DETERMINATION OF PESTICIDE RESIDUES IN COUNTRY BEAN AND BITTER GOURD COLLECTED FROM CUMILLA

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CERTIFICATE

This is to certify that the thesis entitled **"DETERMINATION OF PESTICIDE RESIDUES IN COUNTRY BEAN AND BITTER GOURD COLLECTED FROM CUMILLA"** submitted to the Department of Agricultural Chemistry, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (M.S.)** in **AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bona fide research work carried out by **ANISUR RAHMAN**, Registration No. **12-05104** 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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The Author

DETERMINATION OF PESTICIDE RESIDUES IN COUNTRY BEAN AND BITTER GOURD COLLECTED FROM CUMILLA

Abstract

The study was carried out to analyze pesticide residues in two common vegetables (country bean and bitter gourd) collected from five different locations of Cumilla district during January to April 2018. The collected samples were carried to the Pesticide Analytical Laboratory, Entomology Division of Bangladesh Agricultural Research Institute (BARI), Gazipur on the same sampling day. The collected samples were analyzed using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD) for the determination of pesticide residues in 40 samples of country bean and 40 samples of bitter gourd. Among the 40 analyzed samples of country bean, 11 samples (22.50% of the total number of samples) contained residues of diazinon and dimethoate, where two (2) samples contained multiple residues and 7 samples contained single residue and all the contaminated samples (9 samples) contained residue above the maximum residue limits (MRLs). Out of 40 samples of bitter gourd, 4 samples (10% of the total number of samples) contained residues of dimethoate, diazinon and chlorpyrifos, where 1 sample contained multiple residues and 3 samples contained single residue and all the contaminated samples (4 samples) contained residue above the maximum residue limits (MRLs). This study reflects the overall scenario of pesticide residue remain in country bean and bitter gourd collected from different markets of Cumilla district, which will help the consumer and policy makers 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 Thermionic 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 Quantification
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

Vegetables are important components of the human diet since they provide essential nutrients that are required for most of the reactions occurring in the body. It makes up a major portion of the diet of humans in many parts of the world and plays a significant role in human nutrition, especially as sources of vitamins, minerals, dietary fiber and phytochemicals (Quebedeaux *et al*., 1990). 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.

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 (Ahmed *et al*., 2014). Pesticide application is an essential component of modern crop production technology (Kabir *et al*., 1996). Their use has been contentiously increasing over the past decades. Pesticide use in Bangladesh is under over doses in some cases (cereals, fruit and vegetables) which resulted adverse effect on human health (Rahman *et al*., 2014) followed by Pakistan, the pesticides application is at maximum on cotton crop followed by fruits and vegetables (Usman *et al*., 2009). Insecticides, herbicides and fungicides are commonly used for crop protection throughout the country but the overdose of pesticides makes the residue problem, which might pollute our food and be harmful for our health (Hossain *et al*., 2000). Indiscriminate use of pesticides by the unskilled persons, only a small portion of applied pesticides reaches the targeted species; remainder enters in food chain and is indirectly passed on to human beings (Yadav, 2010). Amongst food items, fresh fruits are the most vulnerable part of the diet, as they are mostly consumed directly

after picking as compared to vegetables and grains that are cooked which in turn reduces and metabolizes the pesticide residues. A world vegetable survey showed that 402 vegetable crops are cultivated worldwide, representing 69 families and 230 genera (Kays and Dias, 1995; Dias, 2011).

Country bean is one of the most important, inexpensive and popular vegetable crops in Bangladesh. It is commonly called country bean in Bangladesh, but it has a variety of names at different regions of the country like ‘sheem, ‘chhoi, ‘uri, ‘deshi sheem etc. Internationally also the crop has various other names e.g., hyacinth bean, bonavist bean, Dolichos bean, Indian bean, Egyptian kidney bean, Lima bean, faba bean etc. (Jadhav *et al*., 1987). It is a rich source of essential vitamins which commonly grown during rainy through rabi seasons usually around the homestead by trailing its vine either on trees or by providing different kinds of supports. Bitter gourd (*Momordica charantia* L.) is an important vegetable. It is commonly called bitter in Bangladesh, but it is locally known as ‘korola’, ‘ussay’ etc. Bitter gourd fruits are a good source of carbohydrates, proteins, vitamins, and minerals and have the highest nutritive value among cucurbits. Moreover, the crude protein content (11.42 g kg^{-1}) of bitter gourd fruits is higher than that of tomato and cucumber (Gilden *et al*., 2010).

Despite of being a prospective crop, high incidences of insect pests attack resulted low yield and poor quality. In general, insect pests cause enormous quantity of yield losses in every season and every year. Although no regular statistical records are kept, as per conservative estimate the yield loss in country bean due to insect pests is reported to be about 12-30% (Hossain, 1990). Country bean is attacked by nine different insect species and one species of mite (Alam, 1969). Among these species, four species are considered as major pests and the rest of them as minor pest. Bitter gourd is attacked by many insect pests, among these, red pumpkin beetle and fruit fly are the major insect pests.

Pesticides are substances or mixture of substances intended for preventing, destroying, repelling or mitigating any pest (US Environmental, 2007). The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during crop growing, storage and transport (FAO, 2007). The use of chemicals in modern agriculture has significantly increased productivity. But it has also significantly increased the concentration of pesticides in food and in our environment, with associated negative effects on human health. Annually there are dozens of million cases of pesticide poisonings worldwide (Richter, 2002). Moreover, it is now better understood that pesticides have significant chronic health effects, including cancer, neurological effects, diabetes, respiratory diseases, fetal diseases, and genetic disorders. These health effects are different depending on the degree, and the type of exposure. Typically, the effects are different for farmers who are directly exposed to pesticides, compared to those for farmers' relatives or people living in rural areas who are less directly exposed. There are also effects on consumers through pesticide residues in food.

In order to reduce yield loss and maintain the quality vegetables farmers use pesticides because they have rapid action to control the pests and diseases, and also less laborious than other pest control methods (Gilden *et al*., 2010). High pest infestation forces farmers to apply pesticides intensively to rescue crop loss. It is reported that the crop loss due to pest infestation can be as high as 100% if they are not controlled (Rajabu *et al*., 2017). In Bangladesh it has been reported that farmers sprayed insecticides quite frequently even every day in vegetables which are severely attacked by insect pests. Farmers have no idea about the pesticide residues in the food as well as their ill effect on human health and the environment. Whimsical spray of insecticides and selling of vegetables after one to two days of spray application are assumed to be a normal practice. Serious

consequences of indiscriminate, overuse and misuse of pesticides results in a disruption in the agro-ecosystem, human health hazard and environmental pollution. Food products containing pesticide residues to consumers over time might cause cancer, teratogenesis, genetic damage and suppression of the immune system (Dias, 2011).

According to World Health organization(WHO) unbalanced diets with low vegetable intake and low consumption of complex carbohydrates and dietary fiber are estimated to cause some 2.7 million deaths each year, and were among the top 10 risk factors contributing to mortality (Dias, 2011). Mostly, vegetable crops are cultivated under high pressure for achieving higher production. A survey on pesticide use in vegetables conducted in 1988 revealed that only about 15% and 6% of the farmers received information from the pesticide dealers and extension agents, respectively (Islam *et al*., 2009). In most of the cases, the farmers either forgot the instructions or did not care to follow those instructions and went on using insecticides at their own choice or experience. Some farmers believed that excess use of insecticides could solve the insect pests' problem. They did not follow the rule of economic threshold and economic injury level. Farmers use insecticides frequently without considering the level of infestation. They usually spray insecticides in their field indiscriminately even without thinking the economic return of their investment.

Pesticide being toxic can become a potential hazard to the manufacturers, the users and the environment. Pesticide can produce negative impacts, both socially and economically (Antle *et al*., 1994). The sole reliance on pesticide applications has shown many side effects and limitations (Aktar *et al*., 2017). At present, inappropriate and irrational use of pesticides for the control of insect pest and diseases of vegetables is the common practice in Bangladesh (Kabir *et al*., 2008).

The present pattern of pesticide usage in Bangladesh particularly in vegetables led to assume that majority of marketed vegetables contain pesticide residue more than Maximum Residue Limit (MRL). The problem of food contamination with pesticide residues is a cause of concern for almost everyone and everywhere. Pesticide residues above the Maximum Residue Limit (MRL) in the crop at harvest are a globally and nationally cause of great concerns. The gravity of the problem of residues is augmented by untimely, uneconomical and unscrupulous spraying of pesticides. As a safety measure for the consumers, many developed countries have set Maximum Residue Limit (MRL) based on the Acceptable Daily Intake (ADI) and Potential Daily Intake (PDI) and that should not be exceeded for a food item to be considered safe for consumption (Prodhan *et al*., 2010; Kabir *et al*., 2007).

In Bangladesh, since harvesting and selling of vegetables are done without perplexing for the pre-harvest interval, pesticide residue levels in such vegetables would undoubtedly be above Maximum Residue Limit. Most of the growers are illiterate and they are not able to read and understand what is written on the label of pesticides. They mostly depend on ill motive pesticide dealers/retailers of their respective locations who have no clear idea about insect pests and pesticides but usually recommend insecticides that create serious problems for public health and the environment. Every pesticide has a withholding period, 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 type. The PHI differs from pesticide to pesticide and crop to crop. Food products become safe for consumption only after withholding period has lapsed. By this time, the pesticide residues get dissipated (Prodhan *et al*., 2009; Khatoon, 2004).

Pesticide residue in food has become a consumers' safety issue and the consumers have the right to know how much pesticide get incorporated in the food they eat. The identification and quantification of pesticide in the food are becoming the public interest. Several researchers analyzed pesticide residues in fruit and vegetables in Greece, India, Bangladesh, Spain, China, Japan and other developed country that have been published in the national journals (Dasika *et al*., 2012; (Prodhan *et al*., 2009; Prodhan *et al*., 2010; Kabir *et al*., 2007; Kabir *et al*., 2008; Islam, 2014, Khatoon, 2004). Nevertheless, more research work is needed to find out the actual scenario of pesticide residues present in vegetables.

Keeping this view, the present study was initiated to identify and quantify the level of different pesticides present in country bean and bitter gourd in the different markets of Cumilla district and compare the level of detected pesticide residues (mg kg^{-1}) with the Maximum Residue Limit (MRL) regarding the following objectives.

1. To identify and quantify the level of different pesticides present in country bean and bitter gourd collected from different markets of Cumilla district.
2. To compare whether the level of detected pesticide residues (mg/Kg) were above the Maximum Residue Limit (MRL) or not.

CHAPTER II

REVIEW OF LITERATURE

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

2.1 Determination of pesticide residues in crops

Tankiewicz (2019) noticed that a modified quick, easy, cheap, efficient, rugged and safe (QuEChERS) method coupled to gas chromatography with electron capture detector (GC-ECD) was developed for simultaneous determination of selected electronegative pesticides in fruits and vegetables with high water content. The chosen compounds are commonly detected in fruit and vegetable crops, and some of their metabolites have even been found in human urine. Extraction and clean up parameters were optimized, thus the original QuEChERS

method was modified to decrease solvent usage, in accordance with ‘green chemistry’ principles. The proposed methodology was validated in terms of selectivity, specificity, linearity, precision and accuracy. The obtained limits of detection (LODs) for all investigated pesticides ranged from 5.6 μg kg^{-1} to 15 μg kg^{-1} and limits of quantification (LOQs) from 17 μg kg^{-1} to 45 μg kg^{-1}. The obtained data demonstrated the good reproducibility and stability of the procedure in the tested concentration range up to 10 mg kg^{-1}, with relative standard deviations (RSDs) lower than 10%. Recoveries for spiked pear samples at LOQ level for each pesticide were from 90% to 107% with RSDs lower than 9.6%. The suitability of the developed procedure was tested on various fruit and vegetable samples available on the market at different seasons. The proposed methodology is applicable for detection and monitoring of selected pesticides not only in fruits and vegetables with high water content, but also in samples containing large amounts of pigments and dyes.

Weijian *et al*. (2019) established a method for the determination of five acylpyrazole pesticide residues in edible vegetable oils using gas chromatography-negative chemical ionization-mass spectrometry (GC-NCI-MS). The pesticides were extracted from a sample with acetonitrile under freezing conditions. A simple cleanup step known as QuEChERS was then conducted. After being identified by GC-NCI-MS, the extracts were quantified using an external standard method that employs a matrix correction standard curve. The linearity of the method was good between 20 and 1000 μg/L, and all limits of quantification were less than 2 μg/kg. Recoveries of all pesticides were in the range of 82.7%-112.4% at the three spiked levels of 0.01, 0.02, and 0.05 mg/kg, and all relative standard deviations were not more than 12.3%.

Hadiana *et al*. (2019) studied forty-eight pesticide residues from different chemical structures including organochlorine, organophosphorus, organonitrogen, dicarboximides, strobilurin, triazine, pyrethroids, and other chemical groups.

Pesticide residues in 85 fruits and vegetables were determined and confirmed by GC-MS. The pesticide was extracted with ethyl-acetate, then, the extracts cleaned using high performance gel permeation column chromatography (GPC) and solid phase column (SPE). The mean recoveries of the pesticides were between 81 and 136%. The reproducibility of the relative standard deviation values was 2.1% and 14.8%. Pesticide residues were more frequently found in vegetables (65.5%) than in fruits (26.7%). The limits of detection and quantification of pesticide residues for the method were ranged from 0.003 to 0.06 μg/g and between 0.01 to 0.1 μg/g respectively. The analyzed samples did not contain residues from the monitored pesticides that were higher than the accepted maximum residue limits (MRLs) as adapted by the FAO/WHO Codex alimentarius commission.

Tomas *et al*. (2018) conducted a study to investigate the presence of pesticide residues in nationally produced fruits and vegetables for domestic consumption. A total of 135 of the most widely consumed fruits and vegetables were analyzed for 35 pesticides. The analyses utilized a *QuEChERS* multiresidue extraction kit along with tandem gas chromatography–mass spectrometry. The results were evaluated according to maximum residue limits (MRLs) for each commodity and pesticide according to national regulation. Pesticides were detected in 65% of the total samples, in 44% of the positive samples at or below the MRLs, and in 56% above the MRLs. Oranges had the highest pesticide concentration detected, but carrots had the highest frequency of noncompliance among the produce items sampled. Five pesticides were detected at frequencies above 10%, the highest being chlorpyrifos in 25.9% of the total samples.

Abubakar *et al*. (2018) developed quick, easy, cheap, effective, rugged and safe technique (QuEChERS) coupled with dispersive solid-phase extraction (dSPE) to overcome the setback challenges experienced by the previous technologies for determination of pesticide residue. Conclusively, the reviewed QuEChERS-dSPE techniques and the recent cleanup modifications justifiably prove to be reliable for

routine determination and monitoring the concentration levels of pesticide residues using advanced instruments such as high-performance liquid chromatography, liquid chromatography–mass spectrometry and gas chromatography–mass spectrometry.

Prodhan *et al*. (2018) estimated the variability of pesticide residues in eggplant units by LC–MS/MS. In total, 120 samples from a trial field and 142 samples from different marketplaces in Thessaloniki, Greece. For the field samples, 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.The variability factors obtained in the marketplace samples were higher than those in the samples collected from the field trial.

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.

Magali *et al*. (2018) developed fast ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) method for quantitative determination of pesticide residues in pear. A fast modified acetate QuEChERS method without clean-up was used for sample preparation. Validation was performed according to SANTE guideline. Matrix effect results were significative for most part of compounds and thus a matrix-matched calibration was employed. The linear range of the method was from 2.5 to 100 $\mu g\ kg^{-1}$. Recoveries were between 70 and 120% with precision ≤ 20%. Limit of quantification was 2.5 $\mu g\ kg^{-1}$ for most compounds. Uncertainty results ranged from 22 to 49%. In real sample analyses, 21 compounds were quantified in concentrations between 3.3 and 1427 $\mu g\ kg^{-1}$. Method proved to be simple, robust and effective to be applied in routine analysis.

Stachniuk *et al*. (2018) carried out a study to evaluate pesticide residue contamination of fresh and frozen fruits and vegetables, agricultural raw material, and the estimation of the multiresidue method effectiveness expressed as the proportion of pesticides detected in food samples to the total number of pesticides analyzed by multiresidue methods. A total of 144 samples (of black currants, red currants, raspberries, cherries, strawberries, blackberries, cauliflowers and broccoli) were analyzed using LC-MS/MS method for the determination of 60 pesticides. QuEChERS extraction, matrix-matched calibration and dynamic multiple reaction monitoring method were used. Residues of 15 compounds, mainly fungicides and insecticides, were detected in 46 samples. The percentage of samples with residues above the maximum residue levels (MRL) was 15%,

whereas samples with residues below MRL were 17%. A total of 13 samples contained more than one pesticide residue. Pesticide residues were detected most often in samples of black currants (50%), broccoli (36.4%), raspberries (29%) and red currants (21.8%). The most frequently detected pesticides were carbendazim and acetamiprid. The proportion of pesticides detected during our study to the total number of analyzed pesticides amounted to 25%.

Joseph *et al*. (2018) evaluated the residues of 99 pesticides in 72 samples of 12 agricultural products using QuEChERS method extraction, and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) and gas chromatography with electron capture detection (GC-ECD). 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. Insecticides (39.7%) were the most prevalent group, with all the samples containing at least one pesticide. 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'-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%). Samples with residues above their MRLs represented 38% of all the positive analyses; chili pepper (6.4%) and kidney beans (5.5%) were found to have the most residues above their MRLs.

Ibrahim *et al*. (2018) experimented the persistence patterns of malathion, fenitrothion and deltamethrin in fruits of tomato and cucumber. Residues were determined by gas liquid chromatography. Results confirmed that the initial deposit of malathion and fenitrothion on and in the cucumber fruits (7.603 and

9.043 μg/g) were higher than on and in tomato fruits (5.390 and7.110) respectively. Data also indicated that the initial residue of deltamethrin on and the tomato fruits (3.660) was higher than the initial deposit of deltamethrin on and in the cucumber fruits (3.643). Results showed that, the consumable safety time was found to be 10 and 14 days after application on tomato and cucumber. This was found to be enough to reduce the residue to below the maximum residue limits (MRL).

Lawal *et al*. (2018) carried out a study to determine pesticide residue in fruits and vegetables using conventionally known techniques, which include liquid-liquid extraction, solid-phase extraction (SPE) and the recently used liquid-phase microextraction techniques. Despite the positive technological effects of these methods, their limitations include; time-consuming, operational difficulty, use of toxic organic solvents, low selective property and expensive extraction setups, with shorter lifespan of instrumental performances. Thus, the potential and maximum use of these methods for pesticides residue determination has resulted in the urgent need for better techniques that will overcome the highlighted drawbacks. Conclusively, the reviewed QuEChERS-dSPE techniques and the recent cleanup modifications justifiably prove to be reliable for routine determination and monitoring the concentration levels of pesticide residues using advanced instruments such as high-performance liquid chromatography, liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry.

Ibrahim *et al*. (2018) conducted a study with three green leafy vegetable samples of pumpkin leaves, spinach leaves, and sorrel leaves and were tested for the presence of residues of organochlorine pesticides. The concentrations of all the pesticide residues in the vegetable samples were determined using GC/MS. Among the organochlorine pesticide p,p'-DDT was detected in pumpkin (0.75 mg/kg), spinach (0.319 mg/kg) and sorrel (0.219 mg/kg). α-BHC and γ-BHC were detected only in pumpkin leaves (0.359 mg/kg and 0.647 mg/kg respectively).

Dieldrin was detected in spinach and sorrel (0.124 mg/kg and 0.053 mg/kg respectively). Endrin was detected in pumpkin (0.732 mg/kg) and Aldrin in sorrel (0.095 mg/kg). All these values were above the maximum residue limit (MRL) value of the pesticides. Endosulfan II was detected in sorrel (0.306 mg/kg) below the MRL.

Lawal *et al*. (2018) conducted a study to determine pesticide residue in jackfruit, strawberries, cucumber, pears, and carrots using modified QuEChERS dispersive solid phase extraction coupled with ionic liquid-based dispersive liquid–liquid microextraction were used for the determination of multi-pesticide residues in fruit and vegetable samples. 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%).

Kiwango *et al*. (2018) reported malpractices in the use of pesticides in vegetable production in the horticultural sector in developing countries. This can result in excessive use of pesticides and, subsequently, in unacceptable levels of pesticide residues in foods of horticultural origin. Consumption of vegetables containing unacceptable levels of pesticide residues is of public concern due to its potentially harmful effects on human health. In this work, it was reviewed that pesticides are rarely applied to vegetables following good agricultural practices. Results from this research will allow for the allocation of resources for improvement, monitoring and control practices to minimize the risk of unwanted pesticide residues in vegetables.

Huifen *et al*. (2018) established a multi-residue determination method after optimization of the QuEChERS pretreatment method, combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology, for 105 typical insecticides, bactericides, herbicides, and plant growth regulators in vegetables. The target compounds were extracted by acetonitrile, purified with 150 mg primary secondary amine (PSA), 150 mg EC-C18, and 30 mg graphitized carbon black (GCB) adsorbents. The standard curves of 105 target compounds were linear in the concentration range of 0.010-0.200 mg/L, with correlation coefficients (r)>0.99. The limit of quantification was 0.010 mg/kg, the recoveries were between 68.2% and 108% at three spiked levels, and the RSDs of the method were between 1.02% and 11.8%. The method is suitable for the rapid determination of the common pesticides in vegetables owing to its advantages of rapidity, simplicity, and better purification.

Chen *et al*. (2018) developed a very quick, easy, cheap, effective, rugged and safe (QuEChERS) procedure by using ultra-high performance liquid chromatography coupled with tandem mass spectrometry for simultaneous determination of afidopyropen and its metabolite M440I007 residues in tomato, watermelon, pepper, cucumber, pear, grape, cabbage etc. The target compound was determined in less than 5.0 min using an electrospray ionisation source in positive mode (ESI^+). The limit of quantification was 1 $\mu g\ kg^{-1}$ in different matrices. Two sorbents primary secondary amine and graphitised carbon black were used in the QuEChERS procedure, and matrix-matched standards gave satisfactory recoveries and relative standard deviation (RSD) values in different matrices at three spiked levels (1, 10 and 500 $\mu g\ kg^{-1}$).

Anna *et al*. (2018) invented a Suspect Screening Strategy for Pesticide Metabolites in Fruit and Vegetables by UPLC-Q-Tof-MS for the detection of pesticide metabolites in fruit and vegetable samples. Based on a retrospectively created accurate mass compound database, a suspect screening approach was established

for pesticides of high concern applied to a wide scope of plant-derived commodities. The metabolite database contained a total of 648 pesticide metabolites originating from 58 active compounds. In 500 samples from daily routine analysis, 96 samples with positive detects for a total of 26 pesticides were re-analyzed for the occurrence of corresponding metabolites. Forty-seven different phase-I and phase-II metabolites were identified, respectively. The developed metabolite database can be applied for a suspect screening approach for pesticide metabolites identification in all kinds of fruits and vegetables.

Liang *et al*. (2018) analyzed 420 samples of 10 different types of fresh vegetables for pesticide multi-residue contents using gas chromatograph 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.

Lin *et al*. (2018) developed a rapid and economical method using modified QuEChERS sample pretreatment coupled with high-sensitivity gas chromatography/triple quadrupole mass spectrometry to determine ten pyrethroid pesticides in fruits and vegetables. All pesticides were detected within 20 min of one injection. Concurrent backflushing provided column protection, greatly facilitating instrument maintenance. For quantitation, matrix-matched calibration was used to compensate for signal-enhancement effects and to ensure the precision

of the method. The limit of detection (LOD) was in the range of 0.3–4.9 μg/kg. The recovery rate was from 78.8 to 118.6%, with relative standard deviation (RSD) below 14.8%. The developed method is suitable for rapid and sensitive multi-residue analysis of pyrethroid pesticides in fruits and vegetables.

Pang *et al*. (2018) carried out an experiment to evaluate the behavioral characteristics of MS of 485 pesticides under different conditions using LC-quadrupole-time-of-flight MS technique. A high-throughput screening and confirmation method has been developed for the 485 pesticides in fruits and vegetables. The method features a full scan of fragments, with 80% of pesticide qualitative points over 10, which helps increase pesticide qualitative accuracy. Four different fruits and vegetables-apples, grapes, celery, and tomatoes-were chosen to evaluate the efficiency of the method at three fortification levels of 5, 10, and 20 μg/kg, and satisfactory results were obtained. With this method, 12551 samples of 146 different fruits and vegetables collected from 638 sampling points in 284 counties across 31 provincial capitals/cities directly under the central government, which provided scientific data backup for ensuring pesticide residue safety of the fruits and vegetables consumed daily by the public. Meanwhile, the big data statistical analysis of the new technique also further proves it to be of high speed, high throughput, high accuracy, high reliability, and high informatization.

Li *et al*. (2018) conducted an experiment with 439 pesticides in fruits and vegetables using GC-quadrupole-time-of-flight MS (GC-Q-TOF/MS) technique 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.

Akter *et al.* (2017) have been found pesticide residues in eggplant monitored in Mymensingh district and among the 50 analyzed samples, 11 (22% of the total number of the samples) contained pesticide residues of diazinon, dimethoate, quinalfos, and chlorpyrifos, of which, 2 had multiple pesticide residues and 5 samples contained residue above the European Union maximum residue limit (EU-MRLs). Chlorpyrifos was detected as the most used pesticide in eggplant in the studied area.

Hasan *et al*. (2017) have been detected two types of insecticides (dimethoate and quinalphos) in country bean samples collected from different market places of dhaka. Among the 50 analyzed samples of country bean, 10 samples (20%) contained residues of dimethoate and quinalphos, of which 5 samples were above the maximum residue limits (MRLs). Most of the contaminated samples (8 samples) contained residue of dimethoate.

Elguetaa *et al*. (2017) conducted a study to investigate pesticide residue concentrations and potential human health risk, with 118 vegetable samples. The pesticide residues were determined using the multiresidue QuEChERS method by gas chromatography as well as high-performance liquid chromatography. The results indicated that 27% of the total samples contained pesticide residues above the maximum residue limits of each active ingredient. The maximum estimated daily intake obtained for carbon disulphide (CS_2), methamidophos, azoxystrobin and cypermethrin were 0.57, 0.07, 0.06 and 0.05 mg kg-1, respectively, which was higher than their acceptable daily intake.

Sebastian *et al*. (2017) developed multiresidue QuEChERS method by gas chromatography as well as high-performance liquid chromatography for the determination of pesticides. The results indicated that 27% of the total samples contained pesticide residues above the maximum residue limits of each active ingredient. The maximum estimated daily intake obtained for carbon disulphide (CS2), methamidophos, azoxystrobin and cypermethrin were 0.57, 0.07, 0.06 and 0.05 mg kg−1, respectively, which was higher than their acceptable daily intake. It is concluded that inhabitants of the North Central agricultural area of Chile are not exposed to health risks through the consumption of leafy vegetables with the exception of methamidophos

Jian *et al*. (2017) established A LC-MS/MS method for determination of eight pesticides (triadimefon, sulfoxaflor, flusilazole, tebuconazole, difenoconazole, amitraz, azoxystrobin, and thiophanate-methyl) in Lycium barbarum. The samples were extracted with acetonitrile, and then cleaned up by primary secondary amine. The extracts were diluted with 0.1% formic acid in water. The results showed that at the fortified levels of 0.01–10 mg/kg, the average recoveries of these pesticides ranged from 82.1% to 96.2% with the relative standard deviations lower than 7%. The half-lives of eight pesticides were 1.3–5.0 days in fruits. The pre-harvest interval of all pesticides mentioned above Lycium barbarum were investigated. Tebuconazole (14 days), sulfoxaflor (14 days) and flusilazole (28 days) have longer pre-harvest interval than the others which have 7 days. The dietary risks, assessed as hazard quotients, were far below 100%. The results showed that the eight pesticides applied to Lycium barbarum were comparably safe for the consumer.

Peng *et al*. (2017) developed A novel carboxylated multi-walled carbon nanotubes (MWCNTs-COOH) dispersive solid phase extraction (d-SPE) method combined with gas chromatography (GC) with an electron capture detector (ECD) for the determination of seven pyrethroid pesticides in cucumber, spinach, eggplant,

tomato and carrot. We optimised d-SPE conditions including the type and volume of the extractant, the type and amount of the sorbent, and shaking time. Under the optimal conditions, the linear range was from 2.0 to 2000 μg kg^{-1}. The recoveries were from 88.5% to 108.2%, with the corresponding RSDs <6%, correlation coefficients 0.9987–0.9998, LOD 0.5–2.9 μg kg^{-1} and LOQ 1.5–9.7 μg kg^{-1}.

Mbulaheni *et al*. (2016) conducted a study to determine pesticide residue levels in fruits and vegetables sold at two of the biggest fresh produce markets. They observed that in most countries, fresh produce sold at local markets is usually not analyzed for agricultural chemical residues as export products are, which raises concerns about the perceived safety levels of local food supplies in contrast with exported products. A total of 199 fruit and vegetable samples were collected and analyzed for 74 pesticides commonly used in the horticultural sector. Of the samples analyzed, 91% were compliant with set maximum residue levels (MRLs). The remaining samples either contained unregistered chemicals (8%) or exceeded set MRL values (1%). Products containing more than one pesticide residue constituted 4.02% of all samples tested. Imazalil and iprodione were found to be the most frequently detected pesticides (12 samples each). Boscalid, endosulfan, profenofos, and procymidone were associated with the most noncompliance, including exceeding MRL values or being unregistered for the specific crop.

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, Cumilla, 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 Cumilla 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.

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

Zhang *et al*. (2016) developed a rapid, efficient, and environmentally friendly method using quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method combined with ionic liquid-based dispersive liquid-liquid micro extraction (QuEChERS-IL-DLLME) prior to high-performance liquid chromatography coupled with photodiode array detection (HPLC-PDA) has been developed for the determination of six triazole fungicides (triazolone, triadimenol, epoxiconazole, flusilazole, tebuconazole, and diniconazole) in various fruits (pear, apple, and grapefruit). And the proposed method was successfully applied for the determination of trace amounts of triazole fungicides in various fruits including pear, apple, and grapefruit.

Andrascikova and Hrouzkova (2016) developed a fast, efficient, and simple method for determination of pesticide residues in pumpkin seeds by combining QuEChERS and dispersive liquid–liquid micro extraction (DLLME) followed by gas chromatography and mass spectrometry (GC-MS). The developed and validated method was successfully applied for the extraction and determination of pesticide residues in 16 real samples with 2 positive findings below maximum residue limits (MRL). Limits of detection (LODs) of the proposed method are below the MRLs established by the European Union.

Rai *et al*. (2016) conducted a research using quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction method combined with dispersive liquid-liquid micro extraction (DLLME) for the quantitative determination of 36 multiclass, multiresidue pesticides (13 organochlorines, 11 organophosphates, and 12 synthetic pyrethroids) in different vegetables and fruits without primary and secondary amine (PSA) cleanup step followed by gas chromatography-mass spectrometry (GC-MS) analysis. The samples collected from Lucknow City, India, were analyzed for the presence of pesticides and only three pesticides β-cypermethrin, λ-cyhalothrin, and chlorpyrifos were found to have value above PFA-1954/CODEX-MRL values.

Zanella *et al*. (2016) conducted a research on different extraction procedures based on the QuEChERS method 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 of C18, PSA, NaOH and CH_3COONa to optimize the sample preparation step. The validation results of the method were satisfactory, since the method presented recoveries between 70% and 118%, with RSD lower than 19% for spike levels between 10 and 100 µg/L. The method limit of detection (LOD) and limit of quantification (LOQ) ranged from

3.0 to 7.6 µg/L and from 4.9 to 26 µg/L respectively. The method developed was adequate for the determination of 74 pesticide residues in orange juice.

Prodhan *et al*. (2016) have been detected three insecticides (chlorpyrifos, cypermethrin and deltamethrin) and two fungicides (fluopicolide and propamocarb hydrochloride) in the cabbage samples collected from different market places in Thessaloniki, Greece. Among 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.

Stocka *et al*. (2016) used QuEChERS sample preparation method for the determination of ten pesticides and their metabolites in fruits (apples, grapes) and vegetables (tomatoes, peppers), coupled to gas chromatography with an electron capture detector (GC-ECD). The GC-ECD method was validated in terms of its linearity, selectivity and recovery. The limits of detection for all investigated analytes ranged from 0.003 to 0.011 mg kg^{-1} and limits of quantification ranged from 0.009 to 0.03 mg kg^{-1}. The mean recoveries from four matrices for development method ranged from 70 to 120%, with relative standard deviations in the range of 3.9 to 7.2% for all ten test compounds. The QuEChERS approach takes advantages of the wide analytical scope and high degree of selectivity and sensitivity, It is simple, rapid and requires low solvent consumption, which, in the era of green chemistry, represents a significant advantage.

Park *et al*. (2016) investigated a total of 230 pesticide residues in 8496 samples of leafy vegetables (e.g. *Brassica* sp. namai, leafy lettuce, spinach, perilla leaves, crown daisy, marshmallow, aster scaber, pimpinella brachycarpa and chinese

chive). The result showed that among 8496 samples, 61 different pesticides were detected in 890 samples, of which 118 samples exceeded the Korean maximum residue limits (KMRLs).

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 chilli, tomato, grape and mango fruits using liquid chromatography tandem mass spectrometry. The method comprised of extraction with ethyl acetate and cyclohexane 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 84.36 to 95.64 % 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 chilli, tomato, grape and mango fruits were determined as 4.76, 3.90, 4.06 and 10.74 days respectively.

Islam *et al*. (2015) carried a study to explore the chemical contamination of vegetables available in local market of Mymensingh, Bangladesh for detection and quantification of the presence of pesticides. Standard pesticides and GC (Gas Chromatography) were used to confirm their retention times and area of eluted peaks. By comparing the retention times of standards and samples, confirmation of residual presence of pesticides were studied. A result revealed that residue of a fungicide (Common Name: mancozeb, a.i: symoxanil 72 wp) was found in one Cucumber sample out of three where 50 mg/Kg quantified. On the other hand, out of 3 spinach samples, 1 of them showed presence of a insecticide (Common name: imidachloropid; a.i.: imidachloropid 20 SL) residues, which quantified as 35

mg/Kg. Residual quantity determined in cucumber sample of BAUSesh More and spinach sample of Kewatkhali market, Mymensingh.

Abdulhamid *et al*. (2015) conducted a study for the determination of organochlorine and pyrethroid pesticide residues in *Amaranthu shybridus* (spinach), *Hibiscus esculentus* (okra) and *Telfairiaocci dentalis* (fluted pumpkin leaves) by QuEChERS Method and Gas Chromatography Triple Quadrupole Mass Spectrometry. The concentration of organochlorine and pyrethroid pesticide residues in *Amaranthus hybridus* (spinach), hibiscus esculentus (okra) and telfairiaoccidentalis (fluted pumpkin) sampled from seven farms in Minna, Nigeria were investigated using gas chromatographytriple quadrupole mass spectrometry. The analysis showed the presence of cypermethrin in concentration range of 0.51 to 9.95 µg/mL in two samples of spinach. The presence of heptachlor was however not confirmed in these samples.

Hossain *et al*. (2015) studied that among organophosphorus pesticides, diazinon and chlorpyriphos were detected in collected samples from Bogra district. Detectable amount of diazinon was found in one bringal sample (BS-4 at 0.32 mg/Kg) among ten bringal samples. It was also detected in one Cucumber sample CS-5 (0.18 mg/Kg) among ten Cucumber samples and one Tomato sample (TS-3 at 0.57 mg/Kg) among five Tomato samples. Chlorpyriphos was being found in one bringal sample (BS-7 at 0.4 mg/Kg). It was also detected in three Cucumber samples among ten Cucumber samples. The IDs of chlorpyriphos positive were CS-2 (0.05 mg/Kg), CS-7 (0.02 mg/Kg).

Biziuk *et al*. (2015) applied multiresidue methods for determination of currently used pesticides in fruits and vegetables using QuEChERS technique. They stated that all over the world, applied pesticides are some of the most common pollutants of the environment because of their stability, mobility, their consequent long-term adverse effects on living organisms in general and human health in particular. The

analysis of food samples for the presence of pesticides causes a lot of difficulties in consideration of specificity of sample preparation based on multistage operations of purification of sample containing vestigial amount of analyte with simultaneous large amount of interferents.

Neufeld *et al*. (2015) used acetylcholinesterase (AChE) inhibition as a simple colorimetric test for organophosphates/carbamates (OP/C), and was tested with extracts from the widely-used QuEChERS extraction method. QuEChERS extraction of chlorpyrifos-spiked tomato, spinach and lettuce samples indicated a high sensitivity to OP/C, with AChE inhibition occurring in the ppb range. The applicability of this method combination was tested by screening tomatoes from 18 different sources, including private gardens, farmer's market venders, and local supermarkets. Tomatoes from one private garden, three "certified naturally grown" farmer's market venders and two "organic" supermarket source had AChE inhibition significantly above nominally pesticide-free controls, suggesting the presence of OP/C residue. These residues were likely below levels of health concern, as indicated by lack of complete AChE inhibition, and the absence of inhibition upon sample dilution. This study demonstrates that the combination of QuEChERS extraction and AChE-inhibition detection provides a relatively simple and inexpensive alternative for detection of OP/C in vegetable samples.

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*. (2015) determine seven insecticides (chlorpyrifos, dimethoate, deltamethrin, thiamethoxam, thiacloprid, pirimicarb and indoxacarb) and three fungicides (azoxystrobin, fluopicolide and propamocarb hydrochloride) in 122 fresh melon samples which was collected from different market places in Thessaloniki, Greece They found the average recoveries of the selected pesticides ranged from 82% to 106% with RSDr ≤6% in four fortification levels of 0.01, 0.05, 0.1 and 0.2 mg/kg and the correlation coefficient (R^2) was ≥0.997 for all the selected pesticides The LOD values ranged from 0.001 to 0.003 mg/kg, and the LOQ was determined at 0.01 mg/kg for all the analytes. Among the 122 analysed samples, 32 (26% of the total no. of samples) were found to have pesticide residues.

Satpathy *et al*. (2014) conducted 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 and spiking levels for the recovery experiments as 0.005, 0.01 and 0.1 mg/kg for GC-MS/MS analyses. 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) conducted a survey on three major vegetables like eggplant, cauliflower, and country bean total 42 samples collected from fields and market by Gas Chromatography (GC) with Flame Thermionized Detector (FTD) and Electron Capture Detector (ECD). On the basis of questionnaires, under their supervision 23 farmers were interviewed. They record 8.33 to 45.00 percent farmers in two selected locations of Narsinghdi to apply different pesticides every day and in some cases even twice in a day on vegetables. They found out of 42 samples, 27 had pesticide residue. Among these 27 samples, 14 samples had pesticide residues above the Maximum Residue Limit (MRL). The detected pesticides were diazinon, malathion, quinalphos, fenitrothion, cypermethrin, fenvalerate and propiconazole.

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

Corteas *et al*. (2013) developed a method for the determination of organophosphorus pesticides in vegetables. Pesticide residues are extracted from samples with a small amount of ethyl acetate and anhydrous sodium sulfate. Analyses are performed by large volume GC injection using the through oven transfer adsorption desorption (TOTAD) interface. The calculated limits of detection for each pesticide injecting 50 μL of extract which is much lower than the maximum residues levels (MRLs). Repeatability studies yielded a relative standard deviation lower than 10% in all cases. The method was applied to the analysis of eggplant, lettuce, pepper, cucumber, and tomato.

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

Cho *et al*. (2013) conducted a research on the “Evaluation of QuEChERS Method for the Determination of Pesticide Residues Using GC/NPD and GC/ECD” where the modified QuEChERS method was evaluated for rapid determination of

pesticide residue in spinach by gas chromatography-nitrogen phosphorus detector and electron capture detector. They selected fifty GC amenable pesticide and found that the detector response linear with determination coefficient higher than 0.995.They also found that the LODs for most compound ranged between 0.001 and 0.1µg/g and about 90% of the compound had LODs of less than 0.05 µg/g. The recoveries 80-120% and relative standard deviation (less than 20%) were within acceptable level except for dichlorvos, propamocarb, chlorothalonil, dichlofluanid, cyhalothrin and fenvalerate.

Milhome *et al*. (2013) carried out a research on the "Validation and Uncertainty of the method for multiresidue analysis of 35 pesticides in melon using Gas Chromatography Coupled to Quadropole Mass Spectrometry (GC-QP/MS)" and determined various validation parameters such as (selectivity, linearity, LOD, LOQ, accuracy and precision) according ABNT NBR 14029:2005. The recoveries rate for all the pesticide they studied 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.

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

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

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

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

Farag *et al*. (2011) carried out a research on the ''Monitoring of pesticide residues in some Egyptian herbs, fruits and vegetables''. He collect One hundred thirty two samples of fruits, vegetables, herbs and spices from Egyptian local markets and analyzed for pesticide residues. He found that contamination with pesticide

residues reached 54.55% while samples free from contamination reached 45.45%. He observed only one sample from 132 analyzed samples violated the Maximum Residue Limits (MRLs) of the Codex Committee. From the 132 analyzed samples, 72 samples (54.55%) were contaminated, from which 43.18% contaminated with residues from one pesticide residue, 6.06% with 2 residues and 5.3% with more than 2 residues.

Sahoo *et al*. (2011) conducted a research work on the ‘‘Development and Validation of QuEChERS Method for Estimation of Propamocarb Residues in Tomato *(Lycopersicon esculentum)* Mill and Soil’’. In his study an easy, simple and efficient analytical method was standardized and validated for the estimation of residues of propamocarb in tomato and soil. QuEChERS method included extraction of the sample with ethyl acetate and cleanup by treatment with PSA and graphitized carbon. Final clear extracts of ethyl acetate were concentrated under vacuum to almost dryness and reconstituted into hexane. The residues of propamocarb were estimated using gas chromatograph-mass spectrometry (GC-MS). They found that propamocarb presented a distinct peak at retention time of 8.962 min. and the consistent recoveries of propamocarb ranging from 87 to 92 percent were observed when they spiked the sample at 0.10, 0.50 and 1.00 mg·kg–1 levels. They also determined the limit of quantification (LOQ) of their method was 0.10 mg/kg.

Charan and Sharma (2010) monitored pesticide residues in vegetables to find out severity of such synthetic agrochemicals on human being. A total of 182 samples of six vegetables were collected for pesticide residue analysis from different agricultural fields of central Aravalli region, when they were ready for transportation to market. The analysis of samples for different pesticide residues were carried out on GCECD and GC-NPD systems equipped with capillary columns by using a multiple residue method. About 40.11% of total analyzed samples were contaminated with different pesticide residues, among which

35.62% of total contaminated samples were exceeded the maximum residual limit (MRL) values.

Schreiber and Wittrig (2010) carried out a research on “Enhanced LC/MS for the Quantitation and Identification of Pesticide in Food Sample” where he collected a variety of fruit and vegetable samples including apple, banana, carrot, cucumber, curry powder grapes, grapefruit, hazelnut, lemon, nectarine, orange, pear, raspberry, red pepper, raisin, salad, spinach and tomato from a supermarket and extracted using QuEChERs procedure. They injected the extracted sample into a liquid chromatography tandem mass spectrometry system where a total number of 12 pesticides were detected. They found 70-120% recovery for most of the pesticide with %CV<15%. They also found methamidophos 130µg/kg, omithoate 42µg/kg, thiamethoxam 48 µg/kg, dimethoate 54µg/kg, clothianidin 14µg/kg, imadacloprid 2.4µg/kg, promamocarb 98µg/kg, carbyl 499µg/kg, metalaxyl 5.1µg/kg, myclobutanil 3.4µg/kg, spinosyn A 6.1µg/kg, spinosyn D 6.8 µg/kg.

Chapter III

Materials and Methods

The samples of vegetable (country bean and bitter gourd) were collected from different markets of Cumilla district. Five upazilla were considered for sample collection. Eight samples were collected from each upazilla. The collected samples were carried to the 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, the required procedures are described below.

3.1 Study area

The study area included major five markets of Cumilla district. It is a district of Bangladesh located about 100 kilometres south east of Dhaka. Cumilla is bordered by Brahmanbaria and Narayanganj district to the North, Noakhali and Feni district to the south, Tripura of India to the east and Munshiganj and Chandpur districts to the west. Cumilla has a total area of 3085.17 square kilometers with 17 Upazilas.

In this study, country bean and bitter gourd were selected to determine pesticide residues that were collected from different upazila market of Cumilla district. Central market of Chandina, Debidwar and Daudkandi upazila and Kangshanagar Bazar under Burichang upazila, and Maynamati Bazar under Cumilla Sadar upazila was considered for sample collection. These markets are famous for vegetables. The vegetables of these markets come from different places of Cumilla district and also from some places of nearby districts.

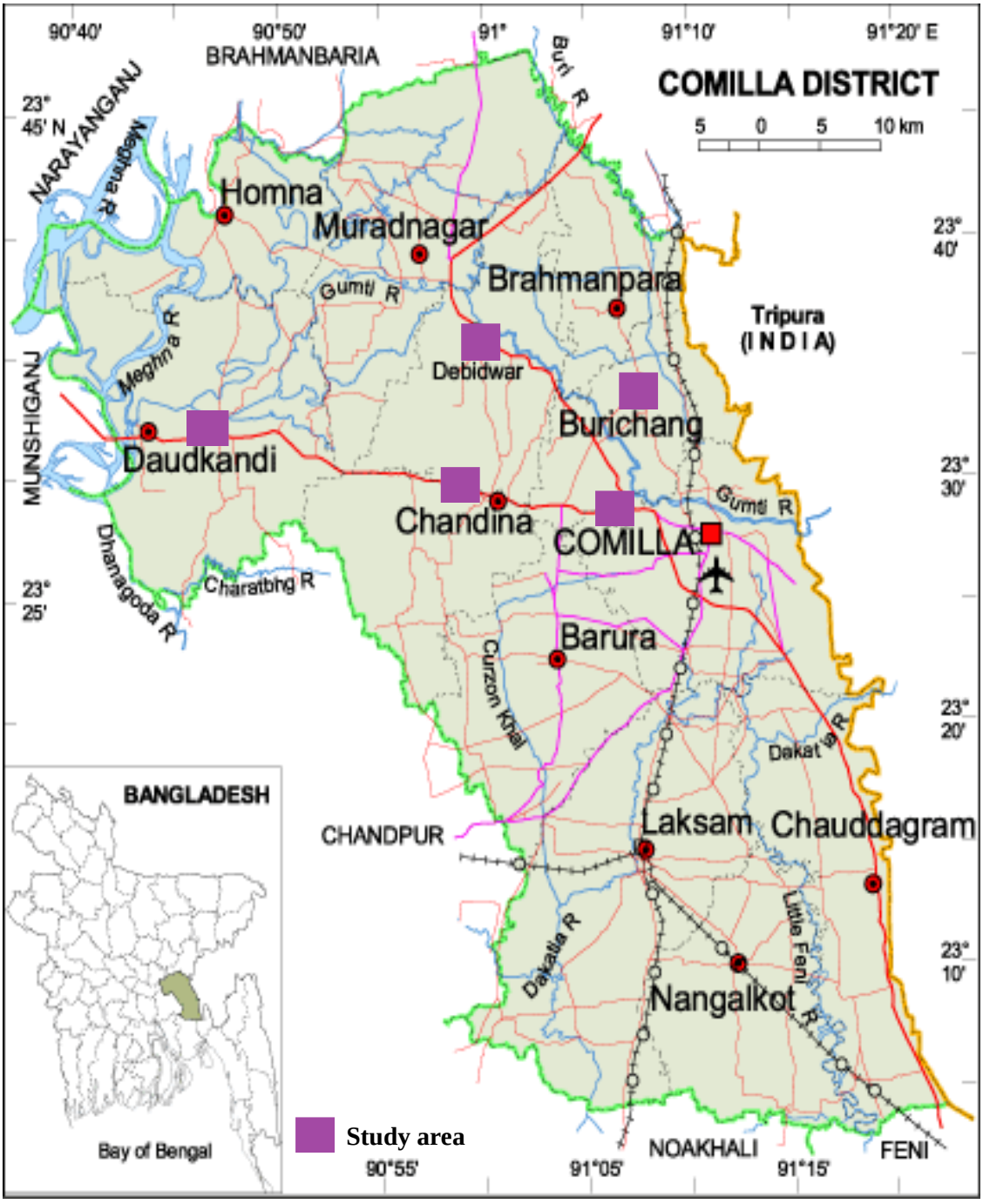


Figure 1: Map showing the places of sample collection in Cumilla district.

3.2 Sample collection

A total of 80 samples (40 country bean and 40 bitter gourd) were collected for this study. Eight samples of country bean and eight samples of bitter gourd were collected from each market (Chandina, Debidwar, Daudkandi, Kangshanagar and Maynamati) presented in Table 1 and Table 2. 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.

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

Area of collection	Sample ID	Source
Chandina	CuChBe-1 CuChBe-2 CuChBe-3 CuChBe-4 CuChBe-5 CuChBe-6 CuChBe-7 CuChBe-8	Different vendors of Chandina upazill market under Cumilla district
Debidwar	CuDeBe-1 CuDeBe-2 CuDeBe-3 CuDeBe-4 CuDeBe-5 CuDeBe-6 CuDeBe-7 CuDeBe-8	Different vendors of Debidwar upazill market under Cumilla district
Kangshanagar	CuKBe-1 CuKBe-2 CuKBe-3 CuKBe-4 CuKBe-5 CuKBe-6 CuKBe-7 CuKBe-8	Different vendors of Kangshanagar market in Burichang upazila under Cumilla district
Daudkandi	CuDaBe-1	Different vendors of

	CuDaBe-2 CuDaBe-3 CuDaBe-4 CuDaBe-5 CuDaBe-6 CuDaBe-7 CuDaBe-8	Daudkandi upazila market under Cumilla district
Maynamati	CuMBe-1 CuMBe-2 CuMBe-3 CuMBe-4 CuMBe-5 CuMBe-6 CuMBe-7 CuMBe-8	Different vendors of Maynamati market in Cumilla Sadar upazill under Cumilla district

Table 2: Sources and places of collection of bitter gourd samples

Area of collection	**Sample ID**	**Source**
Chandina	CuChBg-1 CuChBg-2 CuChBg-3 CuChBg-4 CuChBg-5 CuChBg-6 CuChBg-7 CuChBg-8	Different vendors of Chandina upazill market under Cumilla district
Debidwar	CuDeBg-1 CuDeBg-2 CuDeBg-3 CuDeBg-4 CuDeBg-5 CuDeBg-6 CuDeBg-7 CuDeBg-8	Different vendors of Debidwar upazill market under Cumilla district
Kangshanagar	CuKBg-1 CuKBg-2 CuKBg-3 CuKBg-4 CuKBg-5 CuKBg-6 CuKBg-7 CuKBg-8	Different vendors of Kangshanagar market in Burichang upazila under Cumilla district
Daudkandi	CuDaBg-1 CuDaBg-2	Different vendors of Daudkandi upazila market

	CuDaBg-3	under Cumilla district
	CuDaBg-4	
	CuDaBg-5	
	CuDaBg-6	
	CuDaBg-7	
	CuDaBg-8	
	CuMBe-1	
	CuMBe-2	
Maynamati	CuMBe-3	Different vendors of Maynamati market in Cumilla Sadar upazill under Cumilla district
	CuMBe-4	
	CuMBe-5	
	CuMBe-6	
	CuMBe-7	
	CuMBe-8	

3.3 Sample preparation for analysis

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

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

3.5.1 Some pictorial view related to sample preparation:

Plate 5a: Homogenization of collected Sample

Plate 5b: Homogenization of collected sample

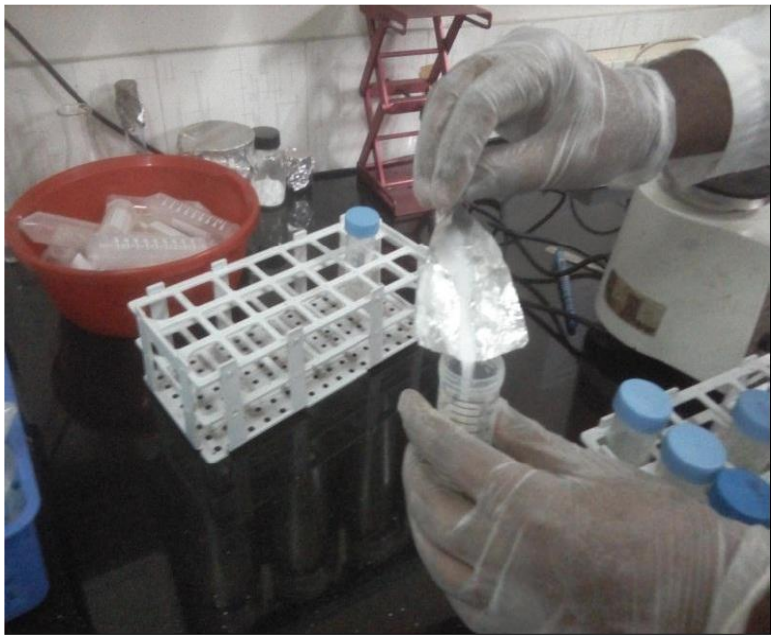


Plate 6: Adding of salt (NaCl and $MgSO_4$)

Plate 7: Shaking of sample

Plate 8: Centrifuging the sample

Plate 9 : Filtration through PTFE Filter

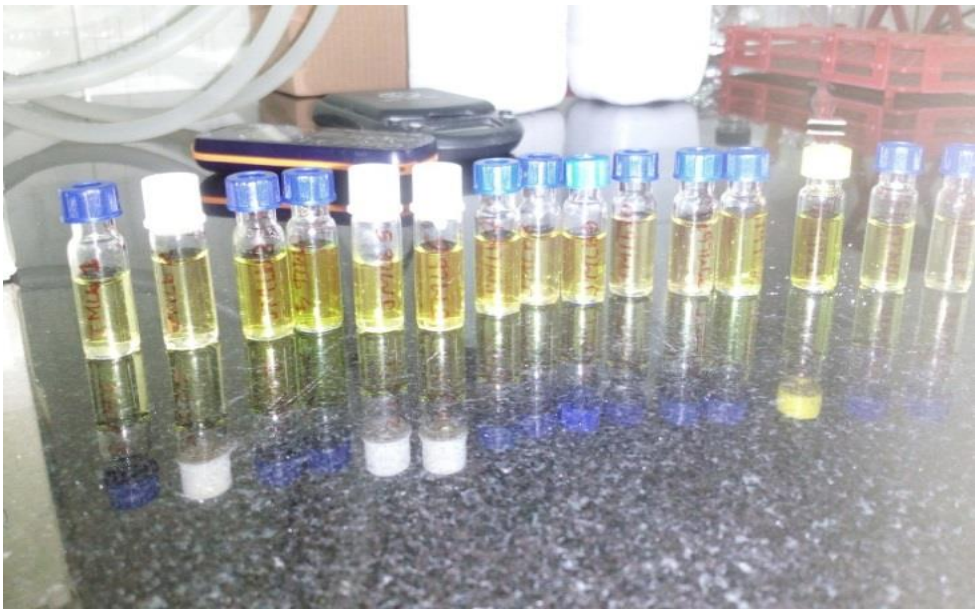


Plate 10. Final sample extract ready for injection

3.6 Preparation of pesticide standard solution

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

3.7 Extraction and clean up

QuEChERS extraction method is one of the latest extraction and clean up techniques for pesticide residue analysis in food matrices which is an anagram for Quick, Easy, Cheap, Effective, Rugged and Safe. This techniques was first introduced by Anastassiades *et al*. (2003), which is gaining popularity day by day compared to the other existing techniques such as Supercritical Fluid Extraction (SFE), Liquid-liquid extraction (LLE), Solid phase extraction (SPE), Solid phase micro extraction (SPME), Stir bar sorptive extraction (SBSE), and Microwave assisted extraction (MAE). The technique uses a single extraction in acetonitrile and requires a very small amount of (10-15 gm) sample. A large excess of salts or buffers are added to extract to aid in the extraction of both polar and non-polar pesticide. This sample initial step simultaneously extract the pesticide from the samples and prepares it for the next dispersive solid phase extraction (d-SPE), the

salts and SPE sorbents chosen for the d- SPE step serve to remove residual water and further remove matrix interference from the sample. The resulting acetonitrile extract is typically analyzed directly by gas chromatography (GC), gas chromatography-mass spectrometry (GC/MS) or liquid chromatography tandem mass spectrometry (LC/MS/MS) with proper dilution.

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

3.8 Detection and quantification of pesticide residue in samples

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

of suspected pesticide was performed by peak retention times in samples to those of peaks in the pure analytical standards. A typical chromatogram containing seven selected organophosphorus insecticides is presented in Figure 2. The instrument conditions are described in Table 3 and Table 4.

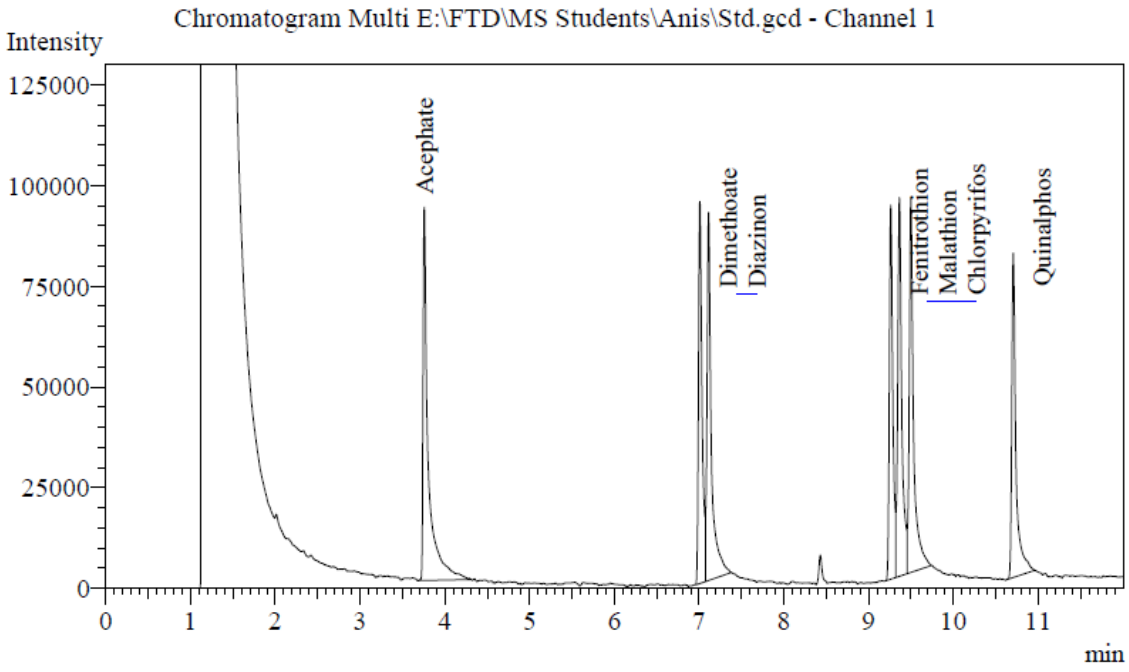


Figure 2: Typical chromatogram of seven organophosphorus insecticide standard run by GC-FTD.

Table 3: The instrument parameters for GC-FTD

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

Table 4: Conditions for column oven temperature for FTD

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

3.9 Preparation of calibration curve

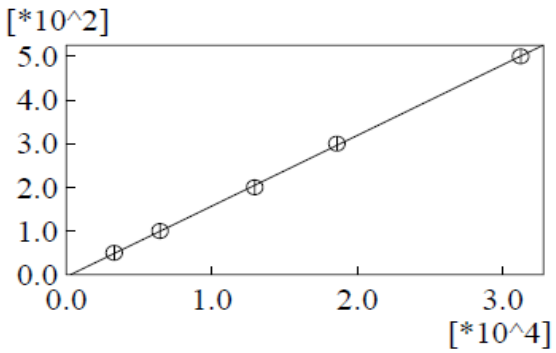
Prior to the injection of the sample extract, standard solutions of different concentrations of each pesticide group were prepared and injected with suitable instrument parameters. The samples were calibrated (retention time, peak area etc.) against five pointed calibration curve of standard solution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

Calibration Curve - Analytical Line 1 - Channel 1

ID#:1 Name:Acephate

f(x)=1.61756310498e-002*x-4.67442357312
R=0.999987242049 R^2=0.999892507739
MeanRF:1.56431705673e-002 RFSD:4.11691966763e-004 RFRSD:2.63176806129
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



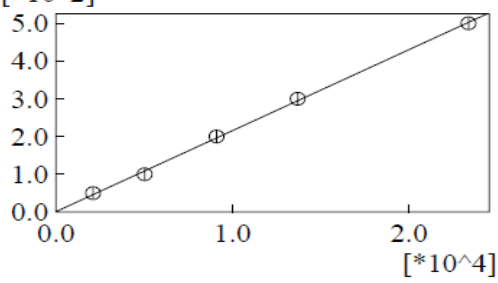
No.	Conc.	Area
1	50.000	3211
2	100.000	6906
3	200.000	13578
4	300.000	19584
5	500.000	32548

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)=2.14476109468e-002*x+1.27409837292
R=0.999894212457 R^2=0.999926113376
MeanRF:2.17625864931e-002 RFSD:1.36709257517e-003 RFRSD:6.28184786586
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



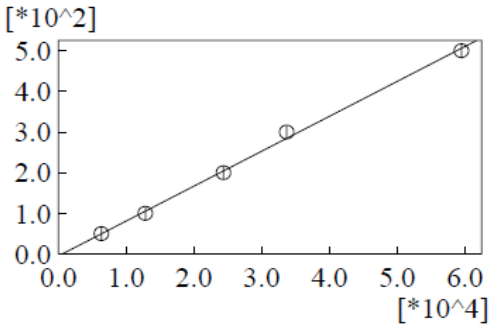
No.	Conc.	Area
1	50.000	2874
2	100.000	5247
3	200.000	9487
4	300.000	14587
5	500.000	22985

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)=8.58182478378e-003*x-4.76864750449
R=0.9989993676 R^2=0.997890349706
MeanRF:8.23177212761e-003 RFSD:4.50915039717e-004 RFRSD:5.47773957694
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



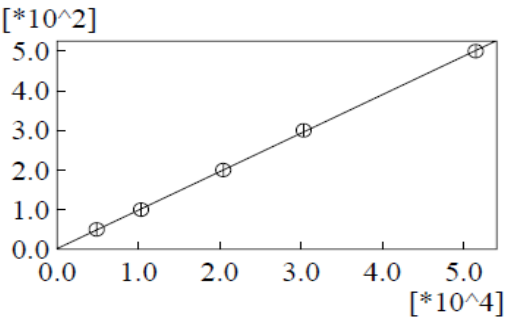
No.	Conc.	Area
1	50.000	6874
2	100.000	13214
3	200.000	23254
4	300.000	32354
5	500.000	58741

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

External Standard



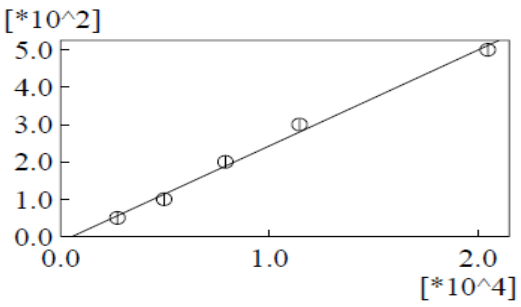
No.	Conc.	Area
1	50.000	4982
2	100.000	11425
3	200.000	21245
4	300.000	32301
5	500.000	50412

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

ID#:5 Name:Malathion

f(x)=2.5662119724e-002*x-13.8726059301
R=0.996793718496 R^2=0.993979245023
MeanRF:2.28770289157e-002 RFSD:3.45826768136e-003 RFRSD:15.116769289
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



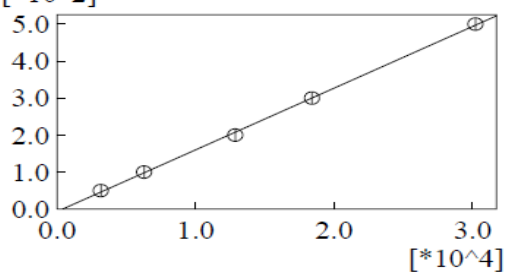
No.	Conc.	Area
1	50.000	2985
2	100.000	4475
3	200.000	7689
4	300.000	12543
5	500.000	21201

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)=1.66718969691e-002*x-6.50897807754
R=0.999687866504 R^2=0.999299859839
MeanRF:1.60231310913e-002 RFSD:4.02450756721e-004 RFRSD:2.51168610197
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



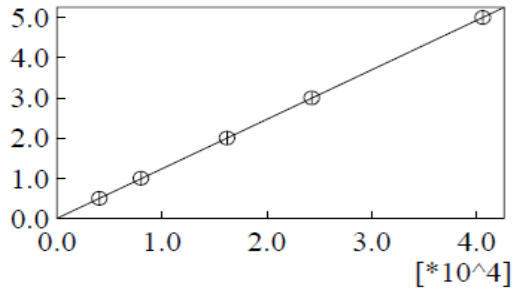
No.	Conc.	Area
1	50.000	3321
2	100.000	6684
3	200.000	13210
4	300.000	18987
5	500.000	31024

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)=1.2305211624e-002*x+0.670992116044
R=0.999994954248 R^2=0.999989878522
MeanRF:1.23562145806e-002 RFSD:7.05710649982e-005 RFRSD:0.571138227959
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



No.	Conc.	Area
1	50.000	4248
2	100.000	8325
3	200.000	16895
4	300.000	23985
5	500.000	41002

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 (country bean and yard long bean) were collected from 5 different markets of Cumilla district (Chandina, Debidwar, Kangshanagar, Daudkandi and Maynamati) to detect and quantify pesticide residues. The results obtained from this study are presented and described in this chapter using figures and tables.

4.1 Pesticide residues in country bean

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

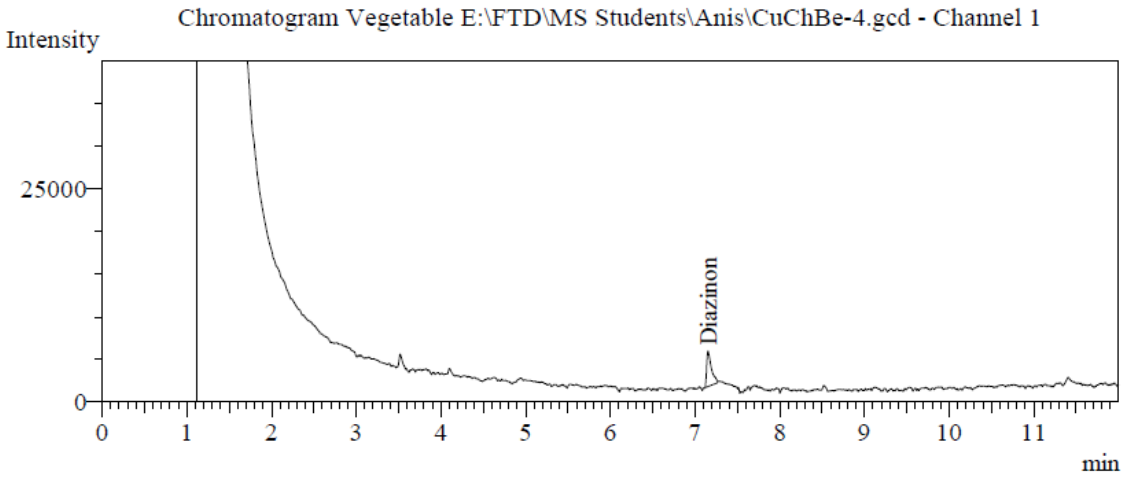


Figure 10: Chromatogram of diazinon found in one of the country bean sample (CuChBe-4) collected from Chandina

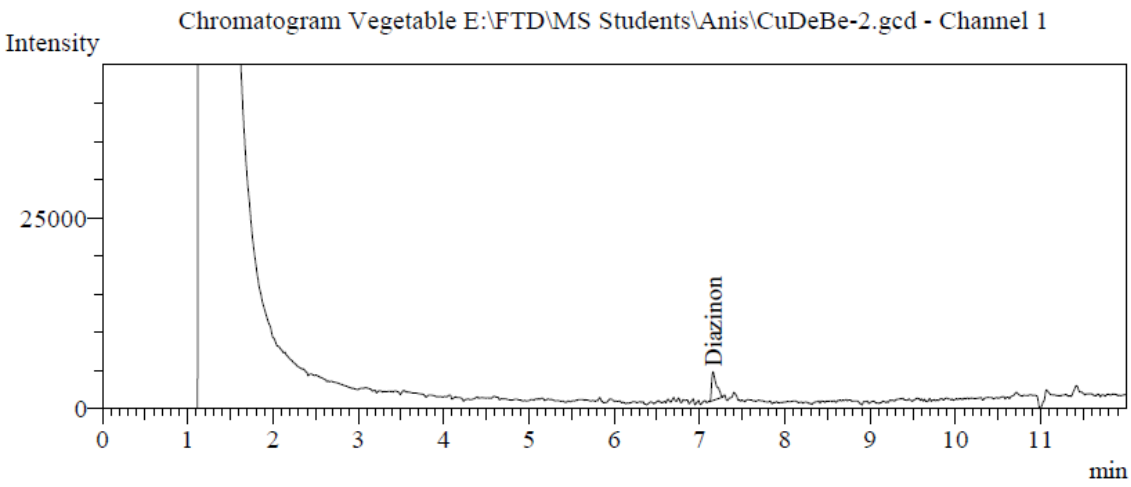


Figure 11: Chromatogram of diazinon found in one of the country bean sample (CuDeBe-2) collected from Debidwar

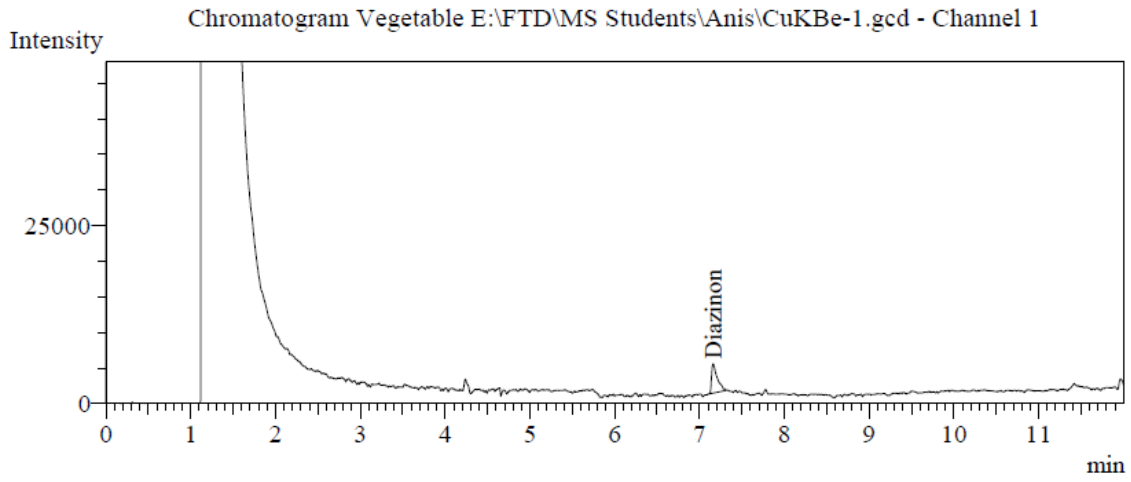


Figure 12: Chromatogram of diazinon found in one of the country bean sample (CuKBe-1) collected from Kangshanagar

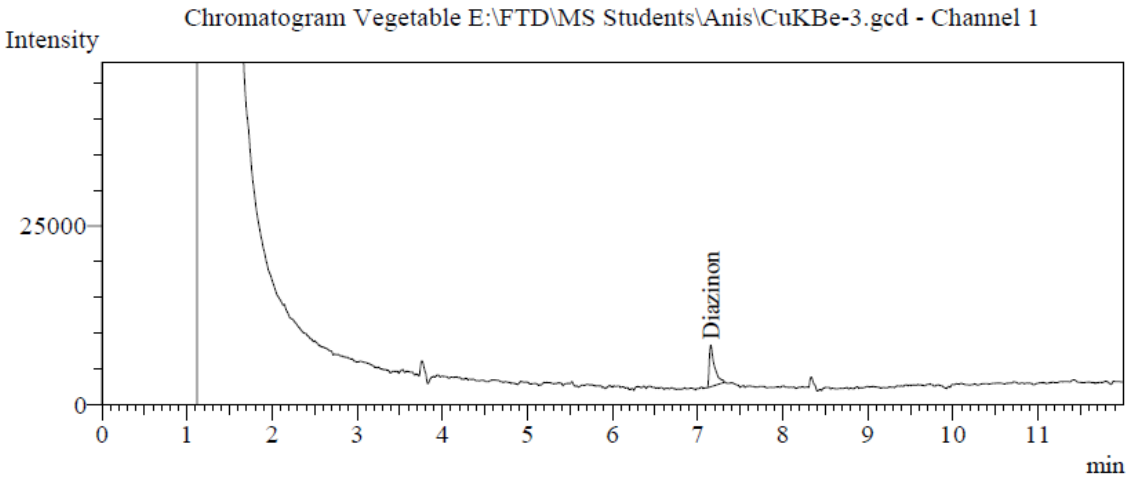


Figure 13: Chromatogram of diazinon found in one of the country bean sample (CuKBe-3) collected from Kangshanagar

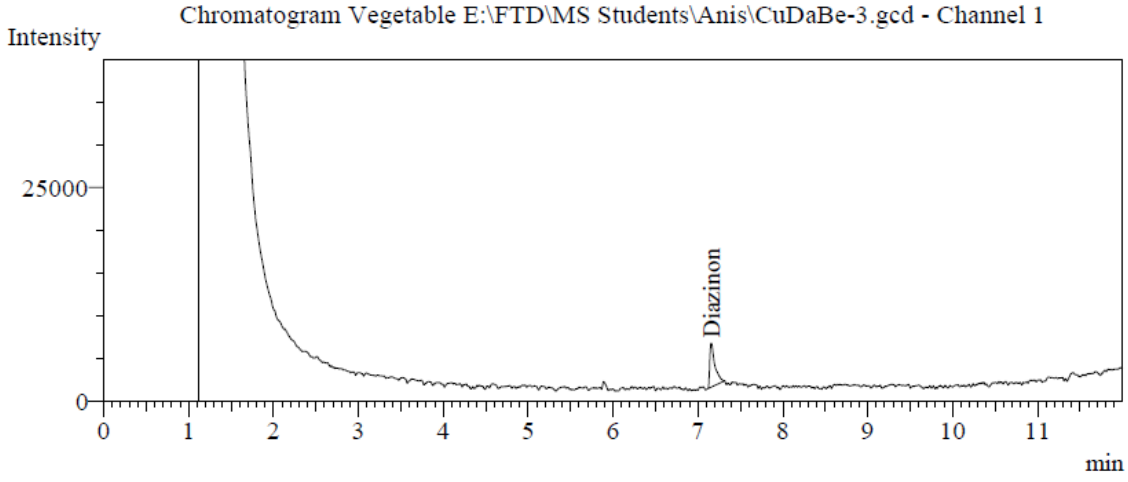


Figure 14: Chromatogram of diazinon found in one of the country bean sample (CuDaBe-3). collected from Daudkandi

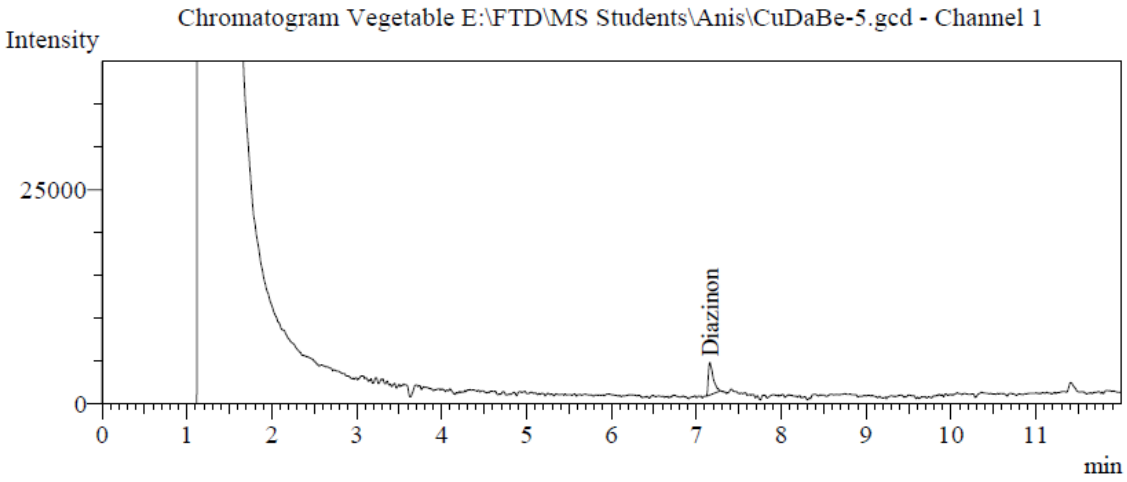


Figure 15: Chromatogram of diazinon found in one of the country bean sample (CuDaBe-5). collected from Daudkandi

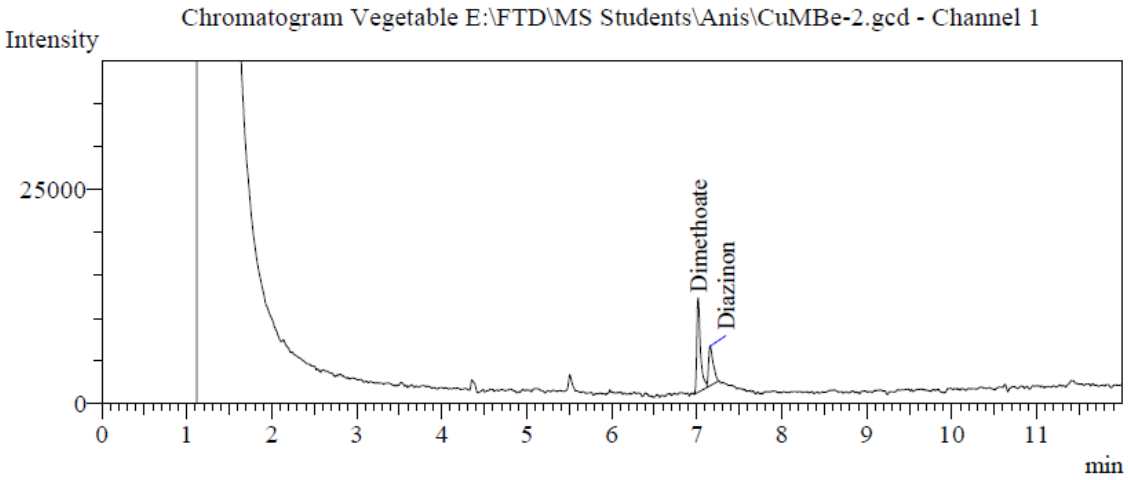


Figure 16: Chromatogram of dimethoate and diazinon found in one of the country bean sample (CuMBe-2) collected from Maynamati

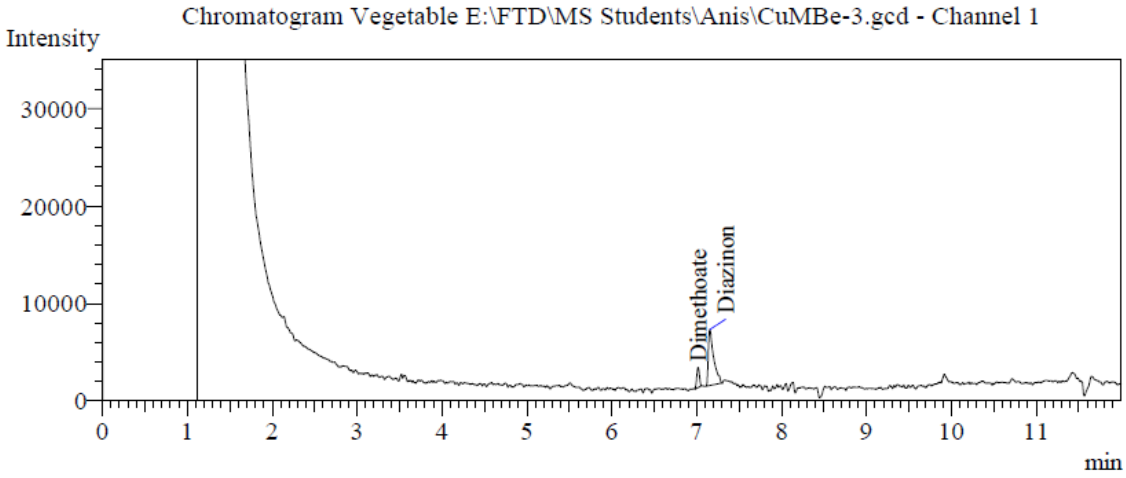


Figure 17: Chromatogram of dimethoate and diazinon found in one of the country bean sample (CuMBe-3) collected from Maynamati

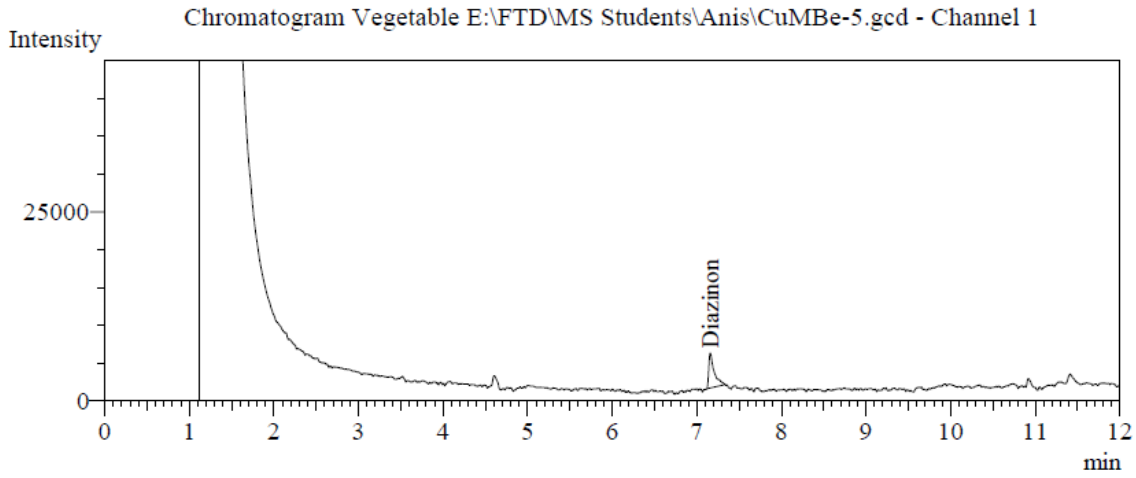


Figure 18: Chromatogram of diazinon found in one of the country bean sample (CuMBe-5) collected from Maynamati

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

Table 5. The level of residues (mg/Kg) of different pesticides found in the analyzed country bean samples.

Area of collection	Sample ID	Name of detected pesticide	Level of residue (mg/Kg)	MRLs (mg/Kg)
Chandina	CuChBe-1	ND	-	-
	CuChBe-2	ND	-	-
	CuChBe-3	ND	-	-
	CuChBe-4	Diazinon	0.089	0.04*
	CuChBe-5	ND	-	-
	CuChBe-6	ND	-	-
	CuChBe-7	ND	-	-
	CuChBe-8	ND	-	-
Debidwar	CuDeBe-1	ND	-	-
	CuDeBe-2	Diazinon	0.079	0.04*
	CuDeBe-3	ND	-	-
	CuDeBe-4	ND	-	-
	CuDeBe-5	ND	-	-
	CuDeBe-6	ND	-	-
	CuDeBe-7	ND	-	-
	CuDeBe-8	ND	-	-
Kangshanagar	CuKBe-1	Diazinon	0.105	0.04*
	CuKBe-2	ND	-	-
	CuKBe-3	Diazinon	0.138	0.04*
	CuKBe-4	ND	-	-
	CuKBe-5	ND	-	-
	CuKBe-6	ND	-	-
	CuKBe-7	ND	-	-
	CuKBe-8	ND	-	-
Daudkandi	CuDaBe-1	ND	-	-
	CuDaBe-2	ND	-	-

	CuDaBe-3	Diazinon	0.119	0.04*
	CuDaBe-4	ND	-	-
	CuDaBe-5	Diazinon	0.082	0.04*
	CuDaBe-6	ND	-	-
	CuDaBe-7	ND	-	-
	CuDaBe-8	ND	-	-
Maynamati	CuMBe-1	ND	-	-
	CuMBe-2	Dimethoate	0.247	0.02*
		Diazinon	0.096	0.04*
	CuMBe-3	Dimethoate	0.034	0.02*
		Diazinon	0.138	0.04*
	CuMBe-4	ND	-	-
	CuMBe-5	Diazinon	0.114	0.04*
	CuMBe-6	ND	-	-
	CuMBe-7	ND	-	-
	CuMBe-8	ND	-	-

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

Forty (40) samples of country bean collected from 5 different markets of Cumilla district (Chandina, Debidwar, Kangshanagar, Daudkandi and Maynamati) 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 country bean, nine (9) samples (22.50% of the total number of samples) contained pesticide residues and thirty one (31) samples (77.50% 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. Ahmed *et al.* (2016) analyzed 170 samples collected from Jessore, Cumilla, Narsingdi, Tangail, Rangpur, Jamalpur, Gazipur and Dhaka. Residues of fenvalerate, diazinon, quinalphos, fenitrothion, acephate, chlorpyrifos, cypermethrin and malathion were

found 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 residue, in which 18.26% samples had residues above MRL. The findings of the present study can also be compared with Akter *et al.* (2017). They have been monitored pesticide residues in eggplant collected from Mymensingh district and found that among the 50 analyzed samples, 11 (22% of the total number of the samples) contained pesticide residues of diazinon, dimethoate, quinalfos, and chlorpyrifos, of which, 2 had multiple pesticide residues and 5 samples contained residue above the European Union maximum residue limit (EU-MRLs). Chlorpyrifos was detected as the most used pesticide in eggplant in the studied area. The results of this study are in a good agreement with Hasan *et al*. (2017). They have been detected two types of insecticides (dimethoate and quinalphos) in country bean samples collected from different market places of dhaka. Among the 50 analyzed samples of country bean, 10 samples (20%) contained residues of dimethoate and quinalphos, of which 5 samples were above the maximum residue limits (MRLs). Most of the contaminated samples (8 samples) contained residue of dimethoate.

Eight (8) country bean samples were collected from central market of Chandina upazila under Cumilla district, among them, one samples (CuChBe-4) contained diazinon at a level of 0.089 mg/Kg, which was above the EU-MRL (0.04 mg/Kg) (European Commission 2015). The other seven (7) samples contain no detectable pesticide residues.

From central market of Debidwar upazila under Cumilla district, eight (8) country bean samples were collected. Among them, one sample (CuDeBe-2) contained residues of diazinon (0.079 mg/Kg). But other seven (7) samples contained no detectable pesticide residues. The level of detected residue of diazinon was above MRL (0.04 mg/Kg).

From Kangshanagar under Burichang upazila, eight (8) country bean samples were collected.Among them two samples (CuKBe-1 and CuKBe-3) were contained residues of diazinon (0.105 mg/Kg and 0.138 mg/Kg, respectively). But other six (6) samples contained no detectable pesticide residues. The level of detected residue of diazinon was above MRL (0.04 mg/Kg).

Among eight (8) country bean samples collected from central market of Daudkandi upazila under Cumilla district, two samples (CuDaBe-3 and CuDaBe-5) were contained residue of diazinon (0.119 mg/Kg and 0.082 mg/Kg, respectively). Other six (6) samples contained no detectable pesticide residues. The level of detected residue of diazinon was above MRL (0.04 mg/Kg).

From Maynamati under Cumilla Sadar upazila, eight (8) samples were collected. Out of eight (8) samples, three samples (CuMBe-2, CuMBe-3 and CuMBe-4) contained residues. The sample (CuMBe-2) contained multiple residues of dimethoate (0.247 mg/Kg) and diazinon (0.096 mg/Kg). Another sample (CuMBe-3) also contained multiple residues of dimethoate (0.034 mg/Kg) and diazinon (0.138 mg/Kg). The other sample (CuMBe-4) contained residue of diazinon (0.114 mg/Kg). But other five (5) samples contained no detectable pesticide residues. The level of detected residue of dimethoate for the sample of CuMBe-2 and CuMBe-3 was above MRL (0.02 mg/Kg) and the level of detected residue of diazinon for the samples of CuMBe-2, CuMBe-3 and CuMBe-4 was above MRL (0.04 mg/Kg).

4.2 Pesticide residues in bitter gourd

The concentrated extracts of bitter gourd samples collected from different markets of Cumilla district were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. Figure 18-21 shows the chromatograms of the injected extracts of bitter gourd sample containing detected pesticides.

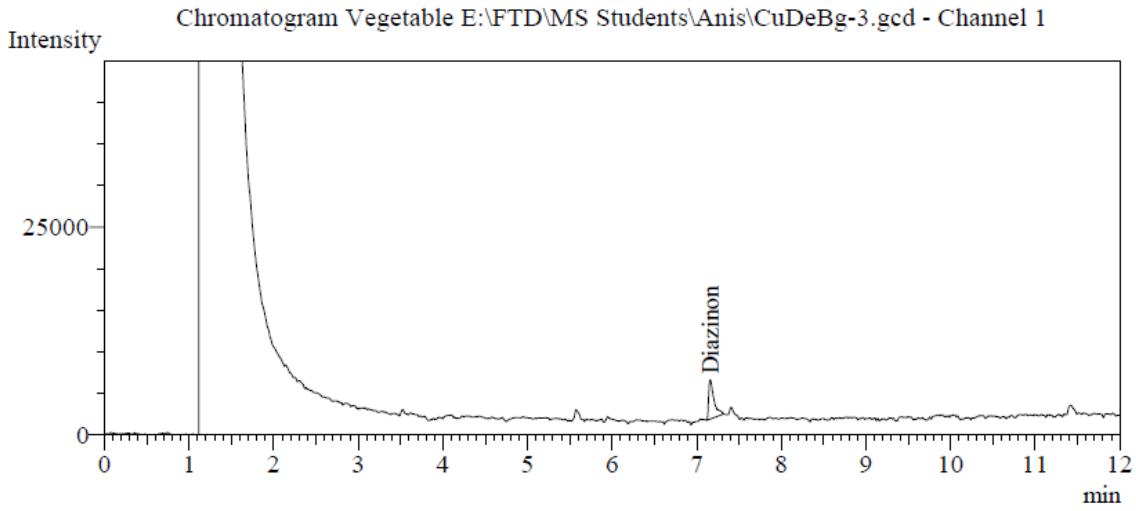


Figure 18: Chromatogram of diazinon found in one of the bitter gourd sample (CuDeBg-3) collected from Debidwar

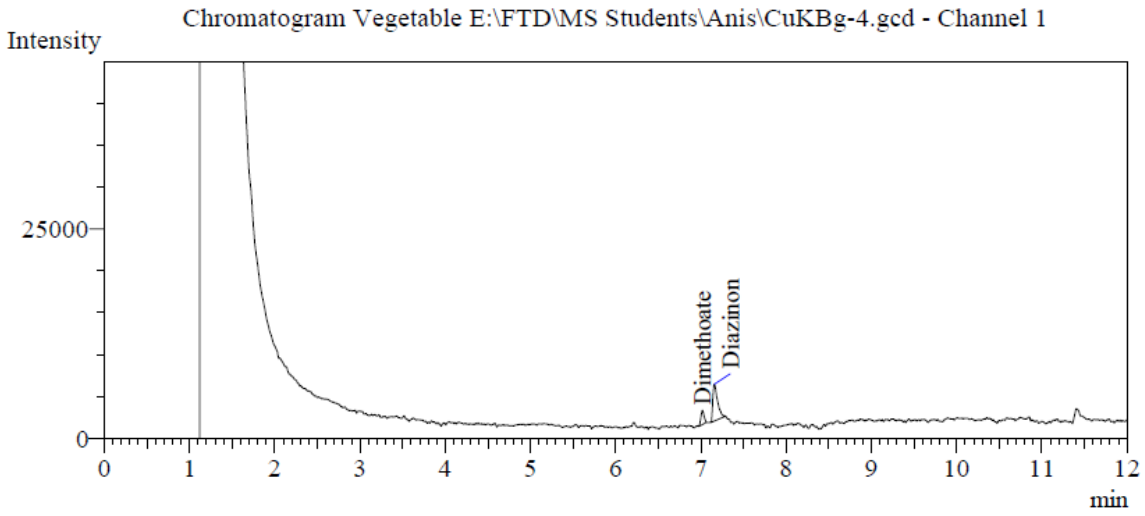


Figure 19: Chromatogram of dimethoate and diazinon found in one of the bitter gourd sample (CuKBg-4). collected from Kangshanagar

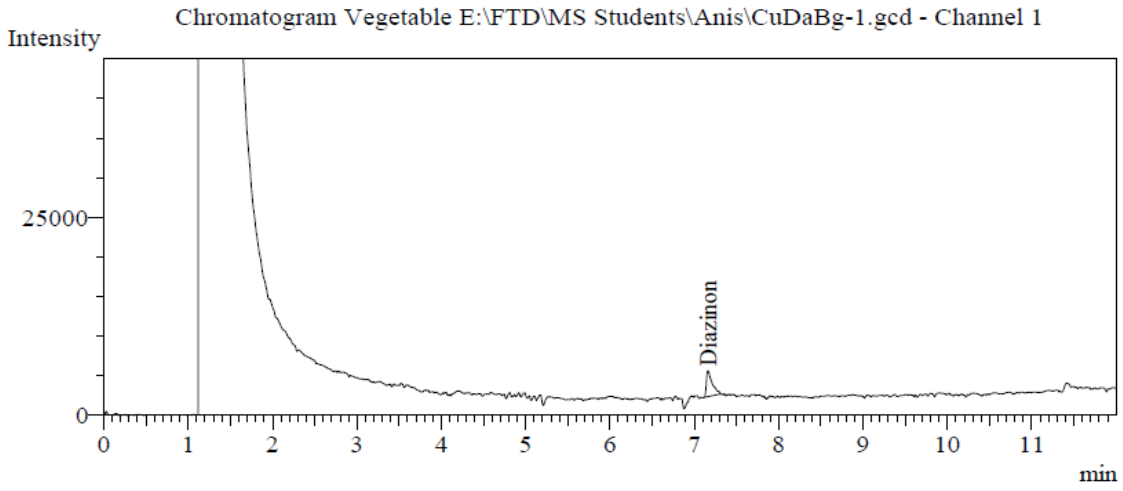


Figure 20: Chromatogram of diazinon found in one of the bitter gourd sample (CuDaBg-1) collected from Daudkandi

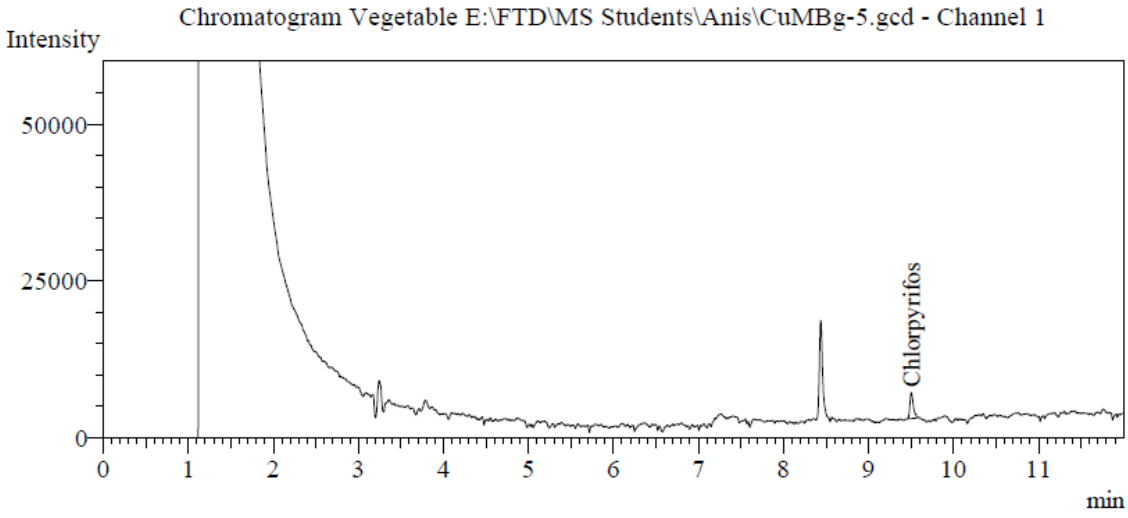


Figure 21: Chromatogram of chlorpyrifos found in one of the bitter gourd sample (CuMBe-5) collected from Maynamati

The level of pesticide residues found in the analyzed bitter gourd samples and their maximum residue levels are outlined in Table 6.

Table 6: The level of residues (mg/Kg) of different pesticides found in the analyzed bitter gourd samples

Area of collection	Sample ID	Name of detected pesticide	Level of residue (mg/Kg)	MRLs (mg/Kg)
Chandina	CuChBg-1	ND	-	-
	CuChBg-2	ND	-	-
	CuChBg-3	ND	-	-
	CuChBg-4	ND	-	-
	CuChBg-5	ND	-	-
	CuChBg-6	ND	-	-
	CuChBg-7	ND	-	-
	CuChBg-8	ND	-	-
Debidwar	CuDeBg-1	ND	-	-
	CuDeBg-2	ND	-	-
	CuDeBg-3	Diazinon	0.113	0.04*
	CuDeBg-4	ND	-	-
	CuDeBg-5	ND	-	-
	CuDeBg-6	ND	-	-
	CuDeBg-7	ND	-	-
	CuDeBg-8	ND	-	-
Kangshanagar	CuKBg-1	ND	-	-
	CuKBg-2	ND	-	-
	CuKBg-3	ND	-	-
	CuKBg-4	Dimethoate	0.032	0.02*
		Diazinon	0.093	0.04*
	CuKBg-5	ND	-	-
	CuKBg-6	ND	-	-
	CuKBg-7	ND	-	-
	CuKBg-8	ND	-	-
Daudkandi	CuDaBg-1	Diazinon	0.086	0.04*
	CuDaBg-2	ND	-	-
	CuDaBg-3	ND	-	-
	CuDaBg-4	ND	-	-
	CuDaBg-5	ND	-	-
	CuDaBg-6	ND	-	-

	CuDaBg-7	ND	-	-
	CuDaBg-8	ND	-	-
Maynamati	CuMBe-1	ND	-	-
	CuMBe-2	ND	-	-
	CuMBe-3	ND	-	-
	CuMBe-4	ND	-	-
	CuMBe-5	Chlorpyrifos	0.056	0.04*
	CuMBe-6	ND	-	-
	CuMBe-7	ND	-	-
	CuMBe-8	ND	-	-

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

Forty (40) samples of bitter gourd collected from five (5) different markets of Cumilla district (Chandina, Debidwar, Kangshanagar, Daudkandi and Maynamati) were analyzed to find out the presence of left over residues of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos). Out of forty (40) samples, 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.

From Debidwar upazila central market under Cumilla district, eight (8) bitter gourd samples were collected. Among them, one sample (CuDeBg-3) contained residue of diazinon (0.113 mg/Kg). The level of detected residue of diazinon was above MRL (0.04 mg/Kg). But other seven (7) samples contained no detectable pesticide residues.

From Kangshanagar under Burichang upazila, eight (8) bitter gourd samples were collected. Of which one sample (CuKBg-4) contained multiple residues of dimethoate (0.032 mg/Kg) and diazinon (0.093 mg/Kg). The level of detected residue of dimethoate was above MRL (0.02 mg/Kg). The level of detected residue of diazinon was also above MRL (0.04 mg/Kg). Other seven (7) samples contained no detectable pesticide residues.

Among eight (8) bitter gourd samples collected from central market of Daudkandi upazila under Cumilla district, one sample (CuDaBg-1) contained residue of diazinon (0. 0.086 mg/Kg). The level of detected residue of diazinon was above MRL (0.04 mg/Kg).Other seven (7) samples contained no detectable pesticide residues.

From Maynamati under Cumilla Sadar upazila, eight (8) samples of bitter gourd were collected. Out of eight (8) samples, one (1) sample (CuMBe-5) contained residue of chlorpyrifos (0.056 mg/Kg). The level of detected residue of chlorpyrifos for the sample of CuMBe-5 was above MRL (0.02 mg/Kg). Another seven (7) samples contained no detectable pesticide residues.

CHAPTER V

SUMMARY AND CONCLUSION

The purpose of this study was intended to identify and quantify the pesticide residue level present in the vegetables collected from various local markets of Cumilla district. Regarding this, forty (40) samples of country bean and forty (40) samples of bitter gourd were collected from five different locations (Chandina, Debidwar, Kangshanagar, Daudkandi and Maynamati) of Cumilla district and carried to the Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The QuEChERS extraction technique was applied for the extraction and cleanup of the collected sample. Gas chromatography associated with flame thermionic detector (FTD) was used to identify and quantify the level of pesticide residues present in the extracted samples. Seven most commonly used pesticides i.e. diazinon, acephate, chlorpyrifos, malathion, fenitrothion, dimethoate and quinalphos were selected for this study.

Among the forty (40) analyzed samples of country bean, 9 samples (22.50% of the total number of samples) contained residues of diazinon and dimethoate and 32 samples (77.50% of the total number of samples) contained no detectable residues of the target pesticides. Among these 9 contaminated samples, 7 contained single residue which were above the maximum residue limits (MRLs) and 2 contained multiple residues which were also above the maximum residue limits (MRLs).

Regarding 40 samples of bitter gourd, 4 samples (10% of the total number of samples) contained residues of dimethoate, diazinon and chlorpyrifos, and 36 samples (90% of the total number of samples) contained no detectable residues of the sought pesticides. Among these 4 contaminated samples, 3 had single residue

and 1 had multiple residues. All of them were above the maximum residue limits (MRLs).

It is a serious matter of concern that pesticide residues remain 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 Cumilla district. The results of the present study indicate that the farmers of Cumilla district are using diazinon, dimethoate and chlorpyrifos indiscriminately. This study will help to increase public awareness as well.

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