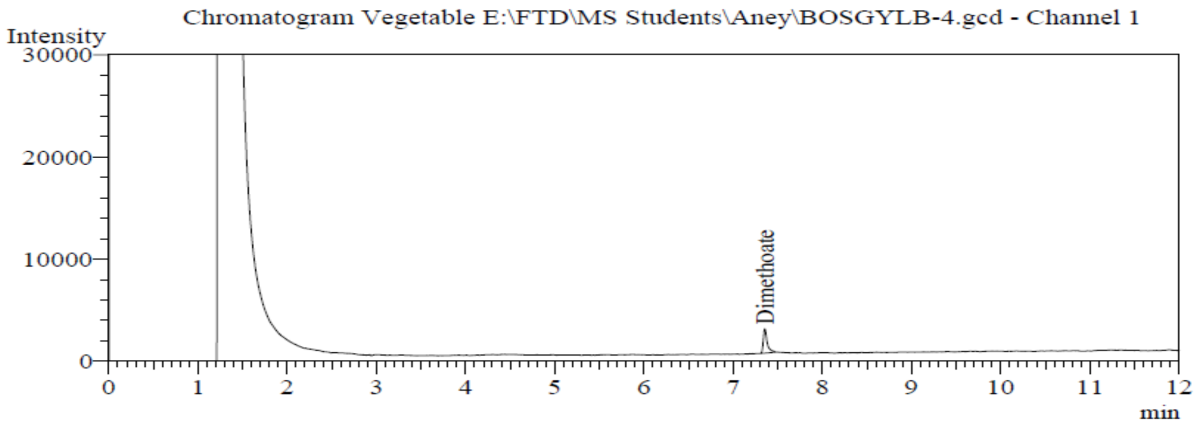


Figure 17. Chromatogram of Dimethoate found in one of the yard long bean sample BOSGYLB-4 showing retention time

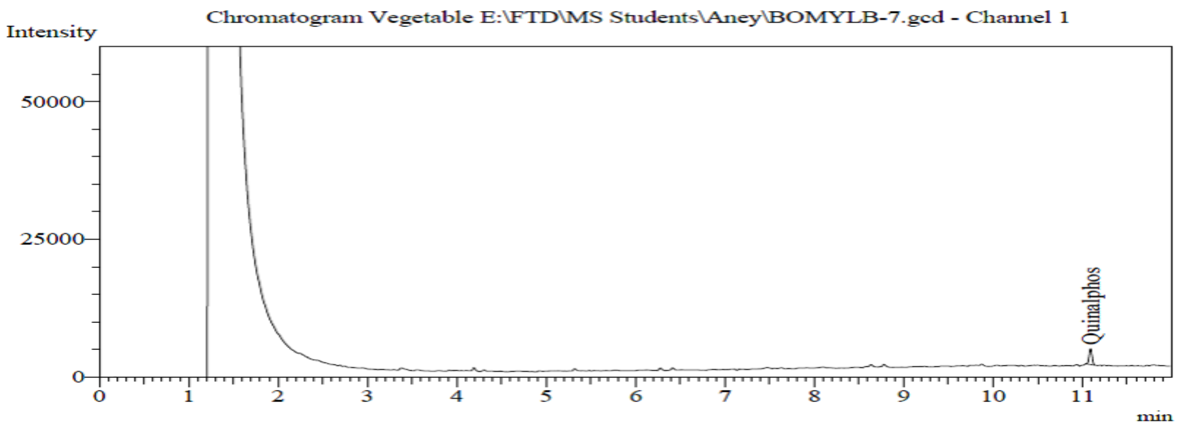


Figure 18: Chromatogram of Quinalphos found in one of the yard long bean sample (BOMYLB-7) showing retention time.

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

Area of collection	**Sample ID**	**Name of detected pesticide**	**Level of residue (mg/kg)**	**MRLs (mg/kg)**
Raja Bazar	BORYLB-1	ND		
	BORYLB-2	ND		
	BORYLB-3	ND	-	
	BORYLB-4	ND	-	
	BORYLB-5	ND	-	
	BORYLB-6	ND	-	
	BORYLB-7	ND		
Gabtali Bazar	BOGYLB-1	ND		
	BOGYLB-2	ND	-	
	BOGYLB-3	ND	-	
	BOGYLB-4	ND	-	
	BOGYLB-5	ND	-	
	BOGYLB-6	ND	-	
	BOGYLB-7	Dimethoate	0.008	0.01*
Sonatola Bazar	BOSOYLB-1	Dimethoate	0.062	0.01*
	BOSOYLB-2	ND	-	
	BOSOYLB-3	ND	-	
	BOSOYLB-4	ND	-	
	BOSOYLB-5	ND	-	
	BOSOYLB-6	ND	-	
	BOSOYLB-7	ND		
Mohasthangor Bazar	BOMYLB-1	ND	-	
	BOMYLB-2	ND	-	
	BOMYLB-3	Dimethoate	0.321	0.01*
		Chlorpyrifos	0.028	0.05*
	BOMYLB-4	ND	-	
	BOMYLB-5			
	BOMYLB-6	ND	-	
	BOMYLB-7	Quinalphos	0.059	0.01*
Shibganj Bazar	BOSGYLB-1	ND	-	
	BOSGYLB-2	ND	-	
	BOSGYLB-3	ND	-	
	BOSGYLB-4	Dimethoate	.009	0.01*
	BOSGYLB-5	ND	-	
	BOSGYLB-6	ND	-	
	BOSGYLB-7	ND		

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

Thirty five samples of yard long bean were collected from 5 different markets of Bogura district (Raja Bazar, Gabtali Bazar, Sonatola Bazar, Mohasthangor Bazar and Shibganj Bazar) and were analyzed to find out the presence of left over residues of seven pesticides (Acephate, Diazinon, Dimethoate, Malathion, Fenitrothion, Chlorpyrifos and Quinalphos).

Out of 35 samples of yard long bean, 5 samples (14% of the total number of samples) contained pesticide residues and 30 samples (86% of the total number of samples) contained no detectable residues of the sought pesticides. The present results can be compared to Rahman (2010). They have collected 94 samples of brinjal, hyacinth bean, cauliflower and yard long bean from fields and markets of different regions like Barisal, Bogra, Chittagong, Comilla, Dhaka, Dinajpur, Gazipur, Jessore, Khagrachuri, Narsingdi, Rajshahi and Rangpur district, Bandgladesh, where they found 48 samples (above 51% of total samples) contained no residues of the sought pesticides.

Seven yard long bean samples were collected from Raja Bazar area, none of the samples contained no detectable residues of the sought pesticides. From Gabtoli Bazar, seven samples were collected. Of which one sample (BOGYLB-7) contained Dimethoate residue (0.008 mg/kg). But other six samples contained no detectable pesticide residues. The level of detected Dimethoate residue (0.008 mg/kg) was below EU-MRL (0.01 mg/kg).

One sample (BOSOYLB-1) of yard long bean contained residue of Dimethoate (0.0.062 mg/kg) among the seven samples collected from Sonatola Bazar, which was above EU-MRL (0.01 mg/kg). The other 6 samples contain no detectable pesticide residues.

One sample (BOSGYLB-4) of yard long bean contained residue of Dimethoate (0.009 mg/kg) among the seven samples collected from Shibgonj Bazar, which was below EU-MRL (0.01 mg/kg). The other 6 samples contain no detectable pesticide residues.

Among the seven samples collected from Mohasthan Bazar, two samples contained pesticide residues. One sample (BOMYLB-3) contained residues of Dimethoate (0.321 mg/Kg) and Chlorpyrifos (0.028 mg/Kg). The level of detected dimethoate residue was above EU-MRL, while the level of detected chlorpyrifos residue was below EU-MRL in this contaminated sample. The another sample (BOMYLB-7) contained Quinalphos residue (0.059 mg/Kg), which was above EU-MRL.

CHAPTER V

SUMMARY AND CONCLUSION

The demand of food has increased in Bangladesh due to the rapid population growth. To cope with this situation, the agricultural productivity needs to be increased. Being an over populated country food shortage and malnutrition are major problems of Bangladesh. Vegetables are one of the major part and sources of vitamin and others nutritional elements of our daily diet, but it contributes a very little portion of our daily intake because of its short supply. The main obstacle of vegetables production in our country is insect pest infestation. In this connection farmers are always relying to apply pesticides in their fields to improve crop yields. High yielding variety is highly susceptible to pests and diseases. So the use of pesticide is now an inherent part of agriculture for pest control. It has been found that many countries of the world including Bangladesh have been extensively using chemical pesticides to cultivate agricultural crops and a major portion of these pesticides are intercepted by the plant leaves during application. As a result, pesticide residues remain in the vegetable which pose a threat to human body. Consumers, who intakes vegetables with high residual contamination in regular basis for long time will be affected by various types of chronic diseases eg. cancer, kidney failure, heart attack etc.

All upazila under Bogura district are famous for vegetable production. But now a day's application of pesticides in Bogura district is very high for increasing crop yield. Moreover, there are no considerable works have yet been done for the determination of different residue levels of pesticide. The purpose of this study is to detect and quantify pesticide residues in country bean and yard long bean and to compare them with the international standards. Regarding this, 35 samples of country bean and 35 samples of yard long bean were collected from five different locations (Raja Bazar, Gabtali Bazar, Sonatola Bazar, Mohasthangor Bazar and Shibganj Bazar) of Bogura district and carried to the Pesticide Analytical Laboratory, Division of Entomology, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The QuEChERS extraction technique was applied for the extraction and cleanup of the collected samples. Gas Chromatography associated with Flame Thermionic Detector (FTD) was used to identify and quantify the level of pesticide residues present in the extracted samples. Seven most commonly used pesticides i.e. Acephate, Diazinon, Dimethoate, Malathion, Fenitrothion, Chlorpyrifos and Quinalphos were considered for this study.

Among the 35 analyzed samples of country bean, 4 samples (11% of the total number of samples) contained pesticide residues of Quinalphos. Among these 4 samples, 2 (5.7%) had residues above the Maximum Residue Limit (MRL) and 2 had residues below the Maximum Residue Limit (MRL) set by European Commission. On the other hand 31 samples (89% of the total number of samples) contained no detectable residues of the sought pesticides.

Out of 35 samples of yard long bean, 5 samples (14% of the total number of samples) contained pesticide residues of Chlorpyrifos, Dimethoate and Quinalphos. Among this 5 samples, 2 (5.7%) had residues above the Maximum Residue Limit (MRL) and 2 had the residues below the Maximum Residue Limit (MRL) set by European Commission and 1 sample contained two different types of pesticide residues such as Dimethoate and Chlorpyrifos. The level of detected Dimethoate residues was above the Maximum Residue Limit (MRL), while the level of Chlorpyrifos residues was below the Maximum Residue Limit (MRL) and the other 30 samples (86% of the total number of samples) contained no detectable residues of the sought pesticides.

At present days pesticide residues in vegetables are becoming a major food safety concern for the consumers and the governments. Therefore, the generated knowledge on pesticide residues level in vegetables at local markets of Bogura district will help to increase public awareness and the policy planners to take necessary action inorder to minimize the pesticide residues level in vegetables at Bogura district in Bangladesh.

CHAPTER VI

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