DETERMINATION OF PESTICIDE RESIDUES IN CAULIFLOWER AND TOMATO COLLECTED FROM DIFFERENT AREAS OF RANGPUR DISTRICT

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BY

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CERTIFICATE

This is to certify that the thesis entitled **"DETERMINATION OF PESTICIDE RESIDUES IN CAULIFLOWER AND TOMATO COLLECTED FROM DIFFERENT AREAS OF RANGPUR 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 **MASTER OF SCIENCE (M.S.)** in **AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bona fide research work carried out by **KAZI MASJIDUN NAHAR**, Registration No. **12-05092** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

June, 2018
Dhaka, Bangladesh

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Dedicated to

My

Beloved Parents

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DETERMINATION OF PESTICIDE RESIDUES IN CAULIFLOWER AND TOMATO COLLECTED FROM DIFFERENT AREAS OF RANGPUR DISTRICT

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Abstract

The study was conducted to analyze pesticide residues in two common vegetables (cauliflower and tomato) collected from five different areas (Paura Bazaar, Station Bazar, Tetultola Bazar, Terminal Bazar and Lalbag Bazar) of Rangpur city from September 2017 to March 2018. The collected samples were carried to the Pesticide Analytical Laboratory, Entomology Division of Bangladesh Agricultural Research Institute (BARI), Gazipur. The collected samples were analyzed using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and Gas Chromatography (GC) coupled with Flame Thermionized Detector (FTD) for the determination of pesticide residues in 40 samples of cauliflower and 40 samples of tomato. Among the 40 analyzed samples of cauliflower, 6 samples (15% of the total number of samples) contained pesticide residues of diazinon and quinalphos. Among these 6 samples, 5 had residues above the Maximum Residue Limit (MRL) and 1 sample contained two different types of pesticide residues such as diazinon and quinalphos. The level of detected diazinon residues was above MRL, while the level of quinalphos residues was below the MRL and the other 34 samples (85% of the total number of samples) contained no detectable residues of the sought pesticides. Similarly out of 40 analyzed samples of tomato, 4 samples (10% of the total number of samples) contained pesticide residues of acephate, dimethoate, chlorpyrifos and quinalphos. Among these 4 samples, 3 had residues above the MRL and 1 had residues below the MRL set by European Commission. This study reflects the overall scenario of pesticide contamination in vegetables especially in cauliflower and tomato available in the local markets of Rangpur District, which will help the consumer to be aware of their health and safety.

Key words: Cauliflower, Tomato, Gas Chromatography, Pesticide residues, QuEChERS Extraction

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LIST OF ABBREVIATIONS

ADI	Acceptable Daily Intake
ACh	Acetylcholine
AChE	Acetycholinesterase
APCI	Atmospheric Pressure Chemical Ionization
ASE	Accelerated solvent extraction
AOAC	Association of Official Analytical Chemists
BARI	Bangladesh Agricultural Research Institute
CAS	The Chemical Abstracts Service
CCD	Colony collapse disorder
CBS	Compound Based Scanning
CSN	Committee for Standardization
DAS	Days After Spray
DDT	Dichloro Diphenyle Trichloroethen
DLLME	Dispersive Liquid–Liquid Micro Extraction
DV	Daily Value
d-SPE	dispersive solid phase extraction

ECD	Electron Capture Detector
EPA	Environmental Protection Agency
ESI	electrospray ionization
et al.	*et alibi* (and others)
etc.	*et cetra* (and so on)
ECD	Electron capture Detector
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FID	Flame Ionization Detector
FPD	Flame photometric Detector
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GC-MS	Gas Chromatograph-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
HRI	Hazard Risk Index
IARC	The International Agency for Research on Cancer
IUPAC	Intrenational Union of Pure and Applied Chemistry
KMRL	Korean Maximum Residue Limits
LC-MS	Liquid Chromatography-Mass Spectrometry
LLE	Liquid-liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
MAE	Microwave Assisted Extraction
MCS	Multiple Chemical Sensitivity Syndrome
MDQ	Minimum Detectable Quantity
MRL	Maximum Residue Limit
MRM	multiple reaction monitoring
NPD	Nitrogen-phosphorus Detector
NPTN	National Pesticides Telecommunications Network
NTE	Neuropathy Target Esterase
OCPs	Organochlorin Pesticides
OPPs	organophosphorus Pesticides

PDA	Photodiode Array detection
PDI	Potential Daily Intake
PSA	Primary Secondary Amine
PHI	Pre-Harvest Interval
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RSM	Response Surface Methodology
RTL	Retention Time Locked
SAU	Sher-e-Bangla Agricultural University
SBSE	Stir Bar Sorptive Extraction
SE	Solvent Extraction
SFE	Supercritical fluid extraction
SF	Supercritical Fluid
SIM	Selected Ion Monitoring
SRM	Selected Reaction Monitoring
SPME	Solid Phase Micro Extraction (SPME)
SPE	Solid phase extraction
TOTAD	Through Oven Transfer Adsorption Desorption
TCD	Thermal conductivity Detector
UHPLC-MS/MS	Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry
VFs	Variability Factors
WHO	World Health Organization

CHAPTER I

INTRODUCTION

Vegetables are herbaceous plants that are cultivated for an edible part or any part of a plant which is normally eaten. The amount of vegetables needed for a person depends on age, sex and levels of physical activity. Vegetables are poorer in calories than a lot of other foods, but they do contain some amount of calories which is essential for human health. Their consumptions can also play a key job in neutralizing the acids created during digestion of greasy and proteins foods and moreover offer important roughages that assist in forwarding of food in intestine (Sarwar, 2012). They are important sources of many nutrients, including potassium, folate (folic acid), vitamin A, vitamin C and others. In the daily diet vegetables have been closely associated with the improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some other forms of cancer (Dias *et al.*, 2012).According to the World Health Report (2007) unbalanced diets with low vegetable intake and low consumption of carbohydrates which is complex and dietary fiber are estimated to cause some 2.7 million deaths each year and among the top 10 risk factors contributing to mortality (J. S. Dias, 2011). Vegetables are valuable in maintaining alkaline reserve of the human body. They are valued mainly because of their vitamin and mineral contents.

Cauliflower, *Brassica oleracea botrytis,* is a vegetable of the family of Brassicaceae. Normally only the head is eaten. The head is composed of a white inflorescence meristem. Cauliflower is an excellent source of vitamin B6, vitamin C, vitamin K, pantothenic acid and folate. It is a source of omega-3 fatty acids, biotin, phosphorus, and manganese. Furthermore, it is a good source of vitamin B1, B2, and B3, the minerals potassium and magnesium, and protein. Raw white cauliflower provide calories, low in fat, carbohydrates, dietary fiber and protein (whfoods.org).

It contains sulforaphane, a sulfur compound that has also been shown to kill cancer stem cells, thereby slowing tumor growth. Some researchers believe eliminating cancer stem cells may be key to controlling cancer.

It also contains a wealth of anti-inflammatory nutrients to help keep inflammation in check, including indole-3-carbinol or I3C, an anti-inflammatory compound that may operate at the genetic level to help prevent the inflammatory responses at its foundational level.

Cauliflower is a good source of choline**,** a B vitamin known for its role in brain development. Choline intake during pregnancy "super-charged" the brain activity of animals in utero, indicating that it may boost cognitive function, and improve learning and memory. It may even diminish age-related memory decline and your brain's vulnerability to toxins during childhood, as well as conferring protection later in life.

For the warm and humid climatic condition of the country, increase use of modern high yielding varieties of crops. Also more use of chemical fertilizers is highly favorable for development and multiplication of pests and diseases. In Bangladesh circumstances, the cauliflower growers have been using pesticides frequently to have the higher yield. But the overdoses of pesticides make the residue problem, which might pollute our food and be harmful for our health. Cauliflower is also very susceptible to insect infestation like other vegetables. The main insects of cauliflower are diamond back moth, leaf webber, stem borer, tobacco caterpillar, aphids, painted bug etc.

Tomato scientifically as *Solanum lycopersicum*, is the berry of a plant from the nightshade family. Despite technically being a fruit, the tomato is generally categorized as a vegetable. They are usually red when mature, but can come in a variety of colors, including yellow, orange, green and purple. There are many subspecies of tomato with different shapes and flavor. The water content of tomato is around 95%. The other 5% consists of mainly carbohydrates and fiber. One medium sized tomato (123 grams) contains only 22 calories. Tomato with carbohydrates make up 4% of raw, which amounts to less than 5 grams of carbs for an average sized tomato (123 grams). It is a good source of fiber, providing about 1.5 grams per average sized tomato. Most of the fibers (87%) in tomato are insoluble, in the form of hemicellulose, cellulose and lignin.

Beside the basic nutritional properties, tomato contain bioactive compounds with antioxidant properties such as ascorbic acid, vitamin E, carotenoids (lycopene), flavonoids and phenolic acids that benefit human health.

Tomato is attacked by many insects and pests such as cutworms, hornsworms, colorado potato beetle, stink bugs, spider mites etc. To control theses insects and pests pesticides are applied in order to avoid significant yield losses. In case of severe infestation in both tomato and cauliflower farmers are like to use chemical control methods as the manual control is costly, time consuming and laborious. So, applying pesticides are being inescapable for economic production as the market value of insect infested tomato fruits and cauliflower heads are very much lower than the un-infested one. As a sub-tropical country, Bangladesh has a wide range of temperature and humidity in different season of the year. That provides a chance of multiplication and development to a wide range of insects. Multiple crops are attacked by many genus/species of insects which already attained resistance because of using some common pesticides year after year. These things may sometimes make confusion and farmers are likely to use overdose more frequently.

It is necessary to maintain improved vegetables production for increasing nutritional status of the community. But the yield per unit area of vegetables is quite low since the insect pests cause 30-40% losses in general and even 100% losses in case of menace if no control measure is applied. A conservative estimate puts about annual yield losses in vegetables at 25% due to insect pests alone (Rahman, 2006).

The high risk groups exposed to pesticides include production workers, formulators, sprayers, mixers, loaders and agricultural farm workers. During manufacture and formulation, the possibility of hazards may be higher because the processes involved are not risk free. In industrial settings, workers are at increased risk since they handle various toxic chemicals including pesticides, raw materials, toxic solvents and inert carriers.

Pesticides have been linked with wide range of health hazards ranging from short term effects such as headaches and nausea to chronic ailments like cancer, reproductive harm and endocrine disruptions. Acute dangers include nerve, skin, eye irritation and damage, dizziness, fatigue, and systematic poisoning. Chronic health effects may occur years after even exposure to pesticides in environment or result from pesticide residues which we take through food and water. It has been linked to all kind of diseases and conditions like Parkinson’s syndrome to cancer. Impacts on environment have been well known. Too much pesticide use can damage agricultural land by harming beneficial insect’s species, soil microorganisms and worms which naturally limit pest populations and maintain soil health. It will also reduce concentrations of essential nutrients in soil like nitrogen and phosphorus. Pesticides can also reach surface water through runoff from treated plants and soils. Contamination of water by pesticides is widespread (Mir, I. U. 2018).

Although the vegetable production is increasing day by day in Bangladesh, various statistical data shows that it is mainly because of area expansion, not because of yield improvement. One potential drawback associated with a shift toward more intensive vegetable production is the reliance of most vegetable producers on heavy application of pesticide (Hossain *et al.*, 2000).

The use of pesticides plays a vital role to eliminate the pests and for economic production. But it may cause threat to the human health, non-target organisms and the ecosystem when used indiscriminately without knowing their hazardous effects. If these pesticides reach the human body directly or via food chain, causing various problems like hazardous health effect, different human and animal diseases by the accumulation of pesticide residues in the body of animals and human beings. Indiscriminate use of pesticides may cause the contamination of natural sources, air, water, and food, the risk to humans may be short term as well as long term. The intensity of the risk depends on the persistence of the pesticide, the exposure period, and other environmental conditions. The pesticides should be used in such a way that there must not any residues in food product or if any, the amount must not exceed the ADI (Acceptable Daily Intake) or the MRL's (Maximum Residue Level).

Every pesticide has a withholding period such as waiting period, lapse period or pre-harvest interval (PHI), which is defined as the number of days required to lapse, between the date of final pesticide application and harvest, for residues to fall below the tolerance level established for that crop or for a similar food items. The PHI differs from pesticide to pesticide and crop to crop. Only after withholding period has lapsed the food products become safe for consumption. By this time, the pesticide residues get dissipated or 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 dosages and use pesticides at any stage of the crop without any awareness of the residues and their ill effects on human health. They pick/ 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 (Anastassiades *et al*., 2003; Prodhan *et al.,* 2016; Prodhan *et al.,* 2016a; Prodhan *et al.,* 2015; Prodhan *et al.,* 2015a; Schenck *et al*., 2008; Singh *et al.* 2012; Dasika *et al.,* 2012; Lehotay,2010). The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method for pesticide residue analysis was introduced in 2003 (Anastassiades *et al.,* 2003). This method provides high-quality results in a fast, easy, inexpensive approach. Follow-up studies have further validated the method for more than 200 pesticides (Lehotay *et al.,* 2005). The improved results for the remaining few problematic analytes (Lehotay *et al.,* 2005) and tested it in fat-containing matrices (Lehotay *et al.,* 2005). As a result QuEChERS extraction techniques along with Gas Chromatography were used in this study to determine selected pesticides in cauliflower and tomato.

The influence of the pesticides residues at the community level is unknown. The broader research on the pesticide application practices to establish the important practices that have significant association with the occurrence of pesticide residues in vegetables is needed. Results from this research will allow for the allocation of resources for improvement, monitoring and control of these practices to minimize the risk of unwanted pesticide residues in vegetables. Continuous monitoring of pesticide residues in food, as well as the correlated human dietary exposure, is highly recommended in order to inform policymakers and risk managers of the status of the risk of exposure to pesticide residues (Kiwango *et al.*, 2018).

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 Rangpur City. Therefore, the present research work was conducted with the following objectives:

- ✓ To determine pesticide residues (if any) in cauliflower and tomato collected from different areas of Rangpur district.
- ✓ To quantify the level of detected pesticide residues.
- ✓ To compare the level of pesticide residues in the selected vegetables with the MRL of respective pesticide recommended by the EU-MRL.

CHAPTER II

REVIEW OF LITERATURE

2.1 Pesticides

Pesticides are necessary tools used for managing and damaging invasive pests in agriculture, forestry and landscape. Pesticides include all materials that are used to prevent, destroy, repel, attract or reduce pest organisms. Insecticides, herbicides, fungicides and rodenticides are some of the more well-known pesticides. Others include growth regulators, plant defoliants, surface disinfectants and some swimming pool chemicals. This includes all types of pesticides used in both conventional and organic production systems, whether they are manufactured or naturally occurring.

Pesticides are only toxic substances released intentionally into our environment to kill living things. This includes substances that are used to kill weeds (herbicides), insects (insecticides), fungus (fungicides), rodents (rodenticides) and others (Mir, I. U. 2018).

Cambridge dictionary defines pesticide as a chemical substance used to kill harmful insects, small animals, wild plants, and other unwanted organisms.

The term pesticide includes all of the following: herbicide, insecticide, insect growth regulator, nematicide, termiticide, molluscicide, piscicide, avicide, rodenticide, predacide, bactericide, insect repellent, animal repellent, antimicrobial, fungicide, disinfectant (antimicrobial), and sanitizer (Randall *et al*., 2013).

Pesticide, any toxic substance used to kill animals, fungi, or plants that cause economic damage to crop or ornamental plants or are hazardous to the health of domestic animals or humans. All pesticides interfere with normal metabolic processes in the pest organism and often are classified according to the type of organism they are intended to control.

The Environmental Protection Agency (EPA), the government body that regulates pesticides in the U.S., says in Section 2(u) of the Federal Insecticide, Fungicide, and Rodenticide Act.:The term "pesticide" means (1) any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest, (2) any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant.

According to the Food and Agriculture Organization (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 the control of insects, arachnids, or other pests in or on their bodies.

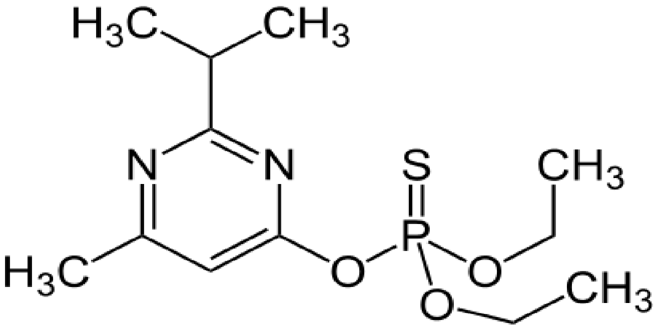
Pesticides are named three ways: by active ingredient, by trade name, and by chemical name. The active ingredient is the chemical that controls the pest. The name of the active ingredient is also known as the common name and is written beside the guarantee on a pesticide label. The trade name is the brand name that the manufacturer gives to the pesticide. It is the prominent name on the front of a pesticide label. Pesticides with different trade names can contain the same active ingredient and some pesticides have more than one active ingredient. The chemical name is the name of the chemical structure of the active ingredient and is used by scientists.

The product that one buys or is exposed to is actually a pesticide formulation that contains a number of different materials such as active and inert ingredients, as well asimpuritiesand contaminants. Besides, pesticides, when subject to various environmental conditions, break down to other materials which are sometimes more toxic than the parent materialknown as metabolites.

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

2.2. Description of the selected pesticides analyzed the present experiment 2.2.1. Diazinon

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



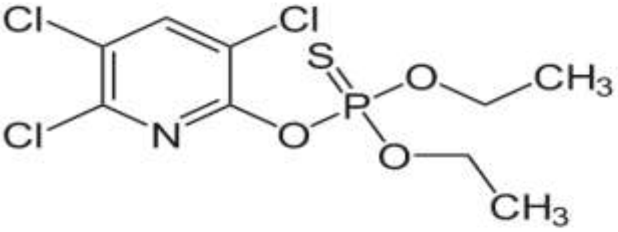
Structural formula of diazinon

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

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

2.2.2. Chlorpyrifos

Chlorpyrifos is a broad-spectrum organophosphate (OP) pesticide, which is a non-systemic insecticide designed to be effective by direct contact, ingestion, and inhalation (Tomlin*et al.*, 2006). The Chemical Abstracts Service (CAS) registry number is 2921-88-2 and the IUPAC name is *O,O*-dimethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate (US.EPA, 2006). It is a colorless to white crystalline solid (US.EPA, 2006; Tomlin*et al.*, 2006). It has a mild mercaptan (thiol) odor, similar to the smell of sulfur compounds found in rottenonions, garlic,eggs and skunks (Tomlin*et al.*, 2006; Lewis*et al.*, 1998).

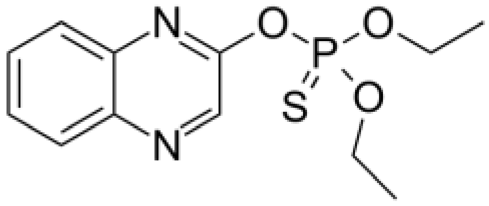


Structural formula of chlorpyrifos

The United States Department of Agriculture (USDA) Pesticide Data Program collects data on pesticide residues in food products and compiles those data on an annual report of the findings. The 2007 annual summary reported 9735 samples of fruit and vegetable commodities tested for chlorpyrifos residues. Chlorpyrifos was detected in 330 (3.48%) of these samples (USDA, 2014). Chlorpyrifos residues were found in 18.0% of peaches tested (100 detections), in 15.6% of nectarines tested (89 detections), in 6.7% of broccoli tested (50 detections) and in 5.3% of kale greens (20 detections). Chlorpyrifos residues were also examined in almonds (46% of samples tested, 167 detections) and corn grain (30% of samples tested, 194 detections) (USDA, 2014). Chlorpyrifos was detected at levels exceeding the U.S. EPA tolerance in one sample each of collard greens (354 samples, 10 with detectable residues) and summer squash (741 samples, 5 with detectable residues). In collard greens, residues were detected in one sample at 6.2 ppm (tolerance of 2.0 ppm). In summer squash, residues were detected in one sample at 0.32 ppm (tolerance 0.10 ppm) (USDA, 2014).

2.2.3. Quinalphos

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

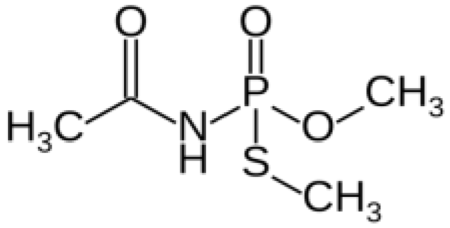


Structural formula of quinalphos

It is classified as a yellow label (highly toxic) pesticide in India and widely used in the following crops: wheat, rice, coffee, sugarcane, and cotton (Pramila*et al.*, 2004).

2.2.4. Acephate

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



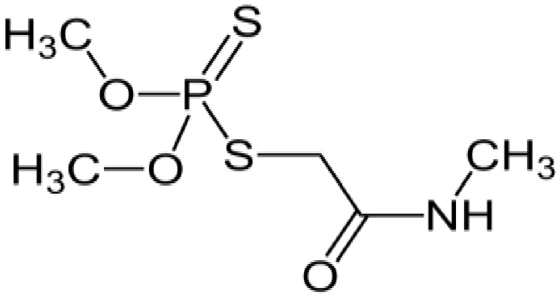
Structural formula of acephate

IUPAC name of acephate is O, S-Dimethyl acetylphosphoramidothioate2 and the Chemical Abstracts Service (CAS) registry number is 30560-19-1(EPA, 2006).Molecular weight is 183.15 g/mol and the solubility (water)is 79 - 83.4 g/100 ml (Davy *et al.*, 2007). In plants, soil and insects it is converted to methamidophos. Methamidophos is another organophosphate insecticide that is registered by the U.S. EPA (EPA, 2006).

Like other organophosphates acephate also bind to and inhibit the enzyme acetylcholinesterase (AChE) in nervous system tissues. Therefore, the neurotransmitter acetylcholine accumulates and constantly activates cholinergic receptors (Klaassen*et al.*, 2001; Reigart *et al.*, 1999).

2.2.5. Dimethoate

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



Structural formula of dimethoate

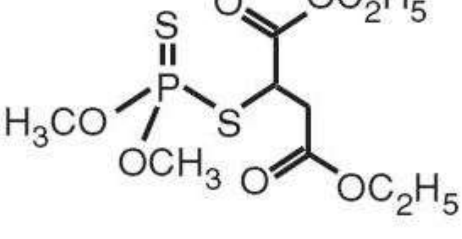
The CAS No. of dimethoatc is 60-51-5 and the IUPAC name is O,O-dimethyl.9-[2- (rnethvlamino)-2-oxoethyl] dithiophosphate (EPA 2006). Dimethoate is a colorless crystalline solid with a camphor-like (mercapian) odor (Worihing, 1987). It will breakdown rapidly when heated to temperatures above 80° C. creating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

(Meister, 1992). Dimethoate is possiblycarcinogenic (Hayes, 1982; Hallenbeck and Cunningham, 1985). An increase in malkznani tumors was reported in rats given oral closes of 'S. IS or 30 mg/kg dimethoate for 511 to 627 clays (Hayes and Laws, 1990). Dimethoate is available in aerosol spray, dust, emulsifiable concentrate and ULV concentrate formulations (Hayes and Laws, 1990: Meister, 1992).

2.2.6. Malathion

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

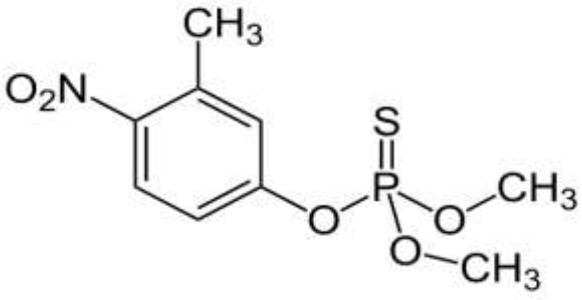
The Chemical Abstracts Service (CAS) registry number is 121-75-5 and the International Union of Pure and Applied Chemistry (IUPAC) name for malathion is O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate (EPA, 2006). Malathion is a colorless to amber liquid with a skunk- or garlic-like odor (US.DHHS, 2008). The vapor pressure of malathion is 1.78×10^{-4} mmHg at 25°C or 5.3 mPa at 30°C and also 1.2×10^{-4} to 8×10^{-6} mmHg at 20 °C(HSDB,US.DHHS, 2008; Tomlin *et al.*, 2006; Hornsby *et al.*, 1006), the molecular weight is about 330.4 g/mol (Tomlin *et al.*, 2006) and the solubility (water)is 145 mg/L(Tomlin *et al.*, 2006).



Malathion

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

2.2.7. Fenitrothion



Structural formula of fenitrothion

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

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

2.3. Instrument used for pesticide residues determination

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

2.3.1. Liquid Chromatography-Mass Spectrometry (LC-MS)

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

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

2.3.2. Gas chromatography–mass spectrometry (GC–MS)

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

2.3.3. Gas Chromatography (GC)

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

2.3.4. High Performance Liquid Chromatography (HPLC)

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

2.4. Pesticides residues

According to WHO pesticide residue is any substance or mixture of substances in food for man or animal resulting from the use of a pesticide and includes any specified derivatives such as degradation and conversion products, metabolites, reaction products and impurities that are considered to be of toxicological significance.

Pesticide residues are the very small amounts of pesticides that can remain in or on a crop after harvesting or storage and make their way into the food chain. Pesticide residues also include any breakdown products from the pesticide.

2.4.1. Acceptable daily intake or ADI

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

2.4.2. Maximum Residue Level (MRL)

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

2.4.3. Determination of Pesticide Residues in Food

Amrollahi *et al.* (2018) conducted a research on pesticide multi-residue analysis in tomato and cucumber samples collected from fruit and vegetable markets in Tehran, Iran. They used gas chromatography-mass spectrometry (GC-MS) and solid phase extraction to detect the pesticides with a sufficient extraction method. The lowest level of pesticide residues were concerning carbaryl, fenpropat and endosulfan in cucumber samples selected from greenhouses and fields with 11.1%, while the highest level was concerning diazinon in cucumber samples of the fields with 55.5%. Carbaryl and permethrin showed the high residue means of 0.37 and 0.72 µg/g in tomato and cucumber samples respectively.

Chiu *et al.* (2018) conducted a research onassociation between pesticide residue intake from consumption of fruits and vegetables and pregnancy outcomes among women undergoing infertility treatment with assisted reproductive technology. Their analysis included 325 women who completed a diet assessment and subsequently underwent 541 ART cycles in the Environment and Reproductive Health (EARTH) prospective cohort study (2007-2016) at a fertility center at a teaching hospital. They found that greater intake of high–pesticide residue FVs was associated with a lower probability of clinical pregnancy and live birth. Compared with women in the lowest quartile of high-pesticide FV intake (<1.0 servings/d), women in the highest quartile (≥2.3 servings/d) had 18% (95% CI, 5%-30%) lower probability of clinical pregnancy and 26% (95% CI, 13%-37%) lower probability of live birth. Intake of low–pesticide residue FVs was not significantly related to ART outcomes.

Lawal *et al.* (2018) worked a research on determination of pesticide residues in fruit and vegetables by high-performance liquid chromatography–tandem mass spectrometry with multivariate response surface methodology. They used modified QuEChERS dispersive solid phase extraction coupled with ionic liquid-based dispersive liquid–liquid microextraction for the determination of multi-pesticide residues in fruit and vegetable samples. They analysed jackfruit, strawberries, cucumber, pears, and carrots sample. The resulting linearity range (5–400 µg/kg) and regression coefficient (>0.99) results were satisfactory. The 94.2 and 95.8% accuracy (89–138%) and precision (0–25%) results were satisfactory and within the recommended ranges (≤20%) and (70–120%), respectively. The limits of detection (0.01–0.54 µg/kg) and quantitation (0.03–1.79 µg/kg) were excellent. The matrix effects (≤−87%) for all analysed samples were not significant. The estimated measurement uncertainties (≤27%) were within the acceptable range (≤50%).

Arain *et al.* (2018) carried out a research on analysis of chlorpyrifos pesticide residues in surface water, ground water and vegetables through gas chromatography. They used USEPA 3510C and USEPA 3620C the standard analytical methods for the extraction and clean-up of pesticide residues. In continuation to this the Gas Chromatography-Flame Ionization detector (GC-FID) was used to analyse the samples. Results revealed that in ground water samples the selected pesticide residual concentration was not more than maximum residual limit (MRL), the minimum value 6.6µg/L was found in UC2 while maximum was 11.2µg/L in UC5. Whereas, in vegetable samples the pesticide residual concentration in 20% of potato samples and 15% of okra samples were within MRL values and rest were found with higher concentrations. However, the higher residual concentration was observed in surface water samples having minimum and maximum values 43.46 µg/L and 79.7 µg/L, respectively.

Akhtar *et al.* (2018) conducted a research on determination of pesticide residues in selected vegetables and fruits from a local market of Lahore, Pakistan. They determined selected pesticides namely bifenthrin, difenoconazole, paraquat, dimethomorph, imidacloprid, deltamethrin residual in fruit (guava) and vegetables (eggplant and round guord) collected from shops in commercial market, Lahore. These samples were prepared and subjected to High Pressure Liquid Chromatography (HPLC) for detection of pesticide residues. The results showed that in guava fruit concentration of bifenthrin, difenoconazole, paraquat, diomethomorph and imidacloprid were 5.13, 81.5, 6.6, 0.48 and 1.65 mg/kg respectively. In eggplant sample, bifenthrin, difenoconazole, paraquat, diomethomorph and deltamethrin's detected residues were 3.53, 5.62, 4.58, 0.25 and 0.005 mg/kg respectively while imidacloprid residues were not found. In round gourd, the values of bifenthrin, difenoconazole, paraquat, diomethomorph residues were 3.87, 61.53, 5.01 and 0.15 mg/kg respectively.

Galani *et al.* (2018) hold a research on evaluation of 99 pesticide residues in major agricultural products from the western highlands zone of cameroon using QuEChERS method extraction and LC-MS/MS and GC-ECD analyses. They used QuEChERS method extraction and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) and gas chromatography with electron capture detection (GC-ECD) for the determination of pesticides residues. This method was suitable for detecting the targeted compounds: For 81 pesticides by LC-MS/MS, the Limit of Quantification (LOQ) was between 0.0004 and 0.0537 mg/kg; and for 18 halogenated pesticides by GC-ECD, it ranged from 0.0012 to 0.2180 mg/kg. The residues of 62 pesticides, including 12 banned compounds, were found in the samples. Twenty-one pesticides (34.4%) exceeded their European Union maximum residue limits (MRLs) and 22 pesticides (34.4%) were found in all 6 sampling locations. Malathion and p,p 0 -DDT were the most distributed pesticides, found in almost all the samples and sampling sites. Food items with the highest rates of positive results were chili pepper (23.2%), white pepper (20.2%), kidney beans (17.3%), and soybeans (17.2%).

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

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

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

Prodhan *et al.* (2018c) has been developed and validated a simple and efficient multiple organochlorine pesticide residues analytical method using quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction technique and Gas Chromatography coupled with Electron Capture Detector (ECD) for the determination of 19 organochlorine pesticides (alpha BHC, delta BHC, beta BHC, gama BHC, heptachlor, aldrin, heptachlor epoxide, gama chlordane, alpha chlordane, alpha endosulfan, 4,4 DDE, dieldrin, endrin, 4,4 DDD, beta endosulfan, 4,4 DDT, endrin aldehyde, endosulfan sulphate, methoxychlor and endrin

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

Lozano *et al.* (2018) carried out a research on pesticide residue analysis in fruit and vegetable based baby foods using GC-Orbitrap MS. method validation which was carried out on 15 selected GC-amenable pesticides covered by Commission Directive No. 2006/125/EC in three different baby food matrixes. Recovery studies were performed at 0.003 and 0.006 mg/kg, with 96% of the cases falling within the 70-120% range and with RSDs <15% for all the pesticides assayed. Linearity over 3 orders of magnitude was verified, with residuals <16% and correlation coefficient values >0.995. In general, matrix effect values were >100%. The LOQ was 0.003 mg/kg for 97% of the cases. The validated method was applied to 20 real baby food samples from Spain and to the European Union Proficiency Test FV-BF01 sample, in which the z-scores obtained were <1, thus demonstrating that this instrumentation has good quantitation capabilities.

Yan *et al.* (2018) conducted a research on successive monitoring surveys of selected banned and restricted pesticide residues in vegetables from the northwest region of China. A total of 32 pesticide residues were detected in 518 samples from 20 types of vegetables in this study. 7.7% of the detected pesticide residues exceeded the MRLs. The percentages of residues that exceeded the MRLs for leafy, melon and fruit, and root vegetables were 11.2%, 5.1%, and 1.6%, respectively. There was no seasonal difference in the proportion of samples that exceeded the MRLs in different vegetables. A total of 84.3% (27/32) pesticides were detected at concentrations that exceeded MRLs. And of the 27 pesticides that exceeded the MRLs, 11 (40.7%) were banned for use in agriculture. The most frequently detected pesticides were malathion (9.4%), dichlorvos (8.7%), and dimethoate (8.1%).

Kiwango *et al.* (2018) carried out a research on pesticide residues in vegetables: practical interventions to minimize the risk of human exposure in Tanzania. They explained that consumption of vegetables containing unacceptable levels of pesticide residues is of public concern due to its potentially harmful effects on human health. In that work, they reviewed the current status of pesticide application, the occurrence and exposure of pesticide residues in vegetables as well as factors influencing the problem of pesticide exposure in Tanzania. The review has realized that pesticides are rarely applied to vegetables following good agricultural practices. Further, pesticide residues in vegetables are not monitored and exposure studies are limited. Studies on the influence of vegetable processing on pesticide residues at household level have been done at laboratory scale. They suggested that continuous monitoring of pesticide residues in food, as well as the correlated human dietary exposure, is highly recommended in order to inform policymakers and risk managers of the status of the risk of exposure to pesticide residues.

Jain *et al.* (2018) carried out a study onscreening of 439 pesticide residues in fruits and vegetables by gas chromatography-quadrupole-time-of-flight mass spectrometry based on TOF accurate mass database and Q-TOF spectrum library. They used GC-quadrupole-time-of-flight MS (GC-Q-TOF/MS) for pesticide residue analysis. The methodology verification results show that more than 70 and 91% of pesticides, spiked in fruits and vegetables with concentrations of 10 and 100 µg/kg, respectively, saw recoveries that conform to the European Commission's criterion of between 70 and 120% with RSD ≤20%. Eighty-one percent of pesticides have screening detection limits lower than 10 µg/kg, which makes this a reliable analysis technology for the monitoring of pesticide residues in fruits and vegetables.

Ishaq, Z. and Nawaz, M. A. (2018) hold a research on analysis of contaminated milk with organochlorine pesticide residues using gas chromatography. They determined pesticide residues with the help of gas chromatograph equipped with electron detector. These pesticides were confirmed by using gas chromatography-mass spectrometry (GC-MS). Analysis showed non-significant results ($p > 0.05$) with large variation among all milk samples regarding pesticide residues. The mean values of DDT, DDE were found to be below the MRL value. The mean values of Dieldrin, Ɣ-HCH were also below MRL value. On the other hand mean values of αEndosulphan, β-Endosulphan, and Endosulphan sulphate were slightly higher than MRL in few milk points.

Liang *et al.* (2018) carried out a research on multi-residue determination of pesticides in vegetables on Dalian market by gas chromatography. They used gas chromatography and NY/T 761-2008 pesticide multi-residue screen methods. The residues exceeded MRLs of forbidden pesticides found were: carbofuran 0.110 mg/kg (kidney bean) and methamidophos 0.037 mg/kg (celery), methamidophos 0.037 mg/kg (tomato), aldicarb 0.013 mg/kg (kidney bean), omethoate 2.200 mg/kg (celery), carbofuran 0.052 mg/kg (green pepper), parathion 0.056 mg/kg (celery) and carbofuran 0.030 mg/kg (celery). Also, chlorpyrifos used as unforbidden pesticide was most frequently found above MRL, rape (0.820 mg/ kg) and celery (0.365 mg/kg), celery (0.330 mg/kg), lettuce (0.298 mg/kg), rape (0.910 mg/kg) and lettuce (0.230 mg/kg). In addition, cypermethrin used as unforbidden pesticide was found above MRL only once in rape (1.270 mg/kg) and none of unforbidden pesticides above MRL was found. Most of the samples (96%) were up to the national standard.

Tosi *et al.* (2018) conducted a research on a 3 year survey of Italian honey bee collected pollen of reveals widespread contamination by agricultural pesticides. They analysed 554 pollen samples for pesticide residues, 62% contained at least one pesticide. The overall rate of multiresidual samples (38%) was higher than the rate of single pesticide samples (24%), reaching a maximum of 7 pesticides per sample (1%). Over 3 years, 18 different pesticides were detected (10 fungicides and 8 insecticides) out of 66 analysed. The pesticide which was most frequently detected was the insecticide chlorpyrifos (30% of the samples overall, exceeding ARfD, ADI, or MRL in 99% of the positive ones), followed by the fungicides mandipropamid (19%), metalaxyl (16%), spiroxamine (15%), and the neonicotinoid insecticide imidacloprid (12%). Imidacloprid had also the highest HQ level (5054, with 12% of its positive samples with HQ higher than 1000).

Mahugija *et al.* (2017) carried out a research on assessment of pesticide residues in tomatoes and watermelons (fruits) from markets in Dar es Salaam, Tanzania. They analyzed the tomatoes and watermelons samples for eighteen organochlorine, organophosphorus and pyrethroid pesticide residues. They used acetone followed by dichloromethane: cyclohexane mixture for extraction and the extracts were cleaned up using florisil. The compounds were determined by GC–MS. Pesticides and metabolites were detected in 95.8% of the samples. The compounds detected included chlorpyrifos, α-endosulfan, β-endosulfan and cypermethrin and their highest concentrations were 3810 ± 50, 370 ± 20, 120 ± 6 and 50 ± 4 ng/g, respectively. Others were p,p'- DDD, o,p'-DDD, p,p'-DDE and α-HCH with highest concentrations varying from 1 ± 0.5 to 20 ± 1.2 ng/g. The concentrations of the contaminants were above the maximum residue limits (MRLs) in 41.7% to 50% of the tomatoes and watermelons indicating risks and concerns for public health.

Njoku *et al.* (2017) hold a research onassessment of pesticide residue levels in vegetables sold in some markets in Lagos State, Nigeria. They determined pesticide residue levels by using gas chromatography/mass spectrometry. Data obtained were statistically analyzed using graph pad prism 6.0 software and were compared with WHO limits. The vegetables from all the markets had pesticide residue level above the WHO lower limit (0.02 mg/kg). The two vegetables from Oyingbo and Mile's 12 markets had total pesticide residue levels above the WHO upper limit (0.7 mg/kg). *T. occidentalis* from Oyingbo market had the highest total pesticide residue level (2.35mg/kg) and the highest number of pesticides while *C. argentea* from Agege market had the least total pesticide residue level (0.08mg/kg). Alen *et al.* (2017) conducted a research on determination of profenofos pesticide residue in tomato (*Solanum lycopersicum* L.) using GC technique. For the determination of profenofos pesticide residue in tomato they used GC technique with FPD. They divided the samples into three groups: unwashed tomato, washed with tap water and washed with detergent. Samples were extracted once by ultrasonication for 10 minutes with 100 mL ethyl acetate. Results showed that the tomato contained profenofos pesticide residue. The unwashed tomato, washed with tap water and washed with detergent contains 0.159±0.0079; 0.070±0.0009 and 0.067 ±0.0016 ppm respectively. The profenofos residue levels do not exceed the MRL that established by Indonesian National Standard (in ppm).

Mustapha *et al.* (2017) hold a research on monitoring of pesticide residues in commonly used fruits and vegetables in Kuwait. A total of 150 samples of different fresh vegetables and fruits were analyzed for the presence of 34 pesticides using the QuEChERS multi-residue extraction, followed by GC-MS or LC-MS/MS. Pesticide residues above the MRL were detected in 21% of the samples and 79% of the samples had no residues of the pesticides surveyed or contained residues below the MRL. Multiple residues were present in 40% of the samples with two to four pesticides and four samples were contaminated with more than four pesticide residues. Of the pesticides investigated, 16 were detected, of which imidacloprid, deltamethrin, cypermethrin, malathion, acetamiprid, monocrotophos, chlorpyrifos-methyl, and diazinon exceeded their MRLs. aldrin, an organochlorine pesticide, was detected in one apple sample, with residues below the MRL.

John *et al.* (2017) carried out a study on the determination of levels of organochlorine, organophosphorus and pyrethroid pesticide residues in vegetables from markets in Dar es Salaam by GC-MS. They extractedthe samples by using acetone followed by dichloromethane : cyclohexane mixture and the extracts were cleaned up using florisil. The compounds were determined by gas chromatography-mass spectrometry (GC-MS). Pesticides and metabolites were detected in 72.2% of the samples. The detected pesticide residues and their highest mean concentrations were DDT $4.00 \times 10{-}3$ mg/kg, α-endosulfan $6.00 \times 10{-}1$ mg/kg, β-endosulfan $2.10 \times 10{-}1$ mg/kg, chlorpyrifos 3.00 mg/kg, and cypermethrin $4.00 \times 10{-}2$ mg/kg. The most frequently detected compounds were chlorpyrifos. The order of contamination was spinach > cabbage > onions.. The concentrations of contaminants were above the maximum residue limits (MRLs) in 33.3–50% of the samples.

Mohammed, M. and Boateng, K.K. (2017) conducted a research on evaluation of pesticide residues in tomato (*Lycopersicum esculentum*) and the potential health risk to consumers in urban areas of Ghana. They analyzed the samples for high percentage of organophosphorous pesticide residues (45%) in Ayigya market with Bantama market recording high percentage of organochlorines and pyrethroid. Laboratory analysis of tomato samples for various pesticides residues has indicated that all the pesticide residues pose no threat to human's health with all estimated hazard indices being below 1; however, heptachlor (HI=0.85) and dieldrin (HI=0.74) have shown the highest risk levels in children.

Grewal *et al.* (2017) hold a research on pesticide residues in food grains, vegetables and fruits: a hazard to human health. They observed from this research that the pesticide residues in food and crops are directly related to the irrational application of pesticides to the growing crops. Accumulated pesticide residues in food products have been associated with a broad variety of human health hazards, ranging from short-term effects to long term toxic effects. They suggested that the impact of pesticide residues can be minimized by taking certain measures such as the rational use of pesticides, promoting organic farming, exploiting natural and bio pesticides, and proper implementation and amendment of pesticide-related laws.

Wittayanan *et al.* (2017) conducted a research on multiresidue method for determination of 20 organochlorine pesticide residues in fruits and vegetables using modified QuEChERS and GC-ECD/GC-MSD. Analytical determinations of the 20 OCs were carried out by gas chromatography, using electron capture detector (GC-ECD) and confirmed by mass spectrometry detector (GCMSD). Recovery studies were performed at 0.05, 0.15 and 1.5 mg kg−1 fortification levels and the results of validation parameters were acceptable (accuracy ranged from 61.2% to 117.6%, precision expressed as %RSD were less than 20 and measurement uncertainty was lower than 50%). The limit of detection (LOD) and the limit of quantitation (LOQ) for the OCs tested in apple and lettuce were 0.02 and 0.05 mg kg−1 respectively. No residual OCs was detected in any vegetable sample. However, detectable pesticides residues were found in 0.4% (3 apple samples) of the fruit samples.

Koch *et al.* (2017) hold a research on pesticide residues in food: attitudes, beliefs, and misconceptions among conventional and organic consumers. A representative sample of the population aged 14 years and older (n = 1,004) was surveyed via computer-assisted telephone interviewing on their attitudes and knowledge with regard to pesticide residues. Based on questions regarding their typical consumer behavior, respondents were classified into conventional and organic consumers to identify differences as well as similarities between these two consumer types. Evaluating the risks and benefits of pesticide use, more than two-thirds of organic consumers (70%) rated the risks as greater than the benefits, compared with just over one-half of conventional consumers (53%).

Jankowska *et al.* (2016) carried out a study on the dissipation of six fungicides in greenhouse-grown tomatoes with processing and health risk where evaluate the dissipation rate kinetics and estimate the behavior of selected pesticides after washing, canning, peeling and simmering of tomato expressed as processing factor (PF). Two varieties (Marissa and Harzfeuer) were treated by six fungicides: chlorothalonil, pyraclostrobin, boscalid, cyprodinil, azoxystrobin and fludioxonil at single and double dose and risk assessment defined as hazard quotient was performed. The QuEChERS method was used for sample preparation subsequently liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

The dissipation of fungicides approximately fitted to a first-order kinetic model, with half-life values ranging from 2.48 and 2.66 days (cyprodinil) to 5.00 and 5.32 days (chlorothalonil) for Marissa and Harzfeuer variety, respectively. Results from processing studies showed that treatments have significant effects on the deletion of the studied fungicides for both varieties. The PFs were generally less than 1 (between 0.01 and 0.90) which did not depend on variety. The dietary exposure assessed based on initial deposits of application at single and double dose on tomatoes. The concentration after each process with PF correction showed no concern to consumer health. The results would be a useful tool for monitoring of fungicides in tomatoes and provide more understanding of residue behavior and risk posed by these fungicides.

Zhou *et al*. (2016) used the analysis of 48 crude pollen samples collected from 8 provinces of China. The main detection rates of each compound were 77.2% for carbendazim, 58.2% for fenpropathrin, 56.3% for chlorpyrifos, 50.0% for fluvalinate, 31.3% for chlorbenzuron, and 29.2% for triadimefon in crude pollen samples. The maximum values of each pesticide were 4516 ng/g for carbendazim, 162.8 ng/g for fenpropathrin, 176.7ng/g for chlorpyrifos, 316.1 ng/g for fluvalinate, 437.1 ng/g for chlorbenzuron, 79.00 ng/g for triadimefon.

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

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

Tiele *et al.* (2015) carried out a stuy on the optimization of a QuEChERS based method by means of central composite design for pesticide multi residue determination in orange juice by UHPLC–MS/MS. Different extraction procedures based on the QuEChERS method were compared for the multi residue determination of pesticides in orange juice by ultra high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS). After choosing preliminary conditions, an experimental design was carried out with the variables C18, PSA, NaOH and CH3COONa to optimize the sample preparation step. The validation results of the validation were satisfactory, since the method presented recoveries between 70% and 118%, with RSD lower than 19% for spike levels between 10 and 100 lg L1 . The method limit of detection (LOD) and limit of quantification (LOQ) ranged from 3.0 to 7.6 lg L1 and from 4.9 to 26 lg L1 , respectively. The method developed was adequate for the determination of 74 pesticide residues in orange juice.

Marek, B. and Jolanta, S. (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 vegetable and fruitsamples, as are the techniques most commonly used in these processes. They also outlined the difficulties occurring at all stage in the analytical procedure.

Anand *et al.* (2015) carried out a research on pesticide residue analysis in fruits and vegetables collected from Kolkata, West bengal, India. They studied with 35 samples including fruits (grape, banana, apple, and guava) and vegetables (tomato, brinjal, and cucumber) from three sampling locations Kancharapara, Jaguli and Barasat. They used Gas Chromatograph coupled with Mass spectrophotometer (GC-MS) to determine pesticides residues. They identified four major pesticides belonging to synthetic pyrethroid and carbamates. The identified pesticides were aldicarb, allethrin, oxamyl and molinate. The typical percent recoveries were found to be 95 Â± 10 for all pesticides. The average level of pesticides concentration of aldicarb, allethrin, oxamyl and molinate were 0.36, 3.93, .18 and 2.83 ppm respectively for fruits and 0.86, 5.57, 5.55 and 3.67 ppm respectively for vegetables were found. The concentration of three pesticides was above the prescribed maximum residue levels (MRL) except for aldicarb.

Prodhan *et al.* (2015) conducted a research on Determination of Multiple Pesticide Residue in Eggplant with Liquid Chromatography-Mass Spectrometry where a simple and efficient multiple pesticide residue analytical method using quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction and liquid chromatography triple quadrupole-mass spectrometry was developed and validated for the determination of ten insecticides and three fungicides in eggplant. The method was validated by evaluating the accuracy, precision, linearity, limit of detection, and limit of quantification. They evaluated matrix effect and it was found that thiamethoxam, cypermethrin, and deltamethrin had pronounced matrix effect (-69, +57, and +93 %, respectively). They applied this method for the residue analysis of 72 fresh eggplant fruit samples collected from different market places in Thessaloniki, Greece. Among the 72 analyzed samples, 34 (47 % of the total no. of samples) had pesticide residues, of which, 5 had multiple pesticide residues and 29 had single pesticide residue. Only one sample contained residue above the EU-MRLs.

Prodhan *et al.* (2015a), where they use a rapid, precise and efficient method for the determination of seven insecticides (chlorpyrifos, dimethoate, deltamethrin, thiamethoxam, thiacloprid, pirimicarb and indoxacarb) and three fungicides (azoxystrobin, fluopicolide and propamocarb hydrochloride) in melon by employing quick, easy, cheap, effective, rugged and safe extraction method coupled with liquidchromatography triple quadrupole mass spectrometry which was developed and validated by evaluating the accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ). They evaluated matrix effect and it was found considerable for thiamethoxam and deltamethrin (-53% and +135%, respectively). Finally, this method was applied for the residue analysis of 122 fresh melon samples collected from different market places in Thessaloniki, Greece. Among the 122 analysed samples, 32 (26% of the total no. of samples) were found to have pesticide residues. None of the samples contained residues above the European Union Maximum Residue Levels. The most frequently detected pesticides were fluopicolide and thiamethoxam.

Ishibashi *et al.* (2015) examined the screening method for multi-residue pesticide analysis, which is simple, accurate and quick and has a reliable performance, is becoming increasingly important for food safety and international trade. This paper proposes a high through put screening methodology that enables the detection of multi-residue pesticides using supercritical fluid chromatography coupled to a high performance bench top quadrupole orbitrap mass spectrometry (SFC/Q Exactive) and an automated library based detection. A total of 444 chemicals covering a wide polarity range (log Pow from -4.1 to 7.7) and a wide molecular weight range (from 99.0 to 871.5) were analyzed simultaneously through a combination of high mass resolution (a value of $m/\Delta m = 70000$), high mass accuracy (<5 ppm) with positive/negative polarity switching, and highly efficient separation by SFC. A total of 372 pesticides were detected in QuEChERS spinach extracts without dispersive solid phase extraction at the 10 µg kg^{-1} level (provisional maximum residue limits in Japan).

Mukherjee *et al.* (2015) carried out a research on Analytical method validation and comparison of two extraction techniques for screening of azoxystrobin from widely used crops using LC–MS/MS where a simple analytical method was developed and validated in tomato, grape, mango and chilli fruits using liquid chromatography tandem mass spectrometry. The method comprised of extraction with cyclohexane and ethyl acetate mixture followed by d-SPE cleanup employing modified quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction method and quantified in LC–MS/MS using gradient elution. The method was validated in concentration ranging from 0.01 to 0.1l µg g^{-1}. The recovery of azoxystrobin in different crops was ranging from 84.35to 95.63% at three different concentration levels of analytes with relative standard deviation of 4–14 %. The global uncertainty was calculated at limit of quantification level i.e. 0.011 µg g^{-1}. The PHI values of azoxystrobin in tomato, mango, grape and chilli fruits were determined as 4.76, 3.90, 4.06 and 10.74 days respectively.

Shasha *et al.* (2014) conducted a study on determination of organophosphorus pesticide residues in cabbages from bindura market place by solid phase extraction and gas liquid chromatography. Gas Chromatography-Nitrogen Phosphorus Detector (GC-NPD) coupled to solid phase extraction on florisil column (500mg/8ml) cartridges was used to determine organophosphate pesticides levels. Percentage recovery was above 76%. Limit of detection and quantification was 0.1ng/ml and 2.35ng/ml respectively. The highest level, 0.81 ± 0.02 was obtained from Aerodrome and the lowest level, 0.02 ± 0.00 was recorded for Magobo cabbages. Overally a total of 51 pesticides residues were detected in 180 samples corresponding to detection rate of 28%. The implicated organophosphate pesticides include; acephate, dimethoate; malathion, chlopyriphos-methyl and monocrotophos.

Satpathy *et al.* (2014) carried out a research on development and validation of multi-Residue analysis of 82 pesticides in grapes and pomegranate as per the requirements of the European Union (EU) and codex alimentarius using GC-MS/MS with compound based screening. They validated the (QuEChERS) multi-residue method for the extraction of 82 pesticides belonging to various chemical classes from grapes and pomegranate (commodities with high sugar and low lipid contents). They found that matrix-matched calibration results have demonstrated good reproducibility, robustness and linearity. They also found the mean recoveries mostly ranged between 70 and 110 % (91% on average) and RSD were generally below 12% (7.3% on average).For all compounds LODs were 0.001 to 0.005 mg/kg and LOQs were 0.005 to 0.020 mg/kg.

Islam *et al.* (2014) undertook a research on detection and quantification of pesticide residues in selected vegetables of Bangladesh. They took interview of 23 farmers and noted that fourteen pesticides belonging to different groups were found to be commonly used on the selected vegetables by the respondent farmers to control the major pests. In two selected locations of Narsinghdi 8.33 to 45.00 percent farmers were recorded to apply different pesticides every day and in some cases even twice in a day on vegetables. They collected 42 samples from fields and markets and analysised multiple pesticide residue by Gas Chromatography (GC) with Flame Thermionized Detector (FTD) and Electron Capture Detector (ECD). Out of 42 samples, 27 had pesticide residue. Among these 27 samples, 14 samples had pesticide residues above the Maximum

Residue Limit (MRL). The detected pesticides were diazinon, malathion, quinalphos, fenitrothion, cypermethrin, fenvalerate and propiconazole.

Prodhan et al.(2014) conducted a research on determination of multiple pesticide residue in eggplant with liquid chromatography-mass spectrometry by adopting (QuEChERS) extraction and liquid chromatography triple quadrupole-mass spectrometry was developed and validated for the determination of three fungicides andten insecticides in 72 fresh eggplant fruit samples collected from different market places in Thessaloniki, Greece. The method was validated by evaluating the linearity, accuracy, precision, LOD, and LOQ and the average recoveries of the selected pesticides ranged from 71.8 to 112 % with precision in case of repeatability (RSDr) ≤16.2 % in four fortification levels of 0.01, 0.05, 0.1, and 0.5 mg/kg. They found the linearity about ≥0.997 for all of the selected pesticides. The limit of detection (LOD) ranged from 0.001 to 0.003 mg/kg and the limit of quantification (LOQ) was 0.01 mg/kg, which was lower than the Maximum residue levels set by European Union (EU-MRLs). Among the 72 analyzed samples, 34 (47 % of the total no. of samples) had pesticide residues, of which, 5 had multiple pesticide residues and 29 had single pesticide residue. Only one sample contained residue above the EU-MRLs.

Panhwar *et al.* (2014) hold a research on residue removal of pesticides from brinjal using different processing methods. The study was conducted on the residual removal of six pesticides (bifenthrin, profenofos, endosulfan, imidacloprid difenthiuron and emamectin benzoate) from brinjal by using various house hold processing methods such as washing, detergent washing, peeling, drying and cooking/frying etc. From water soluble pesticides diafenthiuron reduced more in blanching as compared with imidacloprid and emamectin benzoate. Frying reduced the residues more effectively, rendering it fit for human consumption. Washing followed by frying reduced the fat soluble residues >70% whereas water soluble pesticide residues >80%. It was concluded that each processing was found to be effective in reduction of pesticides residues.

Mantzos *et al.* (2013) conducted a research work on QuEChERS and solid phase extraction methods for the determination of energy crop pesticides in soil, plant and runoff water matrices where QuEChERS and solid phase extraction (SPE) methods were applied for determining four herbicides (quinmerac, oxyfluorfen, quizalofop-p-ethyl, metazachlor) and one insecticide (α (±)-cypermethrin) in runoff water, soil, sunflower and oilseed rape plant matrices. Determination was performed using GC-MS, whereas HPLC-MS was used for quinmerac. In all substrates linearity was evaluated using matrix-matched calibration samples at five concentration levels (50–1000 ng L^{-1} for water, 5–500 μg kg^{-1} for soil and 2.5–500 μg kg^{-1} for oilseed rape or sunflower plant). Correlation coefficient was higher than 0.991 for all pesticides in all substrates. Acceptable mean recovery values were obtained for all pesticides in water (65.3–108.7%), soil (70.0–110.0%) and plant (66.1–118.5%), with intra- and inter-day RSD% below 20%. LODs were in the range of 0.250–26.5ng L^{-1} for water, 0.10–1.7 μg kg^{-1} for soil and 0.15–2.0 μg kg^{-1} for plants.

Hossain *et al.* (2013) carried out a research on health risk assessment of pesticide residues via dietary intake of market vegetables from Dhaka, Bangladesh. They used GC with a photo diode array detector (HPLC-PDA) to determine six organophosphorus (chlorpyrifos, fenitrothion, parathion, ethion, acephate, fenthion), two carbamate (carbaryl and carbofuran) and one pyrethroid (cypermethrin) pesticide residues in twelve samples of three common vegetables (tomato, lady’s finger and brinjal). Acephate, chlorpyrifos, ethion, carbaryl and cypermethrin were detected in only one sample, while co-occurrence occurred twice for fenitrothion and parathion.

Apart from chlorpyrifos in tomato and cypermethrin in brinjal, all pesticide residues exceeded the maximum residue limit (MRL). Hazard risk index (HRI) for ethion (10.12) and carbaryl (1.09) was found in lady’s finger and tomato respectively.

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

Milhome *et al.* (2013) carried out a research on validation and uncertainty of the method for multiresidue analysis of 35 pesticides in melon using gas chromatography coupled to quadropole mass spectrometry (GC-QP/MS).

They determined various validation parameters such as (selectivity, linearity, LOD, LOQ, accuracy and precision) according ABNT NBR 14029:2005. The recoveries rate for all the pesticide they studied was from 63-117% with RSD lower than 15% in the concentration range of 0.05-0.20mg/kg. They also found the LOQ for most compounds were below the MRLs established in Brazil.

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

Dasika *et al.* (2012) conducted a study on pesticide residue analysis of fruits and vegetables. This project describes an efficient and effective analytical method to screen pesticides in fruits and vegetable samples using liquid chromatography tandem mass spectrometry (LC-MS/MS). QuEChERS method with acetate buffering (AOAC Official Method 2007.01) was used for sample preparation, which has high-qualityyield results for hundreds of pesticide residues in foods.

Alam *et al.* (2011) carried out a research on determination of pesticide residue (cartap) in brinjal. They sprayed a dose of 2.5g/L of cartap a.i. pesticide at the time of cultivation in the field. They used high performance liquid chromatography (HPLC) to determine residue in the samples. The range of concentrations of cartap in treated samples, (T-1, T-2, T-3, T-4 and T-5) was from 0.954 to 3.300 ppm where cartap levels were found above the Maximum Residue Limit for crops 0.5 ppm recommended by FAO/WHO. The study reveals that use of pesticide to minimize vegetable infestation is increasing and this trend would be detrimental to mankind as well as environment.

Benson, N. U. and Olufunke, A. I. (2011) hold a research on assessment of contamination by organochlorine pesticides in *Solanum lycopersicum L.* and *Capsicum annuum L.*: A market survey in Nigeria. They used Hewlett Packard (HP 6890) gas chromatograph equipped with electron capture detector (GC-ECD) to analyses the detected samples. In *S. lycopersicum*, the total residue concentrations of ΣHCH (0.140 mg/kg), ΣHCB (0.144 mg/kg), ΣDDT (0.572 mg/kg), Σdieldrin (0.073 mg/kg), and Σtrans-nonachlor (0.117 mg/kg) were found. In *C. annuum*, the total residue levels of HCH, HCB, DDT, dieldrin and trans-nonachlor measured were 7.220, 0.096, 0.275, 0.037 and 0.117 mg/kg respectively. The α-HCH isomer was not detected in *S. lycopersicum* and p,p’-DDD found only in *C. annuum* samples.

Lazartigues *et al.*, (2011) carried out a research on the Multiresidue method to quantify pesticides in fish muscle by QuEChERS-based extraction and LC-MS/MS.They used QuEChERS method coupled to liquid chromatography tandem mass spectrometry (Scheduled MRM-5500 QTRAP) to quantify 13 pesticides (azoxystrobin, dimethachlor, clomazone, diflufenican, carbendazim, iprodion, isoproturon, mesosulfuron-methyl, metazachlor, napropamid, quizalofop and thifensulfuron-methyl) in muscle of fish. Quantification limits were below 1 ng g−1 except for clomazone (1.8 ng g−1) and quizalofop (7.4 ng g−1). Best recoveries were observed for perch (>80%) and roach (>68%), except for thifensulfuron-methyl. Lower recoveries had been observed for carp (6% to 86%).

Prodhan M.D.H. *et al.*, (2010) conducted a study on the determination of pre harvest interval for quinalphos, cypermethrin, diazinon and malathion in major vegetables. They conducted six supervised field trials and sprayed the field dose (2 ml/L of water) of each pesticide except cypermethrin (1 ml/L of water). They collected the samples at 0, 1, 3, 5, 7, 10 and 12 days after spray. In all the cases, the left over residue of tested insecticides were detected up to 10 DAS except cypermethrin in tomato which was 7 DAS and the quantities were above MRL up to 7 DAS for malathion in cauliflower, quinalphos in cabbage, 3 DAS for cypermethrin in tomato, cypermethrin in yard long bean, ciazinon in brinjal and 5 DAS for quinalphos in yard long bean. No residue was detected from any of the tested samples collected at 12 DAS except cypermethrin in tomato which was 10 DAS. The PHI may be selected as 10 DAS for quinalphos in cabbage and malathion in cauliflower; cypermethrin in yard long bean and 7 DAS for quinalphos and diazinon in brinjal; 5 DAS for cypermethrin in tomato.

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

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

Yamagami *et al.*(2009) conducted a research on multi-residue method for determination of 85 pesticides in vegetables, fruits and green tea by stir bar sorptive extraction and thermal desorption GC-MS. They used a multi-residue method to determine five groups of 85 pesticides - chlorinated, carbamate, phosphorous, pyrethroid and others - in vegetables, fruits and green tea has been developed using stir bar sorptive extraction (SBSE) coupled to thermal desorption and retention time locked (RTL) GC-MS. Pre-extraction with methanol and dilution with water prior to SBSE (60 min) were performed. Dilution of methanol extract for SBSE was examined to obtain high sensitivity and to compensate the effect of adsorption to the glass wall of extraction vessel and to sample matrix for the compounds with high log Ko/w values (e.g. pyrethroid). The methanol extracts were diluted twofold and fivefold and were simultaneously SBSE-enriched. The two stir bars were placed in a single glass thermal desorption liner and were simultaneously desorbed. The versatility of the method was exhibited by its good linearity (4-100 µg/kg, r2 >0.9900) for 66 pesticides and limit of detection (LOD: < 5 µg/kg) for most of the analytes. The method enables to determine pesticides at low µg/kg in tomato, cucumber, green soybeans, and spinach, grape and green tea.

CHAPTER III

MATERIALS AND METHODS

The vegetable (cauliflower and tomato) samples were collected from 5 different markets of Rangpur City and carried to the Pesticide Analytical Laboratory, Entomology Division, BARI, Joydebpur, Gazipur for pesticide residue analysis during September 2017 to March 2018. From the collection of samples to the final analysis, all way obligatory a number of processes which are described below.

3.1. Study area

The study area included major five markets of Rangpur City. The area of Rangpur City is about 845 sq.mi, located at North and east hemisphere. In this study, vegetables were collected from Paura Bazar, Tetultola Bazar, Lalbag Bazar, Termilan Bazar and Station Bazar of Rangpur City.

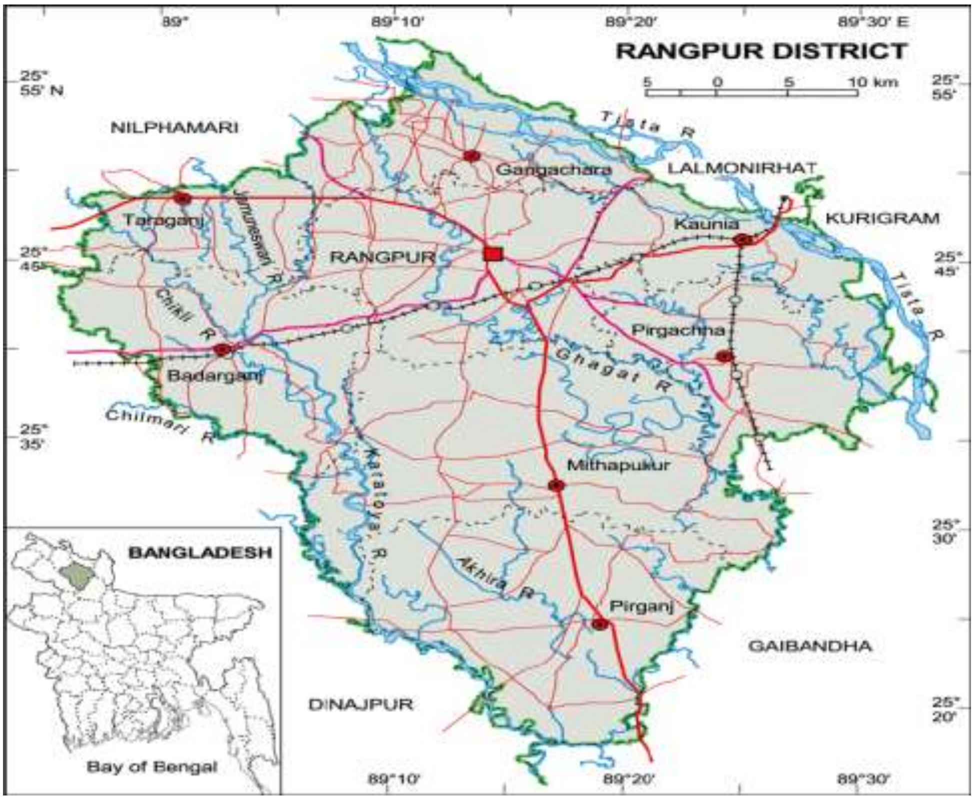


Figure 1: Map showing the different places of Rangpur District.

3.2. Sample collection

A total of 80 samples (40 cauliflower and 40 tomato) were collected for this study. Eight samples of cauliflower and eight samples of tomato were collected from each market. The amount of each sample was 1 Kg for all the selected vegetables. The samples were collected in clean transparent air tight polyethylene bag and each bag was properly labeled with sample number and sources. Sample was collected in individual polyethylene bag to avoid cross contamination.

Table 1: Sources and places of collection of cauliflower samples

Area of collection	Sample ID	Source
Paura Bazar	RPBC-1	Gangachara
	RPBC -2	Kaunia
	RPBC -3	Pirgachha
	RPBC -4	Taragonj
	RPBC -5	Badarganj
	RPBC -6	Mithapukur
	RPBC -7	Pirganj
	RPBC -8	Pirgachha
Tetultola Bazar	RTBC -1	Pirgachha
	RTBC -2	Shathibari
	RTBC -3	Taragonj
	RTBC -4	Jaigirhat
	RTBC -5	Ranipukur
	RTBC -6	Mithapukur
	RTBC -7	Kaunia
	RTBC -8	Barodorga
Lalbag Bazar	RLBC -1	Ranipukur
	RLBC -2	Badlipukur
	RLBC -3	Jaigirhat
	RLBC -4	Taragonj
	RLBC -5	Barodorga
	RLBC -6	Jaigirhat
	RLBC -7	Badlipukur
	RLBC -8	Mithapukur
Station Bazar	RSBC -1	Badlipukur
	RSBC -2	Shathibari
	RSBC -3	Barodorga
	RSBC -4	Jaigirhat
	RSBC -5	Ranipukur
	RSBC -6	Mithapukur
	RSBC -7	Taragonj
	RSBC -8	Barodorga
Terminal Bazar	RTEBC-1	Pirganj
	RTEBC -2	Ranipukur
	RTEBC -3	Barodorga
	RTEBC -4	Jaigirhat
	RTEBC -5	Gangachara
	RTEBC -6	Buriganj
	RTEBC -7	Badlipukur
	RTEBC -8	Shathibari

Table 2: Sources and places of collection of tomato samples

Area of collection	Sample ID	Source
Paura Bazar	RPBT -1	Ranipukur
	RPBT -2	Badlipukur
	RPBT -3	Jaigirhat
	RPBT -4	Taragonj
	RPBT -5	Barodorga
	RPBT -6	Jaigirhat
	RPBT -7	Badlipukur
	RPBT -8	Mithapukur
Tetultola Baza	RTBT-1	Badlipukur
	RTBT -2	Shathibari
	RTBT -3	Barodorga
	RTBT -4	Jaigirhat
	RTBT -5	Ranipukur
	RTBT -6	Mithapukur
	RTBT -7	Taragonj
	RTBT -8	Barodorga
Lalbag Bazar	RLBT -1	Pirgachha
	RLBT -2	Shathibari
	RLBT -3	Taragonj
	RLBT -4	Jaigirhat
	RLBT -5	Ranipukur
	RLBT -6	Mithapukur
	RLBT -7	Kaunia
	RLBT -8	Barodorga
Station Bazar	RSBT -1	Pirganj
	RSBT -2	Ranipukur
	RSBT -3	Barodorga
	RSBT -4	Jaigirhat
	RSBT -5	Gangachara
	RSBT -6	Buriganj
	RSBT -7	Badlipukur
	RSBT -8	Shathibari
Terminal Bazar	RTEBT -1	Ranipukur
	RTEBT -2	Badlipukur
	RTEBT -3	Jaigirhat
	RTEBT -4	Taragonj
	RTEBT -5	Barodorga
	RTEBT -6	Jaigirhat

	RTEBT -7 RTEBT -8	Badlipukur Mithapukur

3.3. Sample preparation for analysis

Just after 1 day of collection all samples were taken to the Pesticide Analytical Laboratory, Division of Entomology, Bangladesh Agricultural Research Institute (BARI), Gazipur. The each sample was cut into small pieces and mixed properly. Air tight clean polythene bags were used to store chopped sample in refrigerator at -20˚C until extraction and cleanup process started.

3.4. Chemicals and reagents

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

3.5. Analytical Apparatus Used

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

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

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

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

Plate 1. Electric Balance

Plate 2. Vortex Mixer

Plate 3. Centrifuge Machine

Plate 4. Gas Chromatograph (GC)

Some pictorial view related to sample preparation

Plate 5. Collection of samples

Plate 6. Chopping of Sample

Plate 7. Adding Acetonitrile **Plate 8. Weighing of Salt**

(NaCl and MgSO$_4$)

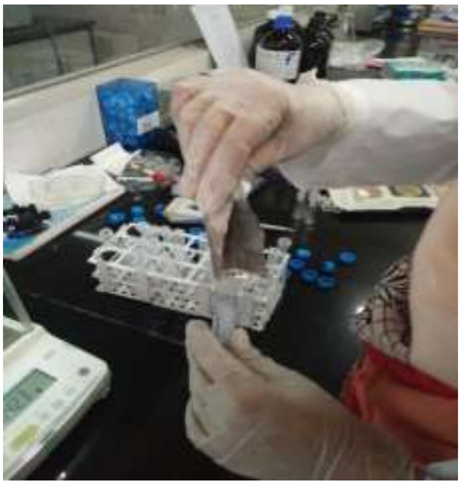


Plate 9. Weighing of PSA **Plate 10. Adding PSA in**
the Sample Extract

Plate 11: Filtration through PTFE Filter   Plate 12. Sample Extract Ready for Injection

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

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

3.6. Preparation of pesticide standard solution

Pesticide standard stock solutions of chlorpyrifos, acephate, diazinon, dimethoate, quinalphos, malathion and fenitrothion were prepared separately in acetone. The concentration was 1000 mg/L. They were stored at -20°C until use. Prepared a mixed standard solution which was about 50 mg/L in acetone containing all the afore mentioned pesticides by adding the appropriate volume of each individual stock solution in a 50 ml volumetric flask and made to volume by addition of acetone. An intermediate mixed standard solution of 10 mg/L in acetone was prepared from the mixed standard solution of 50 mg/L. Then working standard solutions of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 mg/L in methanol were prepared by transferring the proper amount from 10 mg/L intermediate mixed standard solution into ten separate 10-mL 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 is an anagram for Quick, Easy, Cheap, Effective, Rugged and Safe. This techniques was first introduced by Anastassiades *et al.* (2003), which is gaining popularity day by day compared to the other existing techniques such as Supercritical Fluid Extraction (SFE), Liquid-liquid extraction (LLE), Solid phase extraction (SPE), Solid phase micro extraction (SPME), Stir bar sorptive extraction (SBSE), and Microwave assisted extraction (MAE). The technique uses a single extraction in acetonitrile and requires a very small amount of (10-15 gm) sample. A large excess of salts or buffers are added to extract to aid in the extraction of both polar and non-polar pesticide. This sample initial step simultaneously extract the pesticide from the samples and prepares it for the next dispersive solid phase extraction (d-SPE), the salts and SPE sorbents chosen for the d- SPE step serve to remove residual water and further remove matrix interference from the sample. The resulting acetonitrile extract is typically analyzed directly by gas chromatography (GC), gas chromatography-mass spectrometry (GC/MS) or liquid chromatography tandem mass spectrometry (LC/MS/MS) with proper dilution.

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

3.8. Detection and quantification of pesticide residue in samples

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

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

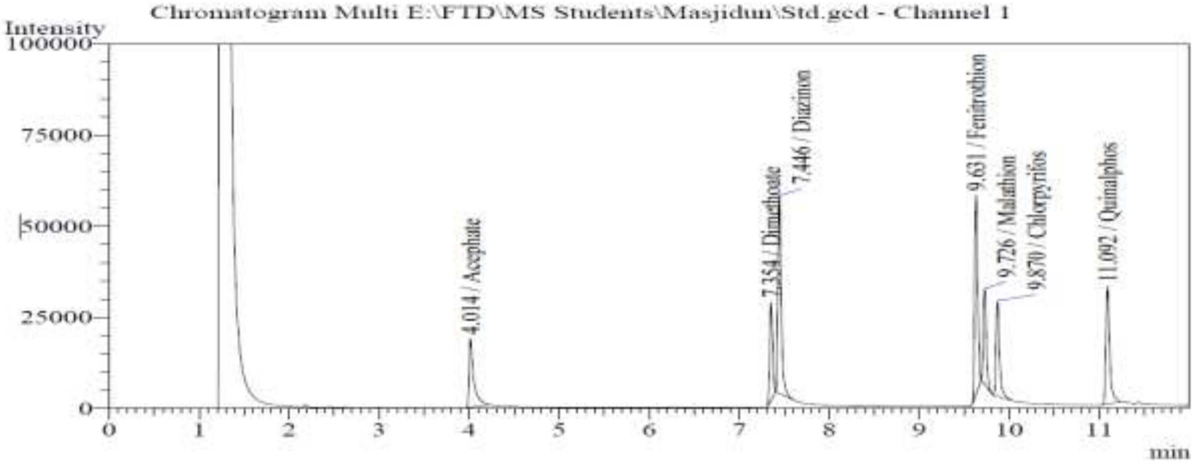


Figure 2: Typical chromatograms of seven organophosphorus insecticide standards run by GC-FTD.

Table 3: The instrument parameters for GC-FTD

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

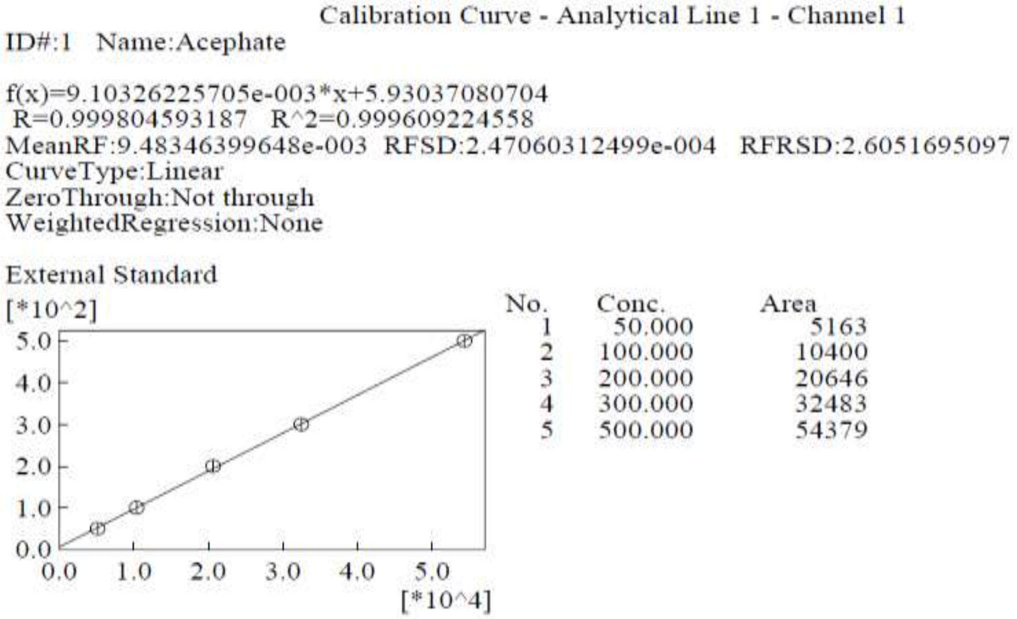
Table 4: Conditions for column oven

Column oven	Rate	Temperature	Hold time(min)

Initial temperature: 150°C	-	150	1
	10	220	2

3.9. Calibration curve preparation

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

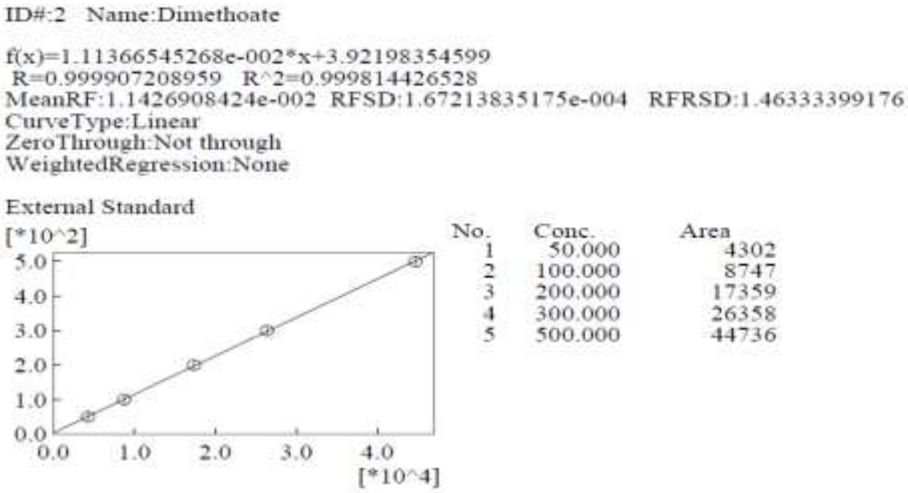


```
                          Calibration Curve - Analytical Line 1 - Channel 1
ID#:1   Name:Acephate

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

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

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

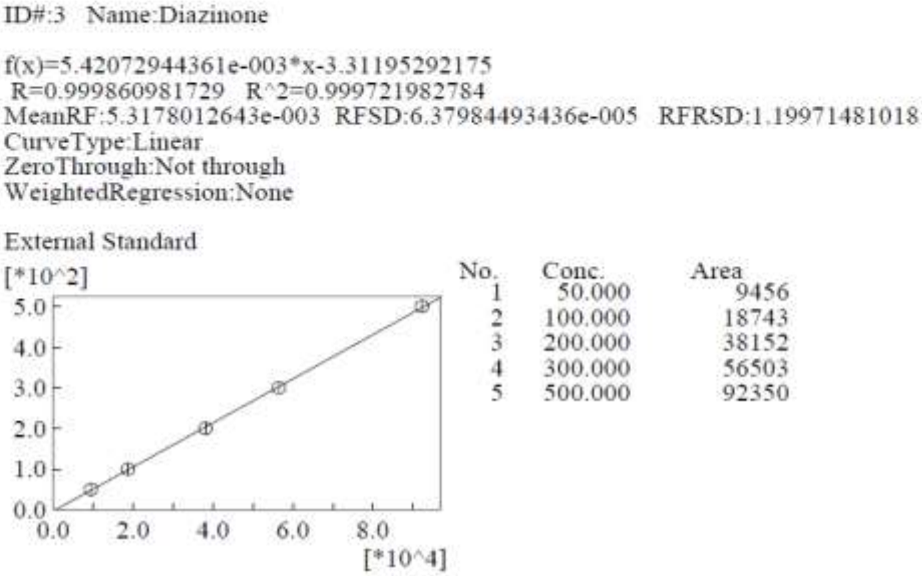


```
ID#:2   Name:Dimethoate

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

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

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

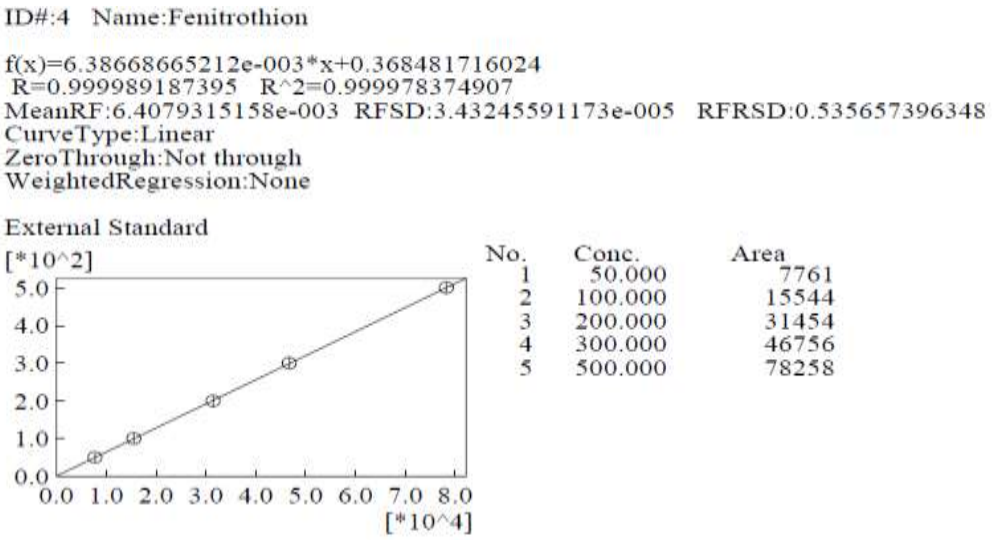


```
ID#:3   Name:Diazinone

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

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

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

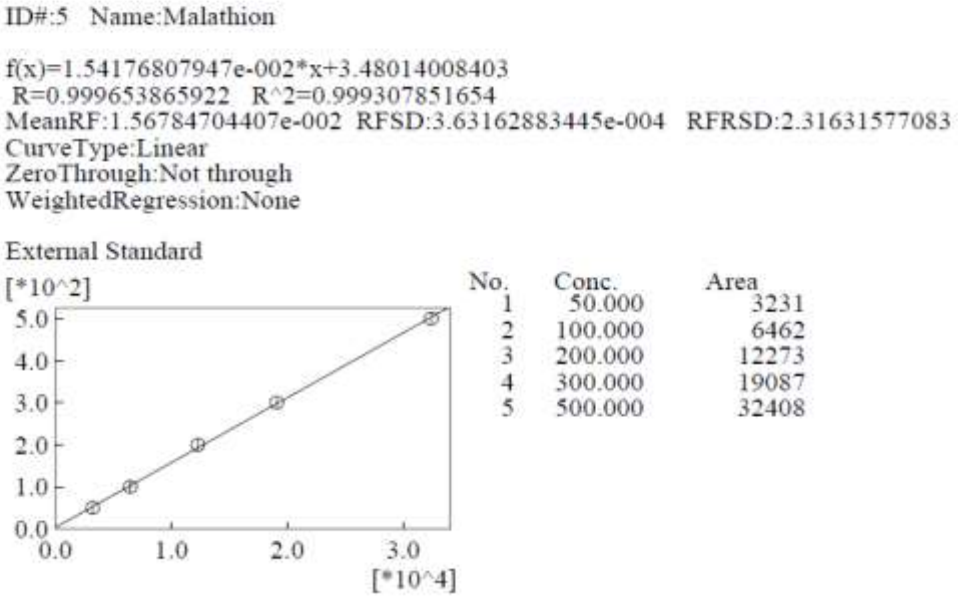


```
ID#:4   Name:Fenitrothion

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

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

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

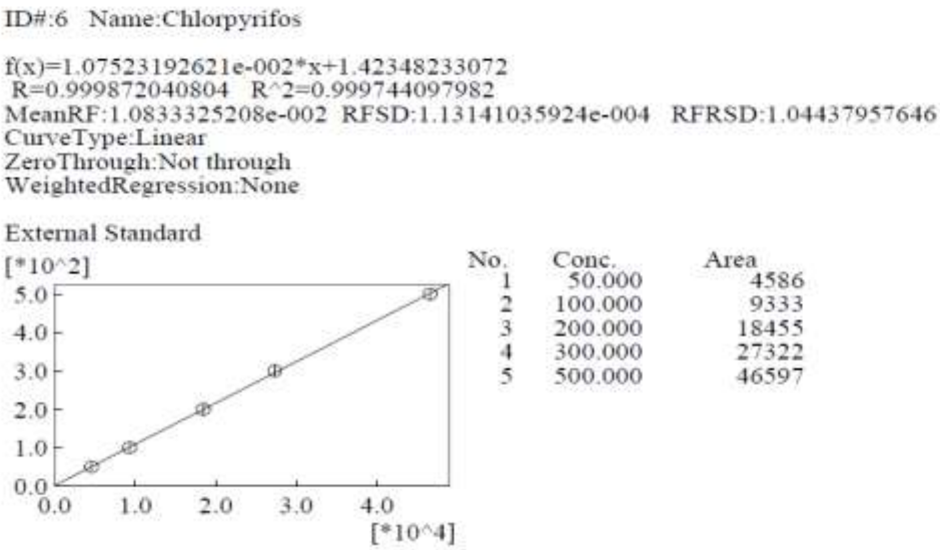


```
ID#:5   Name:Malathion

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

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

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

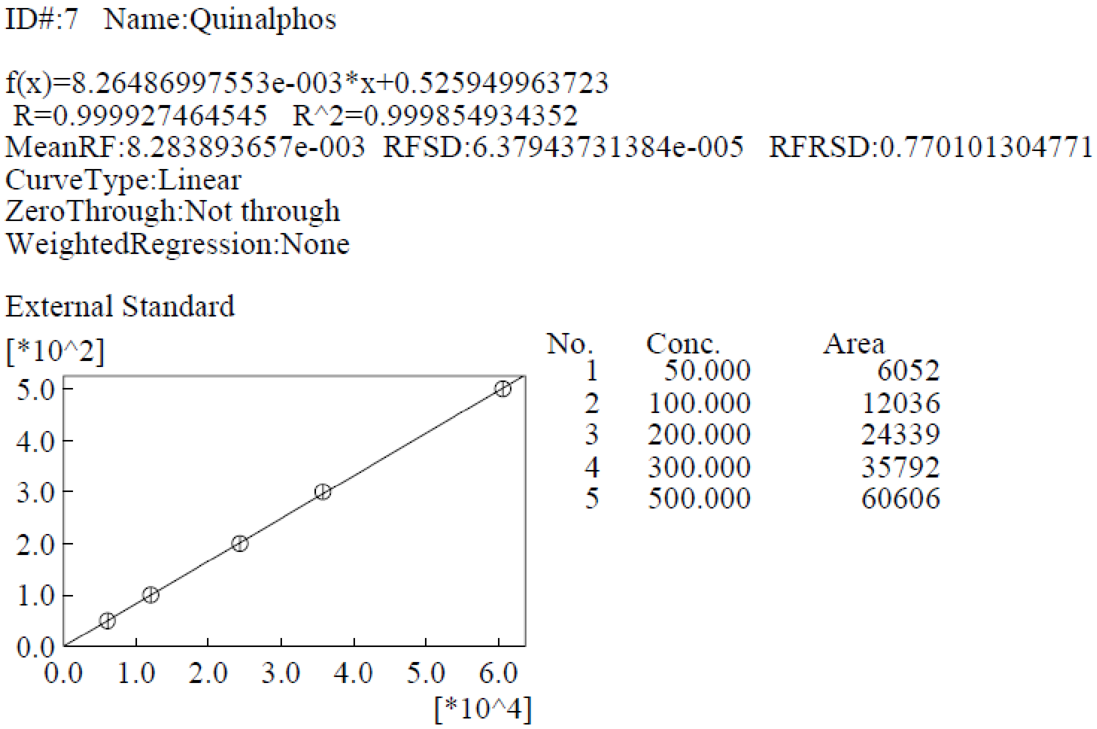


```
ID#:6   Name:Chlorpyrifos

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

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

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



```
ID#:7   Name:Quinalphos

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

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

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

CHAPTER IV

RESULTS AND DISCUSSIONS

Eighty (80) samples of vegetable (cauliflower and tomato) were collected from 5 different markets of Rangpur district (Paura Bazar, Tetultola Bazar, Lalbag Bazar, Termilan Bazar and Station Bazar) to detect and quantify pesticide residues. The results obtained from this study are presented and described in this chapter using figures and tables.

4.1 Pesticide Residues in cauliflower

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

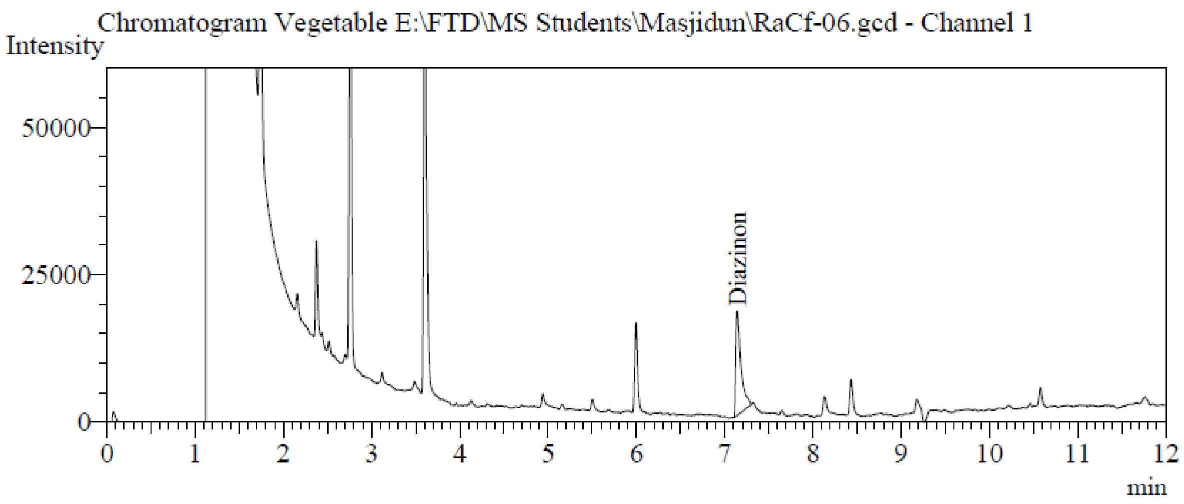


Figure 10. Chromatogram of diazinon found in one of the cauliflower sample (RaCf-06) showing retention time

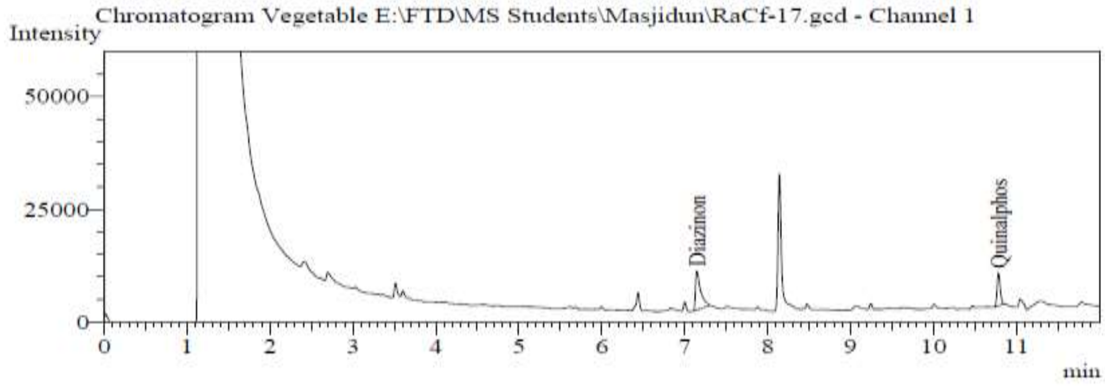


Figure 11. Chromatogram of diazinon and quinalphos found in one of the cauliflower sample (RaCf-17) showing retention time

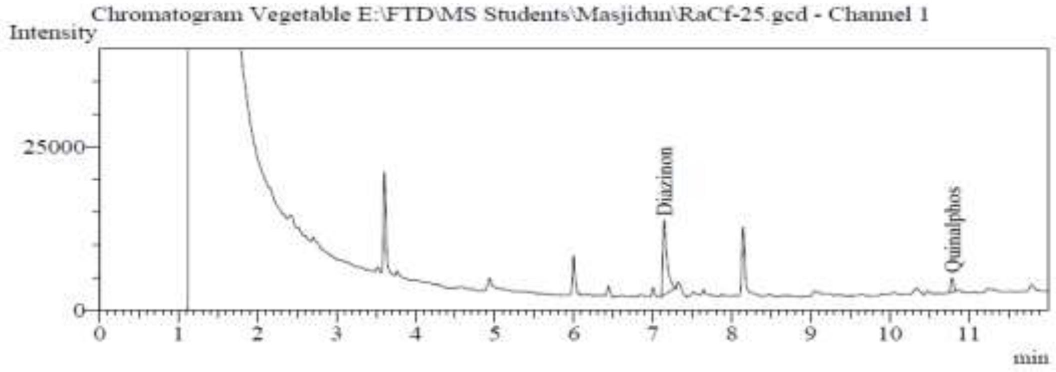


Figure 12. Chromatogram of diazinon and quinalphos found in one of the cauliflower sample (RaCf-25) showing retention time

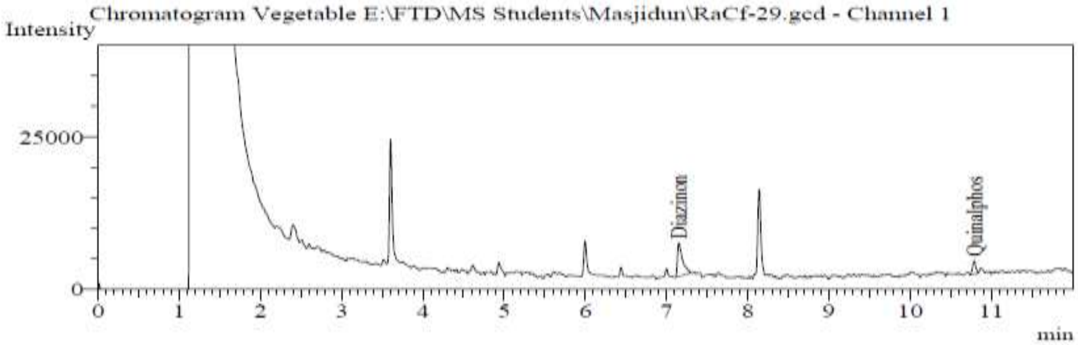


Figure 13. Chromatogram of diazinon and quinalphos found in one of the cauliflower sample (RaCf-29) showing retention time

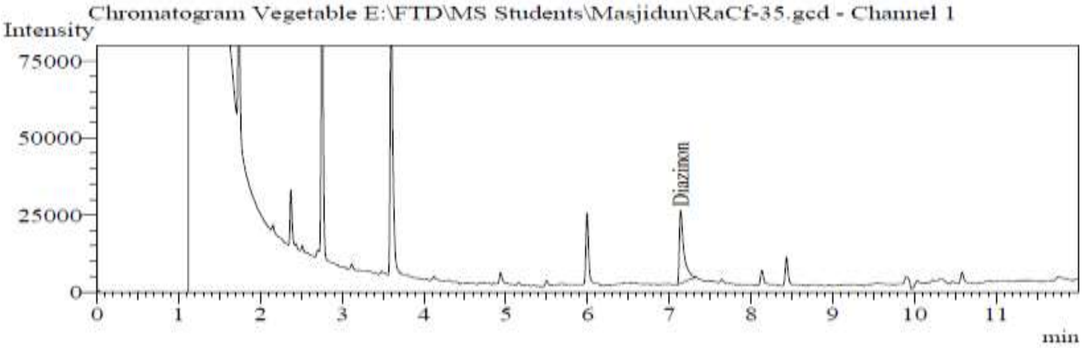


Figure 14. Chromatogram of diazinon found in one of the cauliflower sample
(RaCf-35) showing retention time

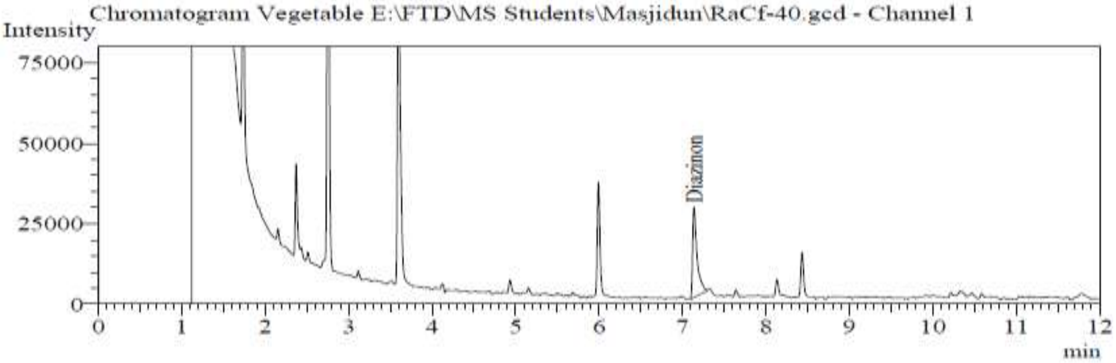


Figure 15. Chromatogram of diazinon found in one of the cauliflower sample
(RaCf-40) showing retention time

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

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

Area of collection	**Sample ID**	**Name of detected pesticide**	**Level of residue (mg/kg)**	**MRLs (mg/kg)**
Paura Bazar	RaCf -1	ND	-	
	RaCf -2	ND	-	
	RaCf -3	ND	-	
	RaCf -4	ND	-	
	RaCf -5	ND	-	
	RaCf -6	Diazinon	0.247	0.01*
	RaCf -7	ND	-	
	RaCf -8	ND	-	
Tetultola Bazar	RaCf -9	ND	-	
	RaCf -10	ND	-	
	RaCf -11	ND	-	-
	RaCf -12	ND	-	
	RaCf -13	ND	-	
	RaCf -14	ND	-	
	RaCf -15	ND	-	
	RaCf -16	ND	-	
Lalbag Bazar	RaCf -17	Dazinon Quinalphos	0.146 0.102	0.01*
	RaCf -18	ND	-	
	RaCf -19	ND	-	
	RaCf -20	ND	-	
	RaCf -21	ND	-	
	RaCf -22	ND	-	
	RaCf -23	ND	-	
	RaCf -24	ND	-	
Station Bazar	RaCf -25	Diazinon Quinalphos	0.162 0.031	0.01*
	RaCf -26	ND	-	
	RaCf -27	ND	-	
	RaCf -28	ND	-	
	RaCf -29	Diazinon Quinalphos	0.093 0.009	0.01*
	RaCf -30	ND	-	
	RaCf -31	ND	-	
	RaCf -32	ND	-	
Terminal Bazar	RaCf -33	ND	-	
	RaCf -34	ND	-	
	RaCf -35	Diazinon	0.281	0.01*
	RaCf -36	ND	-	
	RaCf -37	ND	-	
	RaCf -38	ND	-	
	RaCf -39	ND	-	
	RaCf -40	Diazinon	0.364	0.01*

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

Forty samples of cauliflower collected from 5 different markets of Rangpur (Paura Bazar, Tetultola Bazar, Lalbag Bazar, Termilan Bazar and Station 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 cauliflower 6 samples (15% of the total number of samples) contained pesticide residues and 34 samples (85% 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.

Eight cauliflower samples were collected from Paura Bazar area, among them one samples (RaCf -6) contained diazinon at a level of 0.247 mg/kg, which was above the EU-MRL (European Commission 2015). The other 7 samples contain no detectable pesticide residues.

From Lalbag Bazar, eight samples were collected. One sample (RaCf -17) contained diazinon and quinalphos at a level of 0.146 mg/kg and 0.102 mg/kg respectively which were above the EU-MRL (European Commission 2015). But other seven samples contained no detectable pesticide residues.

One sample (RaCf -25) of cauliflower contained residue of diazinon and quinalphos at a level of 0.162 mg/kg and 0.031 mg/kg respectively collected from Station Bazar, which was above EU-MRL (0.01 mg/kg). The other sample (RaCf -29) contained residue of diazinon and quinalphos at a level of 0.093 mg/kg and 0.009 mg/kg respectively. The amount of quinalphos was below the EU-MRL.

One sample (RaCf -35) of cauliflower contained residue of diazinon (0.281 mg/kg) collected from Terminal Bazar, which was above EU- MRL (0.01 mg/kg). Another sample (RaCf- 40) also contained residure of diazinon (0.364 mg/kg) which was above EU- MRL (0.01 mg/kg).

Among the eight samples collected from tetultola Bazar, none of the samples contained any detectable residues of the sought pesticides.

4.2 Pesticide Residues in tomato

The concentrated extracts of tomato samples collected from different markets of Rangpur were analyzed by GC-2010 (Shimadzu) with Flame Thermonic Detector (FTD) with the pre-set parameters. Figure 16-19 shows the chromatograms of the injected extracts of tomato sample containing detected pesticides.

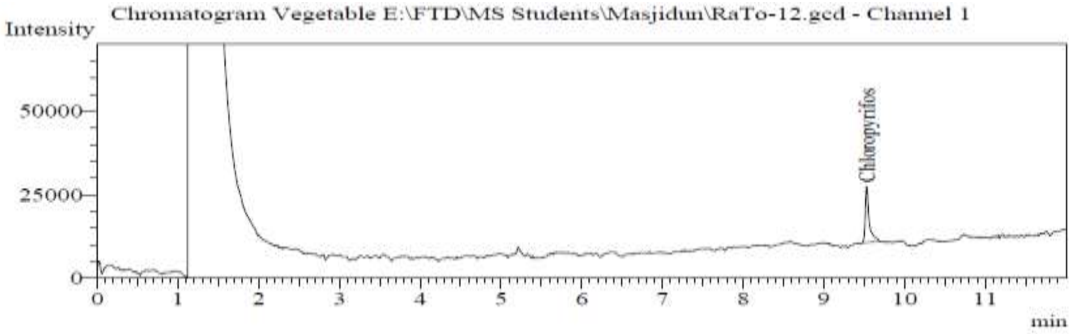


Figure 16. Chromatogram of chloropyrifos found in one of the tomato sample
(RaTo -12) showing retention time

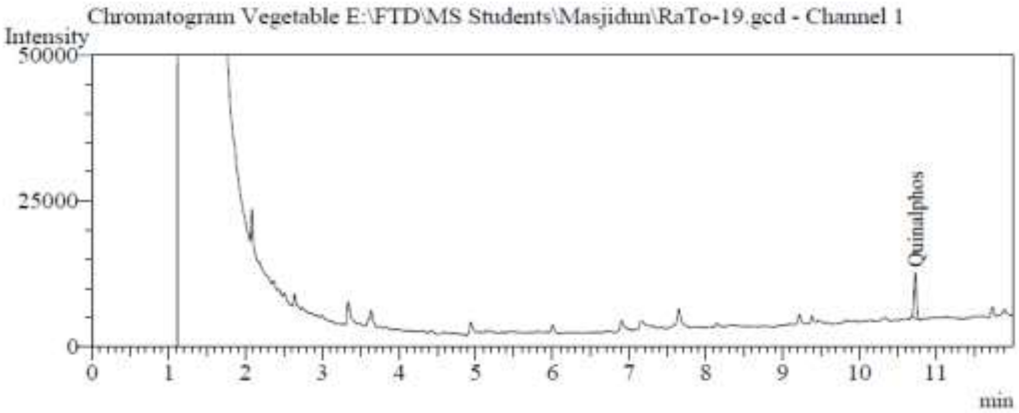


Figure 17. Chromatogram of quinalphos found in one of the tomato sample (RaTo -19) showing retention time

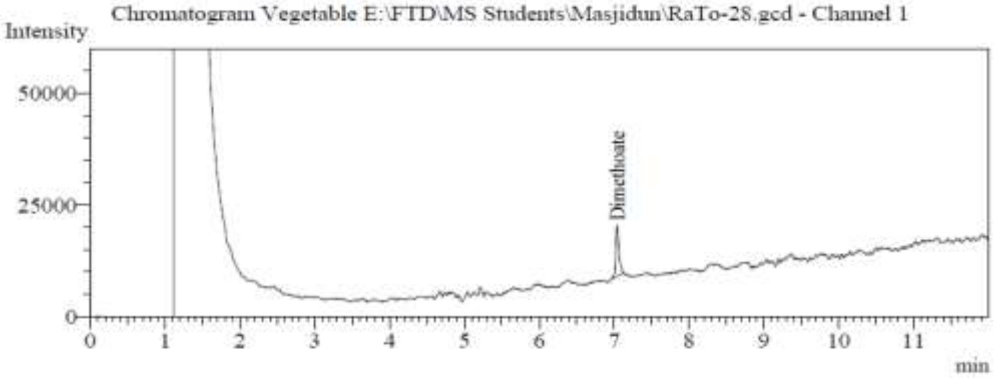


Figure 18. Chromatogram of dimethoate found in one of the tomato sample (RaTo -28) showing retention time

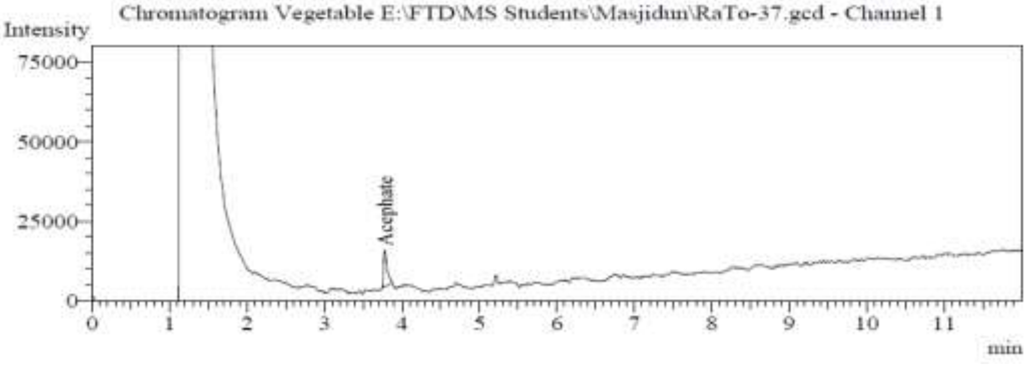


Figure 19. Chromatogram of acephate found in one of the tomato sample (RaTo -37) showing retention time

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

Area of collection	Sample ID	Name of detected pesticide	Level of residue (mg/kg)	MRLs (mg/kg)
Paura Bazar	RaTo -1	ND	-	
	RaTo -2	ND	-	
	RaTo -3	ND	-	
	RaTo -4	ND	-	
	RaTo -5	ND	-	
	RaTo -6	ND	-	
	RaTo -7	ND	-	
	RaTo -8	ND	-	
Tetultola Bazar	RaTo -9	ND	-	
	RaTo -10	ND	-	
	RaTo -11	ND	-	
	RaTo -12	Chloropyrifos	0.096	0.10*
	RaTo -13	ND	-	
	RaTo -14	ND	-	
	RaTo -15	ND	-	
	RaTo -16	ND	-	
Lalbag Bazar	RaTo -17	ND	-	
	RaTo -18	ND	-	
	RaTo -19	Quinalphos	0.095	0.01*
	RaTo -20	ND	-	
	RaTo -21	ND	-	
	RaTo -22	ND	-	
	RaTo -23 RaTo -24	ND ND	-	
Station Bazar	RaTo -25	ND	-	
	RaTo -26	ND	-	
	RaTo -27	ND	-	
	RaTo -28	Dimethoate	0.202	0.01*
	RaTo -29	ND	-	
	RaTo -30	ND	-	
	RaTo -31	ND	-	
	RaTo -32	ND	-	
Terminal Bazar	RaTo -33	ND	-	
	RaTo -34	ND	-	
	RaTo -35	ND	-	
	RaTo -36	ND	-	
	RaTo -37	Acephate	0.273	0.01*
	RaTo -38	ND	-	
	RaTo -39	ND	-	
	RaTo -40	ND	-	

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

Forty samples of tomato were collected from 5 different markets of Rangpur district (Paura Bazar, Tetultola Bazar, Lalbag Bazar, Termilan Bazar and Station 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 tomato, 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.

Eight tomato samples were collected from Paura Bazar, none of the samples contained no detectable residues of the sought pesticides. From Tetultola Bazar, eight samples were collected. Of which one sample (RaTo -12) contained of chloropyrifos residue (0.096 mg/kg) which was below EU-MRL (0.1 mg/kg). But other seven samples contained no detectable pesticide residues.

One sample (RaCf -19) of tomato contained residue of quinalphos (0.095 mg/kg) among the eight samples collected from Lalbag Bazar, which was above EU-MRL (0.01 mg/kg). The other seven samples contain no detectable pesticide residues.

One sample (RaCf -28) of tomato contained residue of dimethoate (0.202 mg/kg) among the eight samples collected from Station Bazar, which was above EU-MRL (0.01 mg/kg). The other seven samples contain no detectable pesticide residues.

Among the eight samples collected from Terminal Bazar, one sample (RaCf-37) contained residues of acephate (0.321 mg/Kg) which was above EU-MRL (0.01 mg/kg).

CHAPTER V

SUMMERY AND CONCLUSION

Vegetables that positive contains pesticide will be dangerous if consumed on and on. The residues will accumulate in the body and influence nerve formation, especially for nerve membrane and it will collect in fat. Residue that had saved in fat cannot excrete by urine and it will be accumulated endlessly and caused tissues damage and cancer. Exposure towards pesticide from organophospate group for long duration and high relative amount will inhibit function of acetylcoline esterase, this can caused salivation, dizziness and even a comma. Inhibition of enzyme works happened because organophospate pesticide was done enzyme phosporilation in stabil component form. This can be obtained with consumed healthy food that will make healthy life.

The purpose of this study is to detect and identify pesticide residues in cauliflower and tomato collected from local markets of Rangpur city and to quantify them for the comparison with the international standards. Regarding this, 40 samples of cauliflower and 40 samples of tomato were collected from 5 different markets of Rangpur City and carried to the Pesticide Analytical Laboratory, Entomology Division, BARI, Joydebpur, Gazipur, Bangladesh. The QuEChERS extraction technique was applied for the extraction and cleanup of these collected samples. Gas chromatography coupled with flame thermionized detector (FTD) was used to identify and quantify the level of pesticide residues present in the extracted samples. Seven most commonly used organophosphorus pesticides i.e acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos were selected for the study.

Out of 40 samples of cauliflower, 6 samples (15% of the total number of samples) contained pesticide residues of diazinon and quinalphos. Among these 6 samples, 5 had residues above the Maximum Residue Limit (MRL) and 1 sample contained two different types of pesticide residues such as diazinon and quinalphos. The level of detected diazinon residues was above MRL, while the level of quinalphos residues was below the MRL and the other 34 samples (85% of the total number of samples) contained no detectable residues of the sought pesticides.

Among the 40 analyzed samples of tomato, 4 samples (10% of the total number of samples) contained pesticide residues of acephate, dimethoate, chlorpyrifos and quinalphos. Among these 4 samples, 3 had residues above the MRL and 1 had residues below the MRL set by European Commission. On the other hand 36 samples (90% of the total number of samples) contained no detectable residues of the sought pesticides.

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

CHAPTER VI
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