DETERMINATION OF PESTICIDE RESIDUES IN EGGPLANT AND CUCUMBER COLLECTED FROM DIFFERENT AREAS OF DINAJPUR DISTRICT

RAWSHONA PARVIN

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

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BY

RAWSHONA PARVIN

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Approved by

Supervisor

Dr. Mohammed Ariful Islam
Professor
Department of Agricultural Chemistry
Sher-e-Bangla Agricultural University
Dhaka-1207

Co-Supervisor

Dr. Mohammad Dalower Hossain Prodhan
Senior Scientific Officer
Entomology Division,
Bangladesh Agricultural Research Institute
Gazipur-1701, Bangladesh.

Dr. Md. Sirajul Islam Khan
Associate Professor
Department of Agricultural Chemistry
Sher-e-Bangla Agricultural University
Chairman
Examination Committee

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

CERTIFICATE

This is to certify that the thesis entitled **"DETERMINATION OF PESTICIDE RESIDUES IN EGGPLANT AND CUCUMBER COLLECTED FROM DIFFERENT AREAS OF DINAJPUR DISTRICT"** submitted to the Department of Agricultural Chemistry, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (M.S.)** in **AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bona fide research work carried out by **RAWSHONA PARVIN**, Registration No. **12-05145** 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.

Reasearch supervisor

Dr. Mohammed Ariful Islam
Professor
Department of Agricultural Chemistry
Sher-e-Bangla Agricultural University
Dhaka-1207

June, 2018
Dhaka, Bangladesh

Dedicated to

My

Beloved Parents

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June, 2018

Dhaka, Bangladesh **The Author**

DETERMINATION OF PESTICIDE RESIDUES IN EGGPLANT AND CUCUMBER COLLECTED FROM DIFFERENT AREAS OF DINAJPUR DISTRICT

Abstract

The study was conducted to analyze seven organophosphorus (OP) and pesticide residues in two common vegetables (eggplant and cucumber) collected from five different areas (Ambari Bazar, Bahadur Bazar, Laxmitola Bazar, Fulbari Bazar and Parbatipur Natun Bazar) of Dinajpur district from September 2017 to March 2018. 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 40 samples of eggplant and 40 samples of cucumber. Among the analyzed 40 samples of eggplant, 5 samples (12.5%) contained residues of diazinon. All of the contaminated samples contained residue above the maximum residue limits (MRLs). In case of cucumber, among the 40 analyzed samples, 4 samples (10%) contained residues of chlorpyrifos, quinalphos, acephate, dimethoate and fenitrothion, where 1 sample contained multiple pesticide residues. Among these 4 samples, 2 samples contained residues above MRLs and 2 samples contained residues below MRLs diazinon were detected in the contaminated eggplant sample, while chlorpyrifos, quinalphos, acephate, dimethoate and fenitrothion were detected in the contaminated cucumber sample. This study reflects the over all scenario of pesticide contamination in vegetables especially in eggplant and cucumber available in the retail markets of Dinajpur district, which will help the consumer to be aware of their health and safety.

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
ACh	Acetylcholine
ADI	Acceptable Daily Intake
AOAC	Association of Analytical Chemist
BARI	Bangladesh Agricultural Research Institute
CAS	Chemical Abstracts Service
CSN	Committee for Standardization
ChE	Cholinesterase
DAS	Days After Spraying
DLLME	Dispersive Liquid–Liquid Microextraction
DPX	Disposable Pipette Extraction
d-SPE	dispersive Solid Phase Extraction
ECD	Electron Capture Detector
et all	*et alibi* (and others)
etc	*et cetra* (and so on)
EU	European Union
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FDA	Food and Drug Administration
FTD	Flame Thermionized Detector
GC-MS	Gas Chromatograph-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
HRI	Hazard Risk Index
LC-MS	Liquid Chromatography-Mass Spectrometry
LOD	Limit Of Detection
LOQ	Limit Of Quantifications
MDQ	Minimum Detectable Quantity
MWCNTs	Multi-Walled Carbon Nanotubes
MRL	Maximum Residue Limit
PDI	Potential Daily Intake

PSA	Primary Secondary Amine
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RSM	Response Surface Methodology
RSD	Relative Standard Deviation
RTL	Retention Time Locked
SAU	Sher-e-Bangla Agricultural University
SIM	Selected Ion Monitoring
SRM	Selected Reaction Monitoring
SPE	Solid phase extraction
SBSE	Stir Bar Sorptive Extraction
SF	Supercritical Fluid
TOTAD	Through Oven Transfer Adsorption Desorption
UHPLC-MS/MS	Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry
VFs	Variability factors
WHO	World Health Organization

CHAPTER I

INTRODUCTION

Vegetables are parts of plants that are consumed by humans as food. The original meaning is still commonly used and is applied to plants refer to all edible plant matter, including the flowers, fruits, stems, leaves, roots, and seeds. Vegetables contribute minerals, vitamins, fiber etc to the diet. Minerals are very important and essential ingredients for diet required for normal metabolic activities. Vegetables can be eaten either raw or cooked and play an important role in human nutrition. Vegetables are important for reducing malnutrition problems in human beings (Ojiewo *et al.*, 2015). Most vegetables include cabbages, tomatoes, black nightshade, cowpea and soybeans which contain essential nutrients. These vegetables contains vitamin A, vitamin E, protein, iron, folate, zinc and calcium which are very essential for human diet (FAO/WHO, 2004). Vegetables are rich in essential micro-nutrients such as vitamin A, C, E, zinc, copper, iron & antioxidants (Afari-Sefa *et al.*, 2016). FAO (2004) recommends that a human being needs to consume 200 g of vegetables/day. However, vegetable consumption is still below 200 g per/day especially for poor and this results in the rising rates of malnutrition (Keatinge *et al.*, 2011). Vegetables being mostly low in fat and carbohydrates, but high in vitamins, minerals and dietary fiber. Many nutritionists encourage people to consume plenty of fruit and vegetables, five or more portions a day often being recommended.

Vegetables are the excellent sources of minerals and essential nutrients. Vitamins are organic compounds occurring in natural foods. Vitamins are needed for maintenance of skin, mucous membranes, bones, teeth and hair, vision and reproduction etc. They help to absorb calcium and phosphorous; needed for bone growth and maintenance. Vitamins are involved in blood clotting, normal functioning of nervous system and endocrine glands. They are needed for metabolism of macro molecules (Chatterjea and Shinde, 1998). When vegetables are included in the diet, there is found to be a reduction in the incidence of cancer, stroke, cardiovascular disease, and other chronic ailments. The nutritional content of vegetables varies considerably; some contain useful amounts of protein though generally they contain little fat and varying proportions of vitamins such as vitamin A, vitamin K, and vitamin B_6; provitamins;

dietary minerals; and carbohydrates. Vegetables such as carrot is a good source of vitamin A, needed for normal vision, like wise spinach and tomato contains enough amount of vitamin C to prevent and cure scurvy. Potato is rich in starches and provide high amount of carbohydrates. Some vegetables contain high amount of dietary fibers and help to prevent constipation. However, vegetables often also contain toxins and antinutrients which interfere with the absorption of nutrients these include α-solanine, α-chaconine etc. (Finotti Enrico *et al.* 2006).

A world vegetable survey showed that 402 vegetable crops are cultivated worldwide, representing 69 families and 230 genera. In Bangladesh, there are two seasons for vegetable cultivation – Rabi (winter season) and Kharif (summer/rainy). Summer vegetables include various cucurbits, vegetable cowpea, hyacinth bean, stem amaranth, several aroids Indian spinach etc. Winter vegetables include tomato, cabbage, Chinese cabbage, cauliflower, eggplant, carrot, spinach, bottle gourd, bush bean and radish. Crops like okra, heat-tolerant tomato, eggplant, carrot, spinach, many leafy vegetables, small onion etc. are grown all year round.

Eggplant (*Solanum melongena* L.) is a warm-weather crop mostly cultivated in tropical and subtropical regions of the world. Two other cultivated eggplant species, the scarlet eggplant (*S. aethiopicum* L.) and the gboma eggplants (*S. macrocarpon* L.), are less known but have local importance in sub-Saharan Africa (Schippers, 2000; Daunay and Hazra, 2012). Based on data from 2014, the global production of eggplant is around 50 million tons annually, which makes it the fifth most economically important solanaceous crop after potato, tomato, pepper, and tobacco (FAO, 2014). The top 5 producing countries are China (28.4 million tons; 57% of world's total), India (13.4 million tons; 27% of world's total), Egypt (1.2 million tons), Turkey (0.82 million tons), and Iran (0.75 million tons). In Asia and the Mediterranean, eggplant ranks among the top 5 most important vegetable crops (Frary *et al.*, 2007).

Eggplant has a very low caloric value and is considered among the healthiest vegetables for its high content of vitamins, minerals and bioactive compounds for human health (Raigón *et al.*, 2008; Plazas *et al.*, 2014b; Docimo *et al.*, 2016). In this respect, eggplant is ranked among the top ten vegetables in terms of oxygen radical absorbance capacity (Cao *et al.*, 1996). The bioactive properties of eggplant are mostly associated with the high content in phenolic compounds (Plazas *et al.*, 2013), which are mostly phenolic acids, particularly chlorogenic acid in the fruit flesh (Stommel *et al.*, 2015) and anthocyanins in the fruit skin (Mennella *et al.*, 2012). Both phenolic acids and anthocyanins have multiple properties beneficial for human health (Plazas *et al.*, 2013; Braga *et al.*, 2016). Eggplant fruits contain several antioxidants including the carotenoids lycopene, lutein, and α-caro- tene, the flavonoids myricetin and kaempferol (Miean *et. al.*, 2001; Ben-Amos and Fishler, 1998). It is an excellent source of digestion supportive dietary fiber and bone-building manganese, enzyme-catalyzing molybdenum and heart healthy potassium. It is also a good source of bone-building vitamin K and magnesium and heart-healthy copper, vitamin C, vitamin B6, folate, and niacin (Ensminger *et. al.*, 1986; Wood, 1988).

Cucumbers are scientifically known as *Cucumis sativus*. Cucumber has a relatively low-calorie food just about 15 calories/cup, and are about 95% water. Cucumbers, which are related to melons are a relatively low-calorie food at just about 15 calories per cup, and are about 95% water. They contain high levels of lignans, flavonoids (apigenin, luteolin, quercetin & kaempferol), vitamin K, cucurbitacins and their derivatives (triterpenoids), antioxidants such as beta carotene and vitamin C, and B vitamins, among other trace elements & minerals (Mukherjee *et. al.*, 2013). Cucumbers have been associated with the spa world and topical skin treatment (Griere, 1992; James, 1997). Aside from their cooling effect on skin, cucumber slices offer many benefits to the eyes and surrounding tissues through their hydrating properties, which work to reduce dehydration, their high levels of vitamin K help to reduce dark circles, and the lignans they contain for reducing inflammation (Lopes, 2007). Cucumbers have been used to treat wrinkles and sunburns and have been used as a moisturizer and skin brightener by inhibiting tyrosinase (Hooda, 2015). Cucumber folk medicine includes treatment of diarrhea, gonorrhea, diabetes, hypertension and it is used to detoxify, as an anti-inflammatory, serum lipids regulator, antioxidant, and analgesic (Abu-Reidah, 2012).

The major common constraints to vegetable production are pests and diseases and these limits farmers in obtaining better crop yield and ensuring food security. Some farmers have adopted the use of chemicals to manage insect pests and diseases in vegetable production. However, there is a challenge of insect resistance and this is becoming a constraint to insect pest management and obtaining good crop yields (Jallow *et al*., 2017). Our agriculture faces two major challenges that are production of sufficient food to feed the growing population, and prevention of environmental degradation. Damage by crop pests includes insects, diseases, nematodes and rodents, is one of the major constraints to increase food production. Reduction in losses caused by pests is the obvious strategy for increased food supply. Crop plants of the world are damaged by more than 10,000 species of insects, however, less than 10% of the total identified pest species are considered as major pests. The incidences of several insect pests like mealy bugs, particularly *Phenacoccus solenopsis* on cotton; sugarcane woolly aphid, *Ceratovacuna lanigera* on sugarcane; *Pieris brassicae* on crucifers; and tobacco caterpillar, Spodoptera litura, on several crops, has shown an increasing trend. The sap feeders, viz. aphids, jassids, mirids and mealy bugs are emerging as serious pests. In case of brinjal among the many pest species, the brinjal shoot and fruit borer (BSFB), *leucinodes orbonalis Guenee*, is the most destructive. The pest larvae bore inside tender shoots and reduce plant growth. More severe economical damage caused by the larvae by feeding inside the fruits, which make damaged the fruit and unfit for human consumption. The diamondback moth was one of the serious insect pests of crucifer vegetables worldwide. The major outbreaks are more likely in the fields that are sprayed frequently and heavily with insecticides.

The vegetables are attacked by number of insect pests in every season and these cause high damage to the production. Insects and pests generally attack vegetables for their liking and to complete their life cycle. They damage and use every part of the plant and ultimate cause high economic loss. Insects injure plants by chewing leaves, stems and roots, sucking juices, egg laying and transmitting diseases. Most vegetables are subjected to pest damage particularly seeds, roots, leaves, stems and fruits are all susceptible to damage effecting plant vigor resulting severe crop losses. The cucurbit fruit fly *Bactrocera cucurbitae*, is one of the most important pests of vegetables, and depending on the environment conditions and susceptibility of the crop species, the extent of losses can vary between 30 to 100% (Dhillon *et. al*., 2005; Sapkota *et. al*., 2010; Sarwar *et. al*., 2013). The vegetable crops are heavily attacked by aphids. Among 14 different winter vegetables there are 14 species of aphids found to attack on plants. These pests are a great concern for the farming community, as they could threaten the agricultural and horticultural industries by increasing the price of production and cost.

Some of the more common insecticides use in the vegetables are Acetamiprid (effective against whiteflies, aphids and other pests), Imidacloprid (soil-applied systemic insecticide that is useful against whiteflies, aphids and beetles), Spinosad (microbial insecticide is very effective against most caterpillar pests), Carbaryl (useful against many beetles), and Malathion (especially useful to control aphids, bugs and certain beetles). The chlorinated hydrocarbon, DDT had been used for controlling various insects since the 1950's. Soon after its use began, some pests began to develop resistance to DDT, includes house flies, mosquitos, bed bugs, and body lice etc. (Metcalf, 1989). The use of pesticides has increased many folds over past few decades. According to an estimation, about 5.2 billion pounds of pesticides are used worldwide per year. The use of pesticides for pest mitigation has become a common practice around the world. Their use is not only restricted to agricultural fields, but also employed in homes in various forms such as sprays, poisons and powders for controlling cockroaches, mosquitoes, rats, fleas, ticks and other harmful bugs. That's why pesticides are frequently found in our food commodities in addition to their presence in the air.

Despite the application of insecticides pests destroy a substantial proportion of annual production in individual crops worldwide: as much as 50 % in rice, 41 % in potato, 40 % in coffee, 39 % in maize, 38 % in cotton, 34 % in wheat, 32 % in soybean, 30 % in barley, and 26 % in sugar beet (Oerke et al. 1994; Oerke and Dehne 2004). Losses are heaviest in developing countries. The yield loss varies different environmental conditions but can exceed 65% in Bangladesh (BARI, 1999). Due to the high loss in food production, insecticides are often used to try to combat the insect's problems. It is estimated that direct damage by insects to 33 major crops caused annual agricultural losses of approximately US$ 2.3 billion (Bento *et al*., 1999). More recently this figure is updated to approximately US$ 12 billion (Oliveira *et al*., 2013).

Pests cause a serious damage in terms of yield and high price of chemicals that cost billions of dollars annually and increase the agricultural production budget (Chattopadhyay *et. al*., 2017). Regardless of the high costs, the widespread application of chemical pesticides has been preferred due to benefits, particularly by protecting crops from pest damage. But on the other hand, pesticide poses a serious danger to human health and environment. The overuse of pesticides leads to health problems and impacs environment when not handled properly. Jallow *et al.* (2017) indicated that 65% of farmers agreed that the use of chemicals for insect and pest management is hazardous to environment and 70.5% confirmed that pesticides can be dangerous to human health.

Farmers and especially those directly involved in the handling of pesticides, are at high risk of exposure when mixing and applying pesticides or working in treated fields and from residues on food and drinking water (Soares, 2009). Various human health related concerns are associated with pesticides, short-term impacts such as headaches and nausea and chronic impacts, such as various cancers, birth defects, infertility, and endocrine disruption (Bourguet and Guillemaud, 2016). Children are more endanger by short-term and chronic exposure to pesticides. Pesticide use has resulted in different cases of acute and chronic poisoning, hazard on human health, from mild effects to death (Dawson *et al.*, 2010). About 3 million cases are reported worldwide every year that occur due to acute pesticides poisoning.(Singh *et al.*, 2013). Depending on their chemical properties, pesticides can enter the organism; bioaccumulates in food chains and consequently influences human health. Furthermore, excessive use of pesticides may lead to the destruction of biodiversity, destruction of non target species, secondary pest out-breaks, soil, water, and air contamination (Recena *et al.*, 2006).

Several studies have highlighted the occurrence of chronic health hazards, e.g., cancers, diabetes, depression, respiratory diseases, and fertility problems, due to pesticides (Bourguet and Guillemaud, 2016). Epidemiological studies in humans indicate that there is a possible association between pesticide exposure and infertility, prostate and ovarian cancer breast, and nervous system cancers (Fleming *et. al.*, 2003). Various problems such as Parkinson’s disease, disruption of glucose homeostasis have been linked with pesticides induced oxidative stress (Mostafalou and Abdollahi, 2013).

Many pesticides are not easily degradable they persist in soil, leach to groundwater and surface water and contaminate wider environment. About 80–90 % of the applied pesticides can volatilize within a few days of application (Majewski and Capel, 1995). The volatilized pesticides evaporate into the air and subsequently harmful to non-target organism. The use of herbicides, which volatilise off the treated plants and the vapours are sufficient to cause severe damage to other plants (Straathoff, 1986). Uncontrolled use of pesticides has resulted in reduction of terrestrial and aquatic animal and plant species. Pesticide residues remain for very long time and cause serious toxic effects, and disturb ecological balance by killing unharmed insects, animals and fishes, and also modified their genetics by creating resistance in them (pest) against these pesticides (Andersson *et al.*, 2014).

As crop production and pesticides are closely related and their left over residue might or might not persist in the environment that's why it should be carefully examined and monitored. The monitoring and detection of pesticide residue particularly in vegetables and fruits is being done in regular fashion in many countries (Kumaril et al., 2004; Rajeswaran et al., 2004). Currently in Bangladesh, organophoshates, carbamates and pyrethroids are mostly used while organochlorine insecticides have been banned because of their toxicity, persistence and bioaccumulation in the environment (Molto *et al*., 1991). Knowledge of withholding period becomes important even for less persistent insecticides, specifically in fruits and vegetables since these crops are harvested/picked shortly after pesticide application.

Every pesticide has a withholding period such as waiting period or pre-harvest interval (PHI), which is defined as the number of days required to lapse, between the date of final pesticide application and harvest, for residues to fall below the tolerance level established for that crop or for a similar food items. The pre-harvest interval (PHI) differs from pesticide to pesticide and crop to crop. Only after lapsing the withholding period food products become safe for consumption. By this time, the pesticide residues get degraded. Nevertheless, the extent and rate of dissipation depends on the nature of the pesticide, crop, cultural practices and various environmental conditions (Handa *et al.,* 1999). As the farmers of our country are illiterate, they do not follow the prescribed dose and use pesticides at any stage of the crop without any awareness of the residues and that effects on human health. They harvest the treated fruits and vegetables without taking into account the withholding period.

There are some analytical methods are used to determine multiple pesticide residues for fruits and vegetables (Prodhan and Alam, 2018; Rajapakse *et. al.*, 2018; Balkan, 2017; Mebdoua *et.al.*, 2017; lhag *et. al.*, 2017; Prodhan *et al.*, 2016; Prodhan *et al.*, 2016a; Schenck *et al.*, 2008; Singh *et al.* 2012; Dasika *et al.*, 2012; Lehotay, 2010; Anastassiades *et al.*, 2003;). Anastassiades *et al.* (2003) introduced the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method for pesticide residue analysis. The method provides high-quality results in a fast, easy, inexpensive approach. QuEChERS extraction techniques along with Gas Chromatography were used in that study to determine selected pesticides in eggplant and cucumber.

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

- ✓ To determine pesticide residues (if any) in eggplant and cucumber collected from different areas of Dinajpur district.
- ✓ To quantify the level of detected pesticide residues.
- ✓ To compare whether the level of detected pesticide residues are above the Maximum Residue Limits (MRLs) or not set by EU-MRL.

CHAPTER II

REVIEW OF LITERETURE

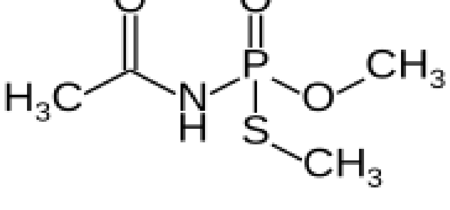
2.1 Pesticides

Pesticides are natural or synthetic agents that are used to kill unwanted plant or animal pests. A pesticide is any substance used to kill, control, or repel certain forms of plant or animal life that are considered to be pests. Pesticide includes herbicides for destroying weeds and other unwanted vegetation, insecticides for controlling a wide variety of insects, fungicides used to prevent the growth of molds & mildew, disinfectants for preventing the spread of bacteria, and compounds used to control mice & rats. Pesticides are chemicals used by the man to control agricultural pests and their correct application is the most accepted and effective for maximum production and quality of crops (Ferrer and Cabral, 1993; Bolognesi, 2003; Mansour, 2004).

According to the FAO "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 control of insects, arachnids, or other pests in or on their bodies". The purpose of all pesticides is to prevent undesirable damage to agriculture, environment and society.

2.2 Description of the selected pesticides

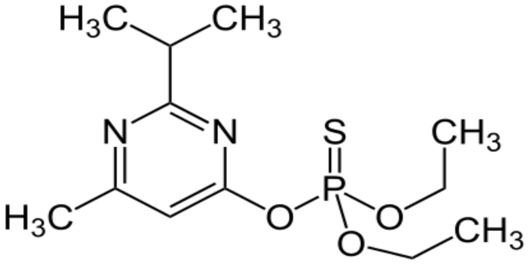
2.2.1 Acephate



Acephate

Acephate is actually a systemic insecticide specially used to control sucking and biting insects by direct contact or ingestion (Tomlin, 2006; Thomson, 1989). Acephate is an organophosphate insecticide. It is a general-use insecticide registered for use on cereals, pulses, vegetables and other crops, agricultural seed and non-bearing plants, horticultural nursery plants, commercial infrastructures and institutions including municipal health facilities, golf course turf, ant mounds etc. (EPA 2006).
IUPAC name of acephate is O, S-Dimethyl 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). Methamidophos is another organophosphate insecticide that is registered by the U.S. EPA. In soil, plants, and insects, acephate is converted to methamidophos. 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).

2.2.2 Diazinon

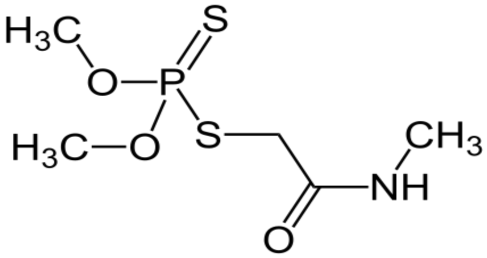


Diazinon

Diazinon (IUPAC name: *O,O*-Diethyl *O*-[4-methyl-6-(propan-2-yl) pyrimidin- 2-yl] phosphoro- thioate, INN - Dimpylate), a colorless to dark brown liquid, is a thiophosphoric acid ester developed in 1952 by Ciba-Geigy, a Swiss chemicalcompany. Diazinon is a contact insecticide which kills insects by altering normal neurotransmission within the nervous system of the insect. 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.

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 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. The initial symptoms of humans are nausea, headache, dizziness, salivation, sweating, lacrimation, and rhinorrhea. The symptoms can progress to vomiting, abdominal cramps, weakness, diarrhea, muscle twitching, 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.

2.2.3 Dimethoate

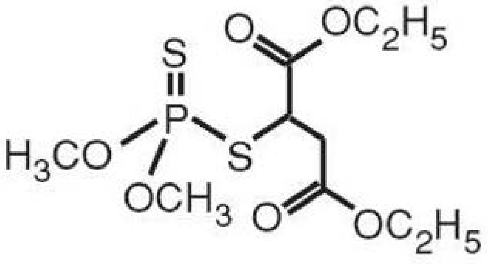


Dimethoate

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

The IUPAC name is O, O-dimethyl. 9-[2- (rnethvlamino)-2-oxoethyl] dithiophosphate and the CAS No. of dimethoatc is 60-51-5 (EPA 2006). Dimethoate is a colorless crystalline solid with a camphor-like (mercapian) odor (Worihing, 1987). 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). It will breakdown rapidly when heated to temperatures above 80° C. creating the possibility of explosion. 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).

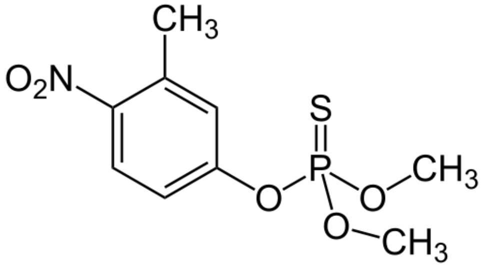
2.2.4 Malathion



Malathion

IUPAC name for malathion is O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate (EPA, 2006). Malathion is a colorless to amber liquid with a skunk- or garlic-like odor (US.DHHS, 2008). Malathion is a non-systemic, wide-spectrum organophosphorus insecticide. It is a widely used insecticide in agriculture, residential landscaping, community recreation areas, and in municipal health pest control programs such as mosquito eradication. In the US, it is the most commonly and widely used organophosphate insecticide (Bonner *et al.*, 2007). 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 *et al.*, 2006).

2.2.5 Fenitrothion

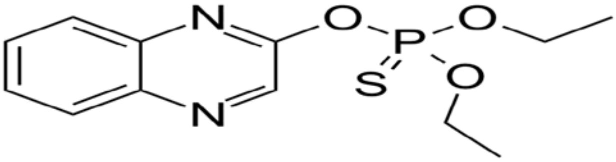


Fenitrothion

Fenitrothion (IUPAC name: O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate) is a phosphorothioate (organophosphate) insecticide; cheap and widely used worldwide. Fenitrothion is effective against a wide range of pests, i.e. penetrating, chewing and sucking insect pests (coffee leafminers, locusts, rice stem borers, wheat bugs, flour beetles, grain beetles, grain weevils) on cereals, cotton, orchard fruits, rice, vegetables, and forests. It may also be used as a fly, mosquito, and cockroach residual contact spray for commercial farms and community health programs (Worthing *et al.,* 1983, Meister *et al.,* 1994; Thomson *et al.,* 1989). 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). The acute toxicity of fenitrothion to mammals is considered to be low (Spencer *et al.,* 1981; Hayes *et al.,* 1990; Hayes *et al.,* 1982).Chronic symptoms in humans include: usual malaise, fatigue, headache, cramps, loss of memory and ability to concentrate, loss of weight, nausea, thirst, loss of weight, anorexia, muscular weakness and tremors. This pesticide produces typical cholinergic poisoning at sufficient dosage (Hayes *et al.,* 1990; Hayes *et al.,* 1982). Preliminary data shows fenitrothion degrades fairly rapidly in soil with a half-life of less than 7 days in non-sterile muck, sandy loam soils. Generally, fenitrothion is intermediately mobile in a variety of soils ranging from sandy loam to clay (Meister *et al.*, 1994; EPA, 1987).

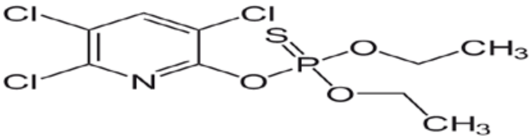
2.2.6 Quinalphos



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).

2.2.7 Chlorpyrifos



Chlorpyrifos

Chlorpyrifos (IUPAC name: O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) is a crystalline organophosphate insecticide, acaricide and miticide. 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.

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.

2.3 Instrument used for determination

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

2.3.1 High Performance Liquid Chromatography

High Performance Liquid Chromatography has been used for manufacturing (e.g. during the production process of pharmaceutical and biological products), research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other), legal (e.g. detecting performance enhancement drugs in urine) and medical (e.g. detecting vitamin D levels in blood serum) purposes.

2.3.2 Gas Chromatography

A gas chromatograph is an analytical instrument that measures the content of various components in a sample. Many detectors can be used in gas chromatography. Flame ionization Detector (FID) is feasible for most organic compounds. Electron capture

Detector (ECD) detector is used for nitriles, halides, nitrates, peroxides, anhydrides, organometallics etc. Thermal Conductivity Detector (TCD) is a universal detector. Nitrogen-Phosphorus Detector (NPD) detector is normally used for nitrogen, phosphorus and Flame Photometric Detector (FPD) is used for phosphorus, sulphur, tin, boron, arsenic, germanium, selenium and chromium.

2.3.3 Gas chromatography–mass spectrometry

In this technique, 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 for monitoring purposes but confidence in confirmation of identity is reduced if the selected ions are affected by matrix effect. GC-MS/MS with ion trap mass spectrometers (Wang *et al*., 2005), triple quadrupole (Patel *et al*., 2005, Garrido Frenich *et al*., 2006) and has been used for pesticide residue analysis on fatty food. Both acquisition mode, the selected reaction monitoring (SRM) (Garrido Frenich *et al*., 2006) and multiple reaction monitoring (MRM) (Patel *et al.*, 2005) mode have been used to analyze multiple pesticide residues from food matrices.

2.4 Pesticides residues

Pesticide residue are the pesticides that may remain on or in food after they are applied to food crops (IUPAC, 1997). Many of these residues, especially derivatives of chlorinated pesticides, exhibit bioaccumulation which could build up to harmful levels in the body and in the environment (*Crinnion, 2009)*. Persistent chemicals can be elevated through the food chain and have been detected in various products such as meat, poultry, fish, vegetable oils, nuts, and various fruits and vegetables (Chung and Chen, 2011).

2.4.1 Determination of pesticide residues in food

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. 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 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*. (2018b) conducted a study to determine the pre harvest interval (PHI) for quinalphos in eggplant, cabbage and yard long bean; cypermethrin in tomato and yard long bean; malathion in eggplant, yard Long bean and cauliflower; 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.* (2018c) has been developed and validated a simple and efficient multiple organochlorine pesticide residues analytical method using quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction technique and Gas Chromatography coupled with Electron Capture Detector (ECD) for the determination of 19 organochlorine pesticides (Alpha BHC, Delta BHC, Beta BHC, Gama BHC, Heptachlor, Aldrin, Heptachlor Epoxide, Gama Chlordane, Alpha Chlordane, Alpha Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, Beta Endosulfan, 4,4 DDT, Endrin Aldehyde, Endosulfan sulphate, Methoxychlor, and Endrin Ketone) in shrimp. The method was validated by evaluating the accuracy, precision and linearity limit of detection (LOD) and limit of quantification (LOQ). The average recoveries of the selected pesticides ranged from 84% to 106% with RSDr ≤ 14% in four fortification levels of 0.05, 0.1, 0.2 and 0.3 mg/Kg. The linearity was ≥0.996 for all of the selected pesticides with matrix matched calibration standards. The LOD ranged from 0.003 to 0.009 mg/Kg and the LOQ was 0.05 mg/Kg.

Suganthi and Bhuvaneswar (2018) A method employing dispersive solid phase extraction and quantification of five neonicotinoid insecticides by liquid chromatography-tandem mass spectrometry/mass spectrometry (LC-MS/MS) was optimized in tomato matrix. QuEChERS method was followed for residue extraction using acetonitrile as the extraction solvent. The validated method showed a linear range, from 0.025 to 0.5 µg mL^{-1} and detection and quantitation limits (LOD and LOQ) of 0.0015 to 0.008 µg g^{-1} and from 0.005 to 0.025 µg g^{-1}, respectively. Validation was based on analysis at five fortification levels and showed satisfactory recoveries (60.00% to 99.14%) and high precision (RSDs between 2.05% to 17.44%). The method is easy, with low consumption of reagents, is characterized by reliability, sensitivity and therefore is suitable for the monitoring the levels of neonicotinoid residues in tomato. Moreover, the developed method was successfully applied to quantify neonicotinoid residues in market samples of tomato.

Rajapakse *et. al.* (2018) conducted a research of pesticide residues in fruits and vegetables in Sri Lanka to determine prevalence of five pesticide residues in locally grown vegetables tomato, cabbage and capsicum in Puttalum, Dambulla and Nuwara Eliya districts. Pesticide residues were analysed by Gas Chromatography with Mass Spectrometry after multi residue extraction procedure (QuEChERS method). The QuEChERS method was validated using 5 pesticides (Diazinon, Chlopyrifos, Fipronil, Prothiofos and Tebuconazole) and their retention times in minutes were 15.948, 19.566, 20.342, 22.308, and 26.201 respectively. Coefficient of detection was obtained near 0.99 for all tested standard pesticides. Either Chlopyrifos, Prothiofos or Tebuconazole were detected in 15 samples out of 45 vegetables samples. However, pesticide residual values were less than Maximum Residual Levels (MRL) for all the tested pesticides. Tebuconazole was the mostly detected pesticide residue with 0.128 ppm and 0.052 ppm in tomato and cabbage.

Sah *et. al.* (2018) evaluated pesticide contamination that was applied during growth period of vegetables in farm gate vegetables samples of Muzaffarpur district of Bihar. 40 samples of seasonal vegetables namely okra (10), brinjal (10), cauliflower (10) and cabbage (10) were monitored during 2012. 30 samples (75 %) were found to be contaminated with different pesticides, 6 samples (15 %) contained residues above maximum residue limit. The residue of cypermethrin and chlorpyriphos were above maximum residue limit (MRL) in 2 samples each and endosulphan and quinalphos in 1 sample each of farmgate vegetables.

Wu *et. al.* (2018) established the GPC - GC - MS detection method for determination of organochlorine pesticide residues in milk powder. After simple processing samples, concentration with multi-position concentrator, and online cleaning with GPC-GC-MS, injection into the mass spectrometer was used for analysis. Results showed that in the linear range of 5～100 ug/L, the correlation coefficient were >0.998. The method detection limits (MDLs) were <0.5 ug/kg. The spiked recoveries at 3 levels of 10, 50, 100 ug/kg were in the range of 70 ~ 95%, the relative standard deviations (RSDs) (n=6) were<10%.

Huifen *et. al*. (2018) established a multi-residue determination method for 105 typical insecticides, bactericides, herbicides, and plant growth regulators in vegetables after optimization of the QuEChERS pretreatment method, combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology. The target compounds were extracted by acetonitrile (ACN), purified with 150 mg primary secondary amine (PSA), 150 mg EC-C_{18}, and 30 mg graphitized carbon black (GCB) adsorbents. With correlation coefficients (*r*)>0.99, the standard curves of 105 target compounds were linear in the concentration range of 0.010-0.200 mg/L. The limit of quantification was 0.010 mg/kg, the recoveries were between 68.2% and 108% at 3 spiked levels, and the RSDs of the method were between 1.02% and 11.8%.

Ucles *et. al*. (2018) studied and evaluated for the determination of this group of thermally troublesome pesticides. Thermally labile pesticides (captafol, captan, dicofol, and folpet) are highly prone to suffer thermal degradation during sample introduction into a gas chromatograph (GC) to tetrahydrophthalimide (THPI), 4,4′-dichlorobenzophenone (DCBP), and phthalimide (PI), respectively. Direct on-column injection (OCI) technique was evaluated as an alternative to avoid or minimize compound alteration during the analysis. The OCI inlet was operated in Btrack oven temperature and connected to a wide-bore deactivated guard column that itself connected to a capillary GC analytical column. Analytical standards spiked were injected in OCI, not observing any thermal degradation rate. This OCI approach was validated to citrate QuEChERS extracts of tomato, apple, and orange matrices for these four compounds and their corresponding transformation products (THPI, DCBP, and PI), evaluating recoveries, repeatability, linearity, and matrix effect. This set-up enabled the correct identification and quantitation for the most compounds at LOQs of 0.010 mg/kg in fruit and vegetable samples.

Ibrahim *et.al*. (2018) collected and tested 3 green leafy vegetable samples of pumpkin leaves, spinach leaves, and sorrel leaves from a farm in Akwanga for the presence of residues of organochlorine pesticides. The concentrations of pesticide residues in the vegetable samples were determined using GC/MS. ∂-BHC and ɣ- BHC were detected in pumpkin leaves (0.359 mg/kg and 0.647 mg/kg respectively). Among organochlorine pesticide p,p’-DDT was detected in pumpkin (0.75 mg/kg), spinach (0.319 mg/kg) and in sorrel (0.219 mg/kg). Dieldrin was detected in spinach & in sorrel (0.124 mg/kg and 0.053 mg/kg respectively). And endrin was detected in pumpkin (0.732 mg/kg) and aldrin in sorrel (0.095 mg/kg). All these values were above the maximum residue limit value of the pesticides. Endosulfan II was detected in sorrel (0.306 mg/kg) below the maximum residue limit (MRL).

Li *et. al.* (2018) conducted a research based on the quick, easy, cheap, effective, rugged, safe (QuEChERS) extraction combined with gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) and ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). That presented for the determination of 113 pesticides in green and ripe mangoes and different buffer systems and purification sorbents were optimized to get better recovery. Extracts from mangoes after acid acetonitrile (ACN) and ammonium acetate extraction were directly injected to UHPLC-MS/MS analysis, whereas other GC-amenable compounds were treated with cleanup approaches with a mixture of sorbents. Recoveries for the pesticides at spike levels of 10, 50, and 100 μg/kg were ranged between 70 and 120% with RSD values below 20%. Limits of detection (LODs) were below 4 μg/kg and limits of quantification (LOQs) were below 10 μg/kg. The matrix effect for 56 pesticides by GC-MS/MS analysis exhibited a non-significant matrix effect (≤ 25%) in green and ripe mangoes. For 57 pesticides used for UHPLC-MS/MS analysis, the optimized method in green mango provided no significant matrix effect (≤ 25%) for 63% of the compounds, whereas only for 16 and 21% compounds, the matrix effect was medium (25–40%) and strong (> 40%). The optimized method in ripe mango provided no significant matrix effect (≤ 25%) for 61% of the compounds, whereas only for 26 and 12% compounds, the matrix effect was medium (25–40%) and strong (> 40%). The application of GC-MS/MS and UHPLC-MS/MS coupled with the modified QuEChERS extraction.

l-Kurdi *et. al.* (2018) developed an easy method for the determination of Nemacur residues in cucumber fruits collected from the central markets and from farm. The method was based on extracting the active ingredients of Nemacur from commercial formulation and used as a standard material to calibrate the HPLC to determine Nemacur residues in cucumber fruits collected from the central market. More than 70% of the active ingredient was extracted from the commercial formulations. Strong positive association between concentration and peak area relationship was showed by standardization of HPLC with extracted materials. Mortality of tested organism (fish) was showed high by bioassay investigation. These demonstrated the effectiveness of extracted Nemacur to calibrate HPLC and in bioassay test. Nemacur residues in Cucumber fruits was collected from the market were below detection limit of HPLC, recovery % of Nemacur from control group of cucumber was above 80%. It can be concluded that the method was easily developed and validated by bioassay and chemo-assay.

Jian-Xun *et. al.* (2018) conducted a research through solid-phase extraction (SPE), whereby pesticides were extracted from fruit and vegetable substrates by using 40 mL 1% acetic acid in acetonitrile (v/v), purified by the Carbon/NH_2 SPE cartridge, and finally detected by GC-Q-TOF/MS, the rapid analysis of 439 pesticides in fruits and vegetables can be achieved. The methodology verification results was shown that more than 70 and 91% of pesticides, spiked in fruits and vegetables with concentrations of 10 and 100 μg/kg, respectively, recoveries that conform to the European Commission's criterion of between 70 and 120% with RSD ≤20%. 81% of pesticides had screening detection limits lower than 10 μg/kg, which made this a reliable analysis technology for the monitoring of pesticide residues in fruits and vegetables. This technology was validated for its characteristics of high precision, high speed, and high throughput through successful detection of 9817 samples during 2013–2015.

Prodhan and Alam (2018) 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 (GC) coupled with Electron Capture Detector (ECD) had been for the determination of 19 organochlorine pesticides (α- BHC, δ- BHC, β- BHC, γ- BHC, Heptachlor, Aldrin, Heptachlor Epoxide, γ- Chlordane, α- Chlordane, α- Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, β- Endosulfan, 4,4 DDT, Endosulfan sulphate, Methoxychlor, and Endrin Ketone) in shrimp. That method was validated by evaluating the accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ). The average recoveries of the selected pesticides was ranged from 84% to 106% with RSDr ≤ 14% in 4 fortification levels of 0.05, 0.1, 0.2 and 0.3 mg/kg. The linearity was ≥ 0.996 for all of the selected pesticides with matrix matched calibration standards. The LOD ranged from 0.003 to 0.009 mg kg-1 and the LOQ was 0.05 mg kg-1. That method was applied successfully for the residue analysis of 40 shrimp samples collected from different regions in Bangladesh.

Dimitrova *et. al.* (2018) conducted a research on development of analytical method for determination of organochlorine pesticides residues in meat by GC-ECD where introduced a new method that performs better analytical characteristics and allows more compounds to be determined, compared to the existing method used for determination of organochlorine pesticides in meat. In the national monitoring program a total of eight organochlorine pesticides had been analyzed: aldrin, heptachlorepoxide– trans isomer, HCH – α, β, γ isomers, p,p'-DDE, p,p'-DDD and p,p'-DDT, by means of a method based on EN 1528:2001– Fatty food – determination of pesticides and polychlorinated biphenyls (PCBs). Using that new method, it was possible 7 additional pesticides to be determined: dieldrin, endosulfan-α and β isomers, endosulfan sulfate, HCB, heptachlor, heptachlorepoxide– cis isomer. The method was based on accelerated solvent extraction technique, liquid – liquid partitioning with acetonitrile– n-hexane, clean-up step on a home-made silica gel column and GC-ECD determination of pesticides. The validation was performed according to the recommendations in the document SANTE/11945/2015 and it meets the acceptability criteria. The method was successfully used in laboratory proficiency test where OCPs included were HCH isomers - β (Z-score of rezult: 0.1), p,p'- DDE (Z-score of rezult: -0.6), Endosulfansulfate (Z-score of rezult: 0.5).

Miliadis *et. al.* (2018) conducted a research of pesticide residues analysis by LC-MS/MS. In total 128 pesticides that belong to 13 different chemical classes were analyzed. Carry over was found for 32 of the 128 studied pesticides (25%), at concentration levels between the LOD & 0.167 μg/mL. For 28, more than two injections of a blank sample were required, as to reduce the effect significantly. Compounds presenting carry over effect were mainly non-polar with logkow values between 4 and 7, characterized with low water solubility, between 0.001 mg/L and 2 mg/L. On the contrary, the vapor pressure did not seem to be obviously related to the effect.

Stachniuk *et. al.* (2017) undertook a research to evaluate pesticide residue contamination of fresh and frozen fruits and vegetables, agricultural raw material, and the estimation of the multiresidue method effectiveness expressed as the proportion of pesticides detected in food samples to the total number of pesticides analyzed by multiresidue methods. Total 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 were detected in 46 samples. The percentage of samples with residues above the maximum residue levels was 15%, whereas samples with residues below MRL were 17%. A total of 13 samples contained more than 1 pesticide residue. Pesticide residues were detected in samples of black currants (50%), broccoli (36.4%), raspberries (29%) and red currants (21.8%). Carbendazim and acetamiprid were most frequently detected pesticides. The proportion was below 50% for methods developed for the analysis of less than 100 pesticides, and below 30% for methods developed for the analysis of more than 100 pesticides.

Mebdoua *et.al.* (2017) conducted a research on evaluation of pesticide residues in fruits and vegetables where a total of 160 samples of 13 types of fresh fruits and vegetables from domestic production and import were analysed to detect the presence of pesticide residues. Residue 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 (MRLs). Risk assessment for long-term exposure was done for all pesticides detected. Except chlorpyrifos and lambda-cyhalothrin, exposure to pesticides was below 1% of the acceptable daily intake. Short-term exposure assessment revealed that in 7 pesticide/commodity combinations, including three pesticides (chlorpyrifos, deltamethrin and lambda-cyhalothrin), the acute reference dose had been exceeded.

lhag *et. al.* (2017) undertook a research on multi-residue analysis of organophosphorus pesticides in cucumbers, potatoes and tomatoes. The samples were minced in a food processor using solid phase extraction (SPE) column. Separation was performed on a Gas Chromatography capillary column (Rtx-5 ms-30 m × 0.25 mm × 0.25 µm). The sample was injected by using splitless mode, helium as the carrier gas followed quadrupole mass spectrometry detection. The mobile phase flow rates, column temperatures, and MS para-meters were all optimized to reach high sensitivity & selectivity. 7 pesticides were detected in tomatoes sample, 6 in cucumber and 4 in potato samples.

Gabrieli *et al.,* (2016) developed an easy and efficient sample preparation method for determination of natamycin residues in wine samples by solvent extraction and ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) analysis was developed. Different amounts of C_{18} and primary secondary amine (PSA) sorbents in order to optimize the clean-up step with the extraction solvent acetonitrile was observed. Validation results were satisfactory. The method was applied in 10 Brazilian wine samples and no residues were detected.

Marilia *et al.* (2016) developed a method based on the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for simultaneous determination of 39 pesticides and 2 plant growth regulators in soy-based beverages by ultra-highperformance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The clean-up procedure was established based on central composite design (CCD), using response surface methodology (RSM). For optimization of the clean-up step, different amounts of sorbents in dispersive solid phase extraction (d-SPE) were evaluated. Validation results were satisfactory, considering that the optimized method presented recoveries between 70 and 112 %, with RSD lower than 19 % for spike levels between 10 and 50 μg L−1. The method limit of detection (LOD) and quantification (LOQ) ranged from 3 to 8 and 10 to 25 μg L−1, respectively. The proposed method combines the advantages of QuEChERS and UHPLC-MS/MS and proved to be compatible for the pesticide multi-residue determination in soy-based beverages in routine laboratory analyses.

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

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

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

Biziuk and Stocka (2015) conducted a research on ‘‘Multi Residue Methods for Determination of Currently used Pesticides in Fruits and Vegetables Using QuEChERS Technique’. They discussed the extraction and determination of pesticide residues in fruit and vegetable samples, as the techniques most commonly used in these processes. They outlined the difficulties occurring at each stage in the analytical procedure.

Portoles *et al.* (2015) developed a method for the detection of pesticides in fruit and vegetables based on gas chromatography coupled to a hybrid quadrupole time-of-flight mass spectrometer with an atmospheric pressure chemical ionization source (GC-(APCI) QTOF MS). A non-target acquisition was performed through 2 alternating scan events: one at low collision energy and another at higher collision energy ramp (MS (E)). In this way, both protonated molecule and/or molecular ion together with fragment ions were obtained in a single run. Validation was accomplished according to SANCO/12571/2013 by analysing 20 samples (10 different commodities in duplicate), fortified with a test set of 132 pesticides at 0.01, 0.05 and 0.20mg kg^{-1}. For screening, the detection was based on 1 diagnostic ion (in most cases the protonated molecule). Overall, at the 0.01mg kg^{-1} level, 89% of the 2620 fortifications made were detected. The screening detection limit for individual pesticides was 0.01mg kg^{-1} for 77% of the pesticides investigated. The possibilities for identification according to the SANCO criteria, requiring 2 ions with a mass accuracy ≤ ±5 ppm and an ion-ratio deviation ≤ ±30%, were investigated. At the 0.01mg kg^{-1} level, identification was possible for 70% of the pesticides detected during screening. This increased to 87% and 93% @ 0.05 and 0.20mg/kg level, respectively. Insufficient sensitivity for the 2nd ion was the main reason for the inability to identify detected pesticides, followed by deviations in mass accuracy and ion ratios.

Prodhan *et al.* (2015a) determine seven insecticides (chlorpyrifos, dimethoate, deltamethrin, thiamethoxam, thiacloprid, pirimicarb and indoxacarb) and three fungicides (azoxystrobin, fluopicolide and propamocarb hydrochloride) in 122 fresh melon samples which was collected from different market places in Thessaloniki, Greece. They found the average recoveries of the selected pesticides ranged from 82% to 106% with RSDr ≤6% in 4 fortification levels of 0.01, 0.05, 0.1 and 0.2 mg/kg and the correlation coefficient (R2) was ≥0.997 for all the selected pesticides the limit of detection (LOD) values ranged from 0.001 to 0.003 mg/kg, and the limit of quantification (LOQ) was determined at 0.01 mg/kg for all the analytes. Among the 122 analysed samples, 32 (26% of the total no. of samples) were found to have residues.

Lorenz *et. al.* (2014) proposed to investigate the level of pesticides residues clorpyrifos, dimethoate, malathion, mevinphos, pedimentalina, simazine and trifluralin in apple, strawberries and tomatoes produced in Santa Catarina state, South of Brazil. The process involved a 2 level full factorial and Doehlert designs followed by validation of the modified method and its application in 91 real samples collected during the years 2010 and 2011. Modifications in the method increased detectability and satisfactory values of linearity, accuracy and precision. In real samples, residues of at least 1 pesticide (≥ LOQ) were found in 8 (27.6%) apple, 12 (40.0%) strawberry and 17 (53.1%) tomato samples. The results reported the necessity of continuous monitoring for pesticide use in fruits and vegetables.

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. 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.

Chandra *et. al.* (2012) developed a gas chromatography-mass spectrometry method for the determination of pesticide residues monocrotophos, chlorpyriphos and endosulfan in cauliflower and capsicum. Samples were extracted with ethyl acetate, cleaned up and purified through solid-phase extraction with Florisil and activated charcoal. Experiments on 2 fortification concentrations are carried out and the limits of detection are 0.005, 0.007 and 0.002 mg kg^{-1} for monocrotophos, chlorpyriphos and endosulfan respectively. The average recoveries of pesticide residues are 89.0 to 110.0 % in cauliflower and capsicum samples respectively.

Stephen and Lam (2012) reported a novel approach for the detection, confirmation, and quantification of 15 selected pyrethroid pesticides, including pyrethins, and two metabolites of dithiocarbamates in foods by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS–MS). The proposed method makes use of a modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure that combines isolation of the pesticides and sample cleanup in a single step. Analysis of pyrethroids and dithiocarbamate metabolites was performed by UPLC–MS–MS operated with electrospray and atmospheric pressure chemical ionization, respectively. 2 specific precursor–product ion transitions were obtained per target compound in multiple reactions monitoring (MRM) mode. Such acquisition achieved the minimum number of identification points according to European Commission (EC) document no. SANCO/10684/2009, thus fulfilling the European Commission (EC) point system requirement for identification of contaminants in samples. The method was affirmed with a variety of food samples. Calibration curves were linear and covered from 1 to 800 µg per kg in the sample for all target compounds. Average recoveries, measured at mass fractions of 10 and 100 µg per kg for pyrethroids and 5 and 50 µg per kg for dithiocarbamate metabolites, were in the range of 70–120% for all target compounds with relative standard deviations below 20%. Method limits of quantification (MLOQ) were 10 µg per kg and 5 µg per kg for pyrethroids and dithiocarbamate metabolites, respectively. The method has been successfully used to analysis of 600 food samples in the course of the first Hong Kong total diet study with pyrethroids and metabolites of dithiocarbamates being the pesticides determined.

Zhao *et. al.* (2012) developed a modified quick, easy, cheap, effective, rugged and safe (QuEChERS) method with multi-walled carbon nanotubes (MWCNTs) as a reversed-dispersive solid-phase extraction (r-DSPE) material combined with gas chromatography-mass spectrometry for the determination of fourteen pesticides in complex matrices. 4 vegetables (leek, onion, ginger and garlic) were selected as the complex matrices for validating this method. This technique involved the acetonitrile-based sample preparation and MWCNTs were used as r-DSPE material in the cleanup step. It investigated two important parameters influencing the MWCNTs efficiency, the external diameters and the amount of MWCNTs used. Under the optimized conditions, recoveries of 78–110% were obtained for the target analytes in the complex matrices at 2 concentration levels of 0.02 and 0.2 mg/kg. The RSD values ranged from 1 to 13%. LOQs and LODs for fourteen pesticides ranged from 2 to 20 mg/kg and from 1 to 6 mg/kg, respectively.

Xie *et. al.* (2011) reported a new sensitive multi-residue liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for detection, confirmation and quantification of 6 neonicotinoid pesticides (dinotefuran, thiamethoxam, clothiandin, imidacloprid, acetamiprid and thiacloprid) in agricultural samples (chestnut, shallot, ginger and tea). For cleaning up the extracts, activated carbon and HLB solid-phase extraction cartridges were used. Analysis was performed by LC–MS/MS operated in the multiple reaction monitoring (MRM) mode, acquiring 2 specific precursor-product ion transitions per target compound. Quantification was carried by the standard method with D_4 -labeled imidacloprid. The method showed excellent linearity ($R_2 \geq 0.9991$) and precision (relative standard deviation, RSD $\leq$ 8.6%). Limits of quantification (LOQs) were 0.01 mg kg^{-1} for chestnut, shallot, ginger sample and 0.02 mg kg^{-1} for tea. The average recoveries, measured at 3 concentrations levels (0.01 mg kg^{-1}, 0.02 mg kg^{-1} and 0.1 mg kg^{-1} for chestnut, shallot, ginger sample, 0.02 mg kg^{-1}, 0.04 mg kg^{-1} and 0.2 mg kg^{-1} for tea sample), were in the range 82.1–108.5%. The method was validated for analysis of 150 agricultural samples (chestnut, shallot, ginger and tea). Imidacloprid and acetamiprid were detected at concentration ranging from 0.05 to 3.6 mg kg^{-1}.

Sanyal *et al.* (2011) developed and validated simple and efficient multi-residue analytical methods for the determination of thirteen organochlorine and seventeen organophosphorous pesticides from soil, spinach and eggplant. For sample preparations, techniques namely accelerated solvent extraction and dispersive SPE were used. The recovery studies were carried out by spiking the samples at 3 concentration levels (one limit of quantification (LOQ), 5 LOQ, and 10 LOQ). The methods were subjected to thorough validation procedure. The mean recovery for soil, spinach & eggplant were in the range of 70–120% with median CV (%) below 10%. The total uncertainty was evaluated taking four main independent sources i.e. weighing, purity of the standard, GC calibration curve and repeatability under consideration. For most pesticides the expanded uncertainty was well below 10% and the rest fell in the range of 10–20%.

Wang *et. al.* (2011) developed and validated a modified QuEChERS method for analysis of pyrimorph residue in tomatoes, cucumbers and soil residue dynamics and final residues in greenhouse vegetables and soil. At fortification levels of 0.05, 0.1 and 1 mg kg^{-1} in tomatoes, cucumbers and soil method got recoveries ranged from 86.1% to 99.3% with relative standard deviations of 1.0%–7.7%, in agreement with directives for method validation in residue analysis. The limit of determination was 0.05 mg kg^{-1}. The proposed method was successfully applied for the determination of pyrimorph residue levels and dissipation rates in vegetables and soil. At 6 experimental sites, pyrimorph residues in tomatoes and cucumbers showed relatively fast dissipation rates, with half-lives of 5.8–7.7 days and 5.7–7.1 days respectively. Half-lives of pyrimorph in soil was 8.5–11.0 days. The final residues of pyrimorph in tomatoes ranged from 0.19 to 3.66 mg kg^{-1}, 0.18 to 4.35 mg kg^{-1} in cucumbers and 0.22 to 16.5 mg kg-1 in soil with pre-harvest interval of three to seven days. 5 mg kg^{-1} was proposed as MRL of pyrimorph in tomatoes and cucumbers.

Guan *et. al.* (2010) developed simple and rapid methods for the analysis of pesticides in produce samples. The current study involved analysis of nonpolar and polar pesticides using GC-ECD and GC-NPD, respectively. By using disposable pipette extraction (DPX) the nonpolar pesticides were extracted following a preliminary acetone extraction based on the Luke method. Recoveries of the fortified pesticides ranged between 70.5 and 110.0%, with relative standard deviations below 10% for most target analytes. The limits of detection (LOD) were determined to be <0.05 ppm for most studied pesticides with coefficients of determination (r_2) >0.99. A micro-Luke method was used for the analysis of polar pesticides, which greatly reduces the volumes of solvent and time for sample preparation. This new method of analysis, combining DPX and micro-Luke, was compared directly with the modified Luke method for analysis of pesticides in produce samples over a nine weeks period. This study validated the suitability for routine analysis of pesticides in acetone extract of fruits and vegetables using the proposed DPX and micro-Luke method.

Chandra (2010) reported the determination of pesticide residue in cauliflower and brinjal. The use of pesticides in agriculture concern of residue accumulation, which may remain in food and agricultural environment causing human health and damaging ecological balance. Cauliflower and brinjal were purchased from local market and analysed for residual contents of chlorpyrifos and cypermethrin. The pesticide residues were extracted from the cauliflower and brinjal with ethyl acetate and cleaned-up with ethyl acetate and hexane (3:7 v/v) mixture using florisil and charcoal column and determination was perform on GC-ECD. Recoveries of residues are over 90 % with coefficient of variation below 5%. This method is suitable for the analysis of in cauliflower and brinjal with high sensitivity and accuracy.

Wang *et. al.* (2010) developed the applications of liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) and ultra-high-pressure liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC QqTOF MS) for the determination of 148 pesticides in fruits & vegetables. Pesticides were extracted from fruits and vegetables using QuEChERS method. Quantification was achieved by using matrix-matched standard calibration curves with isotopically labeled standards or a chemical analog as internal standards in an analytical range from 5 to 500 µg/kg. The method performance parameters including overall recovery, intermediate precision and measurement uncertainty were evaluated according to statistically designed experiment, i.e., a nested design. For LC/ESI-MS/MS, 95% of the pesticides had recoveries between 81% & 110%; 97% had an intermediate precision ≤20%; and 95% (in fruits) or 93% (in vegetables) showed measurement uncertainty ≤40%. Compared to LC/ESI-MS/MS, UHPLC QqTOF MS showed a relatively poor repeatability & large measurement uncertainty. About 93% (in fruits) or 94%(in vegetables) of the pesticides had recoveries between 81% and 110%; 86% (in fruits) or 90% (in vegetables) had an intermediate precision ≤20%; and 79% (in fruits) or 88% (in vegetables) showed measurement uncertainty ≤40%. LC/ESI-MS/MS proved to be the 1st choice for quantification or pre-target analysis due to its superior sensitivity and good repeatability. UHPLC QqTOF MS provided accurate measurement and isotopic patterns and was an ideal tool for post-target screening and confirmation.

Osman *et al.* (2010) conducted a research where a total of twenty three pesticides from different chemical groups in 160 different domestic vegetables collected from 4 major big supermarkets located in Al-Qassim region, Saudi Arabia, were identified by gas chromatography with mass spectrometry (GC–MS). Residues were found in 89 of the 160 samples and 53 samples were above the maximum residue levels. The most frequent pesticides found were carbaryl followed by biphenyl and then carbofuran. Cabbage was the most positive and violated maximum residue levels (16 & 11 samples), followed by carrot and green pepper (12 & 7 samples), cucumber (12 & 6 samples), egg-plant (12 & 5 samples), squash (11 & 7 samples), lettuce (11 & 6 samples) and tomato (11 & 4 samples). The highest concentrations found in lettuce (ethiofencarb, 7.648), followed by tomato (tolclofos-methyl, 7.312 mg/kg), cabbage (chlropyrifos, 6.207 mg/kg), carrot (heptanophos, 3.267 mg/kg), green pepper (carbaryl, 2.228 mg/kg) and egg-plant (carbaryl, 1.917 mg/kg).

Rahman *et al.* (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 maximum residue limit (MRL). Among 38 analyzed brinjal samples, 18 samples contained residue of Cypermethrin, Diazinon and Quinalphos; and only 8 had above maximum residue limit (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 maximum residue limit (MRL). Of 16 yard long bean samples, 8 samples contained residue of Cypermethrin, Diazinon, Quinalphos and Malathion; and only 2 had above MRL.

Zhang *et. al.* (2010) carried out a research on “Analysis of agricultural residues on tea using d-SPE sample preparation with GC-NCI-MS and UHPLC-MS/MS” that presented new sample preparation and analytical procedures for the quantification of pesticides on processed tea leaves. This method includes extraction and dispersive solid phase extraction (d-SPE) to prepare gas chromatography (GC) and ultrahigh-performance liquid chromatography (UHPLC)-ready samples, providing a fast and cost-effective solution for time-sensitive industrial analysis to fulfill regulatory requirements. Both GC-negative chemical ionization mass spectrometry (GC-NCI-MS) and UHPLC-tandem mass spectrometry (UHPLC-MS/MS) employed to produce highly sensitive and reproducible data. Excellent limits of detection (typically below 1 μg/kg for GC and 10 μg/kg for UHPLC), wide linearity ranges, and good recoveries (mostly >70%) achieved on the selected pesticides. 27 tea samples purchased from local grocery stores were analyzed using the newly developed methods. Endosulfan sulfate and kelthane were the most frequently detected by GC-NCI-MS and imidacloprid and acetamiprid by UHPLC-MS/MS in these teas. The samples were found to be clean, with <1 mg/kg of total pesticide residues. The organic-labeled teas were cleaner than nonorganic ones.

Banerjee *et. al.* (2009) optimized and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based method for the multi residue analysis of 87 pesticides in mango at the ≤ 10 ng g(-1) level. The method involved extraction of ten g of homogenized mango samples (+10 mL of water + 1 g of sodium acetate + 10 g of sodium sulfate) with 10 mL of ethyl acetate; cleanup by dispersive solid-phase extraction with a combination of primary secondary amine (PSA, 50 mg), graphitized carbon black (GCB, 25 mg), and anhydrous sodium sulfate (150 mg). Final estimation by LC-MS/MS with multiple reaction monitoring. Direct analysis resulted in significant suppression in ionization of the majority of the test compounds over electrospray ionization probe. However, clean up with the above combination of PSA + GCB reduced the matrix-induced signal suppressions and the signals in the cleaned extracts were nearly equivalent to the corresponding solvent standards. Also substitution of PSA with florisil gave equivalent clean up effects. The method was quite rugged and evident from a low Horwitz ratio (mostly <0.5) and low measurement uncertainties at 10 ng g(-1). The limit of quantification was <10 ng g(-1) for all the pesticides with recoveries within 70-120% for most pesticides even at 2.5 ng g(-1). The method offers a effective, sensitive, cheaper, and safer alternative to the existing methods of multiresidue analysis.

Moreno *et. al*. (2008) developed and validated a new multiresidue method for the determination of more than 140 pesticide residues in cucumber and orange by gas chromatography coupled to triple quadrupole mass spectrometry (GC–QqQ-MS/MS) in a single run of 25.50 minute. The triple quadrupole (QqQ) analyzer simultaneously operated in the selected reaction monitoring (SRM) and selected ion monitoring (SIM) modes, acquiring 2 or 3 transitions per compound. Samples were extracted by application of a single-phase extraction of 10 g of sample with acetonitrile containing 1% of acetic acid, followed by a liquid–liquid partition formed by the addition of 4 g of $MgSO_4$ and 1 g of NaOAc. A dispersive solid-phase extraction (D-SPE) with primary secondary amine was applied to clean up the extracts. A final concentration step included in order to increase sensitivity in the instrumental analysis. The method validated in each matrix in a wide dynamic range (10–400 µg kg^{-1}): this work relies on a new quantification strategy by the use of 2 calibration curves to increase the dynamic range, which permitted the reduction of sample dilutions and increase in sample throughput. The recovery was studied at 3 concentration levels (11.5, 50.0, and 150.0 µg kg^{-1}), yielding values in the range 70–110% with precision values, expressed as relative standard deviation (RSD), lower than 20 and 25% for intraday and interday precision, respectively. Limits of quantification (LOQs) were established at 10 µg kg^{-1}, the lowest maximum residue level value set by the European Union in vegetables. The method was applied to the analysis of pesticide residues in real samples from the southeastern Spain.

Walorczyk (2008) undertook a research which described the extension and revalidation of a previously published multi-residue method to currently 140 pesticides and 4 pesticide degradation products in cereals and feedingstuffs. The pesticides were extracted using QuEChERS ("quick, easy, cheap, rugged, effective and safe") method and then cleaned up using dispersive solid-phase extraction with Bondesil PSA and C_{18} sorbents, and optionally by a freezing-out clean-up step. The extracts were analyzed in a single injection gas chromatographic-tandem quadrupole mass spectrometric (GC–MS/MS) acquisition method. Thorough optimization of the GC–MS/MS acquisition conditions and application of an effective clean-up procedure resulted in a remarkable enhancement of validation parameters. The linearity of the calibration curves was excellent in matrix-matched standards, and yielded the coefficients of determination (R_2) ≥ 0.99 for approximately 96% of target analytes. Average recoveries of pesticides spiked at 0.01 mg kg^{-1} into a feed mixture and wheat grain were in the range 70–120% with associated RSD values $\leq$20% for approximately 60% and 67% of the compounds, respectively. At higher spiking levels of 0.05, 0.1 and 0.5 mg kg^{-1} average recoveries and RSDs readily met the validation criteria for all the studied pesticides. Based on results, the proposed approach has been proven to be highly efficient and suitable for determinations of multi-class pesticides in a range of cereal and related matrices. Up to now, 145 samples of matrices including cereals grain, bran, whole ears, straw, hay, feed mixtures and other samples such as malt, starch and dry vegetables have been analyzed. 15 different pesticides have been detected, among which pirimiphos methyl (19 cases), deltamethrin (14 cases), tolylfluanid (5 cases), dichlofluanid (5 cases), and tebuconazole (4 cases) were the most frequently encountered ones.

Garrido Frenich *et al.* (2008) developed a rapid, simple, and sensitive multiresidue method for analysis of 53 pesticides in fruit and vegetables by ultra-performance liquid chromatography (UPLC) coupled to triple-quadruple tandem mass spectrometry (MS-MS). Before analysis, analytes were extracted by use of buffered QuEChERS (quick, easy, cheap, effective, rugged, and safe) methodology without further cleanup for non-fatty matrices. Chromatographic conditions were optimized in order to achieve fast separation in multiple reactions monitoring (MRM) mode. Indeed, more than 50 pesticides can be separated in less than ten minute. 4 common representative matrices (cucumber, orange, strawberry, and olive) were selected to investigate the effect of different matrices on recovery and precision. Mean recoveries ranged from 70 to 109% with relative standard deviations lower than 20% for all pesticides examined in the 4 selected matrices. The method has been applied to the analysis of 200 vegetable samples, and imidacloprid was the pesticide most frequently found, with concentrations ranging from 0.01 to 1.00 mg per kg. This methodology combines the advantages of both QuEChERS and UPLC-MS-MS producing a very rapid, sensitive, and reliable procedure which can be applied in routine analytical laboratories.

Poulsen *et. al.* (2007) investigated the differences in residue pattern between Italy and South Africa, the main exporters of table grapes to the Danish market. The results showed no major differences with respect to number of samples with residues, with residues being found in 54–78% of the samples. Exceedances of the European Union maximum residue limit were found in 5 samples from Italy. A number of samples rinsed to study the possible reduction of residues. For copper, iprodione, procymidone and dithiocarbamates a significant effect was found (20–49% reduction of residues). However, no significant effect was found for organophosphorus pesticides and pyrethroids, whereas number of samples with residues of benzilates, phenylamids and triazoles was insufficient to demonstrate any significant effects. An intake calculation showed the average intake from Italian grapes was 3.9 μg day^{-1} for pesticides and 21 μg day^{-1} 1 for copper. The intakes from South African grapes were 2.6 and 5.7 μg day^{-1}, respectively. The exposure were approximately 0.5% for Italian samples and 1% for South African samples, when the total exposure of pesticides from grapes were related to acceptable daily intake, expressed as the sum of Hazard Quotients.

Fenoll *et. al.* (2007) developed an analytical multiresidue method for the simultaneous determination of various classes of pesticides in vegetables (pepper and tomato). Samples are extracted with acetone and the pesticides are partitioned into ethyl acetate/cyclohexane. The final determination was made by gas chromatography with nitrogen–phosphorus detection. Confirmation analysis of pesticides carried out by the gas chromatography coupled with mass spectrometry in the selected ion monitoring

(SIM) mode. The identification of compounds was based on retention time and on comparison of the primary and secondary ions. Recovery studies were performed at 0.05, 0.1 & 0.02 mg kg^{-1} fortification levels of each compound and the recoveries obtained ranged from 70.1% to 128.5% with relative standard deviations lower than 7%. The method was showed good linearity over the range assayed 50–1500 μg kg^{-1} and the detection and quantification limits for the pesticides studied varied from 0.1 to 4.4 μg kg^{-1} and 0.4 to 14.5 μg kg^{-1}, respectively. The proposed method used to determine pesticides levels in peppers and tomatoes grown in experimental greenhouses.

Tseng *et. al.* (2007) developed a fast and easy multiresidue method using the macroporous diatomaceous earth (MDE) column for determining 136 pesticide and metabolite residues in fruits and vegetables. MDE column was used instead of the separation funnel for liquid/liquid partition in sample preparation. Through columns, the preparation of test solution became easier and faster than traditional funnel liquid/liquid partition. The emulsion problem also be avoided. 51 pesticides including acephate were determined by gas chromatography (GC) with a pulsed flame photometric detector (GC/PFPD) and 63 pesticides including aldrin were detected by GC with electron capture detection. 20 carbamate peticides and metabolites including aldicarb were determined by high performance liquid chromatography with a post-column derivaization system and a fluorescence detector (HPLC/FLD) and 2 benzimidazole pesticides including carbendazim and thiabendazole were detected by HPLC with an ultra-violet detector (HPLC/UV). The recovery studies were performed by spiking pesticides (0.05~2.0 ppm) in fruit and vegetable samples, and the results showed satisfactory recoveries and repeatability. The limits ranged from 0.003 to 0.2 ppm. The developed multireside method can be employed to the other pesticides or matrices with an easier and less solvent consumption way to prepare sample solution. It is useful and an environment-friendly method for routine pesticide analysis.

Amvrazi and Albanis (2006) undertook a research on the “Multiresidue Method for Determination of 35 Pesticides in Virgin Olive Oil by Using Liquid−Liquid Extraction Techniques Coupled with Solid-Phase Extraction Clean Up and Gas Chromatography with Nitrogen Phosphorus Detection and Electron Capture Detection”. 3 liquid-liquid extraction (LLE) procedures based on (i) partition of pesticides between acetonitrile and oil solution in n-hexane, (ii) partition of pesticides between saturated ACN with n-hexane and oil solution in n-hexane saturated with ACN, and (iii) partition of pesticides between ACN and oil were tested for optimization of the highest pesticide recoveries with the lowest oil residue in the final extracts. Experimental tests were preformed to study the efficiency of different clean up procedures with N-Alumina, Florisil, C18, and ENVI-Carb solid-phase extraction (SPE) cartridges for the compounds analyzed by GC-nitrogen phosphorus detection. A 2nd step of clean up was also performed for the compounds analyzed by GC-electron capture detection (ECD), by using phenyl-bonded silica (Ph), diol-bonded silica (Diol), cyanopropyl-bonded silica (CN), and amino propyl-bonded silica (NH_2) SPE cartridges. LLE of the oil solution in hexane followed by an ENVI-Carb SPE clean-up of the extract gave the best results for all target compounds. The ACN extract additionally cleaned through a Diol-SPE cartridge for the determination of pesticides analyzed mainly by GC-ECD. Pesticide recoveries form virgin olive oil spiked with 20, 100, and 500 mg/kg concentrations of pesticides ranged from 70.9 to 107.4%. The method featured good sensitivity, pesticide quantification limits were low enough, and the precision, expressed as relative standard deviation, ranged from 2.4 to 12.0%. The method was applied successfully for the residue determination of the selected pesticides in commercial olive oil samples.

Lehotay (2005) conducted validation experiments of a simple, fast, and inexpensive method for the determination of 229 pesticides fortified at 10–100 ng/g in lettuce and orange matrixes. The method is known as quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for pesticide residues in foods. The procedure involved the extraction of a 15 g sample with 15 mL acetonitrile, followed by liquid–liquid partitioning step performed by adding 6 g anhydrous $MgSO_4$ plus 1.5 g NaCl. After centrifugation, the extract was decanted into a tube containing 300 mg primary secondary amine (PSA) sorbent plus 1.8 g anhydrous $MgSO_4$, which constituted a cleanup procedure called dispersive solid-phase extraction. After a 2nd shaking and centrifugation step, the acetonitrile extract was transferred to autosampler vials for concurrent analysis by gas chromatography/mass spectrometry with an ion trap instrument and liquid chromatography/tandem mass spectrometry with a triple quadrupole instrument using electrospray ionization. Each analytical method was designed to analyze 144 pesticides. Recoveries for all but 11 of the analytes in at least

1 of the matrixes were between 70–120% (90–110% for 206 pesticides), and repeatabilities typically <10%were achieved for a wide range of fortified pesticides, including methamidophos, spinosad, imidacloprid, and imazalil. Dispersive SPE with primary secondary amine (PSA) retained carboxylic acids (e.g., daminozide), and <50% recoveries were obtained for asulam, pyridate, dicofol, thiram, and chlorothalonil. Many actual samples and proficiency test samples were analyzed by this method, and the results compared favorably with those from traditional methods.

Boulaid *et al*. (2005) carried out a research on the "Effect of household processing and unit-to-unit variability of pyrifenox, pyridaben, and tralomethrin residues in tomatoes" where the residue levels of pyrifenox, pyridaben, and tralomethrin were determined in unprocessed and processed tomatoes, grown in an experimental greenhouse, to evaluate the effect of 3 different household processes (washing, peeling, and cooking) and the "unit to unit" variability of these pesticides in tomatoes. The study was carried out on 11 greenhouse tomato samples collected during a five week period in which 2 successive treatments with the studied pesticides were applied. Residue levels in tomato samples were determined by means of ethyl acetate extraction and gas chromatography-electron capture detection determination. The washing processing factor results were 0.9±0.3 for pyridaben, 1.1±0.3 for pyrifenox, and 1.2±0.5 for tralomethrin, whereas the peeling processing factors were 0.3 ±0.2 for pyridaben and 0.0±0.0 for both pyrifenox and tralomethrin. The average loss of water in pure tomato samples during cooking process was approximately 50%; the cooking processing factors were 2.1±0.8 for pyridaben, 3.0±1.1 for pyrifenox, and 1.9±0.8 for tralomethrin. The unit-to-unit variability factors were determined on 3 different greenhouse samples analyzing ten different units of unprocessed tomatoes from each sample. In all cases, the results of variability factor of the unit-to-unit were within the range of 1.3-2.2.

Hetherton *et. al.* (2004) developed a multi-residue screening method for the simultaneous analysis of 73 pesticides and their metabolites using high-performance liquid chromatography coupled with electrospray tandem mass spectrometry. Pesticides were determined under a single set of experimental conditions involving a simple acetonitrile extraction without the requirement for a clean-up step. Validation was achieved at 0.01 and 0.1 mg/kg levels in apple, lettuce and orange. Recoveries were in the range 77–124% for majority of pesticides.

Jansson *et. al*. (2004) presented a new multi-residue method for determination of pesticide residues in a wide variety of fruit and vegetables, using the National Food Administration (NFA) ethyl acetate extraction and determination by means of LC–MS/MS. This method includes pesticides normally detected by LC–UV or LC-fluorescence such as benzimidazoles, carbamates, N-methylcarbamates and organophosphorus compounds with an oxidisable sulphide group as well. After extraction with ethyl acetate, the extract was concentrated and an aliquot of the extract was evaporated to dryness and redissolved in methanol before injection on LC–MS/MS. The method had been validated for 57 different pesticides and metabolites. Representative species from different commodity groups were chosen as matrices to study the influence from different matrices on recoveries. The fortification levels studied were 0.01–0.5 mg/kg. They tested for all matrices by means of standard addition to blank extracts. The matrix effect, expressed as signal in solvent compared to signal in matrix, was in general found to be small. The obtained recoveries are in the range 70–100%. The proposed method was quick and straightforward and no additional clean-up steps are needed. The method can be used for the analysis of all 57 pesticides in 1 single determination step at 0.01 mg/kg.

Kontou *et. al*. (2004) undertook a research on “Stability of the dithiocarbamate pesticide maneb in tomato homogenates during cold storage and thermal processing, Food Additives and Contaminants” where the effect of storage at 5°C and of thermal processing by cooking at 100°C and sterilization at 121°C for 15 minute on maneb residues in tomato homogenates was investigated. Remaining maneb and its toxic metabolite ethylenethiourea (ETU) were measured after each treatment by headspace gas chromatography (GC) with flame-photometric detection and by high-performance liquid chromatography with photo-diode array detection, respectively. No significant loss of maneb was observed during cold storage for up to six weeks, taking into account analytical variability. Conversely, thermal treatment resulted in substantial degradation of maneb with extensive conversion to ethylenethiourea After cooking, only 26 ± 1% (± SE, n ¼ 8) of initial maneb residues remained in the samples, while the conversion to ETU was 28±1% (molmol^{-1})(±SE, n ¼ 4).

Martınez Vidal *et. al.* (2004) developed and validated a gas chromatographic–tandem mass spectrometric (GC–MS–MS) method for analysis of 30 multi-class pesticide residues in 4 vegetables (cucumber, marrow, pepper, and eggplant) and 2 fruits (melon and watermelon). The performance characteristics i.e. linearity, detection limit (LOD), quantification limit (LOQ), precision, and recovery were studied. The effect of the matrix on the calibration step was studied by comparing calibration curves and recoveries for food commodity. 2 different food commodities, cucumber and watermelon, were selected as potential reference matrixes for the target vegetables and fruits.

Anastassiades and Lehotay (2003) introduced a simple, fast, and inexpensive method for determination of pesticide residues in fruits and veg-etables. The procedure involves initial single phase extraction of 10 g sample with 10 mL acetonitrile (ACN), followed by liquid–liquid parti-tioning formed by addition of 4 g anhydrous MgSO4 plus one g NaCl. Removal of residual water and cleanup are performed simultaneously by using a rapid procedure called dispersive solid-phase extraction, in which 150 mg anhydrous $MgSO_4$ and 25 mg primary secondary amine (PSA) sorbent are simply mixed with 1 mL acetonitrile (ACN) extract. The dispersive-SPE with PSA effectively removes many polar matrix components, such as organic acids, certain polar pigments, sugars to some extent from the food extracts. Gas chromatography/mass spectrometry was used for quantitative and confirmatory analysis of GC-amenable pesticides. Recoveries between 85 and 101% (mostly >95%) and repeatabilities typically <5% had been achieved for a wide range of fortified pesticides, including very polar and basic compounds such as methamidophos, acephate, omethoate, imazalil, and thiabendazole. By using this method, a single chemist can prepare a batch of 6 previously chopped samples in <30 minute.

Stajnbaher and Zupancic-Kralj (2003) developed a multiresidue method for analysis of 90 pesticides with different physicochemical properties in fruits & vegetables. The method involves a rapid small-scale extraction procedure with acetone using vortex mixing. Solid-phase extraction (SPE) on a highly cross-linked polystyrene divinylbenzene column (LiChrolut EN) was used for clean-up and pre-concentration of the pesticides from the water-diluted acetone extracts. For most fruit & vegetable samples this partial clean-up was sufficient, but some of them with more co-extracting substances need further clean-up (cereals, spinach, carrots, etc.). Diethylaminopropyl modified silica was used for efficient removal of interferences caused by various organic acids, sugars, etc. The pesticide residues determined by gas chromatography with a mass selective detector (GC-MS). The majority of pesticide recoveries for various fruits and vegetables were >80% in the concentration range from 0.01 to 0.50 mg/kg, except for most polar pesticides (methamidophos, acephate, omethoate) which couldn't be determined by this method. The limit of quantitation for most pesticides was 0.01 mg kg^{-1} with majority of relative standard deviations (R.S.D.s) below 10%.

Valverde *et. al.* (2002) determined residue levels of pyridaben and tralomethrin in peppers grown in an experimental greenhouse, during a four week period in which up to 4 successive treatments with both pesticides were applied. In all cases, plants were sprayed with a mixture of pyridaben & tralomethrin at application rates of active ingredients of 140 and 36 g/ha, respectively. Sampling carried out at 1, 3, 7, 8, 10, or 14 days after each application, simulating the typical harvesting practices in greenhouse plantations. Residue levels of pyridaben and tralomethrin were determined by using ethyl acetate extraction and GC-ECD. During study, residue levels in the plantation ranged between 0.22 and 0.04 mg kg^{-1} for pyridaben and between 0.09 and 0.02 mg kg^{-1} for tralomethrin, with median values of 0.14 and 0.06 mg kg^{-1}, respectively. These values represent 28 and 600%, respectively, of the corresponding maximum reside limits currently established in Spain. The application of intensive washing process to the pepper samples did not lead to a significant reduction of the residue level of either pesticide. No significant differences were found between the residue levels in the "edible" and "inedible" parts of the peppers.

Gamon *et. al.* (2001) determined pesticide residues in fruit and vegetables by gas chromatography/tandem mass spectrometry (GC/MS/MS). For 80 compounds, Electron impact (EI)/MS/MS and chemical ionization (CI)/MS/MS were developed, including organochlorine, organophosphorus, organonitrogen, and pyrethroids, providing unambiguous spectral confirmation for these complex matrixes. Residue was extracted from samples with acetone followed by a mixture of dichloromethane–petroleum ether. 2 injections per sample were required for analysis of the entire pesticide list by EI/MS/MS and CI/MS/MS. Initial steps involved cleanup and concentration of extracts were eliminated.

Adou *et. al*. (2001) conducted a research on “Multiresidue Method for the Analysis of Pesticide Residues in Fruits and Vegetables by Accelerated Solvent Extraction and Capillary Gas Chromatography”. They developed an analytical procedure using accelerated solvent extraction and capillary gas chromatography with electron capture and flame photometric detections to determine residues of different pesticides in fruits and vegetables. Single laboratory validation of the method was carried out for 28 compounds selected from 8 pesticide classes, in blank and fortified samples of fresh pear, cantaloupe, white potato, and cabbage. The method meet specific established validation criteria for regulatory purposes applicable to our laboratory. At each of the 2 fortification levels studied, 24 of the 28 pesticides gave recoveries of more than 70% with a coefficient of variation of less than 10%. The method showed acceptable limits of detection (from 0.0019 to 0.14 mg/g depending on the pesticide and matrix) while minimizing environmental concerns, time, and labor.

CHAPTER III

MATERIALS AND METHODS

The vegetable (eggplant and cucumber) samples were collected from different markets of Dinajpur district and carried to the Pesticide Analytical Laboratory, Entomology Division, BARI, Joydebpur, Gazipur for pesticide residue analysis during September 2017 to March 2018. All way required a number of processes from the collection of samples to the final analysis which are described below.

3.1 Study area

The study area included major four markets of Dinajpur district. The area of Dinajpur district is about 3444.30 sq km, located at 25.63° North latitude and 88.64° East longitude with an elevation of 42 meters from the sea level. In this study, vegetables were collected from 5 markets of Ambari Bazar, Bahadur Bazar, Laxmitola Bazar, Fulbari Bazar and Parbatipur Natun Bazar in Dinajpur district.

3.2 Sample collection

A total of 80 samples (40 eggplant and 40 cucumber) were collected for this study. Eight samples of eggplant and eight samples of cucumber were collected from each market. Each sample was 1kg for both eggplant and cucumber. Transparent airtight clean polyethylene bags were used to collect samples and each bag was properly labeled with source, sample number, sample ID, collection date, location etc. To avoid cross contamination each sample was collected in a separate polyethylene bag.

Figure 01. Map showing the places of sample collection

Table 01: Sources and places of collection of eggplant samples

Area of collection	Sample ID	Source
Ambari Bazar	DIAEg-01	Daulatpur
	DIAEg-02	Kutubpur
	DIAEg-03	Raypur
	DIAEg-04	Habra
	DIAEg-05	Bosirbania
	DIAEg-06	Haripur
	DIAEg-07	Mohonpur
	DIAEg-08	Joypur
Bahadur Bazar	DIBEg-09	Kherkati
	DIBEg-10	Goyalhat
	DIBEg-11	Panchbari
	DIBEg-12	Mohonpur
	DIBEg-13	Laxmitola
	DIBEg-14	Ambari
	DIBEg-15	Chirirbandar
	DIBEg-16	Palashbari
Laxmitola Bazar	DILEg-17	Goyalhat
	DILEg-18	Panchbari
	DILEg-19	Mohonpur
	DILEg-20	Laxmitola
	DILEg-21	Ambari
	DILEg-22	Jaliapara
	DILEg-23	Rajapukur
	DILEg-24	Kamarpara
Fulbari Bazar	DIFEg-25	Ambari
	DIFEg-26	Dangarhat
	DIFEg-27	Habra
	DIFEg-28	Choupathi
	DIFEg-29	Bhowanipur
	DIFEg-30	Khrampur
	DIFEg-31	Harirumpur
	DIFEg-32	Khoyerpukur
Parbatipur Natun Bazar	DIPEg-33	Kholahati
	DIPEg-34	Choupathi
	DIPEg-35	Zahanabad
	DIPEg-36	Bosirbania
	DIPEg-37	Monmotpur
	DIPEg-38	Habra
	DIPEg-39	Ambari
	DIPEg-40	Raypur

Table 02: Sources and places of collection of cucumber samples

Area of collection	Sample ID	Source
Ambari Bazar	DIACu-01	Daulatpur
	DIACu-02	Kutubpur
	DIACu-03	Raypur
	DIACu-04	Habra
	DIACu-05	Bosirbania
	DIACu-06	Haripur
	DIACu-07	Mohonpur
	DIACu-08	Joypur
Bahadur Bazar	DIBCu-09	Kherkati
	DIBCu-10	Goyalhat
	DIBCu-11	Panchbari
	DIBCu-12	Mohonpur
	DIBCu-13	Laxmitola
	DIBCu-14	Ambari
	DIBCu-15	Chirirbandar
	DIBCu-16	Palashbari
Laxmitola Bazar	DILCu-17	Goyalhat
	DILCu-18	Panchbari
	DILCu-19	Mohonpur
	DILCu-20	Laxmitola
	DILCu-21	Ambari
	DILCu-22	Jaliapara
	DILCu-23	Rajapukur
	DILCu-24	Kamarpara
Fulbari Bazar	DIFCu-25	Ambari
	DIFCu-26	Dangarhat
	DIFCu-27	Habra
	DIFCu-28	Choupathi
	DIFCu-29	Bhowanipur
	DIFCu-30	Khrampur
	DIFCu-31	Harirumpur
	DIFCu-32	Khoyerpukur
Parbatipur Natun Bazar	DIPCu-33	Kholahati
	DIPCu-34	Choupathi
	DIPCu-35	Zahanabad
	DIPCu-36	Bosirbania
	DIPCu-37	Monmotpur
	DIPCu-38	Habra
	DIPCu-39	Ambari
	DIPCu-40	Raypur

3.3 Sample preparation for analysis

The collected samples were carried to the Pesticide Analytical Laboratory, Division of Entomology, Bangladesh Agricultural Research Institute (BARI) just after 1 day of collection. Each sample cut into small pieces and mixed properly. Clean air tight polythene bags were used to store chopped sample in refrigerator at -20°C until extraction and then cleanup process started.

3.4 Chemicals and reagents

The standard of malathion, diazinon, fenitrothion, quinalphos, chlorpyrifos, cypermethrin, and fenvalerate were obtained from Sigma-Aldrich Laborchemikalien (St Louis, MO, USA) via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. Standards of all the pesticides contained >99.6% purity. Methanol, acetone, gradient grade acetonitrile (MeCN), sodium chloride (NaCl), anhydrous magnesium sulphate ($MgSO_4$) Methanol and Primary Secondary Amine (PSA) were purchased from Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh.

3.5 Analytical apparatus used

a. Centrifuge machine, Model: Sigma 3k 30, Germany (Plate 1).

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

c. Electric balance, Model: AY- 220, Shimadzu Corporation, Japan (Plate 3)

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

Plate 01. Centrifuge Machine

Plate 02: Electric Balance

Plate 03: Vortex Mixer

Plate 04: Gas Chromatograph (GC)

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

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

Some pictorial view related to sample preparation

Plate 05: Chopping of Sample

Plate 06. Weighing of PSA

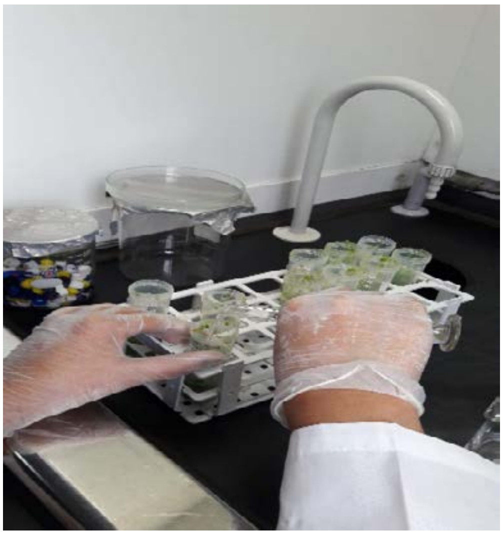


Plate 07: Adding Acetonitrile

Plate 08: Filtration through PTFE Filter

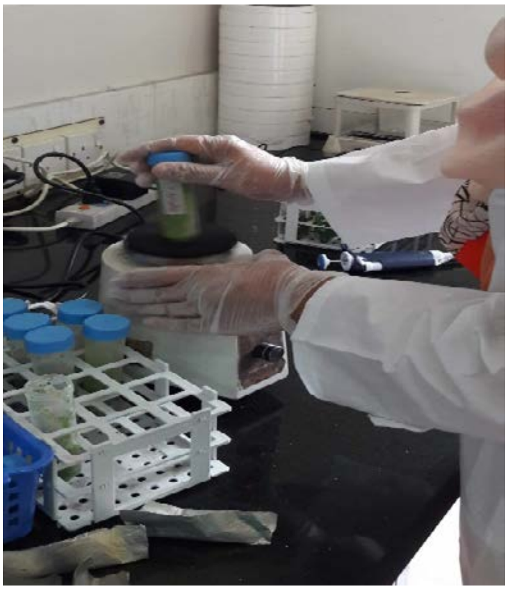


Plate 09: Shaking of sample

Plate 10. Centrifuging the sample

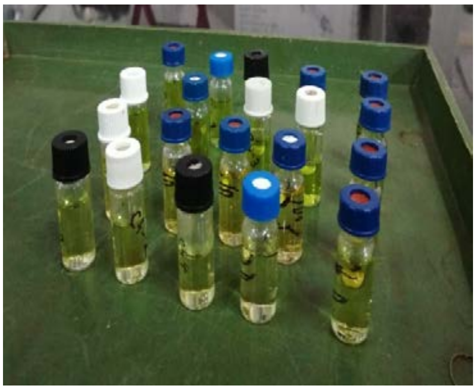


Plate 11. Sample extract ready for injection

3.6 Preparation of pesticide standard solution

Pesticide standard stock solutions of Malathion, Diazinon, Fenitrothion, Quinalphos, Chlorpyrifos, Cypermethrin and Fenvalerate were prepared separately in acetone at a concentration of 1000 mgL^{-1} and they were stored at -20°C until use. A mixed standard solution of 50 mgL^{-1} in 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 then made to volume by addition of acetone. An intermediate mixed standard solution of 10 mgL^{-1} in acetone was prepared from the mixed standard solution of 50 mgL^{-1}. Then working standard solutions of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 mgL^{-1} in acetone were prepared by transferring the appropriate amount from 10 mgL^{-1} of intermediate mixed standard solution into 10 separate 10mL volumetric flasks. All of the standard solutions were kept in a freezer at -20°C until use.

3.7 Extraction and clean up

QuEChERS extraction method is one of the latest extraction and clean up techniques for pesticide residue analysis in food matrices which stands for Quick, Easy, Cheap, Effective, Rugged and Safe. This techniques was first introduced in 2003 by Anastassiades *et al*. and gaining popularity day by day compared to the other existing techniques such as Solid phase extraction (SPE), Liquid-liquid extraction (LLE), Supercritical Fluid Extraction (SFE), Solid phase micro extraction (SPME), Stir bar sorptive extraction (SBSE), and Microwave assisted extraction (MAE). In this study, the QuEChERS extraction technique was used for the extraction and clean-up of samples which was modified in 2015 by Prodhan *et al.* where the chopped samples were grounded thoroughly with the fruit blender. In a 50 mL polypropylene centrifuge tube, a representative 10g portion of thoroughly homogenized sample was weighted and 10 mL of acetonitrile (MeCN) was added into the centrifuge tube. Then the centrifuge tube was closed properly and shaken vigorously for 30 s by the use of a vortex mixer and 1 g of NaCl and 4 g of anhydrous $MgSO_4$ were added into the centrifuge tube, and it was shaken immediately by the vortex mixer for 1 minute to prevent the formation of magnesium sulfate aggregates. Afterwards, the extract was centrifuged at 5000 rpm for 5 min. An aliquot of 3 mL of MeCN layer was transferred into a 15 mL micro centrifuge tube containing 120 mg Primary Secondary Amine

(PSA) and 600 mg anhydrous $MgSO_4$ and then it was thoroughly mixed by vortex for 30 s and centrifuged at 4000 rpm for 5 minutes. (Laboratory Centrifuges, Sigma-3K30, Germany). After centrifugation, a 1 mL supernatant was filtered by a 0.2 µm PTFE filter. Then it was taken in a clean GC vial for injection.

3.8 Detection and quantification of pesticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) for the detection of acephate, chlorpyrifos, dimethoate, diazinon, fenitrothion, malathion and quinalphos. The capillary column was AT-1 length was 30m, ID was 0.25mm and film thickness was 0.25µm. Helium was used as carrier and make up gas for FTD. The identification of suspected pesticide was performed by peak retention times in samples to those of peaks in the pure analytical standards. A typical chromatogram containing seven selected organophosphorus insecticides is presented in Figure 2.

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

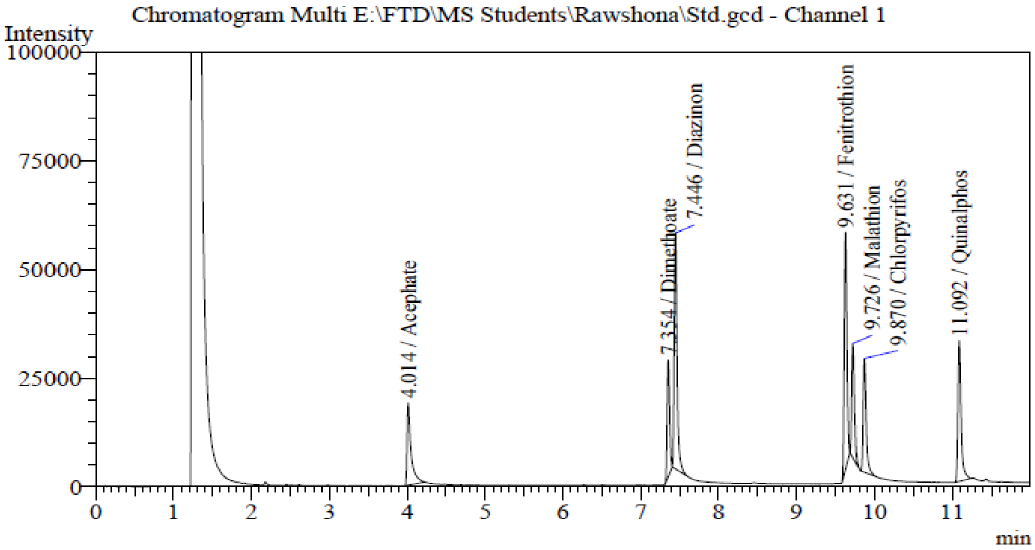


Figure 02: Typical chromatograms of seven organophosphorus insecticide standards run by GC-FTD.

Table 03: The instrument parameters for GC-FTD

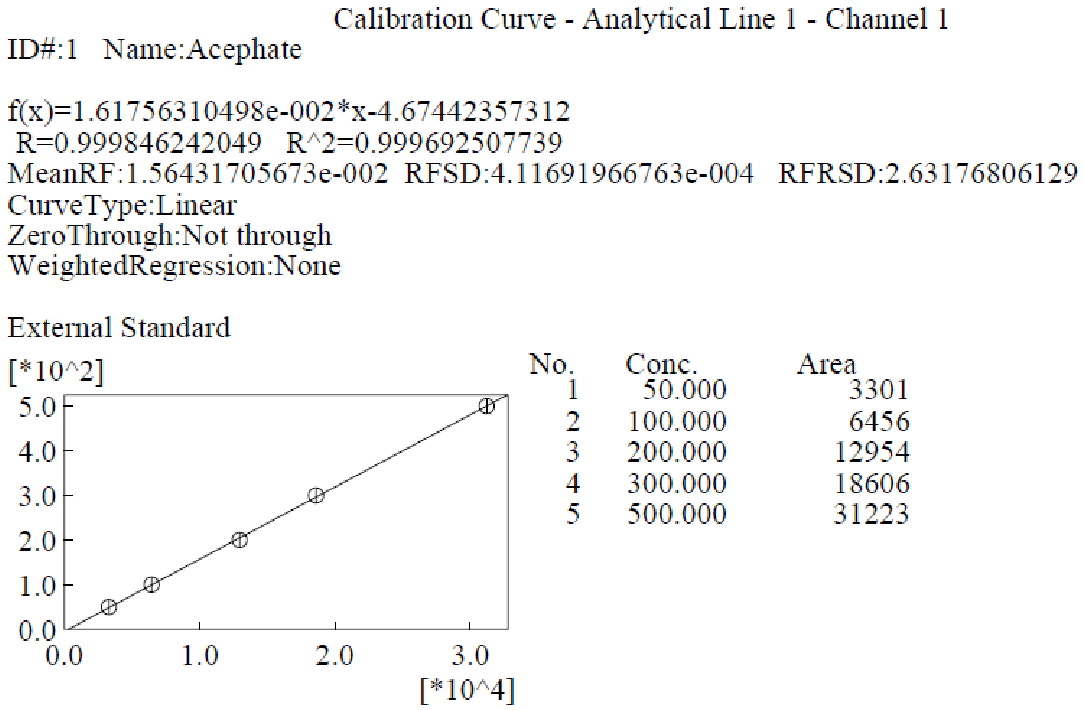
Instruments	**Conditions**
Injection port SPL	Injection mode: split; temperature: 250°C; flow control mode: linear velocity; split ratio: 30:0
Detector channel 1 FTD	Temperature: 280°C; current: 1.00 Pa; H_2 flow: 1.5 mL/min; stop time: 10 min; make up flow: 30 mL/min; air flow: 145 mL/min

Table 04: 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.

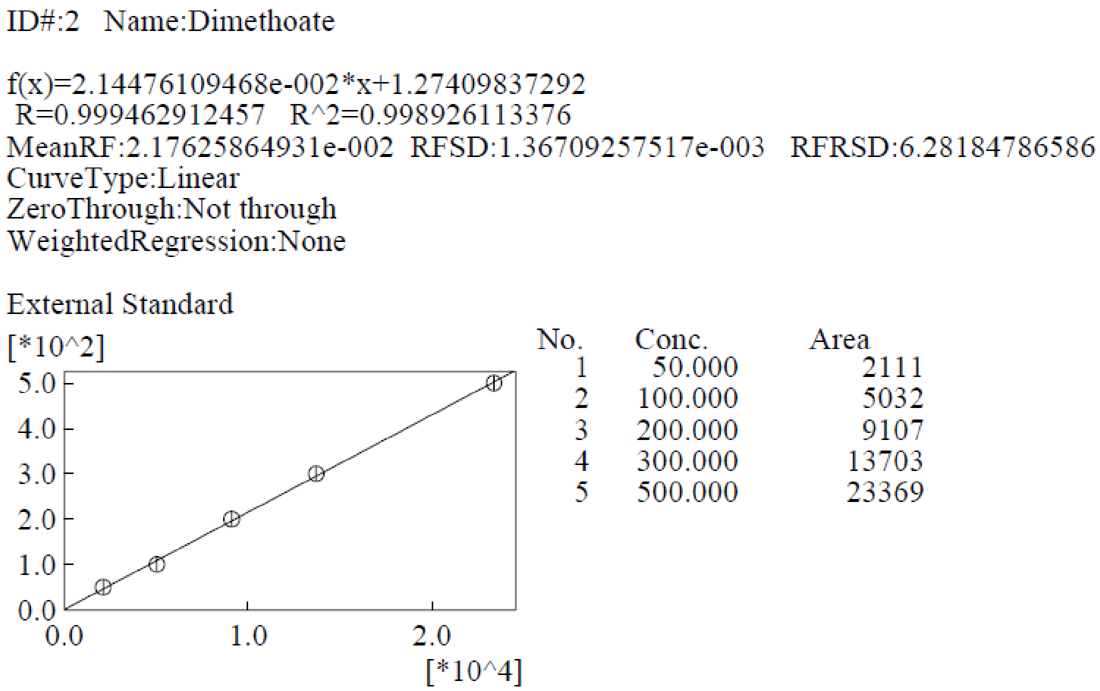


```
                              Calibration Curve - Analytical Line 1 - Channel 1
ID#:1   Name:Acephate

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

External Standard
                                             No.     Conc.          Area
                                              1       50.000          3301
                                              2      100.000          6456
                                              3      200.000         12954
                                              4      300.000         18606
                                              5      500.000         31223
```

Figure 03. Calibration curve prepared for acephate made with different concentrations ranging from 50 µg/L to 500 µg/L.

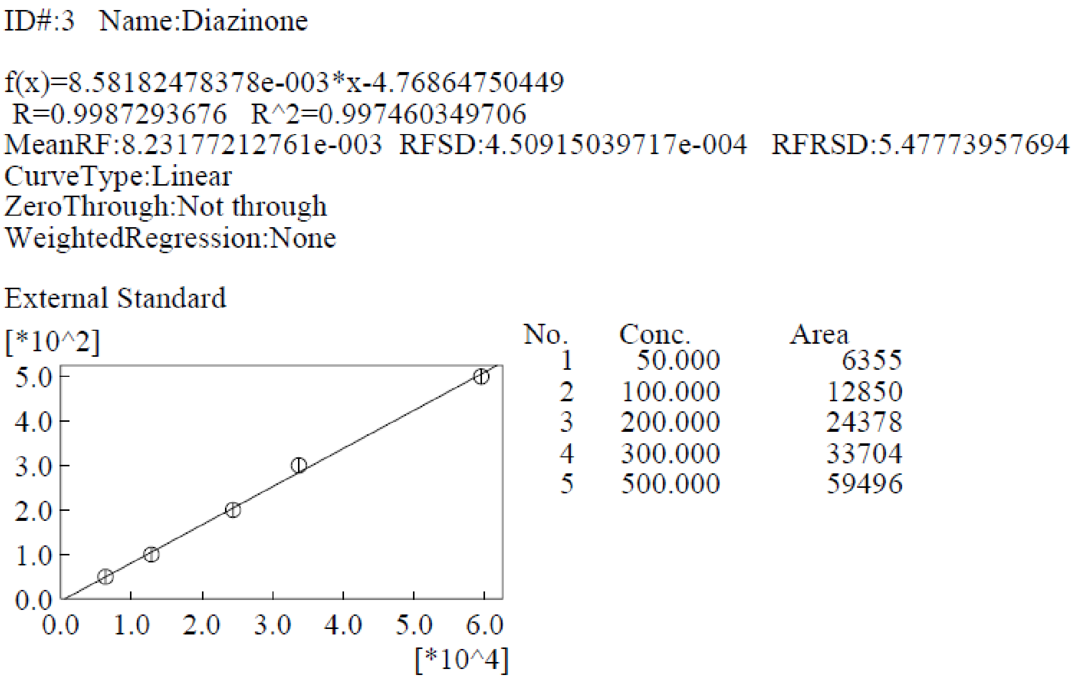


```
ID#:2   Name:Dimethoate

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

External Standard
                                             No.     Conc.          Area
                                              1       50.000          2111
                                              2      100.000          5032
                                              3      200.000          9107
                                              4      300.000         13703
                                              5      500.000         23369
```

Figure 04. Calibration curve prepared for dimethoate made with different concentrations ranging from 50 µg/L to 500 µg/L.



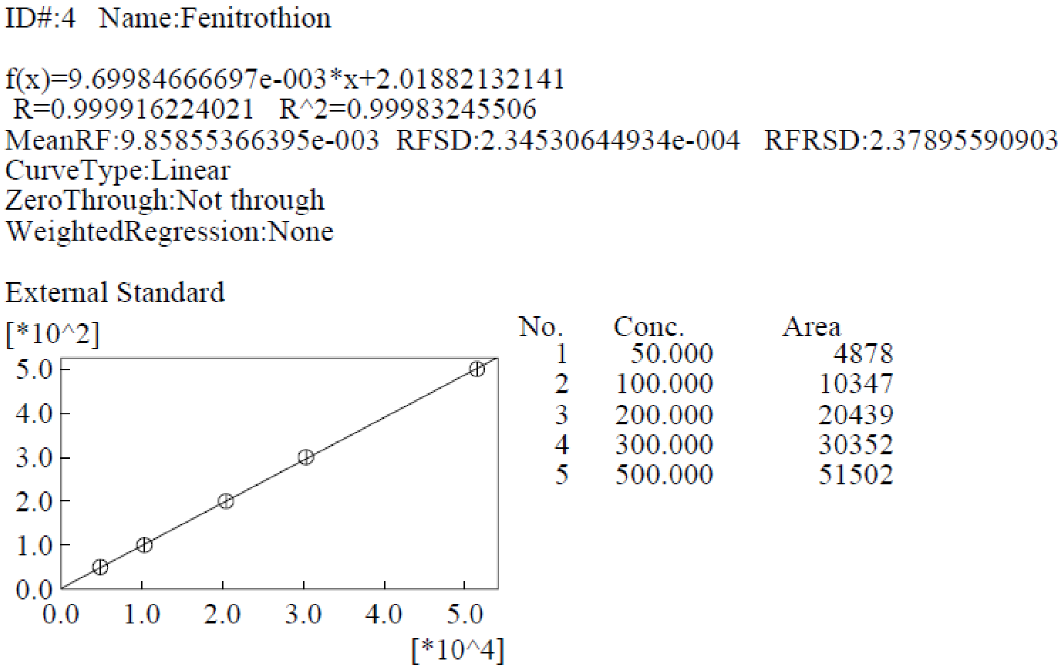
```
ID#:3   Name:Diazinone

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

External Standard
```

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

Figure 05. Calibration curve prepared for diazinone made with different concentrations ranging from 50 µg/L to 500 µg/L.



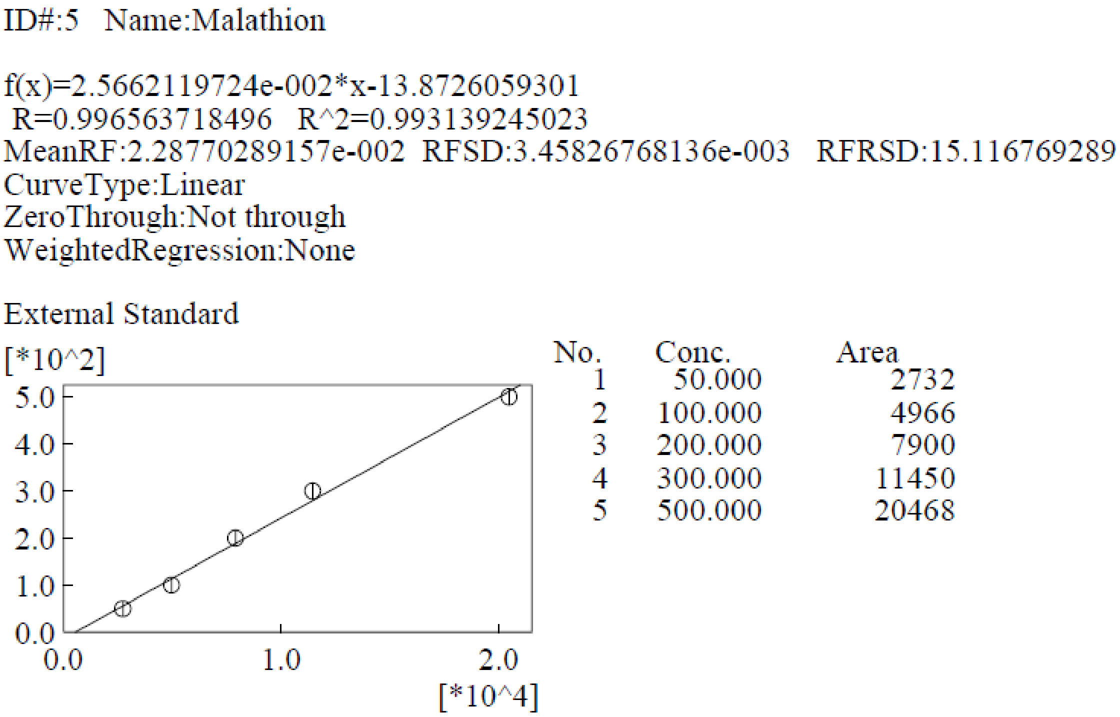
```
ID#:4   Name:Fenitrothion

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

External Standard
```

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

Figure 06. Calibration curve prepared for fenitrothion made with different concentrations ranging from 50 µg/L to 500 µg/L.

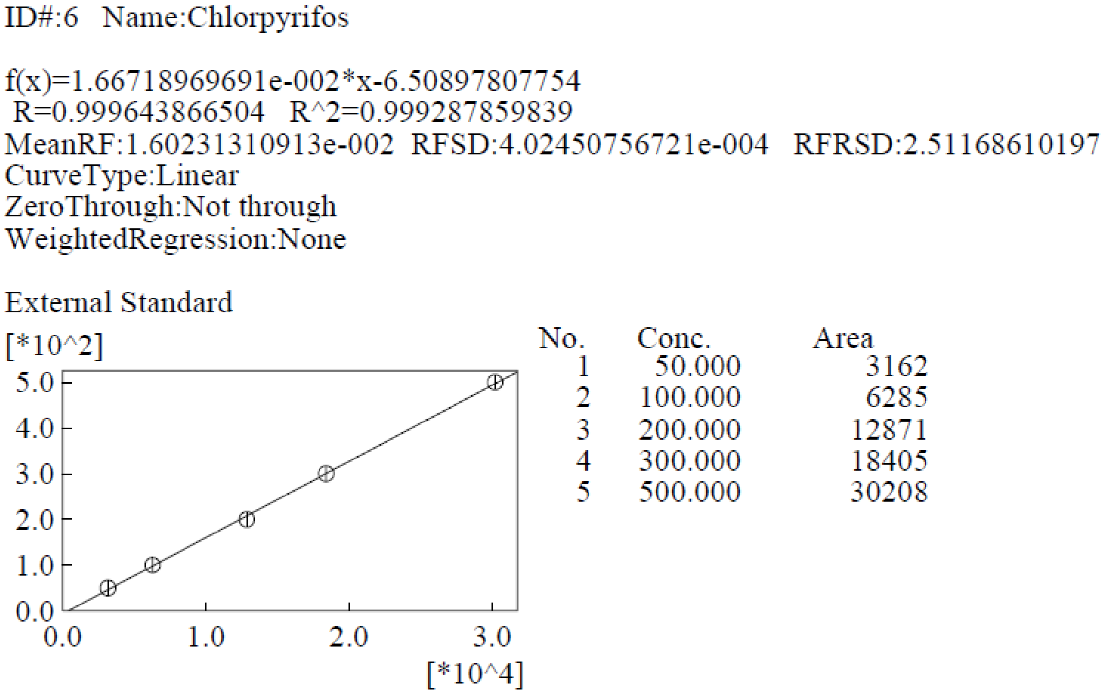


```
ID#:5   Name:Malathion

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

External Standard
                                        No.     Conc.         Area
                                         1       50.000        2732
                                         2      100.000        4966
                                         3      200.000        7900
                                         4      300.000       11450
                                         5      500.000       20468
```

Figure 07. Calibration curve prepared for malathion made with different concentrations ranging from 50 µg/L to 500 µg/L.

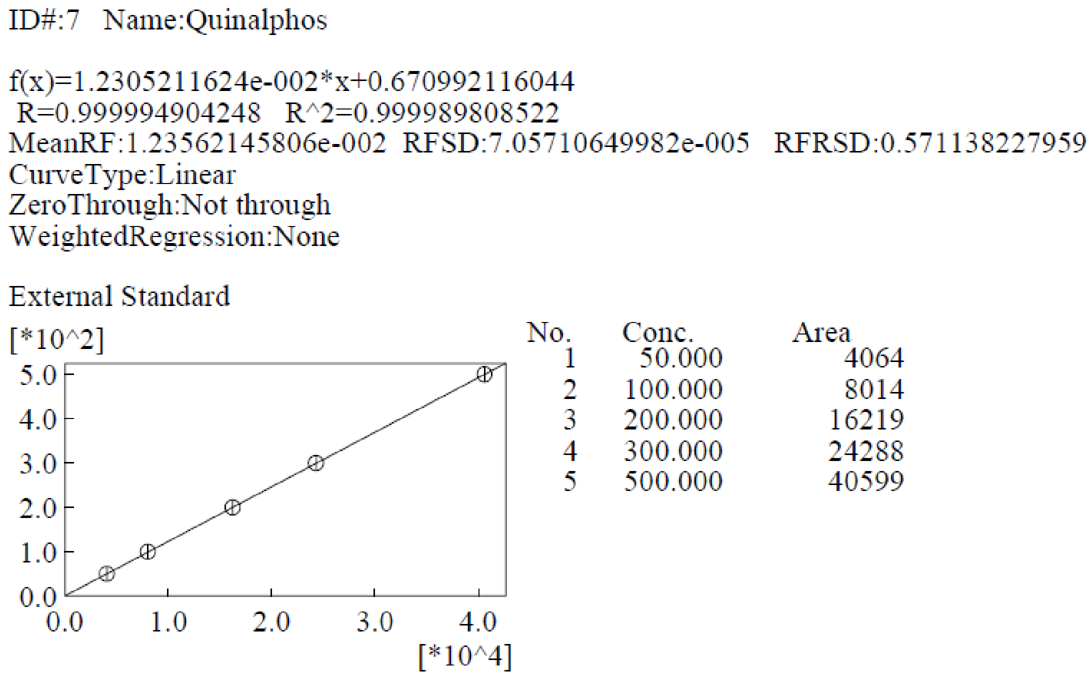


```
ID#:6   Name:Chlorpyrifos

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

External Standard
                                        No.     Conc.         Area
                                         1       50.000        3162
                                         2      100.000        6285
                                         3      200.000       12871
                                         4      300.000       18405
                                         5      500.000       30208
```

Figure 08. Calibration curve prepared for chlorpyrifos made with different concentrations ranging from 50 µg/L to 500 µg/L.



```
ID#:7   Name:Quinalphos

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

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

Figure 09. Calibration curve prepared for quinalphos made with different concentrations ranging from 50 µg/L to 500 µg/L.

CHAPTER IV

RESULTS AND DISCUSSIONS

Eighty (80) samples of vegetable (eggplant and cucumber) were collected from 5 different markets of Dinajpur district (Ambari Bazar, Bahadur Bazar, Laxmitola Bazar, Fulbari Bazar and Parbatipur Natun 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 eggplant

The concentrated extracts of eggplant samples collected from different markets of Dinajpur were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. Figure 10-14 shows the chromatograms of the injected extracts of eggplant sample containing detected pesticides.

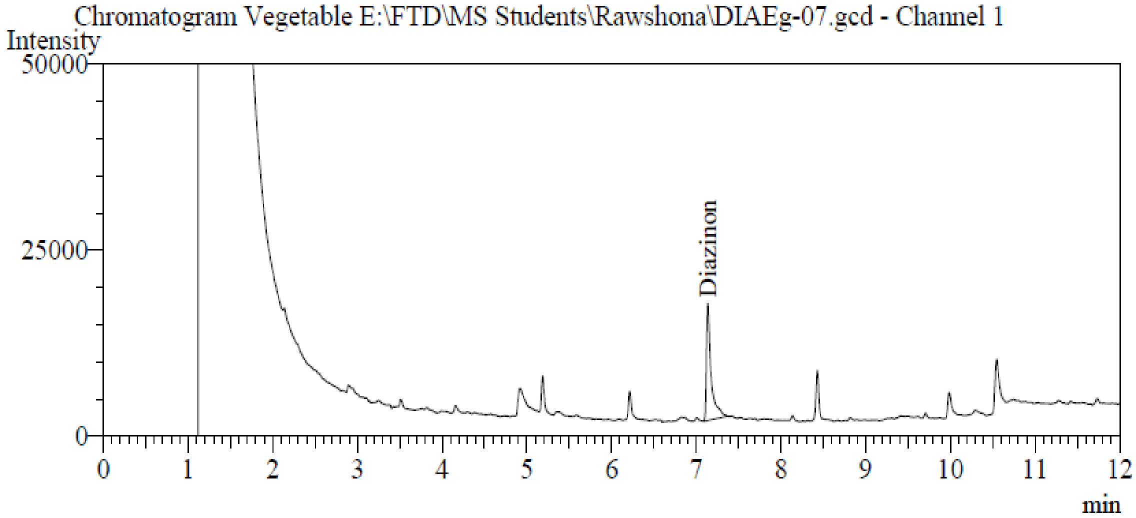


Figure 10. Chromatogram of diazinon found in one of the eggplant sample (DIAEg-07) collected from Ambari Bazar.

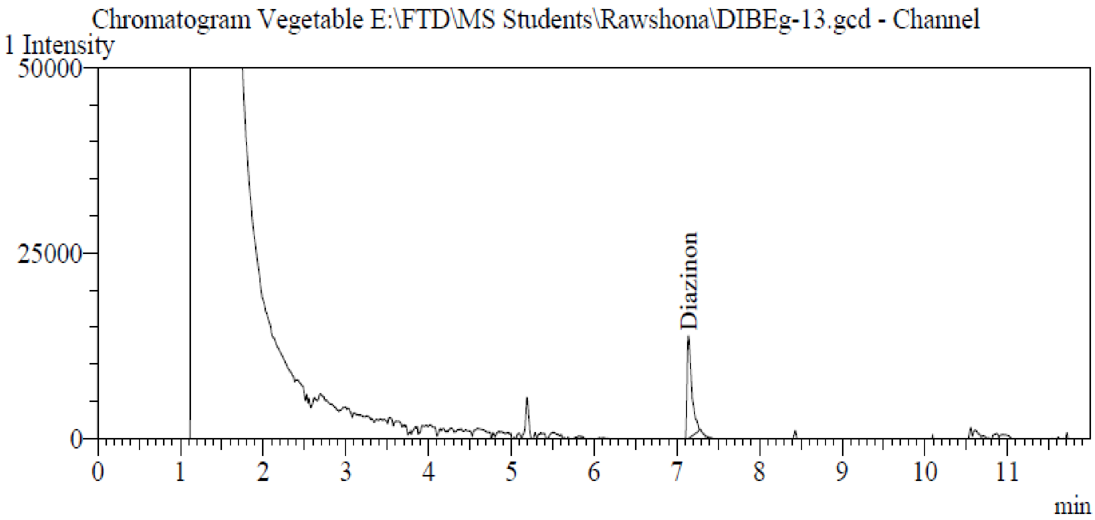


Figure 11. Chromatogram of diazinon found in one of the eggplant sample (DIBEg-13) collected from Bahadur Bazar.

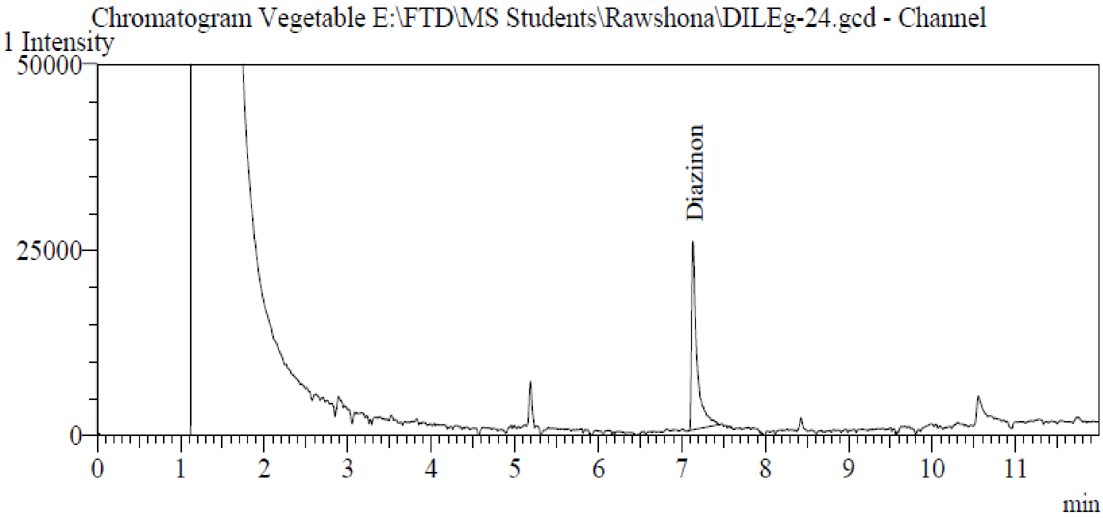


Figure 12. Chromatogram of diazinon found in one of the eggplant sample (DILEg-24) collected from Laxmitola Bazar.

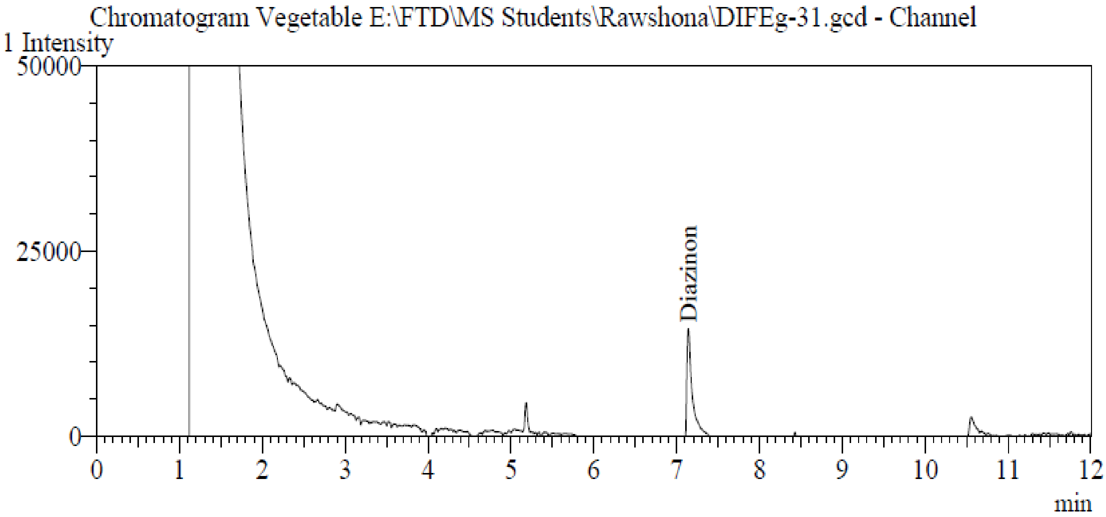


Figure 13. Chromatogram of diazinon found in one of the eggplant sample (DIFEg-31) collected from Fulbari Bazar.

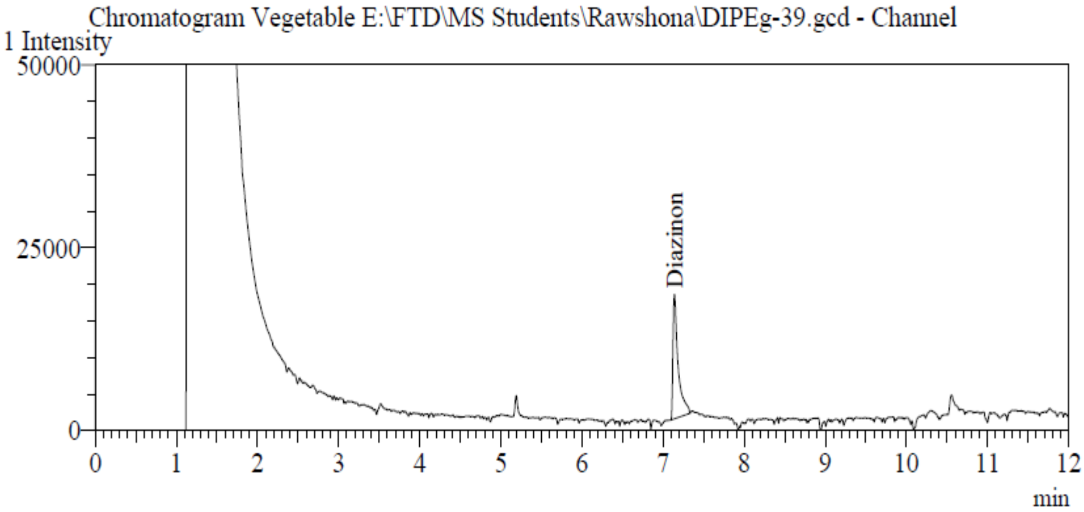


Figure 14. Chromatogram of diazinon found in one of the eggplant sample (DIPEg-39) collected from Parbatipur Natun Bazar.

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

Table 05. The level of residues (mg/kg) of different pesticides found in the analyzed eggplant samples.

Area of collection	**Sample ID**	**Name of detected pesticide**	**Level of residue (mg/kg)**	**MRLs (mg/kg)**
Ambari Bazar	DIAEg-01	ND	-	
	DIAEg-02	ND	-	
	DIAEg-03	ND	-	
	DIAEg-04	ND	-	
	DIAEg-05	ND	-	
	DIAEg-06	ND	-	
	DIAEg-07	Diazinon	0.242	0.01*
	DIAEg-08	ND	-	
Bahadur Bazar	DIBEg-09	ND	-	
	DIBEg-10	ND	-	
	DIBEg-11	ND	-	
	DIBEg-12	ND	-	
	DIBEg-13	Diazinon	0.215	0.01*
	DIBEg-14	ND	-	
	DIBEg-15	ND	-	
	DIBEg-16	ND	-	
Laxmitola Bazar	DILEg-17	ND	-	
	DILEg-18	ND	-	
	DILEg-19	ND	-	
	DILEg-20	ND	-	
	DILEg-21	ND	-	
	DILEg-22	ND	-	
	DILEg-23	ND	-	
	DILEg-24	Diazinon	0.442	0.01*
Fulbari Bazar	DIFEg-25	ND	-	
	DIFEg-26	ND		
	DIFEg-27	ND	-	
	DIFEg-28	ND	-	
	DIFEg-29	ND	-	
	DIFEg-30	ND	-	
	DIFEg-31	Diazinon	0.314	0.01*
	DIFEg-32	ND	-	
Parbatipur Natun Bazar	DIPEg-33	ND	-	
	DIPEg-34	ND	-	
	DIPEg-35	ND	-	
	DIPEg-36	ND	-	
	DIPEg-37	ND	-	
	DIPEg-38	ND	-	
	DIPEg-39	Diazinon	0.298	0.01*
	DIPEg-40	-	-	

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

Fourty samples of eggplant collected from 5 different markets of Dinajpur (Ambari Bazar, Bahadur Bazar, Laxmitola Bazar, Fulbari Bazar and Parbatipur Natun 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 40 samples of eggplant, 5 samples (12.5% of the total number of samples) contained pesticide residues and 31 samples (87.5% 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.

The findings of the present study can also be compared with Akter *et al.* (2017). They have been monitored pesticide residues in eggplant collected from Mymensingh district and found that among the 50 analyzed samples, 11 (22% of the total number of the samples) contained pesticide residues of diazinon, dimethoate, quinalfos, and chlorpyrifos, of which, 2 had multiple pesticide residues and 5 samples contained residue above the European Union maximum residue limit (EU-MRLs). Chlorpyrifos was detected as the most used pesticide in eggplant in the studied area. The results of this study are in a good agreement with Hasan *et al*. (2017). They have been detected two types of insecticides (Dimethoate and Quinalphos) in country bean samples collected from different market places of dhaka. Among the 50 analyzed samples of country bean, 10 samples (20%) contained residues of Dimethoate and Quinalphos, of which 5 samples were above the maximum residue limits (MRLs). Most of the contaminated samples (8 samples) contained residue of Dimethoate.

Eight eggplant samples were collected from Ambari Bazar area, among them one samples (DIAEg-07) contained diazinon at a level of 0.242 mg/kg, which was above the EU-MRL (European Commission 2015). The other 7 samples contain no detectable pesticide residues.

From Bahadur Bazar, eight samples were collected, of which one sample (DIBEg-13) contained diazinon residue (0.215 mg/kg). But other seven samples contained no detectable pesticide residues. The level of detected diazinon residue (0.215 mg/kg) was below EU-MRL (0.01 mg/kg).

One sample (DILEg -24) of eggplant contained residue of diazinon (0.442 mg/kg) among the eight samples collected from Laxmitola Bazar, which was above EU-MRL (0.01 mg/kg). The other 7 samples contain no detectable pesticide residues.

One sample (DIFEg-31) of eggplant contained residue of diazinon (0.314 mg/kg) among the eight samples collected from Fulbari Bazar, which was below EU- MRL (0.01 mg/kg). The other 7 samples contain no detectable pesticide residues.

From Parbatipur Natun Bazar, eight samples were collected of which one sample (DIPEg-39) contained diazinon residue (0.298 mg/kg). But other seven samples contained no detectable pesticide residues. The level of detected diazinon residue (0.298 mg/kg) was below EU-MRL (0.01 mg/kg).

4.2 Pesticide residues in cucumber

The concentrated extracts of cucumber samples collected from different markets of Dinajpur district were analyzed by GC-2010 (Shimadzu) with Flame Thermonic Detector (FTD) with the pre-set parameters. Figure 15-18 shows the chromatograms of the injected extracts of yard long bean sample containing detected pesticides.

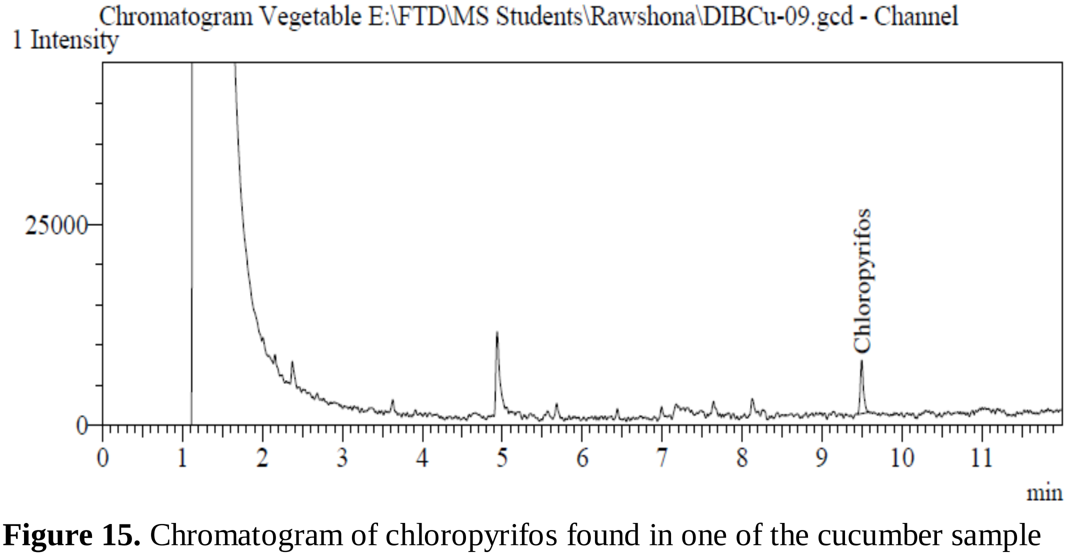


Figure 15. Chromatogram of chloropyrifos found in one of the cucumber sample (DIBCu-09) collected from Bahadur Bazar.

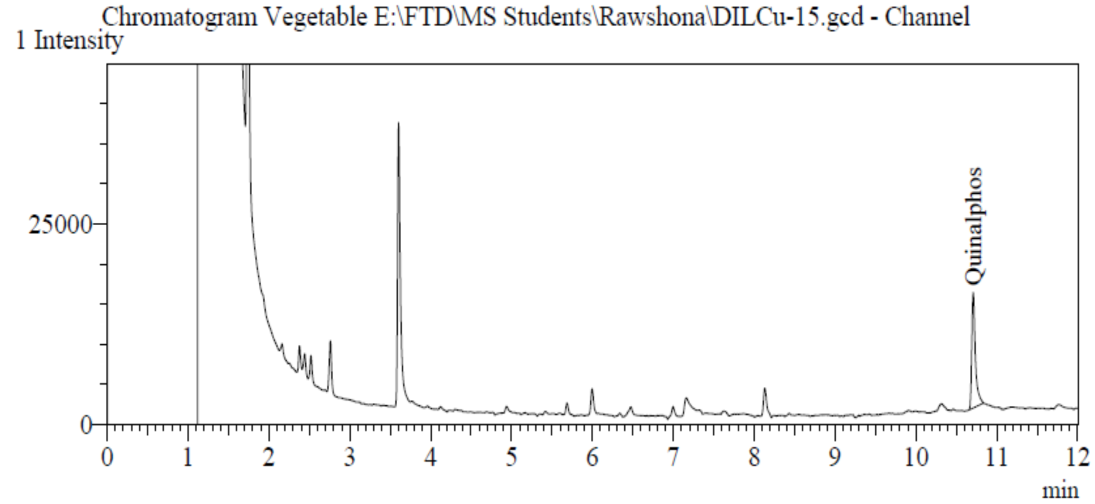


Figure 16. Chromatogram of quinalphos found in one of the cucumber sample (DIBCu -15) collected from Bahadur Bazar.

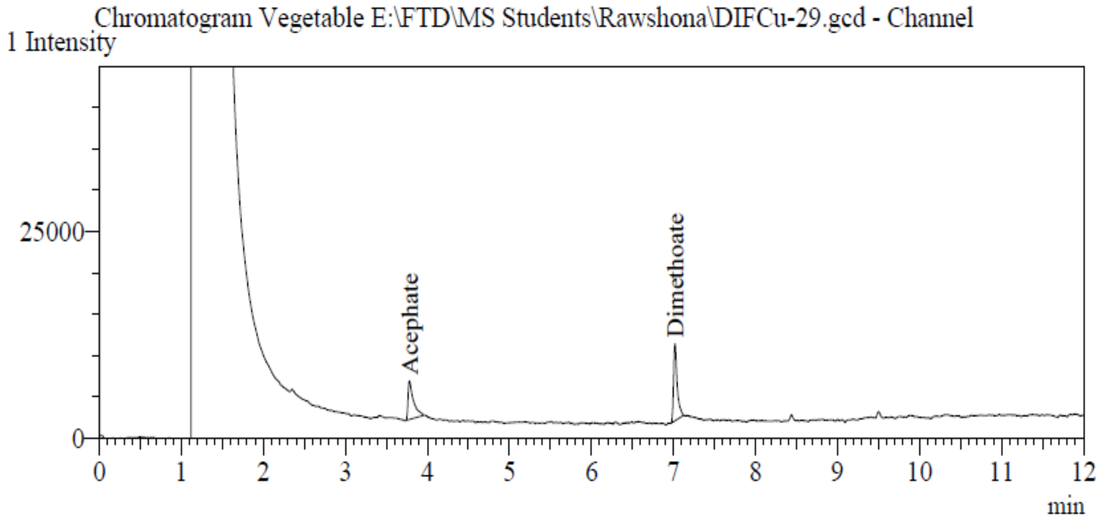


Figure 17. Chromatogram of acephate and dimethoate found in one of the cucumber sample (DIFCu-29) collected from Fulbari Bazar.

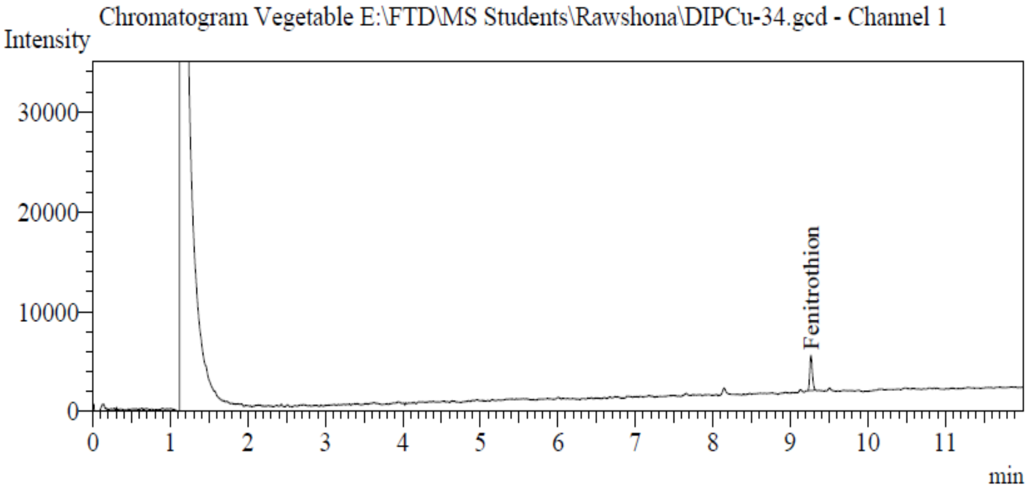


Figure 18. Chromatogram of fenitrothion found in one of the cucumber sample (DIPCu-34) collected from Parbatipur Natun Bazar.

Table 06. The level of residues (mg/kg) of different pesticides found in the analyzed cucumber samples

Area of collection	**Sample ID**	**Name of detected pesticide**	**Level of residue (mg/kg)**	**MRLs (mg/kg)**
Ambari Bazar	DIACu-01	ND	-	
	DIACu-02	ND	-	
	DIACu-03	ND	-	
	DIACu-04	ND	-	
	DIACu-05	ND	-	
	DIACu-06	ND	-	
	DIACu-07	ND	-	
	DIACu-08	ND	-	
Bahadur Bazar	DIBCu-09	Chloropyrifos	0.008	0.01*
	DIBCu-10	ND	-	
	DIBCu-11	ND	-	
	DIBCu-12	ND	-	
	DIBCu-13	ND	-	
	DIBCu-14	ND	-	
	DIBCu-15	Quinalphos	0.241	0.01*
	DIBCu-16	ND	-	
Laxmitola Bazar	DILCu-17	ND	-	
	DILCu-18	ND	-	
	DILCu-19	ND	-	
	DILCu-20	ND	-	
	DILCu-21	ND	-	
	DILCu-22	ND	-	
	DILCu-23	ND	-	
	DILCu-24	ND	-	

Fulbari Bazar	DIFCu-25	ND	-	
	DIFCu-26	ND	-	
	DIFCu-27	ND	-	
	DIFCu-28	ND	-	
	DIFCu-29	Acephate	0.156	0.01*
		Dimethoate	0.168	0.01*
	DIFCu-30	ND	-	
	DIFCu-31	ND	-	
	DIFCu-32	ND	-	
Parbatipur Natun Bazar	DIPCu-33	ND	-	
	DIPCu-34	Fenitrothion	0.009	0.01*
	DIPCu-35	ND	-	
	DIPCu-36	ND	-	
	DIPCu-37	ND	-	
	DIPCu-38	ND	-	
	DIPCu-39	ND	-	
	DIPCu-40	ND	-	

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

Fourty samples of cucumber were collected from 5 different markets of Dinajpur district (Ambari Bazar, Bahadur Bazar, Laxmitola Bazar, Fulbari Bazar and Parbatipur Natun 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 40 samples of cucumber, 4 samples (10% of the total number of samples) contained pesticide residues and 36 samples (90% 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.

Eight cucumber samples were collected from Ambari Bazar and Laxmitola Bazar, none of the samples contained no detectable residues of the sought pesticides.
From Bahadur Bazar, eight samples were collected of which one sample (DIBCu -09) contained chloripyrifos residue (0.008 mg/kg) and another sample (DILCu-15)

contained residue of quinalphos (0.241 mg/kg). But other six samples contained no detectable pesticide residues. The level of detected chloripyrifos residue (0.008 mg/kg) was below EU-MRL (0.01 mg/kg). But quinalphos residue (0.241 mg/kg) was above EU-MRL (0.01 mg/kg).

One sample (DIFCu-29) of cucumber contained residue of acephate (0.156 mg/kg) and dimethoate (0.168 mg/kg) among the eight samples collected from Fulbari Bazar,. The other 7 samples contain no detectable pesticide residues. The level of detected acephate residue (0.156 mg/Kg) and dimethoate residue (0.168 mg/Kg) was above EU-MRL (0.01 mg/Kg).

Among the eight samples collected from Parbatipur Natun Bazar, one sample (DIPCu-34) contained residues of fenitrothion (0.009 mg/Kg). The level of detected fenitrothion residue was below EU-MRL (0.01 mg/Kg).

CHAPTER V
SUMMARY AND CONCLUSION

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. Being an over populated country food shortage and malnutrition are major problems of Bangladesh. 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. 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. 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. 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. 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 Dinajpur district are famous for vegetable production. But now a day's application of pesticides in Dinajpur district is increasing inorder to increase the crop yield and to control of insect pests and diseases. Moreover, there are no considerable works have yet been done for the determination of residue levels of pesticide. The purpose of this study is to detect and quantify pesticide residues in eggplant and cucumber and to compare them with the Maximum residue levels (MRLs). Regarding this, 40 samples of eggplant and 40 samples of cucumber were collected from five different locations (Ambari Bazar, Bahadur Bazar, Laximotola Bazar, Fulbari Bazar and Parbatipur Natun Bazar) of Dinajpur 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 40 analyzed samples of eggplant, 5 samples (12.5% of the total number of samples) contained pesticide residues of diazinon. All the 5 samples had residues above the Maximum Residue Limit (MRL) set by European Commission. On the other hand 35 samples (87.5% of the total number of samples) contained no detectable residues of the sought pesticides.

Out of 40 samples of cucumber, 4 samples (10% of the total number of samples) contained pesticide residues of chlorpyrifos, quinalphos, acephate, dimethoate and fenitrothion and the other 36 samples (90% of the total number of samples) contained no detectable residues of the sought pesticides. Among these 4 samples, 2 samples 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 acephate and dimethoate. The level of detected quinalphos, acephate and dimethoate residues was above the Maximum Residue Limit (MRL).

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 remain in vegetables at local markets of Dinajpur 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 Dinajpur district in Bangladesh.

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