DETERMINATION OF CYPERMETHRIN RESIDUE IN VEGETABLES (TOMATO, OKRA AND EGGPLANT) BY SPECTROPHOTOMETRIC METHOD

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CERTIFICATE

This is to certify that the thesis entitled **"DETERMINATION OF CYPERMETHRIN RESIDUE IN VEGETABLES (TOMATO, OKRA AND EGGPLANT) BY SPECTROPHOTOMETRIC METHOD"** submitted to the Department of Agricultural Chemistry, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (M.S.)** in **AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bonafide research work carried out by **Parves Sardar**, Registration No. **12-04730** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

June, 2019

Dhaka, Bangladesh

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Dedicated to
My
Beloved Parents

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ABSTRACT

A sensitive Spectrophotometric method was initiated for the determination of pesticide residue of cypermethrin, from 75 collected samples of different vegetables (tomato, okra and eggplant). A total of 75 samples (25 samples of tomato, 25 samples of okra and 25 samples of eggplant) were collected from five different locations (Kawran Bazar, Jatrabari, Shanti Nagar, Shyamoli and Mirpur-1) in Dhaka city. The proposed methods have been successfully applied for the determination of cypermethrin in collected samples. Among the 25 analyzed samples of tomato, 2 samples (8% of the total number of samples) contained residues of cypermethrin, where one sample contained above the maximum residue limits (MRLs) and one was below MRLs. Out of 25 samples of okra, 2 samples (8% of the total number of samples) contained residues cypermethrin, where both were contained below the maximum residue limits (MRLs). Among the 25 analyzed samples of eggplant, 3 samples (12% of the total number of samples) contained residues of cypermethrin, where one sample contained above the maximum residue limits (MRLs) and two were below MRLs. This study reflects the overall cypermethrinresidue remain in tomato, okra and eggplantcollected from different markets of Dhaka city, which will help the consumer to be aware of their health and safety.

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LIST OF ABBREVIATIONS

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

CHAPTER I

INTRODUCTION

Bangladesh is predominantly an agricultural country with an area of 1, 47,570 sq. km. It has only 0.31 percent of the total agricultural land in the world but 2.0 percent of total population of the globe (Rasul and Thapa, 2004). Agriculture plays an important role to give food security in this highly populated country. The major crops grown in the country are rice, wheat, jute, potato, sugarcane, vegetables and tea (Islam *et al*., 2009). Chemical fertilizers and pesticides have contributed significantly to improve yields of crops, increasing the production of food grains. But, the widely cultivated high yielding variety is highly vulnerable to pests and diseases. So the use of pesticide is now an inherent part of agriculture for pest control (SuvagataBagchi*et. al*., 2008).

Vegetables are important constituents of Bangladesh diet as they are rich source of carbohydrate, proteins, vitamins, minerals, glucosinolates, antioxidants and fiber etc. Vegetables and fruits are consumed for nutrition, maintenance of health and many for their therapeutic values and prevention of diseases. Intake of vegetables has been encouraged not only to prevent consequences due to vitamin deficiency but also to reduce the incidence of major diseases such as cancer, cardiovascular diseases and obesity.

There are many vegetables which are cultivated and consumed in Bangladesh. Among them, tomato, okra and eggplant are most important vegetables in respect of production and consumption. Bangladesh now grows 3877thousand metric ton (MT) of vegetables annually in the country from the 992 thousand acres of cultivable land and over 1.62 crore farmers cultivate 156 types of vegetables across the country (BBS, 2016). Tomato okra and eggplant are most important vegetables which occupies 67000, 26000 and 78000 acres of land respectively

with production of 368000, 44000 and 310000 MT respectively in 2015-16 (BBS, 2016).

Like other crops, fruits and vegetables are attacked by pests and diseases during production and storage leading to damages that reduce the quality and the yield. In order to reduce the loss and maintain the quality of fruits and vegetables harvest, pesticides are used together with other pest management techniques during cropping to destroy pests and prevent diseases. Pesticide application is an essential component of modern crop production technology. Their use has been contentiously increasing over the past decades.

Pesticides, in general, are chemicals used worldwide in agricultural production to destroy or control weeds, insects, fungi, and other pests. Some of those pesticides remain on food as residues. Bangladesh has been using pesticides since 1950's in agriculture and public health. Now about 272 registered pesticides (Belonging; Miticide = 8, Fungicide = 79, Insecticide = 117, Herbicide = 52, Biopesticide = 10, Strong grain products =4 and Rodenticide = 2) are commonly used in agriculture sector while 88 pesticide are commonly used in public sectors in Bangladesh (BCPA, 2016).

When pesticides are applied improperly, resulting residues can pose significant health risks to consumers (Rodrigues, 2011).The indiscriminate use of agricultural pesticides has created very serious health and environmental problems in many developing countries. Worldwide, one to five million farm workers are estimated to suffer pesticide poisoning every year. Most of the poisonings take place in rural areas of developing countries, where safeguards typically are inadequate or lacking altogether. Although developing countries use 25% of the world's production of pesticides, they experience 99% of the deaths due to pesticide poisoning (WHO, 2004). So, the levels of pesticide residues in various environmental samples should be monitored routinely, and effective measures

must be adopted to control the use of pesticides to minimize human health hazards. Various agencies of United Nations and their established networks are extending co-operation, collaboration expert guidance in devising practical steps for the controlled use of pesticides to minimizing the undesirable levels of residues in food chains and untoward effects on target organisms in the environment (Ambrush, 1997).

The word “pesticide” is used as an umbrella term for all kind of pesticides such as insecticides, herbicides, fungicides, rodenticides, wood preservatives, garden chemicals and household disinfectants. Since pesticides varies in identity, physical and chemical properties, it`s therefore logical to have them classified and their properties studied under their respective groups (Drum, 1980). Based on chemical properties, pesticides are classified into four main groups namely; organochlorines, organophosphorous, carbamates and pyrethrin and pyrethroids (Buchel, 1983).

Pyrethroids are digestive and contact insecticide acknowledged of their fast knocking down effect against insect pests, low mammalian toxicity and facile biodegradation. Although the naturally occurring pyrethrins are effective insecticides, their photochemical degradation is so rapid that their uses as agricultural insecticides become impractical. The synthetic analogues of the naturally occurring pyrethrins (pyrethroids) were developed by the modification of pyrethrin structure by introducing a biphenoxy moiety and substituting some hydrogens with halogens in order to confer stability at the same time retaining the basic properties of pyrethrins (Manahan, 2001). Pyrethroid insecticides are derived from natural compounds (the pyrethrins), isolated from Chrysanthemum genus of plants. Pyrethroid pesticides, widely used on crops like cotton, fruits and vegetables, appear to be a significant source of sediment toxicity in urban and agriculturally dominated streams. Pyrethroids act as neurotoxins and target the central nervous system of insects (Narahashi*et al*., 1998). Pyrethroid pesticides are

toxic to man and animals, carcinogenic to human and exert genotoxic, mutagenic and embryotoxic effects (Weston *et al*., 2005). The use of pyrethroid insecticides is increasing for agriculture, commercial pest control and residential consumer use. These insecticides are intensively used for a variety of crops, its residue enters into inland water sources from the fields where it has been applied and contaminates the adjacent streams, ponds, lakes, wells, etc. The insecticides are highly stable under acidic conditions, though unstable at pH 8.0. The most widely used synthetic pyrethroids include permethrin, cypermethrin and deltamethrin (Manahan, 2001).

Cypermethrin ($C_{22}H_{19}Cl_2NO_3$) commonly used to control various pests, including moth pests of cotton, fruit, and vegetable crops (Meister, 1992). It is also used for crack, crevice, and spot treatment to control insect pests in stores, warehouses, industrial buildings, houses and apartments, greenhouses, laboratories, ships, rail-cars, buses, trucks, and aircrafts. It may also be used in nonfood areas in schools, nursing homes, hospitals, restaurants, hotels, and food processing plants (Anonymous, 1989). Cypermethrin is a moderately toxic material by dermal absorption or ingestion. Symptoms of high dermal exposure include numbness, tingling and itching, burning sensation, loss of bladder control, in coordination, and possible death. It may adversely affect the central nervous system. Symptoms of high-dose ingestion include nausea, prolonged vomiting, stomach pains, and diarrhea which progresses to convulsions, unconsciousness, and coma. Cypermethrin is a slight skin or eye irritant, and may cause allergic skin reactions. It is highly toxic to fish, water insects and aquatic invertebrates. It is also highly toxic to bees and low toxic to birds. It causes genetic damage and chromosome abnormalities increased in bone marrow and spleen cells when mice were exposed to cypermethrin (Janghel and Pervez, 2011).

Consumers expect a product to be free of pesticides (or low concentrations) and other contaminants (Nagel, 2011). Therefore, the pesticides must undergo

extensive efficacy, environmental, and toxicological testing to be registered by governments for legal use in specified applications. The applied chemicals and/or their degradation products may remain as residues in the agricultural products, which becomes a concern for human exposure (Dasika *et al*., 2012).

The indiscriminate use of chemical inputs (pesticides) in agriculture fears/concerns the contamination of foods with agrochemicals and also the pollution of environment, soil and water and therefore made us to think about alternate form of agriculture to produce food free of contaminants. Besides in the present era of global warming and climate change, the face of agriculture has to be more environments friendly, hence the main emphasis should be for development of production technologies which are sustainable in long run. In modern day agricultural practices, the use of pesticides provides unquestionable benefits by increasing the production of crops. However, it has the drawback of pesticide residues which remain on the vegetables, constituting potential health risks to consumers. This leads on the one hand to the establishment of legal directives to control their levels through the maximum residue levels (MRLs) and on the other to continue search for pesticides, which are less persistent and less toxic for human beings (Guan *et al*., 2011). Pesticide residue is becoming a major food safety concern of consumers and governments. In order to remove residual effect of pesticides which are toxic, exact dose and the harvest time after spray of each pesticide which should be recommended to the farmer so that the amount of residual pesticides in vegetables might be lower than the acceptable range (Tashkent, 1998).The tomato, okra and eggplant growers have been using the pesticides frequently to have the higher and insect free yield. But the overdoses of pesticides make the residue problem, which might pollute our food and environment. The pesticides residues can be decreased if recommended dose applied by the vegetable growers (Murthy *et al*., 2011).

Different methods are initiated to detect pesticide residue in the world till date in different vegetables, grain, fruits, water and soil etc. QuEChERS (Quick Easy Cheap Effective Rugged Safe) extraction in combination with gas chromatography–tandem quadrupole mass spectrometry (GC-MS/MS) and liquid chromatography–tandem quadrupole mass spectrometry (LC-MS/MS) are important methods for determination of pesticide residue. Simple Spectrophotometric methods and HPLC methods are also important methods for pesticide analysis. The most of the chemical analysis to determine pesticide residue needs expensive instruments like GC-MS, LC-MS which is not possible to purchase by all laboratory. Therefore, the present study has been introduced an alternative and affordable spectrophotometric method to analysis cypermethrin residue in some selected vegetables.

Considering the above fact, the present study aimed to determination of cypermethrin residue by a simple and affordable spectrophotometric method in some selected vegetables (tomato, okra and eggplant) grown under local agroclimatic conditions.

CHAPTER II

REVIEW OF LITERATURE

Investigation of pesticides in food samples is a difficult task, because of the complexity of the surrounding substances and the low concentrations at which these compounds are usually present. Thus, despite advances in the development of highly efficient analytical instrumentation for their final determination, sample pretreatment remains an important part of obtaining accurate quantitative results. Many choices have been proposed for pretreatment and/or extraction of pesticide residues in vegetables. In most of these the extraction procedure usually involves sample homogenization with an organic solvent, alone or mixed with water or pH-adjusted water, using a homogenizer, blender, or sonicator.

Under the present study, spectrophotometric method was chosen to determine pesticide residues in vegetables (tomato, okra and eggplant) regarding cypermethrin uses. In this chapter challenge has been made to assessment literatures for updating the information regarding the existing status of research and knowledge about the determination of pesticide residues in vegetables. The studies on the measurement of detected pesticide residues below or above the Maximum Residue Limit (MRL) of vegetables in Bangladesh are rarely reported. With this background, the information collected from different sources have been reviewed and presented below:

2.1 Detection of pesticide residues in crops

Weng *et al*. (2019) conducted a study to developed a rapid detection method for fonofos, phosmet, and sulfoxaflor in paddy water through chemometric methods and surface-enhanced Raman spectroscopy (SERS). The obtained spectra from the SERS can detect 0.5 mg/L fonofos, 0.25 mg/L phosmet, and 1 mg/L sulfoxaflor through the appearance of major characteristic peaks. Then, chemometric methods was used to develop models for the intelligent analysis of pesticides, alongside the

SERS spectra. For the quantitative analysis, the partial least squares regression models obtained the best predicted performance for fonofos and sulfoxaflor, and the support vector machine model provided optimal results, with a root-mean-square error of validation of 0.207 and a coefficient of determination of validation of 0.99952, for phosmet.

Hegazya *et al*. (2018) conducted two specific spectrophotometric techniques for assay of four pesticides applied for cucumber; mandipropamid (MAN), metalaxyl (MET), thiophenate methyl (THO) and carbendazim (CAR). First method is derivative method for the quaternary mixture of the studied pesticides; by using the first derivative THO was assayed at 281 nm, while MAN and CAR were assayed using second derivative at 236.4 and 256.2 nm, respectively and MET was assayed using third derivative at 221.6 nm. Second method is double divisor ratio spectrum derivative method that used for assay of MAN at 289 nm and MET at 233.2 nm using a double divisor of 30 μgmL^{-1} of each of THO and CAR, then both THO and CAR were determined at 259 and 267.4 nm, respectively, using a double divisor of 4 μgmL−1 of each of MAN and MET. The obtained results were within the acceptable range regarding to the Egyptian committee of agricultural recommendations.

Altunay *et al*. (2018) applied a new ionic liquid (IL) phase microextraction method using IL, 1-butyl-3-methyli midazoliumhexafluoro phosphate $[C_4mim][PF_6]$, as extracting solvent is proposed for simple and fast determination of low levels of TBZ in fruits and vegetables by spectrophotometry. The method is based on selective complex formation of thiabendazole (TBZ) with Cu (II) ions in presence of PF6– as counter ion at pH 5.5, and then microextraction of the complex into the fine micro-drops of IL phase. After optimisation of variables affecting microextraction efficiency, the analytical parameters of the method were determined by calibration curves. The method exhibits a linear relationship (0.3–280 $\mu g\ L^{-1}$), low detection limit (0.1 $\mu g\ L^{-1}$), good intra- and inter-day precision

(2.4–4.5% as RSDr%, 2.1–5.6% as RSDR%), good recovery (≥95.1–98.2%) and high sensitivity enhancement factor (150) by solvent-based calibration curve. It allows a detection limit of 0.24 μg L^{-1} and a range of 0.8–250 μg L^{-1} by the matrix-matched calibration curve. After validation, the method was successfully applied to the determination of TBZ residues with method quantification limits in fruit and vegetables of 2.0 and 2.5 μg kg^{-1} with and without adding polyvinylpyrrolidone (PVP-15) solution. Recoveries range from 85.5% to 98.2% after spiking (10, 50 and 100 μg kg^{-1}, n: 3).

Elgailani and Alghamdi (2018) used UV/VIS spectrophotometer method to estimate the quantity of the pesticide (acephate) in terms of a reference sample of the pesticide. The pesticide residues in the three vegetable samples were identified by using Thin Layer Chromatography (TLC) by using two different developing systems, one is 5% acetic acid in ethyl acetoacetate and the other is formic acid: acetic acid: methanol 1: 1 : 5. The results of the study showed the presence of acephate pesticide residues in these selected vegetables with a concentration of 14.25 mg/kg in Coriandrumsativum (leaves), 8.10 mg/kg in Eruca sativa and 4.65 mg/kg in Petroselinum crispum.

Li *et al*. (2018) carried out a study on 439 pesticides residue analysjis in fruits and vegetables by GC-quadrupole-time-of-flight MS (GC-Q-TOF/MS). Through solid-phase extraction (SPE), whereby pesticides are extracted from fruit and vegetable substrates by using 40 mL 1% acetic acid in acetonitrile (v/v), purified by the Carbon/NH_2 SPE cartridge, and finally detected by GC-Q-TOF/MS, the rapid analysis of 439 pesticides in fruits and vegetables can be achieved. The methodology verification results 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.

Amin *et al*. (2017) carried out an experiment with two simple, sinsitive and accurate spectrophotometric methods have been developed for the determination of two insecticides (dimethoate and deltamethrin) in pure forms, formulations and environmental samples and investigated the development of indirect redox spectrophotometric reaction for determination of deltamethrin or dimethoate with known excess of cerric (IV) ammonium sulfate as an oxidizing agent in acidic medium, followed by determination of unreacted cerric (IV) by adding a fixed amount of Amaranth (AM) or Rhodamin 6G (Rh6G) and measuring the absorbance at λ_{max} 520 and 525 nm for AM and Rh6G methods, respectively. The beer's law was obeyed in the concentration ranges 0.5-4.0 and 0.5-4.5 μg mL^{-1} for deltamethrin and 0.5-5.0 and 0.5-6.0 μg mL^{-1} for dimethoate using AM and Rh6G methods, respectively with correlation coefficients ≥ 0.9993. The molar absorptivity and Sandell's sensitivity, detection and quantification limits were calculated. Intra-day and inter-day accuracy and precision of the methods have been evaluated. No interference was observed from the additives. The proposed methods have been successfully applied for the determination of deltamethrin and dimethoate in its formulations and environmental samples.

Mashuni andJahiding (2017) conducted this study to develop a method of quantitative analysis for cypermethrin which generally used by gas chromatography technique. It is said that cypermethrin is a pesticide that is widely used in agriculture of residue accumulation, which may remain in food causing concern of human health. Cypermethrin analysis was developed with ultra violet-visible spectrophotometry. The method is based on hydrolysis of cypermethrin in alkaline solution to cyanide ion and reacts with iron (III) ions to form coloured complex compounds and the concentration of cypermethrin can be measured quantitatively with a spectrophotometer. Ferricyanide complex ions are formed by

adding iron (III) chloride, then it is measured the absorption to determine of cypermethrin concentration. In this experiments, maximum wavelength absorption w?as obtained at 420 nm and the method has a working range of concentration from 0.076 to 1.60 ppm with r > 0.9998. Limits of detection and limits of quantitation are 0.023 and 0.076 ppm, respectively. Based on accuracy and precision analysis, this methods can be used accurately and have good precision with value of recovery tested is 101.10 % and RSD is 0.25 %.

Dimitra and Triantafyllos (2016) observed that the methods based on chromatographic separation with mass spectrometric detection have resulted in greater likelihood of identification and are acknowledged to be extremely useful and authoritative methods for determination of pesticide residues. Even with such powerful instrumental techniques, however, the risk of interference increases with the complexity of the matrix studied, so sample preparation before instrumental analysis is still mandatory in many applications, for example food analysis. This article summarizes the analytical characteristics of the different methods of sample-preparation for determination of pesticide residues in a variety of food matrices, and surveys their recent applications in combination with chromatographic mass spectrometric analysis.

Ahmed *et al*. (2016) carried out the study to detect and quantify the left over residues of eight commonly used insecticides (fenvalerate, diazinon, quinalphos, fenitrothion, acephate, chlorpyriphos, cypermethrin and malathion) in brinjal, yard long bean, bitter gourd, snake gourd, pointed gourd, okra, tomato, hyacinth bean and cabbage samples collected from local market of eight different regions like Jessore, Comilla, Narsingdi, Tangail, Rangpur, Jamalpur, Gazipur and Dhaka during 2011-2012 seasons. Among the 170 analyzed samples, 21.78% were contaminated with four insecticides (chlorpyriphos, quinalphos, acephate and cypermethrin) either single or multiple product residue, in which 18.26% samples had residue above MRL. The vegetables of Norsingdi, Jessore and Comilla had

more insecticide residue in comparison to other locations. Most of the samples contain chlorpyriphos (13.53%) and quinalphos (8.4%) residue. Acephate, chlorpyriphos and quinaphos were also found as multiple product residues expressing 2.35% of the total samples which were above MRL and 19.41% sample contained single product residue with chlorpyriphos, quinaphos and cypermethrin where 15.88% were of above MRL. Cypermethrin residue was detected only in two samples (brinjal and yard long bean) which were below MRL.

Amin *et al*. (2015) developed three sensitive spectrophotometric methods for the determination of deltamethrin as synthetic pyrethroid in its formulations and environmental samples. The proposed methods were based on the hydrolysis of deltamethrin with ethanolic KOH to form 3-phenoxy benzaldehyde which condensation with anthranilic acid (Method A), 4-aminoantipyrine (method B) and 2-chlorophenyl hydrazine (method C) to form color product having λ_{max} at 480, 495 and 552 nm, for methods A, B and C, respectively. The color derivatives were stable for 3, 5 and 6 days for methods A, B and C, respectively. The Beer's law was obeyed over the concentration range of 0.2–2.0, 0.5-3.0 and 0.5–4.0 μg mL^{-1} for methods A, B and C, respectively. The molar absorptivity and Sandell's sensitivity, detection and quantification limits were calculated. The proposed methods have been successfully applied for the determination of deltamethrin in its formulations and environmental samples.

Yasmeen *et al*. (2015) described a modified and sensitive spectrophotometric method for the determination of nitrile insecticides i.e., cypermethrin, fenvalerate, deltamethrin, in sub-microgram levels. During alkaline hydrolysis of pyrethroids to produce cyanide ion, which react with bromine-water to form cynogen bromide, the pyridine rings are spilt by Konig reaction and the reaction products are coupled with diazotised p-aminobenzoic acid. The colour system obeys Beer's law in the following working range in ppm - cypermethrin 0.13-0.93, fenvalerate 0.27-2.0 and deltamethrin 0.2-1.33 respectively. The Molar absorptivity, Sandell's

sensitivity, Correlation coefficient have been determined. Other pyrethroids not containing a hydrolysable nitrile group (permethrin, resmethrin, allenthin, etc), should not interfere. Moreover organochlorine, organophosphorous and carbamate insecticides do not give colour spot. The method is highly reproducible and have been successfully applied for determination of nitrile containing insecticides in environmental and biological samples.

Hossain *et al*. (2015) conducted a study to determine the residual level present in the vegetable samples by gas chromatography mass spectrophotometry. Twenty five vegetable samples which are three types- bringal samples (BS), cucumber samples (CS) and tomato samples (TS) were collected from different Upazilla of Bogra district in Bangladesh. Out of them eight samples were contaminated with pesticides residues, of which five samples exceeded the Maximum Residue Level (MRL) recommended by FAO/WHO. Moreover, eight samples contaminated with organophosphorus (Diazinon with BS-4, CS-5, TS-3 and Chlorpyriphos with BS-7, CS-2, CS-7, CS-9, TS-3), while only one (BS-3) with carbamate pesticide (Carbaryl). The detected pesticide concentration in various vegetable samples were 4.43 ppm in BS-3, 0.32 ppm in BS-4, 0.4 ppm in BS-7, 0.05 ppm in CS-2, 0.18 ppm in CS-5, 0.02 ppm in CS-7, 0.33 ppm in CS-9, 0.57 ppm inTS-3 and 0.025 ppm in TS-3. The highest concentration for carbaryl was found in BS-3 is 4.43 ppm and the highest level for Diazion in CS-5, TS-3 was estimated 0.18 ppm, 0.57 ppm respectively and for Chlorpyriphos in BS-7, CS-9 was detected 0.4 ppm, 0.33 ppm accordingly. Whereas, no organochlorine pesticides such as DDT, endrin, aldrin, dieldrin, endosulfanetc were found in the samples studied. The study reveals that application of different pesticides is increasing day by day in Bangladesh and this trend would be detrimental to mankind as well as environment.

Hernandes *et al*. (2014) conducted a study with food samples to Determine Residual Cypermethrin and Deltamethrin by a simple Method of GC-mass

spectrometry (MS). Average recoveries from fortified food samples were in the range of 60 to 81%, with relative standard deviation (RSD) from 9 to 18%. Method limits of quantification (LOQ) were 0.010 μg g^{-1} for both pyrethroids and limits of detection (LOD) were 0.007 and 0.002 μg g^{-1} for cypermethrin and deltamethrin, respectively. The main advantage of the proposed method is the reduced number of steps involved, besides being simple, rapid and inexpensive. The method was applied to whole food samples collected. Deltamethrin was not detected in the twenty analyzed samples, and cypermethrin was detected in four samples (20%) at trace levels (< LOQ).

Debbab *et al*. (2014) applied the HPLC and LC-ESIMS techniques to detect cypermethrin residues in five fresh vegetables, when applicable with illegal doses by some farmers. For this purpose, samples of fresh bio-vegetables were bought and divided into three groups, one of which was treated with an illegal dose of cypermethrin. All samples (blank, treated with legal and illegal doses) were then cut into small pieces, frozen and lyophilized. Freeze-dried samples were extracted with ethyl acetate, filtered and evaporated till dryness. Interestingly, comparison of antioxidant activity of organic fennel, cabbage, and celery samples which were not exposed to pesticide treatment with treated samples (propylgallate positive control having 100% antioxidant activity) showing a reduction of antioxidant activity for the treated samples which was very obvious in the cabbage sample but slighter in fennel and celery samples. These results indicate a lower nutritional quality of the treated vegetables with illegal doses.

Chowdhury *et al*. (2013) investigated with fourteen tomato samples collected from local markets of SavarUpazila, Dhaka, Bangladesh and analyzed to identify the level of widely applied cypermethrin, chlorpyrifos and diazinon residues by using high performance liquid chromatography (HPLC) technique. It was found that out of 14 samples, five were found contaminated with cypermethrin and one with chlorpyrifos, but none residue of diazinon was detected in any of the tested

samples. Only 7% of the samples were found contaminated with cypermethrin insecticide residue which was above the maximum residue level (MRL) adopted by the FAO/WHO Codex Alimentarius Commission (CAC), although cypermethrin (0.065 ± 0.07mg/kg) and chlorpyrifos (0.024 ± 0.041mg/kg) residues were detected in samples.

Cui *et al*. (2013) conducted a study for the determination of 193 pesticide residues in vegetables and fruits on the basis of optimization of solid phase extraction adsorbent, eluting solvent types and amounts, a gas chromatography-mass spectrometric method was used. The analyses were extracted from the samples using acetonitrile. The extract was cleaned-up with a C18/PSA solid-phase extractor, eluted by acetonitrile and analyzed by GC-MS under selected ion monitoring (SIM) mode using triphenyl phosphate (TPP) as internal standard. The linear range was from 10 to 1 000 microg/L for 130 pesticides, from 20 to 1 000 microg/L for 34 pesticides, from 50 to 1 000 microg/L for 26 pesticides, from 100 to 1 000 microg/L for the other 3 pesticides with the good correlation coefficients (r > or = 0.996 7). The limits of detection were 0.04 - 8.26 microg/kg. The mean recoveries of the pesticides were 71.6% -117.9%. The relative standard deviations were 3.0% - 11.8%. This method is simple, rapid, sensitive and specific. It is appropriate for the simultaneous identification and quantification of the multi-residues in fruits and vegetables.

Chowdhury *et al*. (2013) investigated the presence of 19 different agricultural pesticides in 210 samples of eight types of domestic vegetables collected from several vegetable-growing regions in Bangladesh. A multiresidue method was developed to detect the pesticide levels in the collected samples using gas chromatography with mass spectrophotometry (GC–MS). Pesticide residues were detected in 51.30% of the total samples, and among the positive samples, 38.89% contained levels above the maximum residue levels (MRLs). The most frequently detected pesticides were chlorpyrifos (34) followed by carbofuran (17), diazinon

(16), carbaryl (14), malathion (11), endosulfan (8), cypermethrin (7) and dimethoate(6). Some (10.47%) of the samples contained multiple residues. It is concluded that the continuous monitoring and strict regulation of pesticide use on food crops, especially vegetables, are necessary.

Venugopal *et al*. (2012) found a new reaction system for spectrophotometric determination of chlorpyrifos pesticide. This is based on reaction of chlorpyrifos with diazotized anthranilic acid in alkaline medium to form an orange-red color. The solution containing 200ppm chlorpyrifos pesticide and 2 ml of decinormal sodium hydroxide is colorless and with the addition of diazotized anthranilic acid, an immediate orange-red dye is formed. The method is rapid, simple and easy to carry out. The absorbance maximum was observed at 450nm.The Beers law is obeyed up to 8.18 ppm for chlorpyrifos standard solution. Water and vegetable samples were collected in different areas of Visakhapatnam district, Andhra Pradesh, India to determine the chlorpyrifos and found low levels in the range up to 0.04ppm. Interference study was carried for other pesticides and ions.

Selim *et al*. (2011) conducted this study to monitor the pesticide residues in leafy vegetable and to develop the efficient Liquid Chromatography for the extraction and GC/MS for the analysis of investigated 86 pesticides in leafy vegetables. More than 550 samples of leafy vegetables have been collected Saudi Arabia. A multi-residue analysis method was developed and described for simultaneously determination of 86 pesticides commonly used in crops, using a broad range of physico-chemical properties in leafy vegetables related to Organophosphorous, Organochlorines, Pyrethroids and Carbamates which commonly used in agriculture commodities. Good sensitivity and selectivity of the method were obtained with the limits of quantification 0.0001 mg kg^{-1} in almost of all. The method was satisfactorily applied to routine analysis as a complement to traditional GC-MS method and the limit of detection was generally 10-20 times lesser than the Maximum Residue Levels (MRLs) established by Codex

Alimentarius Commission. Pesticide residues were detected in 24.69% of the tested samples (140 samples from the total of 567 samples). Meanwhile, the detected pesticides concentration had been exceeded the MRL in 18.34% of the total tested samples under this investigation.

Khan *et al*. (2011) conducted the study to determine the pesticide residues on selected summer vegetables. Five vegetables were grown with three replicates in a split plot randomized complete block design. Pesticides were sprayed on vegetables thrice at regular intervals each after 15 days. At maturity the pesticides residues were extracted from edible and leaf portions using anhydrous sodium sulfate and ethyl acetate while adsorption chromatography technique was used for cleanup. The extracts were subjected to high performance liquid chromatography (HPLC) for separation and analysis of the compounds. Significant differences ($p<0.05$) were found in the pesticides residues on edible portions whereas highly significant differences ($p<0.001$) were observed for the leafy portions. The residual level of cypermethrin was highest (16.2 mg kg^{-1}) in edible portion of bitter gourd, while Lambdacyhalothrin and Mancozeb residues were detected high (4.50 mg kg^{-1}, 6.26 mg kg^{-1}) in edible portion of bitter gourd and Cucumber respectively. Cypermethrin residues were high (1.86 mg kg^{-1}) in Okra leaves. Mancozeb and Lambdacyhalothrin residual level was high (1.23mg kg^{-1}, and 0.0002 mg kg^{-1}) in chili and tomato leaves. Cypermethrin residues were readily detected in edible and leaf portion of the selected vegetables.

Ayman *et al*. (2010) applied three rapid, simple, reproducible and sensitive spectrophotometric methods (A, B and C) are described for the determination of two organophosphorus pesticides, (malathion and dimethoate) in formulations and vegetable samples. The methods A and B involve the addition of an excess of Ce^{4+} into sulphuric acid medium and the determination of the unreacted oxidant by decreasing the red color of chromotrope 2R (C_2R) at a suitable λ_{max} = 528 nm for method A, or a decrease in the orange pink color of rhodamine 6G (Rh6G) at a

suitable λ_{max} = 525 nm. The method C is based on the oxidation of malathion or dimethoate with the slight excess of N-bromosuccinimide (NBS) and the determination of unreacted oxidant by reacting it with amaranth dye (AM) in hydrochloric acid medium at a suitable λ_{max} = 520 ηm. A regression analysis of Beer-Lambert plots showed a good correlation in the concentration range of 0.1-4.2 μg mL^{-1}. The apparent molar absorptivity, Sandell sensitivity, the detection and quantification limits were calculated. For more accurate analysis, Ringbomoptimumconcentration ranges are 0.25-4.0 μg mL^{-1}. The developed methods were successfully applied to the determination of malathion, and dimethoate in their formulations and environmental vegetable samples.

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

Ferrer *et al*. (2005) carried out this study to detect pesticide residue for different types of fruit and vegetables: pepper, broccoli, tomato, orange, lemon, apple and melon using liquid chromatography–time-of-flight mass spectrometry (LC–TOF-MS) for the quantitative (routine) analysis of 15 pesticide residues has been developed. The accurate mass measurements were compared in different matrices at significantly different concentration levels (from 0.01 to 0.5 mg/kg) obtaining accuracy errors lower than 2 ppm, which is well within the accepted limits for

elemental confirmation. Linearity of response over two orders of magnitude was demonstrated ($r > 0.99$). Matrix effects resulting in suppression or enhancement of the response were frequently observed, most notably in broccoli and citrus. Instrumental limits of detection (LOD) were between 0.0005 and 0.03 mg/kg depending on the commodity and pesticide studied, all being within European Union regulations for food monitoring program. Finally, the methodology was applied to the analysis of two samples from an inter-laboratory exercise. This study is a valuable indicator of the potential of LC–TOF-MS for quantitative multi-residue analysis of pesticides in vegetables and fruits.

CHAPTER III

MATERIALS AND METHODS

The samples of vegetable (tomato, okra and eggplant) were collected from different markets of Dhaka city. Five different important market places were considered for sample collection. Five samples were collected from each market place. The collected samples were carried to the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, and also to Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur for pesticide residue analysis during January to April 2018. From sampling to final analysis, required procedures are described below.

3.1 Study area

The study area included major five markets of Dhaka city namely Kawran Bazar, Jatrabari, Shanti Nagar, Shyamoli and Mirpur-1 (Fig. 1). These markets are famous for vegetables. As capital city of Bangladesh, various kinds of vegetables are brought here from different districts of Bangladesh. Tomato, okra and eggplant are important vegetables with huge consumption.

3.2 Sample collection

A total of 75 samples (25 tomatoes, 25 okraand 25 eggplant) were collected for this study presented in Table 1, Table 2 and Table 3. The amount of each sample was 1 Kg for 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.

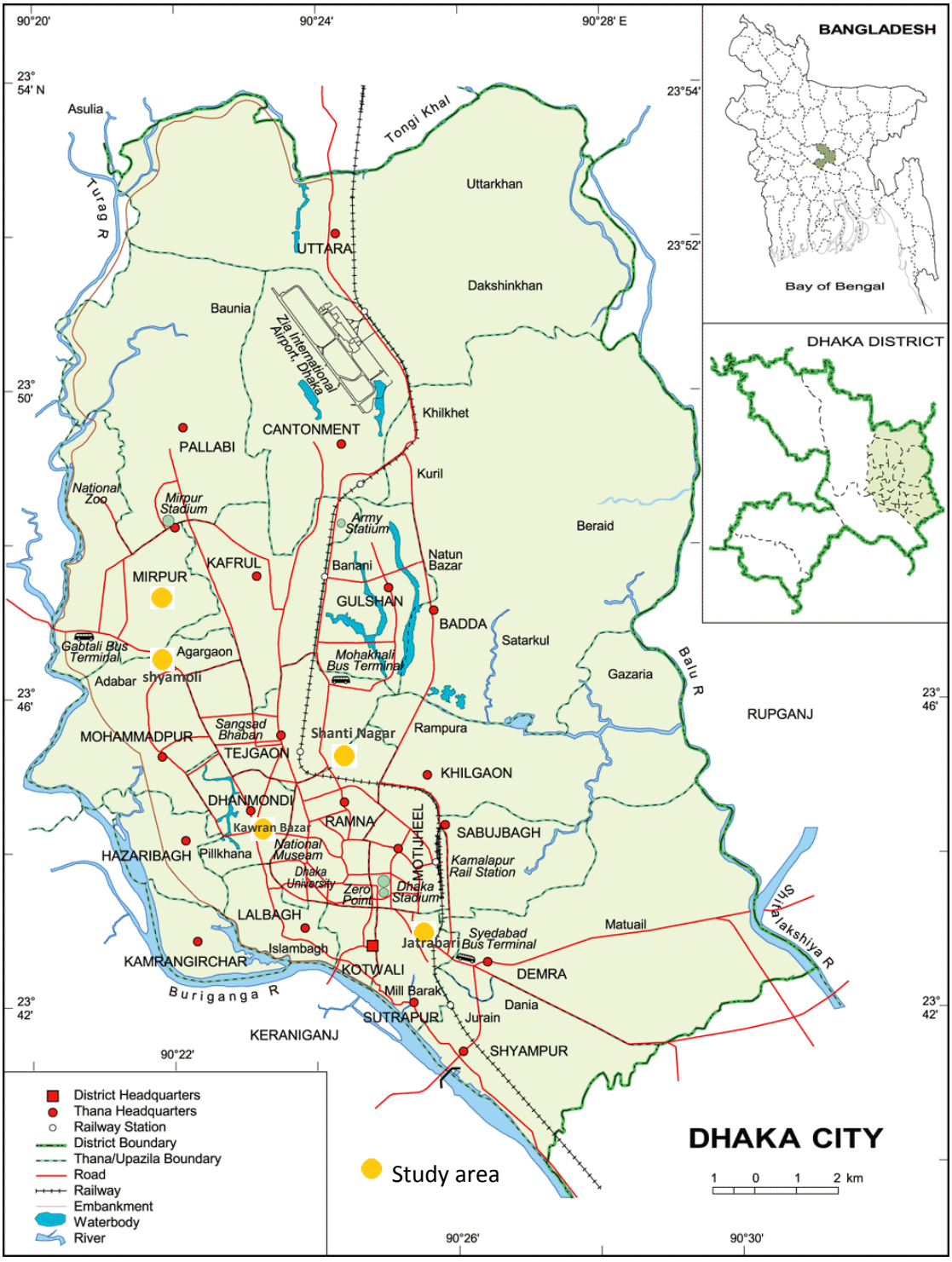


Figure 1: Map showing the places of sample collection in Dhaka city

Table 1: Sources and places of collection of tomato samples

Area of collection	Sample ID	Source
Kawran Bazar	DhKBTo -1 DhKBTo -2 DhKBTo -3 DhKBTo -4 DhKBTo -5	Kawran Bazar vegetable market (wholesale and retail market)
Jatrabari	DhJbTo -1 DhJbTo -2 DhJbTo -3 DhJbTo -4 DhJbTo -5	Jatrabari vegetable market (wholesale and retail market)
Shanti Nagar	DhSNTo -1 DhSNTo -2 DhSNTo -3 DhSNTo -4 DhSNTo -5	Shanti Nagar vegetable market (wholesale and retail market)
Shyamoli	DhShTo -1 DhShTo -2 DhShTo -3 DhShTo -4 DhShTo -5	Shyamoli vegetable market (wholesale and retail market)
Mirpur-1	DhMiTo -1 DhMiTo -2 DhMiTo -3 DhMiTo -4 DhMiTo -5	Mirpur-1 vegetable market (wholesale and retail market)

Table 2: Sources and places of collection of okra samples

Area of collection	Sample ID	Source
Kawran Bazar	DhKBOk -1 DhKBOk -2 DhKBOk -3 DhKBOk -4 DhKBOk -5	Kawran Bazar vegetable market (wholesale and retail market)
Jatrabari	DhJbOk -1 DhJbOk -2 DhJbOk -3 DhJbOk -4 DhJbOk -5	Jatrabari vegetable market (wholesale and retail market)
Shanti Nagar	DhSNOk -1 DhSNOk -2 DhSNOk -3 DhSNOk -4 DhSNOk -5	Shanti Nagar vegetable market (wholesale and retail market)
Shyamoli	DhShOk -1 DhShOk -2 DhShOk -3 DhShOk -4 DhShOk -5	Shyamoli vegetable market (wholesale and retail market)
Mirpur-1	DhMiOk-1 DhMiOk -2 DhMiOk -3 DhMiOk -4 DhMiOk -5	Mirpur-1 vegetable market (wholesale and retail market)

Table 3: Sources and places of collection of eggplantsamples

Area of collection	**Sample ID**	**Source**
Kawran Bazar	DhKBEp -1 DhKBEp -2 DhKBEp -3 DhKBEp -4 DhKBEp -5	Kawran Bazar vegetable market (wholesale and retail market)
Jatrabari	DhJbEp -1 DhJbEp -2 DhJbEp -3 DhJbEp -4 DhJbEp -5	Jatrabari vegetable market (wholesale and retail market)
Shanti Nagar	DhSNEp -1 DhSNEp -2 DhSNEp -3 DhSNEp -4 DhSNEp -5	Shanti Nagar vegetable market (wholesale and retail market)
Shyamoli	DhShEp -1 DhShEp -2 DhShEp -3 DhShEp -4 DhShEp -5	Shyamoli vegetable market (wholesale and retail market)
Mirpur-1	DhMiEp -1 DhMiEp -2 DhMiEp -3 DhMiEp -4 DhMiEp -5	Mirpur-1 vegetable market (wholesale and retail market)

3.3 Sample preparation for analysis

The collected samples were carried to the Department of Agricultural Chemistry of Sher-e-Bangla Agricultural University on the day of collection. The whole unit of each sample cut into small pieces and mixed properly. Clean air tight polythene bags were used to store chopped sample in refrigerator at -20°C until extraction and cleanup process started.

3.4 Chemicals and reagents

The standard of Cypermethrin was obtained from Sigma-Aldrich (St Louis, MO, USA) via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. Standards of all pesticides contained >99.6% purity.

Phosphoric acid (H_3PO_4), Sodium hydroxide (NaOH), Potassium iodide (KI), Leuco crystal violet (LCV) (2g) and Ethanol 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) A Systronics UV-VIS spectrophotometer (model 104) with matched silica cells was used for all spectral measurements (Plate 2).

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

A stock solution of 1 mg mL^{-1} was prepared in ethanol. Pesticide standard stock solutions in different concentration (working standard solutions) were by diluting stock solution with deionized water. Dissolve LCV (250 mg) in water (200 ml) containing (3 ml 85%) phosphoric acid and make up the final volume to the mark in a 1 L standard flask with water. The chemical name and structure of LCV are 4,4,4-methyldynetris (N,N-dimethyl aniline) respectively.

3.7 Some pictorial view related to sample preparation:

Plate 1. Electric Balanc**e**

Plate 2. Single Beam UV-VIS Spectrophotometer

Plate 3: Chopping of Sample

Plate 4: Shaking of sample

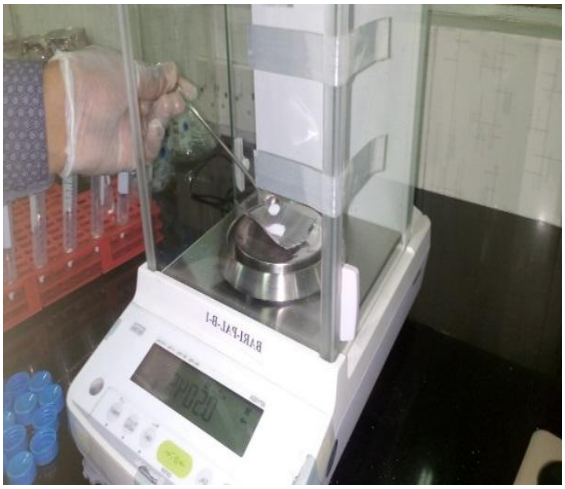
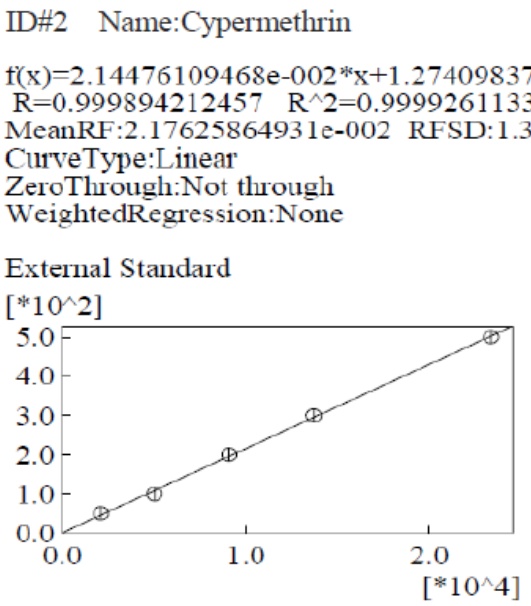


Plate 5. Weighing of PSA

Plate 6: Preparation of standard curve

3.8 Preparation of calibration curve:

Prior to the injection of the sample extract, standard solutions of different concentrations of cypermethrin 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 2). Each peak was characterized by its retention time. Sample results were expressed in mg/kg automatically by the GC software.



No.	Conc.	Area
1	50.000	2874
2	100.000	5247
3	200.000	9487
4	300.000	14587
5	500.000	22985

Figure 2: Calibration curve prepared for cypermethrin made with different concentrations ranging from 50 µg/L to 500 µg/L.

3.9 Determination of cypermethrin:

An analyte containing 3.0 to 17µg of cypermethrin in a 25 ml graduated cylinder was taken and 1 ml of 20% NaOHwas added to it. Keep the solution at room temperature for complete hydrolysis. Neutralize the reaction mixture, make slightly acidic with 4M phosphoric acid, and treat with 1 ml 0.1% KI to liberate iodine. Then 1 ml LCV, shake thoroughly, and keep for 15 minutes for color

development of the crystal violet dye. Make up the colored solution up to the mark with water. Measure the absorbance at 595ηm against a reagent blank.

Collected samples were macerate and 25 g sample was taken with two 20ml of ethanol/deminerialized water (1:1), filter through a thin cotton cloth and Centrifuge the filtrate at 1850g for 10 minutes. Then transfer the filtrate quantitatively into 50ml volumetric flask and fill up the mark with 50% ethanol. Take the aliquots of supernatant in a 25ml graduated cylinder add 0.1ml of 20% NaOH and keep it room temperature for 10 minutes for complete hydrolysis. Neutralize the reaction mixture and make it acidic with 4M phosphoric acid treat with 1 ml of 0.1% KI and 1 ml of LCV solution mix thoroughly keep for 15minutes for full color development. Make up the solution to the mark with water and measure the absorbance at 595ηm against reagent blank by spectrophotometer.

CHAPTER IV

RESULTS AND DISCUSSION

Samples of vegetable (tomato, okra and eggplant) were collected from 5 different markets of Dhaka city (Kawran Bazar, Jatrabari, Shanti Nagar, Shyamoli and Mirpur-1) to detect and quantify pesticide residues. Five (5) samples for each vegetable from each mentioned place were collected. As a result, 25 samples for tomato, 25 samples for okra and 25 samples for eggplant were collected from 5 selected places of Dhaka city. The results obtained from this study are presented and described in this chapter using differentTables.

4.1 Pesticide residues in tomato

The concentrated extracts of tomato samples collected from different markets of Dhaka city were analyzed by Spectrophotometric Methodwith the pre-set parameters. The level of pesticide residues found in the analyzed tomato samples and their maximum residue levels are outlined in Table 4.

Five (5) different markets of Dhaka city (Kawran Bazar, Jatrabari, Shanti Nagar, Shyamoli and Mirpur-1), twenty-five (25) samples of tomato were collected and analyzed to find out the presence of left over residue of cypermethrin.

Out of 25 samples of tomato, two (2) samples (8% of the total number of samples) contained pesticide residues and twenty three (23) samples (92% of the total number of samples) contained no detectable residues of the sought pesticides. The present results can be compared to the study of Ahmed *et al*. (2016) from 170 vegetable samples analysis collected from Jessore, Comilla, Narsingdi, Tangail, Rangpur, Jamalpur, Gazipur and Dhaka where commonly used insecticides were fenvalerate, diazinon, quinalphos, fenitrothion, acephate, chlorpyriphos, cypermethrin and malathion which were used in brinjal, yard long bean, bitter gourd, snake gourd, pointed gourd, okra, tomato, hyacinth bean and cabbage

samples. Among the 170 samples, 21.78% were contaminated with insecticides either single or multiple product residue, in which 18.26% samples had residue above MRL.

Five (5) tomato samples were collected from Kawran Bazar under Dhaka city, among them, one sample (DhKBTo-3) contained cypermethrin at a level of 0.62 mg kg^{-1}, which was above the EU-MRL (0.5 mg kg^{-1}) (European Commission 2015). The other four (4) samples contain no cypermethrin residue.

From JatrabariKacha Bazar under Dhaka city, five (5) tomato samples were collected. Among them, no sample contained residues of cypermethrin.

From Shanti Nagar Kacha Bazar under Dhaka city, five (5) tomato samples were collected. Of which one sample (DhSNTo-3) contained residues of cypermethrin (0.16 mg kg^{-1}). But other four (4) samples contained no cypermethrin residue. The level of detected residue of cypermethrin was below MRL (0.5 mg kg^{-1}).

From ShyamoliKacha Bazar and Mirpur-1 Kacha Bazar under Dhaka city, ten (10) tomato samples (5 samples from each Kacha Bazar) were collected. Among them, no sample contained residue of cypermethrin.

Table 4. The level of residues (ppm) of cypermethrin found in the analyzed tomato samples.

Area of collection	Sample ID	Level of residue (mg kg^{-1})	MRLs (mg kg^{-1})
Kawran Bazar	DhKBTo -1	-	-
	DhKBTo -2	-	-
	DhKBTo -3	0.62	0.5*
	DhKBTo -4	-	-
	DhKBTo -5	-	-
Jatrabari	DhJbTo -1	-	-
	DhJbTo -2	-	-
	DhJbTo -3	-	-
	DhJbTo -4	-	-
	DhJbTo -5	-	-
Shanti Nagar	DhSNTo -1	-	-
	DhSNTo -2	-	-
	DhSNTo -3	0.16	0.5*
	DhSNTo -4	-	-
	DhSNTo -5	-	-
Shyamoli	DhShTo -1	-	-
	DhShTo -2	-	-
	DhShTo -3	-	-
	DhShTo -4	-	-
	DhShTo -5	-	-
Mirpur-1	DhMiTo -1	-	-
	DhMiTo -2	-	-
	DhMiTo -3	-	-
	DhMiTo -4	-	-
	DhMiTo -5	-	-

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

4.2 Pesticide residues in okra

The concentrated extracts of okra samples collected from different markets of Dhaka city were analyzed by Spectrophotometric Method with the pre-set parameters. The level of pesticide residues found in the analyzed okra samples and their maximum residue levels are outlined in Table 5.

Five (5) different markets of Dhaka city (Kawran Bazar, Jatrabari, Shanti Nagar, Shyamoli and Mirpur-1), twenty five (25) samples of okra were collected and analyzed to find out the presence of left over residue of cypermethrin.

Out of 25 samples of okra, two (2) samples (8% of the total number of samples) contained cypermethrinresidue and twenty three (23) samples (92% of the total number of samples) contained no detectable residue of the sought pesticides.

From Kawran Bazar under Dhaka city, five (5) okra samples were collected, among them, one sample (DhKBOk-4) contained cypermethrin at a level of 0.22 mg kg^{-1}, which was below the EU-MRL (0.5 mg kg^{-1}) (European Commission 2015). The other four (4) samples contain no cypermethrin residue.

From Mirpur-1 Kacha Bazar under Dhaka city, five (5) okra samples were collected. Of which one sample (DhMiOk-4) contained residues of cypermethrin (0.44 mg kg^{-1}). But other four (4) samples contained no cypermethrin residue. The level of detected residue of cypermethrin was below MRL (0.5 mg kg^{-1}).

From JatrabariKacha Bazar, Shanti Nagar Kacha Bazar and Shyamoli Kacha Bazar under Dhaka city, fifteen (15) okra samples (5 samples from each Kacha Bazar) were collected. Among them, no sample contained residue of cypermethrin.

Table 5. The level of residues (ppm) of cypermethrin found in the analyzed okra samples.

Area of collection	Sample ID	Level ofresidue (mg kg^{-1})	MRLs (mg kg^{-1})
Kawran Bazar	DhKBOk -1	-	-
	DhKBOk -2	-	-
	DhKBOk -3	-	-
	DhKBOk -4	0.22	0.5*
	DhKBOk -5	-	-
Jatrabari	DhJbOk -1	-	-
	DhJbOk -2	-	-
	DhJbOk -3	-	-
	DhJbOk -4	-	-
	DhJbOk -5	-	-
Shanti Nagar	DhSNOk -1		
	DhSNOk -2	--	-
	DhSNOk -3	-	-
	DhSNOk -4	-	-
	DhSNOk -5	-	-
Shyamoli	DhShOk -1	-	-
	DhShOk -2	-	-
	DhShOk -3	-	-
	DhShOk -4	-	-
	DhShOk -5	-	-
Mirpur-1	DhMiOk-1	-	-
	DhMiOk -2	-	-
	DhMiOk -3	-	-
	DhMiOk -4	0.44	0.5*
	DhMiOk -5	-	-

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

4.3 Pesticide residues in eggplant

The concentrated extracts of eggplant samples collected from different markets of Dhaka city were analyzed by Spectrophotometric Method with the pre-set parameters. The level of pesticide residues found in the analyzed eggplant samples and their maximum residue levels are outlined in Table 6.

Five (5) different markets of Dhaka city (Kawran Bazar, Jatrabari, Shanti Nagar, Shyamoli and Mirpur-1), twenty five (25) samples of eggplant were collected and analyzed to find out the presence of left over residue of cypermethrin.

Out of 25 samples of eggplant, three (3) samples (12% of the total number of samples) contained cypermethrin residue and twenty two (22) samples (88% of the total number of samples) contained no detectable residue of the sought pesticides.

From Kawran Bazar under Dhaka city, five (5) eggplant samples were collected, among them, one sample (DhKBEp-2) contained cypermethrin at a level of 0.56 mg kg^{-1}, which was above the EU-MRL (0.5 mg kg^{-1}) (European Commission 2015). The other four (4) samples contain no cypermethrin residue.

From Jatrabari Kacha Bazar under Dhaka city, five (5) eggplant samples were collected. Of which one sample (DhJbEp-2) contained residues of cypermethrin (0.32 mg kg^{-1}). But other four (4) samples contained no cypermethrin residue. The level of detected residue of cypermethrin was below MRL (0.5 mg kg^{-1}).

From Mirpur-1 Kacha Bazar under Dhaka city, five (5) eggplant samples were collected. Of which one sample (DhMiEp-3) contained residues of cypermethrin (0.13 mg kg^{-1}). But other four (4) samples contained no cypermethrin residue. The level of detected residue of cypermethrin was below MRL (0.5 mg kg^{-1}).

From Shanti Nagar Kacha Bazar and Shyamoli Kacha Bazar under Dhaka city, fifteen (15) eggplant samples (5 samples from each Kacha Bazar) were collected. Among them, no sample contained residue of cypermethrin.

Table 6. The level of residues (ppm) of cypermethrin found in the analyzed eggplant samples.

Area of collection	**Sample ID**	**Level ofresidue (mg kg^{-1})**	**MRLs (mg kg^{-1})**
Kawran Bazar	DhKBEp -1	-	-
	DhKBEp -2	0.56	0.5*
	DhKBEp -3	-	-
	DhKBEp -4	-	-
	DhKBEp -5	-	-
Jatrabari	DhJbEp -1	-	-
	DhJbEp -2	0.32	0.5*
	DhJbEp -3	-	-
	DhJbEp -4	--	-
	DhJbEp -5	-	-
Shanti Nagar	DhSNEp -1	-	-
	DhSNEp -2	-	-
	DhSNEp -3	--	-
	DhSNEp -4	-	-
	DhSNEp -5	-	-
Shyamoli	DhShEp -1	-	-
	DhShEp -2	-	-
	DhShEp -3	-	-
	DhShEp -4	-	-
	DhShEp -5	-	-
Mirpur-1	DhMiEp -1	-	-
	DhMiEp -2	-	
	DhMiEp -3	0.13	0.5*
	DhMiEp -4	-	-
	DhMiEp -5	-	-

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

CHAPTER V

SUMMARY AND CONCLUSION

5.1 Summary

The purpose of this study was proposed to determination of cypermethrin residue in vegetables (tomato, okra and eggplant) by Spectrophotometric method collected from different local markets of Dhaka city. Concerning this, twenty five (25) samples of tomato, twenty five (25) samples of okra and twenty five (25) samples of eggplant were collected from five different locations (Kawran Bazar, Jatrabari, Shanti Nagar, Shyamoli and Mirpur-1) of Dhaka city and carried to Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, and also to Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur for pesticide residue analysis during January to April 2018.

The Spectrophotometric method was applied to identify and quantify the level of selected pesticide residue present in the extracted samples. The most commonly used pesticide cypermethrin was selected for this study.

Among the twenty five (25) analyzed samples of tomato, 2 samples (8% of the total number of samples) contained cypermethrinresidue and 23 samples (90% of the total number of samples) contained no cypermethrinresidue. Among two (2) contained residues samples, one (1)was contained above the maximum residue limits (MRLs) and another one (1) contained below MRLs.

Considering, the twenty five (25) analyzed samples of okra, 2 samples (8% of the total number of samples) contained cypermethrinresidue and 23 samples (90% of the total number of samples) contained no cypermethrin residue. Among 2 contained residues samples, both were contained below the maximum residue limits (MRLs).

Regarding twenty five (25) samples of eggplant, 3 samples (12% of the total number of samples) contained residues of cypermethrin, and 22 samples (88% of the total number of samples) contained no cypermethrin residue. Among the detected 3 samples contained pesticides residue of cypermethrin, two (2) were contained below the maximum residue limits (MRLs) and one (1) was contained above the maximum residue limits (MRLs).

5.2 Conclusion

It is a serious matter of concern that pesticide residues are in vegetables and other foods and it is a safety issue for the consumers. The findings of the present study reflect the overall scenario of pesticide residue load in the selected vegetables collected from different markets of Dhaka city. The results of the present study indicate that the vegetables available in Dhaka city stored from different parts of Bangladesh are contaminated with cypermethrin pesticide.

5.3 Recommendations

1. This method is good alternative to some reported costly instrumental method.
2. Many other places can be considered for sample collection including out of Dhaka city.
3. Some other important vegetables can be selected to determine pesticide residue for final recommendations.

5.4 Limitations

1. This method is not a good approach to determine pesticide residue in vegetables because of color development process (using chemicals) for the preparation of standard curve is not easy.
2. This method (Spectrophotometric method) is cost effective but not more accurate compared to GC-MS methods.
3. The collected vegetable samples were also carried out to Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur for pesticide residue analysis because of the equipments and chemicals available in the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University was not in good condition and sufficient.
4. The color development process in the method need to be optimized as we get color far late compare to the description mentioned by (Nollet and Rathore, 2007)
5. The sensitivity also need to be cross checked with GC-MS for more confidence on the result

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