Monitoring of Pesticide Residues in Major Vegetables Collected from Different Markets of Gazipur and Narsingdi

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DHAKA-1207

DECEMBER, 2016

Monitoring of Pesticide Residues in Major Vegetables Collected from Different Markets of Gazipur and Narsingdi

BY

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Registration No. : 15-06944

A Thesis
Submitted to the Department of Entomology
Sher-e-Bangla Agricultural University, Dhaka,
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
IN
ENTOMOLOGY
SEMESTER: JULY-DECEMBER, 2016

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ACKNOWLEDGEMENT

All praises to the **"Almighty Allah"** who enables me to complete a piece of research work and prepare this thesis for the degree of Master of Science (M.S.) in Entomology.

She feels much pleasure to express his gratefulness, sincere appreciation and heartfelt liability to her venerable research supervisor **Dr. S. M. Mizanur Rahman**, Department of Entomology, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207 for his scholastic guidance, support, uninterrupted encouragement, valuable suggestions and constructive criticism throughout the study period.

She also expresses her gratitude and thankfulness to reverend co-supervisor **Dr. Mohammad Dalower Hossain Prodhan,** Senior Scientific Officer (SSO), Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur and Chairman, **Mst. Nur Mohal Akhter Banu**, Department of Entomology, SAU, Dhaka-1207 for their constant inspiration, valuable suggestions, cordial help, heartiest co-operation and supports throughout the study period.

The author would like to express her grateful thanks to all teachers of the Department of Entomology,SAU and all members of Bangladesh Agricultural Research Institute (BARI), Gazipur, for their constructive suggestions and advice during the study period.

The author deeply acknowledges the profound dedication to her beloved father, mother, husband and in-laws for their moral support, steadfast encouragement and continuous prayer in all phases of academic pursuit from the beginning to the completion of study successfully.

Finally, the author is deeply indebted to her friends and well-wishers for their kind help, constant inspiration, co-operation and moral support which can never be forgotten.

December, 2016 **The Author**
Dhaka, Bangladesh

Monitoring of Pesticide Residues in Major Vegetables Collected from Different Markets of Gazipur and Narsingdi

Abstract

The study was conducted to analyze pesticide residues in three common vegetables (country bean, cauliflower and yard long bean) from 5 different markets of Gazipur and Narsingdi. The collected samples were analyzed using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and Gas Chromatography (GC) coupled with Flame Thermionized Detector (FTD) for the detection and determination of pesticide residue in country bean, cualiflower and yard long bean of 30 samples in each case. Out of 15 samples of country bean, 2 samples contained pesticide residues with above MRL in Gazipur, whereas it was 3 samples above MRL in Narsingdi and no detectable residues of the sought pesticides were identified from remaining samples. In case of cauliflower, among the same number of analyzed samples, 2 samples from each location contained pesticide residues with above MRL and rest of the samples had no detectable pesticide residues. From 15 yard long bean samples from each surveyed area, pesticide residues with above MRL was identified and quantified in 3 samples from each region. No pesticide residue was found from remnant samples. This study reflects the overall scenario of pesticide contamination in vegetables specially in country bean, cauliflower and yard long bean available in the local markets of Gazipur and Narsingdi city, which will help the consumer to be aware of their health and safety.

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CERTIFICATE

This is to certify that the thesis entitled,"**Monitoring of Pesticide Residues in Major Vegetables Collected from Different Markets of Gazipur and Narsingdi**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in ENTOMOLOGY (M.S.),** embodies the results of a piece of bona- -fide research work carried out by **NUZAT TASNIM** ,Registration No. **15-06944** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged**.**

Dated: December-2016
Dhaka, Bangladesh

Prof. Dr. S. M. Mizanur Rahman
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LIST OF ABBREVIATIONS

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

CHAPTER I

INTRODUCTION

Vegetables have been part of the human diet from time immemorial. Some are staple foods but most are accessory foodstuffs, adding variety to meals with their unique flavors and at the same time, adding nutrients necessary for health. Vegetables are considered essential for well-balanced diets since they supply vitamins, minerals, dietary fiber, and phytochemicals. They make up a major portion of the diet of humans in many parts of the world and play a significant role in human nutrition, especially as sources of phytonutrients: vitamins (A, B1, B6, B9, C, E), minerals, dietary fiber and phytochemicals (Dias *et al*., 2011). Not only vegetables are valuable in maintaining alkaline reserve of the body but also improve gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some forms of cancer. Having so much importance a world vegetable survey showed that 402 vegetable crops are cultivated worldwide, representing 69 families and 230 genera (Kays *et al*., 2011).

As responsive to world in Bangladesh, as in most developing countries, agriculture plays a key role in the overall economic performance of the country, not only in terms of its contribution to gross domestic product (GDP, 20.01%), but also as a major source of foreign exchange earnings, and in providing employment (47.3%) to a large segment of the population, particularly the poor In Bangladesh around 1.8% lands are using for vegetable cultivation and about 528 thousand hector land are used for Rabi seasonal vegetable cultivation. According to FAO (2012), vegetable cultivation has increased five times in past 40 years and currently about 16200 farmers are involved in vegetables production in our country.

In Bangladesh, there are two seasons of vegetable cultivation – Rabi (winter season) and Kharif (summer/rainy). Of the summer vegetables, various cucurbits, vegetable cowpea, hyacinth bean, stem amaranth, several aroids and Indian spinach are predominant. Winter vegetables include tomato, cabbage, Chinese cabbage, cauliflower, eggplant, carrot, spinach, bottle gourd, bush bean and radish. Crops like okra, heat-tolerant tomato, eggplant, carrot, spinach, many leafy vegetables and small onion are grown all year round.

A disturbing thing is that 40 per cent of the total amount of vegetable, according to a survey report, is wasted from production to the consumers' level. The main barriares, farmers face for vegetable cultivation are availability of seed, pest management, diseases management, field management, marketing and extension media contact, storage and transport facilities. Among these, insect and pest infestation is a major concern now-a-days.

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

Owing to the massive damage caused by pests to agricultural fields and crops, production often declines below the level of subsistence for farmers, which can eventually have adverse effects on the national economy. In the process of checking and killing pests over the years, pesticide application in Bangladesh has increased manifold from 758 metric tons in 1960 and 3028 metric tons in 1980 to over 19000 metric tons in 2013 and in 2014, the amount of pesticide applied in fields across the country rose to 48690 metric tons (BBS, 2014). Insecticides, being the dominant item, account for 76% of the pesticides applied in year. Most farmers particularly in the developing countries like Bangladesh apply pesticide without knowing its actual requirements and/or effectiveness, and thus there are very high frequencies of pesticides application.

Study shows that farmers spray pesticides 140 times during a cropping season of 180-200 days. 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, 1999). 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. Due to lack of education, the farmers of our country do not follow the prescribed dosages and use pesticides at any stage of the crop without any awareness of the residues and their ill effects on human health and environment.

Pesticide poisoning is a major global health problem, and it is more prevalent in countries like Bangladesh. Intentional and unintentional pesticide poisoning has been acknowledged as a serious problem in many agricultural communities of low and middle-income countries (WHO, 2004). The harmful effects on human beings in the form of acute and chronic toxicity exposed to insecticides are well established. World Health Organization (WHO, 2002) estimated about 849,000 people death globally from acute toxicity of the pesticide in 2002. The incidence of pesticides poisoning is increasing and it is estimated that about 5 million people die every year as a result of intentional, accidental and occupational exposure worldwide (Singh and Gupta, 2009). The World Health Organization and the United Nations Environment Program estimate pesticides poisoning at a rate of 2-3 per minute, with approximately 20,000 workers dying from exposure every year, the majority in developing countries (WHO, 1990; Kishi *et al.*, 1995). The indiscriminate use of agricultural pesticides has created very serious health and environmental problems in many developing countries. Worldwide, one to five million farm workers are estimated to suffer pesticide poisoning every year. Most of the poisonings take place in rural areas, where safeguards typically are inadequate or lacking altogether. Although developing countries use 25% of the world's production of pesticides, they experience 99% of the deaths due to pesticide poisoning (WHO, 2004).

Among major vegetables country bean, yard long bean, cauliflower are the popular Rabi seasonal crops. In spite of being a prospective crop, high incidences of insect pests have limited the crop to its low yield and poor quality. Farmers in our country face significant yield loss of beans every year due to severe attack of various insect pests. Country bean is a rich source of essential vitamins and commonly grown during rainy through Rabi seasons According to Alam *et al.*, (1969), country bean is attacked by nine different insect species and one species of mite. Among these species, four species are considered as major pests and the rest of them as minor pest. But normally the leguminous vegetables are attacked by a number of insect pests. An FAO panel

meeting held in Bangkok in 1975 identified the bean pod borer (*Maruca testulalis* G.) as a legume pod borer (Reddy 1975). Dina *et al.* (1979) and Bakar *et al*. (1980) found *M. testulalis* G. is a serious insect pest of leguminous vegetables. In another work they found aphid as the most common pests all over the world and lepidopterous larvae *M. testulalis* (Geyer) as pests of bean causing damage by boring tender of mature pods.

Yard-long bean is rich in protein, vitamins A and C, and calcium; vitamin B and dry matter of green seeds contains 24.6% protein (Aykroiyd *et al.*, 1974) with high amount of lysine (Ferry *et al*., 1981). One of the major constraints for yard-long bean production in our country is the attack of insect pests among the insect pests, pod borer *Euchrysops cnejus* F. (Lepidoptera: Lycaenidae) is considered the major pests of yard-long been in Bangladesh (Das, 1998; Ali, 2006). In order to protect their crops from damages of pod borer, our farmers often apply traditional insecticides without having sufficient field control of this pest.

Cauliflower is an excellent source of vitamin C, vitamin K, folate, pantothenic acid, and vitamin B6. Like other vegetables, cauliflower is also very susceptible to insect infestation. Diamond back moth, leaf webber, stem borer, tobacco caterpillar, aphids, painted bug etc. are main insects of cauliflower.

For controlling these vegetable pest, farmers use different pesticides like dimethoate, quinalphos, chlorpyrifos, diazinone, acephate, fenitrothion, etc. 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 items. Food products become safe for consumption only after the withholding period has lapsed. By this time, the pesticide residues get dissipated or degraded. Due to lack of education, the farmers of our country do not follow the prescribed dosages and use pesticides at any stage of the crop without any awareness of the residues.

For the determination of these pesticide residues in vegetables many scientists have went through different research in our country. A number of analytical methods are used to determine multiple pesticide residues for fruits and vegetables. But a little

work was done in Gazipur and Narsingdi city. Considering the circumstances the objectives of the present study is given below:

OBJECTIVES

- To identify different pesticides present in the selected vegetables available in the markets of Gazipur and Narsingdi;
- To measure the level of detected pesticide residues (mg/Kg) remain in the selected vegetables; and
- To compare the level of detected pesticide residues (mg/Kg) found in the selected vegetables with the Maximum Residue Limit (MRL).

CHAPTER II
REVIEW OF LITERATURE

To enrich our findings regarding the current status of research and information on the use pattern of insecticides in vegetables and the level of insecticide residues present in the samples collected from Gazipur and Narsingdi market places of vegetables, the quantification of the detected insecticides residues above the Maximum Residue Limit (MRL) or not etc., at home and abroad an effort has been made to review the available literatures. Although the review could not be made so comprehensive due to limited scope and facility, it is hoped that most of the relevant information available in and around Bangladesh was collected and reviewed. It is revealed that most of the information on the aspects searched as mentioned above are mostly available 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.

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.

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.

Jallow *et al.*, (2017) have gone through a research in Kuwait in Fruits and vegetables and found imidacloprid, deltamethrin, cypermethrin, melathion, acetamiprid,

monocrotophos, chloropyrifos-methyl, diazinon pesticide residues with maximum residue limit.

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

Prodhan *et al.,* (2016b) have also been detected four insecticides (chlorpyrifos, cypermethrin, deltamethrin and indoxacarb) in the cauliflower samples collected from different market places in Thessaloniki, Greece. Among the 120 analyzed samples, 48 (40% of the total number of samples) were found to have pesticide residues.

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

Yang *et al.,* (2015) detected the residue levels of imidacloprid, acetamiprid, carbendazim, diflubenzuron, chlorbenzuron, phoxim, pyrimethanil, abamectin and iprodione in 117 samples of wax apple, starfruit and Indian jujube which were collected from Hainan, Fujian, Guangdong and Guangxi province in Chain using ultra performance liquid chromatography tandem mass spectrometry. They found carbendazim was the most frequently detected residue in 51 samples in the concentration range of 34.0-443.8 µg/kg. Diflubenzuron, phoxim, pyrimethanil and abamectin were not found in any sample. They also found that 29 samples contaminated with multiple pesticide residue.

Prodhan *et al.,* (2015a) have been analyzed seven insecticides (chlorpyrifos, dimethoate, deltamethrin, thiamethoxam, thiacloprid, pirimicarb and indoxacarb) and three fungicides (azoxystrobin, fluopicolide and propamocarb hydrochloride) in the fresh melon samples which was collected from different market places in

Thessaloniki, Greece. Among the 122 analyzed samples, 32 (26% of the total number of samples) were found to have pesticide residues.

Zhang *et al.,* (2015) analyzed 14 β-Agonist (banbuterol, brombuterol, cimaterol, cimbuterol, clenbuterol, clenpeterol, clorpenaline, isoxsuprine, mabuterol, mapenterol, ractopamine, salbutamol, terbutaline and tulobuterol) and two β-Blocker (propranololand penbutolol) in procine muscle. They pretreated the sample by QuEChERS method and ultra-high performance liquid chromatography coupled with high resolution mass spectrometry for qualification and quantification of 16 targeted compounds.

Prodhan *et al.,* (2015b) experimented an experiment to determine pesticide residue in 72 fresh eggplant samples collected from different market in Thessaloniki, Greece with Liquid Chromatography-Mass Spectrometry by adopting QuEChERS extraction method. Among the 72 analyzed samples, 34 (47 % of the total number 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 (European Union- Maximum Residue Limits).

Hossain *et al.,* (2015) analyzed pesticide residues in twenty five samples of brinjal, cucumber and tomato collected from different Upazilla of Bogra district, Bangladesh using Gas Chromatography and Mass Spectrophotometry (GC-MS). They found that out of twenty-five samples eight samples were contaminated with pesticides residues, of which five samples exceeded the Maximum Residue Level (MRL) recommended by FAO /WHO

Satpathy *et al.,* (2014) analyzed a research for the multi-residue analysis of 82 pesticides in grapes and pomegranate collected from a local market by QuEChERS extraction method and Gas Chromatography-tandem mass spectrometry (GC-MS/MS). They found 82 pesticides for all compound over the entire range of 0.005-0.1mg/kg except grapes in which they found 28 pesticides in 0.005mg/kg level.

Islam *et al.,* (2014) conducted pesticide residues in 42 samples of three major vegetables like eggplant, cauliflower, and country bean collected from fields and market using Gas Chromatography (GC) with Flame Thermionized Detector (FTD) and Electron Capture Detector (ECD). They found out of 42 samples, 27 had pesticide

residue. Among these 27 samples, 14 samples had pesticide residues above the Maximum Residue Limit (MRL). The detected pesticides were diazinon, Malathion, quinalphos, fenitrothion, cypermethrin, fenvalerate and propiconazole.

Akan *et al.,* (2013) have been examined the concentrations of organophosphorus pesticide residues (dichlorvos, diazinon, chlorpyrifos, and fenitrothion) in spinach, lettuce, cabbage, tomato and onion. They determined concentrations of all the pesticides in the vegetables using GC equipped with electron capture detector (ECD). The highest concentrations of diclorvos, diazinon, chlorpiryfos and fenithrothion were observed in tomato, while the lowest concentrations were observed in spinach.

Corteas *et al.,* (2013) have been found pesticide residue in eggplant, lettuce, pepper, cucumber and tomato by large volume GC injection using the through oven transfer adsorption desorption (TOTAD) interface. They calculated the limits of detection (LODs) for each pesticide 50 µg/l of extract which is much lower than the maximum residues levels (MRLs).

Hossain *et al.,* (2013) analyzed a research 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 tomato, lady's finger and brinjal in different markets of Dhaka using gas chromatography with a photo diode array detector (HPLC-PDA). 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).

Panhwar and Sheikh (2013) carried out a study to assess the pesticide residues of bifenthrin, endosulfan, profenofos, emamectin benzoate, imidacloprid and diafenthiuron in cauliflower through gas Chromatography-µECD and high 44 performance liquid Chromatography (HPLC) analysis. The results revealed that the residual level of pesticides in unwashed unprocessed cauliflower samples are beyond their recommended MRLs, the amount of bifenthrin, endosulfan, profenofos, emamectin benzoate, imidacloprid and diafenthiuron and the respective values were 0.151, 0.671, 0.172, 1.04, 1.011 and 0.052 ppm respectively.

Neetu (2013) experimented a research to investigate the chlorinated pesticide in vegetables, cereals and pulses by Gas Chromatography in East National Capital Region, Delhi, India. Most of the collected samples were found to be contaminated with residues of DDT. In some of detected samples DDT exceeded the limit of tolerance prescribed by WHO and FAO.

Cho *et al.,* (2013) carried out a research to evaluate pesticide residue in spinach by QuEChERS extraction method, gas chromatography-nitrogen phosphorus detector and electron capture detector. They selected fifty GC amenable pesticides and found that the detector response linearly with determination coefficient higher than 0.995. They also found that the level of detection (LODs) for most compound ranged between 0.001 and 0.1µg/g and about 90% of the compound had LODs less than 0.05 µg.

Islam *et al.,* (2013) examined the presence of pesticide residues in cucumber, spinach, and brinjal available in local market of Mymensingh Sadar Upazila using gas chromatography. They found that among the studied 9 samples, only 3 samples responded to two remarkable elusions. Mancozeb 64% + Symoxanil 8% residues occurred in only one cucumber sample which was collected from Seshmore BAU. The quantity of the Mancozeb 64% + Symoxanil 8% residue was about 50 ppm. On the other hand, out of 3 spinach samples, 1 of them showed presence of imidacloprid residues, collected from Seshmore, BAU eluted a small area contained peak which was very minute level (less than 0.1 ppm). Cucumber sample from Seshmore, BAU showed a remarkable peak which was approximately 50 ppm level of Mancozeb 64% + Symoxanil 8% residue.

Cserháti and Szőgyi (2012) have been conducted a research to examine 150 pesticide in tomato, strawberry, potato and oranged lettuce samples by QuEChERS extraction method and GC-MS. Five pesticide residue (gamma HCH, clorothalonil, fenitrothion, chlorpyrifos and pocymidone) were found in tomatoes. They also determined that 5% to 15% of all samples contain pesticide residues.

Chauhan (2012) detected the residues of five commonly used pesticides (endosulfan, carbendazim, chlorpyrifos, cypermethrin and imidacloprid) in different vegetables of Uttarakhand, India. Out of the five pesticides, four of them were insecticides belonging to organochlorine, organophosphate, pyrethroid and 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 samples, cauliflower and tomato had carbendazim residues higher than the recommended MRL's.

Bagyalakshmi *et al.,* (2011) carried out a research on residue determination of dimethoate in leafy vegetables (Spinach) using RPHPLC. They carried out the quantification using RP-HPLC for unprocessed (fresh) and processed (washed, washed and cooked) spinach samples. The extraction was carried out with benzene. They found that unprocessed spinach contains dimethoate residue which was slightly more than the tolerance limit, while the processed sample contains dimethoate residue within the tolerance limit. The washed samples (3 times under ordinary tap water) showed 28-50 % reduction while the cooked samples (boiling for 15 min) showed 58-71 % reduction compared to the unprocessed samples. They said that in case of greens, it should be washed three times with ordinary water and then cooked (cooked for fifteen minutes) so that the pesticide residue is very much reduced and it will be well within the tolerance limit as specified by EPA (Environmental Protection Agency).

Farag *et al.,* (2011) detected pesticide residues in some Egyptian herbs, fruits and vegetables. They collected One hundred thirty-two samples of fruits, vegetables, herbs and spices from Egyptian local markets and analyzed for pesticide residues. They found that contamination with pesticide residues reached 54.55% while samples free from contamination reached 45.45%. 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 pesticides and 5.3% with more than 2 pesticides.

Parveen *et al.,* (2011) analyzed pesticide residues in 120 sample of different fruits including apple, apricot, persimmon, chiku, citrus, grapes, guava, mango, papaya, peach, plum and pomegranate procured from different selling point of Karachi, Pakistan. They analyzed the samples for multiple pesticide residue using GC/FID and

HPLC/UV. They found that 62.5% of samples contained residues of pesticide while 22% exceeded the maximum residue limit (MRL) according to FAO/WHO.

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

Charan and Sharma (2010) detected pesticide residues in a total of 182 samples of six vegetables collected from different agricultural fields of central Aravalli region, India to find out the severity of synthetic agrochemicals on human being. They analyzed pesticide residues using 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% exceeded the maximum residual limit (MRL) values.

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

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

Prodhan *et al.,* (2009) detected organophosphorus and organochlorine insecticide residues in fish sample using electron capture detector (ECD) and flame thermionic detector (FTD) of Gas Chromatograph (GC). They collected sixty-eight samples of fish (Rui, Shrimp and others) from Dhaka, Khulna and Chittagong offices of Department of Fisheries including different fish export companies during August, 2008 to July, 2009. The results revealed that among 68 samples, 13 had insecticide residues. For Dhaka, out of six samples 1 had DDT residue which was 0.28 ppm. For Chittagong, out of 23 samples 3 had diazinon residue ranged from 0.03-0.120 ppm. For Khulna, out of 39 samples 9 had diazinon residue ranged from 0.04-0.205 ppm.

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

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

Kabir *et al.,* (2008) detected the left over residue of diazinon and carbosulfan in brinjal and quinalphos in yard long bean and made a comparison between the detected residue level with maximum residue level (MRL) set by FAO 1970. Three supervised field trials (two for brinjal and one for yard long bean) were carried out sprayed with the field dose (1.5 ml/L of water) of diazinon, carbosulfan, and quinaiphos. They collected sample daily after spraying. They found that in case of diazinon, left over residue was up to 6 days after spray (DAS), and upto 3 DAS, the level of residue was above the MRL. They also detected carbosulfan residue remained till 7 DAS and the detected quantity of residue was above MRL up to 3 DAS. Left over residue of quinalphos in yard long bean sample was detected upto 6 DAS and up to 4 DAS the level of residue was above the MRL.

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

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

Ferrer *et al*., (2005) undertook a research for the quantitative (routine) analysis of 15 pesticide residues in pepper, broccoli, tomato, orange, lemon, apple and melon using liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS). They found residues ranged between 0.0005 and 0.03 mg/kg.

Ortelli *et al.*, (2004) analyzed 74 pesticides in 2500 samples of fruits and vegetables by liquid chromatography-electrospray-tandem mass spectrometry and they found that more than 30% of sample contained multiple pesticide residues, 12 different pesticides in grape sample but all concentrations found were below MRLs.

Kumar and Hosmani (2001) monitored the residue of carbofuran and 3-hydroxy carbofuran in rice in Brazil following Furadan 50G insecticide treatment where they treated rice plants with 3 broadcast application at the nursery (10 days before transplant), tillering and booting (25 and 89 days after transplanting, respectively) stages in India. Plant samples were harvested at 36 days PHI dried in the field for one day and under the sun for 4-6 hours for 3 days in a clean area.

Cornell (2001) conducted a study to detect the magnitude of the residue of carbofuran and 3 hydroxy carbofuran in sweet corn following treatment with Furadan 4F insecticide. No residues of carbofuran (carbofuran + 3-OH-carbofuran) was detected in any sample analyzed (kernels plus the cob with the husks removed).

Holihan (2001) monitored a research to investigate the magnitude of the residue of carbofuran and 3-hydroxy carbofuran in rice in Brazil following Furadan 50G

insecticide. A total of 9 trials were conducted, Total residues of Carbofuran in grain (carbofuran and 3 hydroxy carbofuran) were 0.10 and 0.12 mg/kg.

Kumar and Hosmani (2001) analyzed the residue of carbofuran and 3-hydroxy carbofuran in rice in Brazil following furadan 50G insecticide treatment where they treated rice plants with 3 broadcast application at the nursery (10 days before transplant), tillering and booting (25 and 89 days after transplanting, respectively) stages in India. Plant samples were harvested at 36 days PHI dried in the field for one day and under the sun for 4-6 hours for 3 days in a clean area.

Lee (2001) detected a research to investigate the magnitude of the residue of carbofuran and 3-hydroxy carbofuran in rice in Brazil following Furadan 50G insecticide treatment in South Korea where plants were treated 2 or 3 from transplanting to milk-ripe stage. In one trials, total residues were 0.17 mg/kg and the two other trials, no residue was detected (<0.02 mg/kg) after 63 days of the last application.

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

Salwa *et al.,* (1999) analyzed organophosphorus, dithiocarbamates and some synthetic pyrethroids pesticide residues in 397 Egyptian fruits and vegetables collected from 8 local markets during 1995. Of all analysed samples, 42.8% contained detectable residues, of which 1.76% exceeded their maximum residue limits (MRL's). The rates of contamination with the different pesticides were 0-86%. The most commonly detected residues were dithiocarbarnates as well as dicofol (15.1% of 397 samples), dimethoate (6.8%), tetradifon (4.5%), Malathion (3.3%), profenofos (2.8%), omethoate (2.3%), chlorothalonil (2.0%) and chiorpyrifos-methyl (1.5%). Among all samples, 22 strawberry samples (5.32%) contained 10 pesticide residues, 65 grape samples (15.73%) contained 11 pesticides residues and 62 tomato samples (15.01%)

contained 13 pesticide residues. Cauliflower, onion and guava samples were free from pesticides residues. Samples of carrot and eggplant contained trace amounts of DDT and DDE residues.

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

Dethe *et al.,* (1995) monitored a research to investigate the residues of commonly used pesticide in tomato, eggplant, okra, cabbage in India. They found detectable levels of residues were 33.3% in tomatoes (diazion, endosulfan, dimethoate and monocrotophos), 73.3% in eggplant (endosulfan, diazinon, cypermethrin, fenvalerate, quinalphos, dimethoate and monocrotophos), 14.3% in okras (endosulfan), 88.9% in cabbage (endosulfan, fenvalerate, cypermethrin, dimethoate and monocrotophos). However, the levels of pesticide residues were lower than the maximum residue limits (MRL).

Singh and Karla (1992) estimated the cypermethrin residues in tomato fruits, leaves and soil samples by gas liquid chromatography. The extraction process was done by silica gel column clean-up method and 63 N gas liquid chromatograph. The minimum limits of *cis*-permethrin and *trans*-cypermethrin were 0.008 and 0.006 mg/kg.

Frank *et al.,* (1990) carried out a research to investigate residues of insecticide (organophosphorus, synthetic pyrethroid *N*-methyl carbamate) and fungicide (dithiocarbamate, dicarboximide and organochlorine) in 433 composite samples of eggplant, asparagus, carrots celery, Cole crops, cucumbers, lettuce, onions, peppers, potatoes, radishes and tomatoes in Ontario, Canada. In 64% samples, no pesticide residues were identified to the limits of detection which ranged from 0.005 to 0.05 mg/kg.

Thier *et al.*, (1989) hold a research "Quality assurance in insecticide residue analysis" and reported that during the past few years' pesticide residues of the German Chemical Society has organized 6 laboratory performance tests in which numerous laboratories were involved. In these tests, the choice of analytical methods for the examination of fats or vegetable substance was free. Organochlorine pesticides at over 0.01 mg/kg were most readily identified, whereas in the analysis of organophosphorus residues often only the classic compound such as parathion and diazinon were reported. Many false positive results could have been avoided by using more accurate methods for confirmatory analysis. The quantitative results, however, were generally quite reliable. It can be concluded that the performance of a residue laboratory is not constant, and that it is necessary to assess regularly the quality of the results by participating in such inter laboratory tests.

Singh and Kalra (1989) conducted a research on the "determination of residues of cypermethrin in brinjal fruits, leaves and soil in Ludhiana, India" to determine the residues of permethrin applied for the control of *Leucinodes orbonalis* on eggplant fruit. The compound was sprayed at a rate of 50g a.i/ha at fortnightly intervals. Initial deposits on fruit range from 1.3 to 0.7 mg/kg and maximum residue level 1, 2, 3 and 10 days after spraying was 0.34, 0.2, 0.11 and 0.07 mg/kg respectively. The half-life on insecticides on fruits ranged from 2.1 to 3.0 days. The trans-isomers of permethrin degraded slightly faster than the cis-isomers in leaves and fruits. A 1-day waiting period is recommended for consumption of fruits.

Sattar (1987) reported GC external standard analysis method for DDT and can also be applied for organophosphorus pesticides. Again, GC internal standard analysis method for organophosphorus pesticides universally established (Sattar, 1992). Organophosphorus pesticides are the largely used pesticides in the world where these methods are extensively used for the detection of residues of one or more compounds from soils, crops and food materials .This basic, applied and fundamental contribution is universal and can be treated as pioneer contribution in the history of environmental and pesticide sciences of Bangladesh. Here numerous solvent mixtures were established with recovery of 95-100% covering sensitivity of residues of different organophosphorus pesticides from soils, crops or food materials.

Sattar (1986) established GC external standard analysis method of five organochlorine and four DDT-type organochlorine pesticides were schematically described and their identical chromatograms are listed against respective peaks of organochlorine pesticides and DDT-types pesticides. Different solvent systems were developed with residue recovery upto 100%. The method widely used for the detection of residue in soil lives. This was the basic, applied and fundamental contribution in pesticide and environmental chemistry as well as pioneer work in environmental science of Bangladesh.

Sattar (1985) undertook a research where he used GC external standard and GC internal standard analysis methods to detect phenoxyherbicides, Here, different solvent mixtures and clean up procedures were where sensitivity recorded to ppb levels. This technique is widely recommended to detect the residues of the compounds from soils, crops and food materials.

Sattar (1983) established GC external standard method with 4 chlorinated cresols compounds where 6 solvent systems were developed with recovery upto 100% and sensitivity upto pg (ppb) levels. The examples of chromatographic peaks are reported. Chlorinated cresols come to soils, crops, food materials through various sources and create hazard to human health and other living bodies. This GC method development was the pioneer work as well as applied and fundamental contribution in the environmental sciences of Bangladesh. Even now, still no such method has been developed in Bangladesh. This method also widely applied for the detection of the residues of the compounds.

Sattar and Pavirta (1980) developed the detailed internal standard analysis method (procedure) of MCPA together its two metabolites 4-chlo-cresol and 5-chloro-3-methyl cathechol are schematically presented and the examples of chromatograms are reported as proof records. This was the universal applied fundamental contribution for the detection of the residues of MCPA and/or its metabolites in soils, crops and food, materials. Different solvent/solvent systems were developed including three cleanup procedures (Column, water, toluene-shaking and TLC) where shaking is mostly used. This internal standard procedure of Sattar and Paasivirta 1980 historically invented (lst times in the history of mankind and 1st time in the history of environmental/analytical chemistry of the world) the two metabolites of MCPA by

GC, GC-MS, and NMR applications from foods and vegetables and soils where by applying of only MCPA compound, the recovery recorded up to three compounds like MCPA with its 2 metabolites.

Sattar *et al.,* (1977) established detailed external standard procedure and analysis methods of MCPA and MCPA together with its two metabolites 4-chloro-o-cresol and 5-chloro-3methyl catechol are schernatically described and some examples of chromatograms (peaks) are presented. The method covered different solvent systems for extraction and three clean up procedures like column and water-toluene shaking. This was the excellent basic, applied and fundamental contribution and still largely using in pesticide, agriculture, environmental and analytical chemistry for minimizing residues and hazards and building of peace.

FAO-WHO (1970) reported that the residue of diazinon on/or in plants, in animal tissues, or even in the soil are not highly persistent. From this study, it was revealed that residue of diazinon could be detected up to 6 DAS. The quantities of residue were above MRL upto 3 DAS and these were 2.228 ppm, 1.889 ppm, 1.675 ppm, and 0.761 ppm at 0, 1, 2 and 3 DAS, respectively. Samples of 4, 5, and 6 DAS contained 0.436 ppm, 0.396 ppm and 0.297 ppm. Diazinon residue, respectively, which are below MRL set by FAO-WHO (1970). This results more or less agree with the observation of Geigy (1956-67). He observed the diazinon residue level after spraying the field dose, were < 0.1 ppm in Cabbage at 7 DAS, 0.4 ppm in cauliflower at 5 DAS and < 0.1 ppm in Cucumber at 7 DAS. Adnan *et al.,* (1987) found diazinon residue above MRL upto 8 DAS in sweet pepper grown in green house.

CHAPTER III

MATERIALS AND METHODS

The collected samples of vegetable (country bean, cauliflower and yard long bean) from different markets of Gazipur and Narsingdi carried to the Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur for pesticide residue analysis during July 2016 to January 2017. From sampling to final analysis, required procedures are described below.

3.1 Study area

The sampling area included major ten markets of Gazipur and Narsingdi city. Both Gazipur and Narsingdi is about 35 km and 50km from Dhaka city and both are important area for vegetables cultivation and supply chain in Dhaka city. Gazipur is an commercial as well as industrial area and a major part of vegetable supply and growing zone for Dhaka city. On the other hand, Narsingdi is known as "vegetable basket" and also an important zone of vegetable supply to Dhaka. In this study, vegetables were collected from 5 markets of each city. The samples were collected from Joydebpur Bazar, Harinal Bazar, Laxmipura Bazar, BADC Bazar and Pubail Bazar from Gazipur and Narsingdi samples were collected from Shibpur, C&B Bazar, Marjal, Belabo and Velanagar Bazar.

3.2 Sample collection

A total of 90 samples (30 country bean, 30 cauliflower and 30 yard long bean) were collected for this study. 45 samples were collected from Gazipur and 45 samples were collected from Narsingdi district. The samples were collected from 5 markets of both cities (Table 1).

The amount of each sample was 1Kg for all the 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. Places of sample collection of country bean, cauliflower and yard long bean

Area of collection	Markets	Sample Code*
Gazipur	Joydebpur Bazar	GM 01
		GM 02
		GM 03
	Harinal Bazar	GM 04
		GM 05
		GM 06
	BADC Bazar	GM 07
		GM 08
		GM 09
	Pubail Bazar	GM 10
		GM 11
		GM 12
	Luxmipura Bazar	GM13
		GM 14
		GM 15
Narsingdi	Shibpur Bazar	NM 01
		NM 02
		NM 03
	C&B Bazar	NM 04
		NM 05
		NM 06
	Belabo Bazar	NM 07
		NM 08
		NM 09
	Marjal Bazar	NM 10
		NM 11
		NM 12
	Velanagar Bazar	NM 13
		NM 14
		NM 15

*Each sample code comprises three experimented vegetables, *i.e.* country bean, cauliflower and yard long bean.

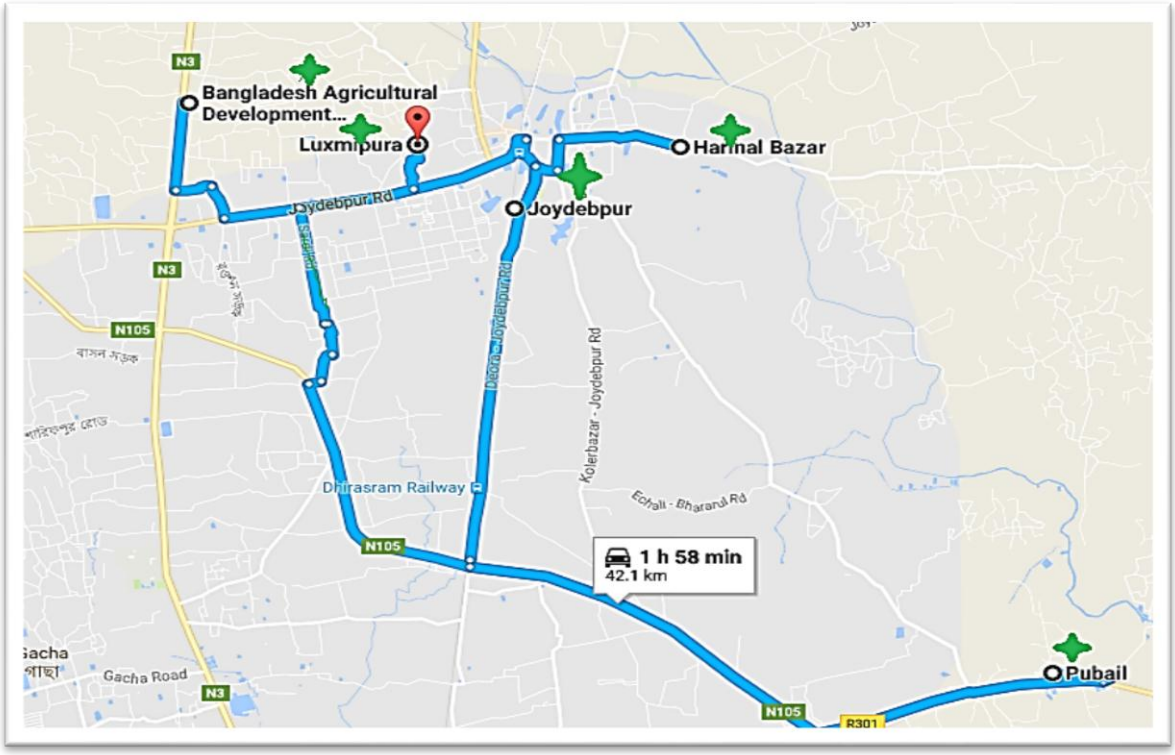


Plate 1. Map showing the places of sample collection in Gazipur

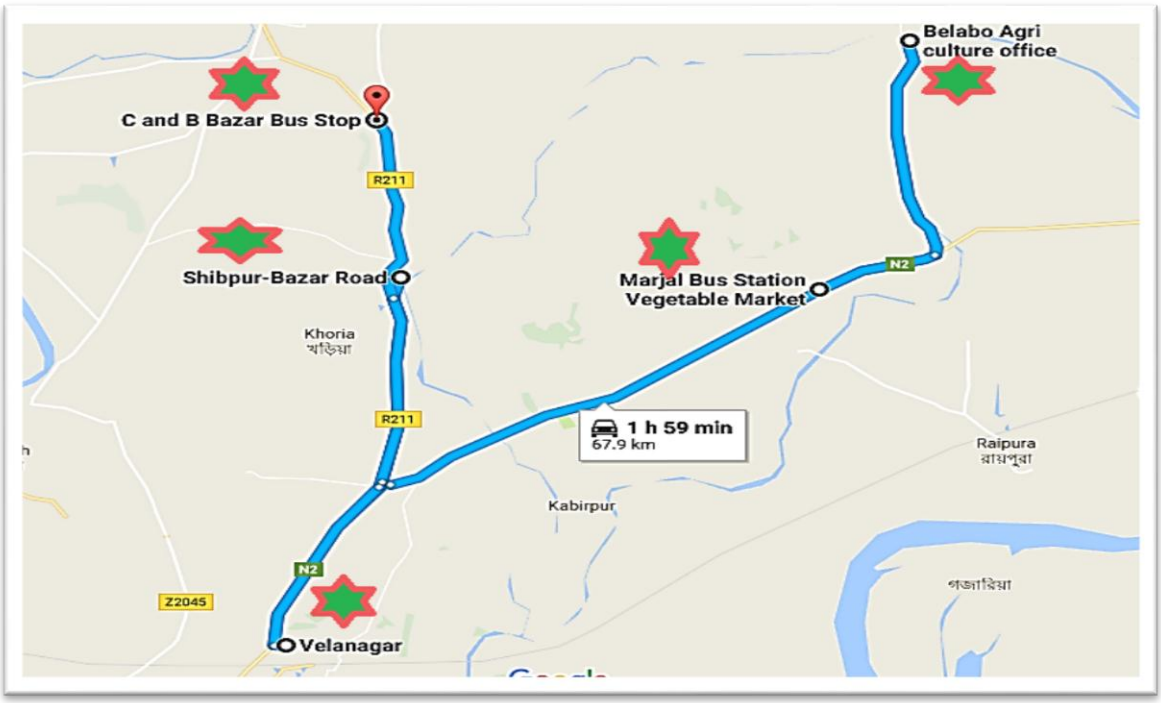


Plate 2. Map showing the places of sample collection in Narsingdi

3.3 Sample preparation for analysis

The samples were taken to the Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI) 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 Laborchemikalien (St Louis, MO, USA) via Bangladesh Scientific Pvt. Ltd. Dhaka, Bangladesh. Standards of all the pesticides contained >99.6%) purity.

Methanol, acetone, gradient grade acetonitrile, 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 required

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

b. Electric balance, Model: AY- 220, Shimadzu Corporation, Japan (Plate 4).

c. Homogenizer, Model: Ultraturax, IKA T18 basic, Germany (Plate 5)

d. Vortex mixer, Model: Maxi max ii, USA (Plate 6)

e. GC-2010, Shimadzu Corporation, Japan (Plate 7).

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

• Scissors	• Funnel
• Measuring cylinder	• Test tube
• Conical flask	• Micro pipette
• Volumetric flask	• Aluminum foil
• Tray	• Para film
• Knife	• Centrifuge tube
• Spatula	• Glass vial, etc.

3.6 Preparation of pesticide standard solution

Pesticide standard stock solutions of chlorpyrifos, acephate, diazinon, dimethoate, quinalphos, Malathion and fenitrothion were prepared separately in acetone at a concentration of 1000 mg/L and stored at -20°C until use. A mixed standard solution of 50 mg/L in acetone containing all the aforementioned pesticides was prepared by adding the appropriate volume of each individual stock solution in a 50 ml volumetric flask and made to volume by addition of acetone. An intermediate mixed standard solution of 10 mg/L in acetone was prepared from the mixed standard solution of 50 mg/L. Then working standard solutions of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 mg/L in acetone were prepared by transferring the appropriate amount from 10 mg/L intermediate mixed standard solution into ten separate 10mL 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 technique 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 theextraction 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 (Anon, 2011).

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 MgSO4 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 MgSO4 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, 1 mL supernatant was filtered by a 0.2 μm PTFE filter, and then it was taken in a clean GC vial for injection. The process of QuEChERS extraction methods of study samples during research is given in Plate 8-15.

Plate 3. Centrifuge Machine

Plate 4. Electric Balanc**e**

Plate 5. Homogenizer

Plate 6. Vortex Mixer

Plate 7. Gas Chromatograph (GC)

Plate 8. Chopping of collected sample

Plate 9. Weighing of sample and salt (NaCl and $MgSO_4$)

Plate 10. Adding acetonitrile (MeCN)

Plate 11. Shaking of samples

Plate 12. Centrifuging the sample

Plate 13. Weighing of PSA

Plate 14. Adding PSA in the sample extract

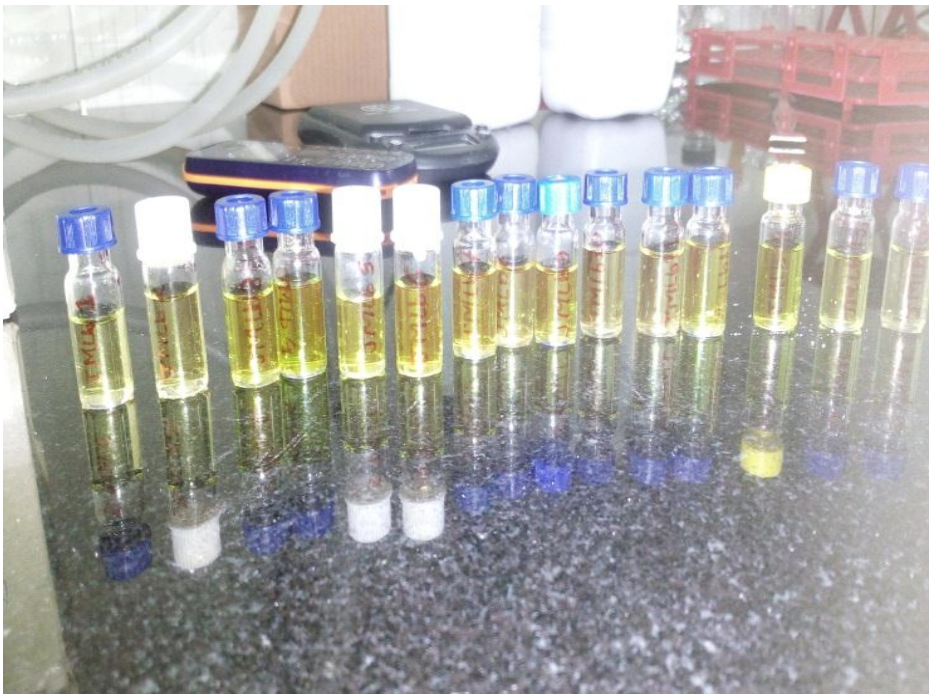
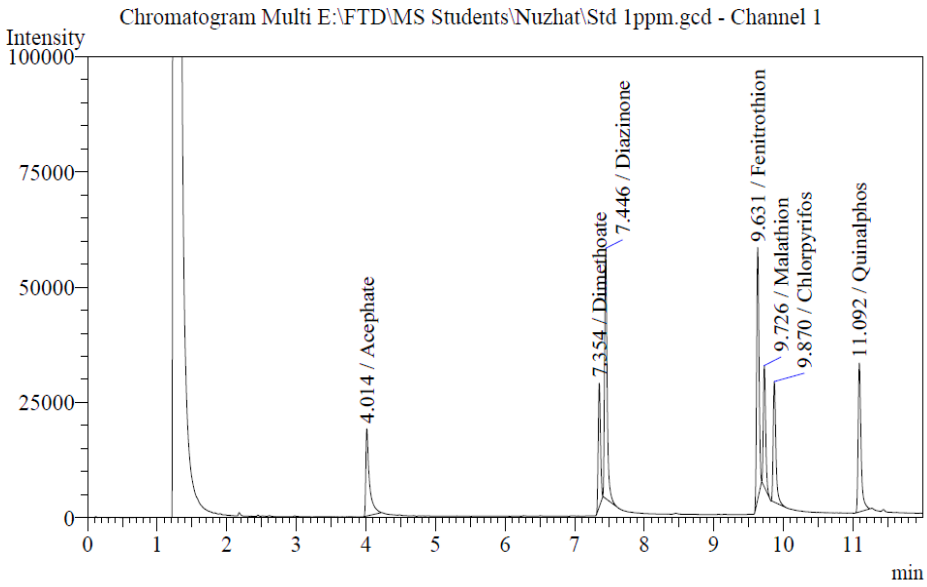


Plate 15. Sample extract ready for injection

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) with Flame Thermionized 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 (Figure 1). The instrument conditions are described in Table 2 and Table 3.



Time (min)

Figure 1. Typical chromatograms of seven organophosphorus insecticide standards run by GC-FTD.

Table 2. 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 3.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 Calibration curve preparation

Prior to the injection of the sample extract, standard solutions of different concentrations of each pesticide group were prepared and injected with suitable instrument parameters. The samples were calibrated (retention time, peak area, etc.) against five pointed calibration curve of standard solution of concerned pesticide (Figure 2-8). Each peak was characterized by its retention time. Sample results were expressed in mg/kg automatically by the GC software.

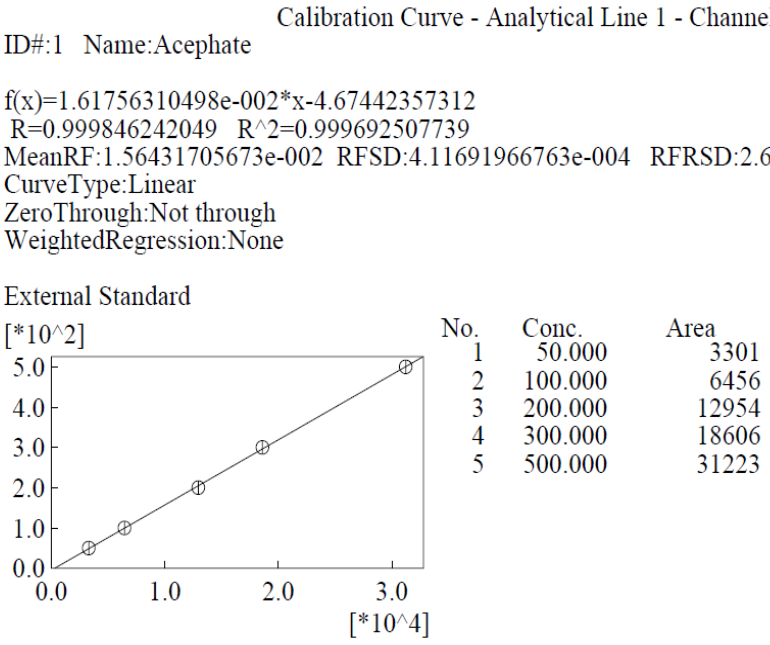
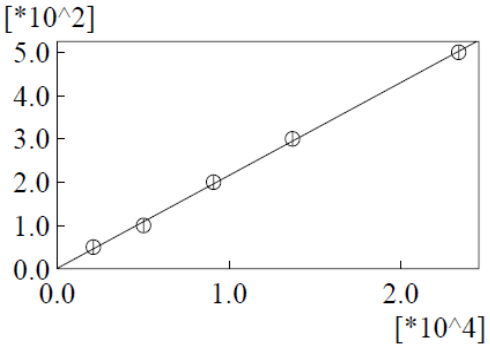


Figure 2. Calibration curve prepared for acephate made with different concentrations ranging from 50 µg/L to 500 µg/L.

ID#:2 Name:Dimethoate

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

External Standard



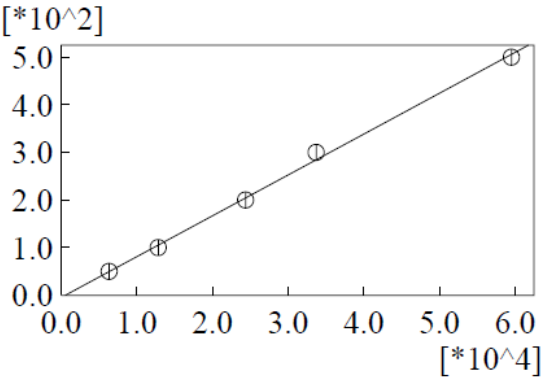
No.	Conc.	Area
1	50.000	2111
2	100.000	5032
3	200.000	9107
4	300.000	13703
5	500.000	23369

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

ID#:3 Name:Diazinone

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

External Standard



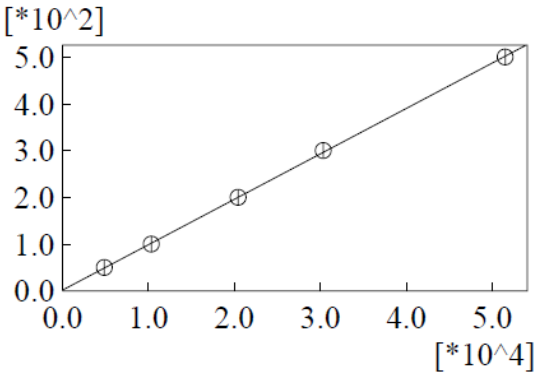
No.	Conc.	Area
1	50.000	6355
2	100.000	12850
3	200.000	24378
4	300.000	33704
5	500.000	59496

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

ID#:4 Name:Fenitrothion

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

External Standard



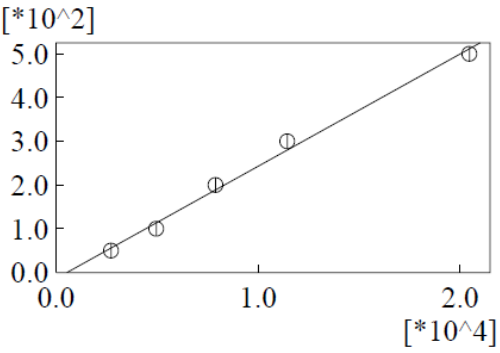
No.	Conc.	Area
1	50.000	4878
2	100.000	10347
3	200.000	20439
4	300.000	30352
5	500.000	51502

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

ID#:5 Name:Malathion

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

External Standard



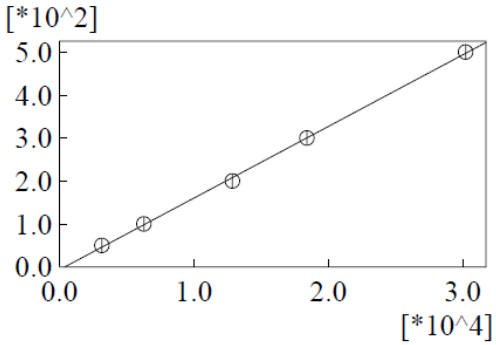
No.	Conc.	Area
1	50.000	2732
2	100.000	4966
3	200.000	7900
4	300.000	11450
5	500.000	20468

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

ID#:6 Name:Chlorpyrifos

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

External Standard



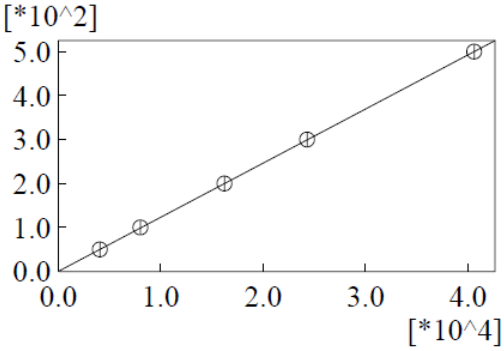
No.	Conc.	Area
1	50.000	3162
2	100.000	6285
3	200.000	12871
4	300.000	18405
5	500.000	30208

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

ID#:7 Name:Quinalphos

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

External Standard



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

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

CHAPTER IV

RESULTS AND DISCUSSION

The collected 90 samples of vegetables (country bean, cauliflower and yard long bean) from different markets of Gazipur and Narsingdi carried to the Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur for pesticide residue analysis. The results obtained from this study are presented and described in this chapter by using figures and tables.

4.1 Pesticide residues in country bean

The concentrated extracts of country bean samples collected from different markets were analyzed by GC-2010 (Shimadzu) with Flame Thermionized Detector (FTD) with the pre-set parameters. Figure 10-19 shows the chromatograms of the injected extracts of country bean sample containing detected pesticides.

4.1.1 Gazipur region

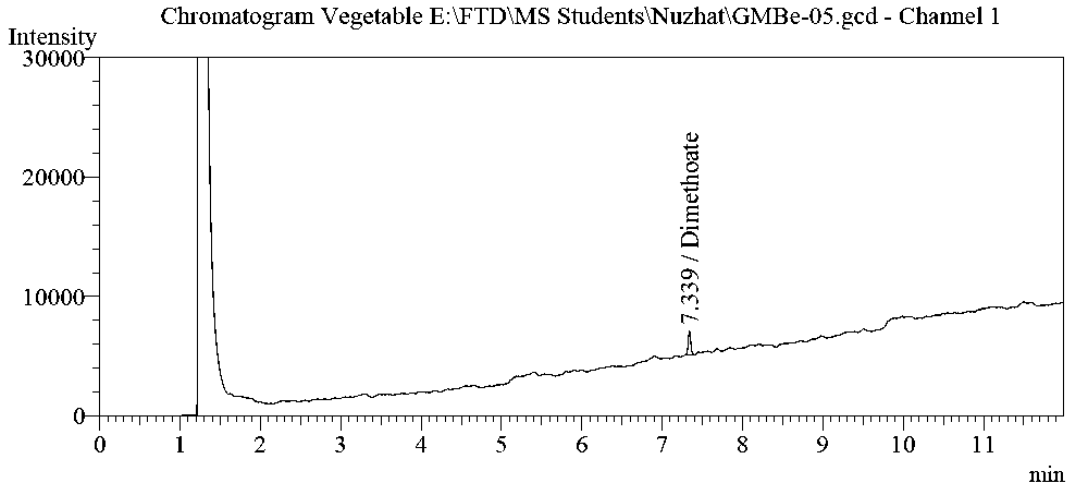


Figure 9. Chromatogram of dimethoate found in one of the country bean collected from Gazipur marketed sample (GMBe-05) showing retention time.

Table 4. Detected pesticide residue of analyzed samples of country bean from Gazipur and their maximum residue level

Area of collection	Sample code	Name of detected pesticide	Level of residue	MRL
Joydebpur	GMBe 01	ND		
	GMBe 02	ND		
	GMBe 03	ND		
Harinal	GMBe 04	ND		
	GMBe 05	Dimethoate	0.074	0.02*
	GMBe 06	ND		
BADC	GMBe 07	ND		
	GMBe 08	ND		
	GMBe 09	ND		
Pubail	GMBe 10	ND		
	GMBe 11	Chloropyrifos	0.171	0.01*
	GMBe 12	ND		
Luxmipura	GMBe13	ND		
	GMBe 14	ND		
	GMBe 15	ND		

Fifteen samples of country bean collected from 5 different markets of Gazipur city (Joydebpur, Harinal, BADC, Laxmipura and Pubail) were analyzed to find out the presence of left over residue of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos. Out of 15 samples, 2 samples (15% of the total number of samples) contained pesticide residues and 13 samples (of the total number of samples) contained no detectable residues out of the sought pesticides.

Among 15 samples of country bean collected from Gazipur region, one sample collected from Harinal bazar (sample code GMBe 05) contained dimethoate pesticide with 0.074 mg/kg residue and that is above its MRL. Another sample collected from Pubail bazar (GMBe 11) contained chloropyrifos with 0.171 mg/kg residue and that is also above MRL.

4.1.2 Narsingdi region

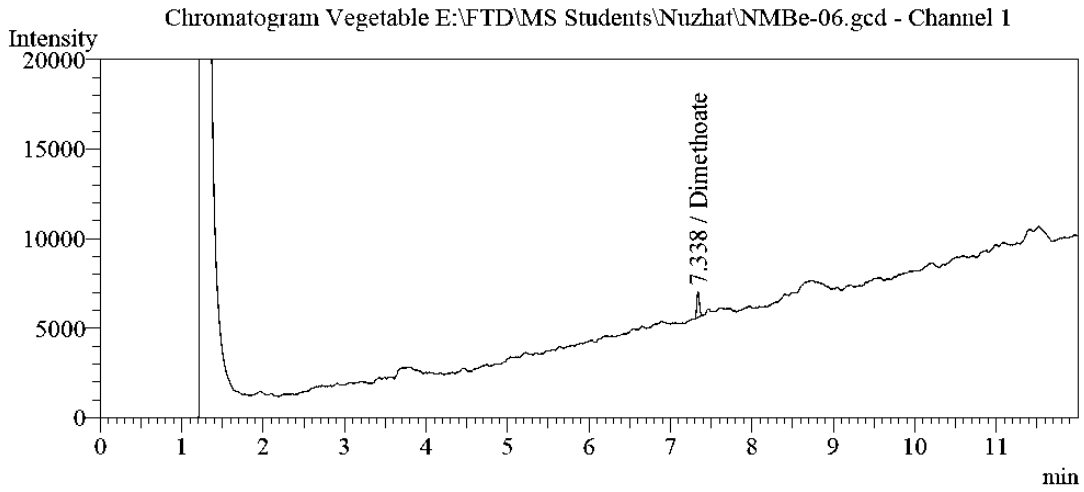


Figure 10. Chromatogram of Dimethoate found in one of the country bean collected from Narsingdi marketed sample (NMBe-06) showing retention time.

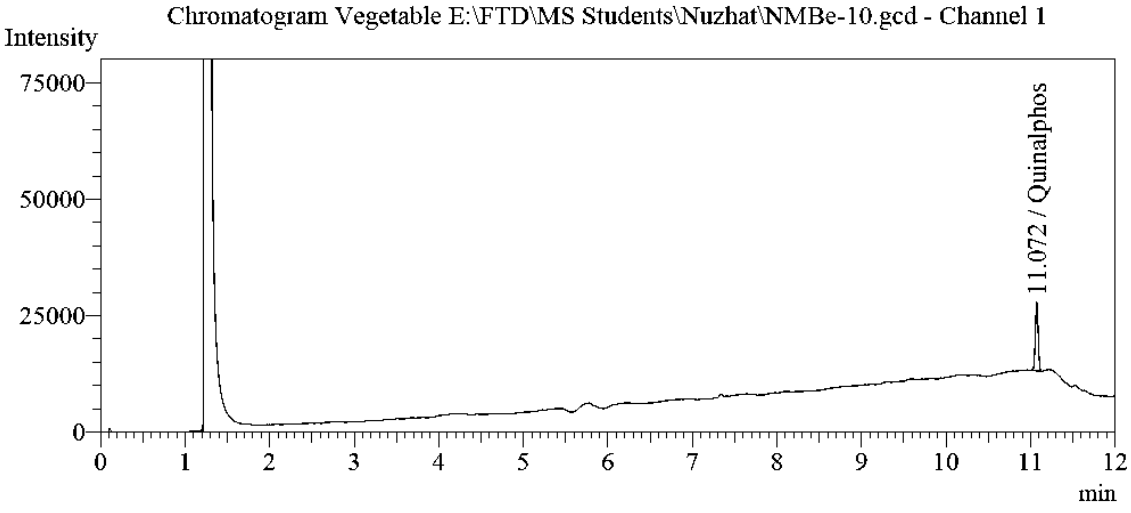


Figure 11. Chromatogram of quinalphos found in one of the country bean collected from Narsingdi marketed sample (NMBe-10) showing retention time.

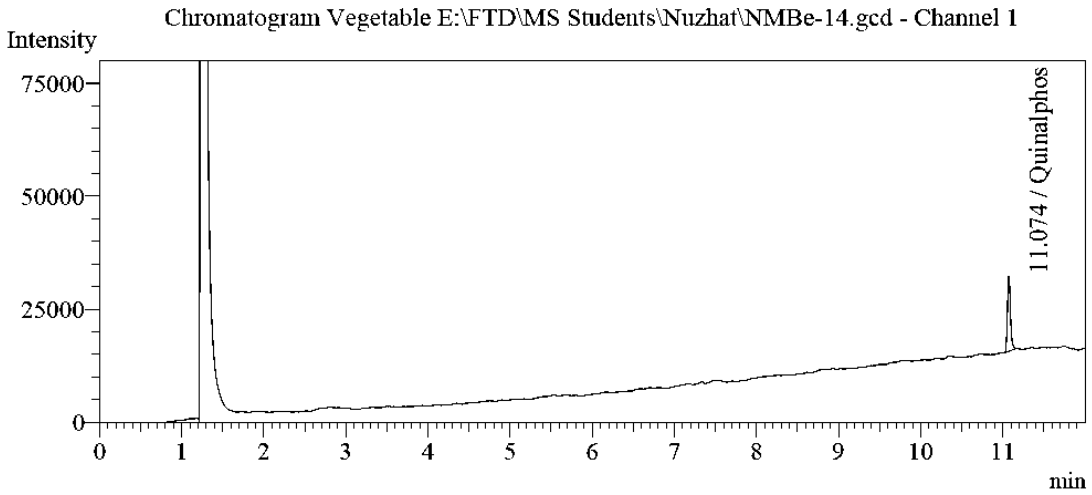


Figure 12. Chromatogram of quinalphos found in one of the country bean collected from Narsingdi marketed sample (NMBe-14) showing retention time.

Table 5. Detected pesticide residue of analyzed samples of country bean from Narsingdi and their maximum residue level

Area of collection	Sample code	Name of detected pesticide	Level of residue	MRL
Shibpur	NMBe 01	ND		
	NMBe 02	ND		
	NMBe 03	ND		
C&B bazar	NMBe 04	ND		
	NMBe 05	ND		
	NMBe 06	Dimethoate	0.053	0.02*
Belabo	NMBe 07	ND		
	NMBe08	ND		
	NMBe 09	ND		
Marjal	NMBe 10	Quinalphos	0.365	0.05*
	NMBe 11	ND		
	NMBe 12	ND		
Velanagar	NMBe 13	ND		
	NMBe 14	Quinalphos	0.454	0.05*
	NMBe 15	ND		

Fifteen samples of country bean collected from 5 different markets of Narsingdi city (Shibpur, C&B, Belabo, Marjal and Velanogor) were analyzed to find out the presence of left over residue of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos). Out of 15 samples, 3 samples (around 20% of total samples) contained pesticide residues and other 11 samples detected no pesticide residue.

Among 15 samples of country bean collected from Narsingdi region one sample collected from CNB bazar (NMBe 06) contained dimethoate pesticide with 0.053 mg/kg residue which was above MRL. Another sample from Marjal Bazar (NMBe 10) contained quinalphos with 0.365 mg/kg residue and that was also above MRL. Sample from Velanagar Bazar (NMBe 14) also contained quinalphos with 0.0454 mg/kg residue and that is also above MRL.

4.2. Pesticide residue in cauliflower

4.2.1. Gazipur region

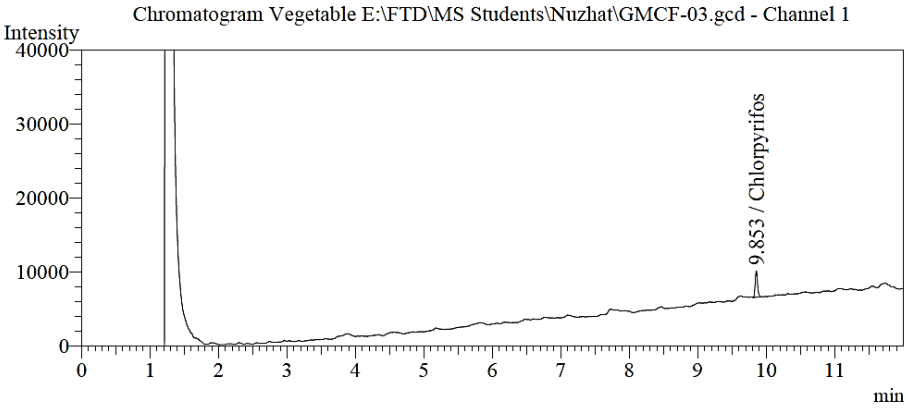


Figure 13. Chromatogram of chlorpyrifos found in one of the cauliflower marketed sample ($GMCF_{03}$) showing retention time.

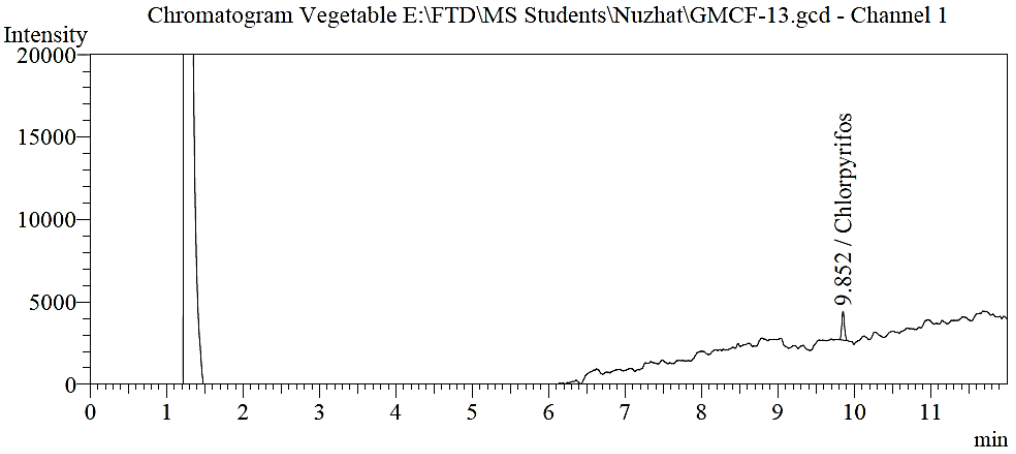


Figure 14. Chromatogram of chlorpyrifos found in one of the cauliflower marketed sample ($GMCF_{13}$) showing retention time.

Table 6. Detected pesticide residue of analyzed samples of cauliflower from Gazipur and their maximum residue level

Area of collection	Sample code	Name of detected pesticide	Level of residue	MRL
Joydebpur	GMCF 01	ND		
	GMCF 02	ND		
	GMCF 03	Chloropyrifos	0.120	0.05*
Harinal	GMCF 04	ND		
	GMCF 05	ND		
	GMCF 06	ND		
BADC	GMCF 07	ND		
	GMCF 08	ND		
	GMCF 09	ND		
Pubail	GMCF 10	ND		
	GMCF 11	ND		
	GMCF 12	ND		
luxmipur	GMCF 13	Chloropyrifos	0.058	0.05*
	GMCF 14	ND		
	GMCF15	ND		

Fifteen samples of cauliflower collected from 5 different markets of Gazipur city (Joydebpur, Harinal, BADC, Laxmipura and Pubail) were analyzed to find out the presence of left over residue of seven pesticides (acephate, diazinon, dimethoate,

malathion, fenitrothion, chlorpyrifos and quinalphos. Out of 15 samples, 2 samples (13% of the total number of samples) contained pesticide residues and 13 samples (of the total number of samples) contained no detectable residues out of the sought pesticides. Among 15 samples of cauliflower collected from Gazipur region, one sample collected from Joydebpur bazar (sample code GMBe 03) contained chloropyrifos pesticide with 0.120 mg residue and that was above its MRL. Another sample collected from Pubail bazar (GMBe 13) also contained chloropyrifos with 0.058mg residue and that was also above MRL.

4.2.2. Narsingdi region

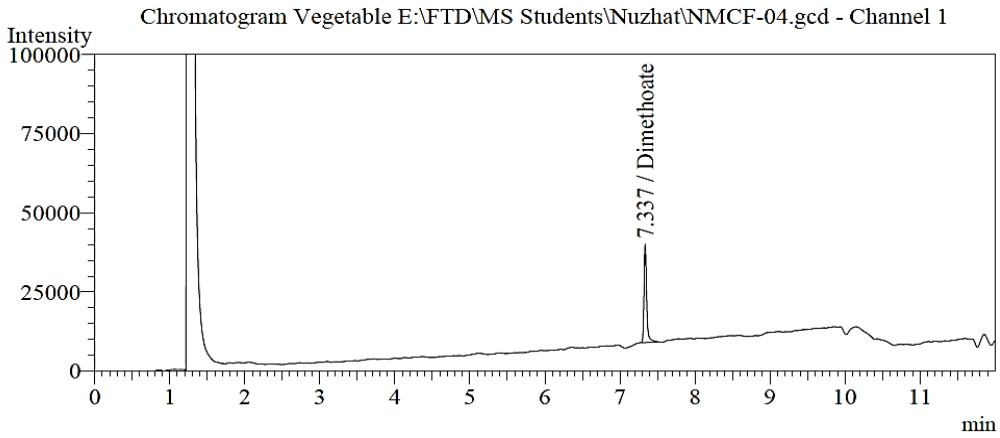


Figure 15. Chromatogram of dimethoate found in one of the cauliflower marketed sample ($NMCF_{04}$) showing retention time

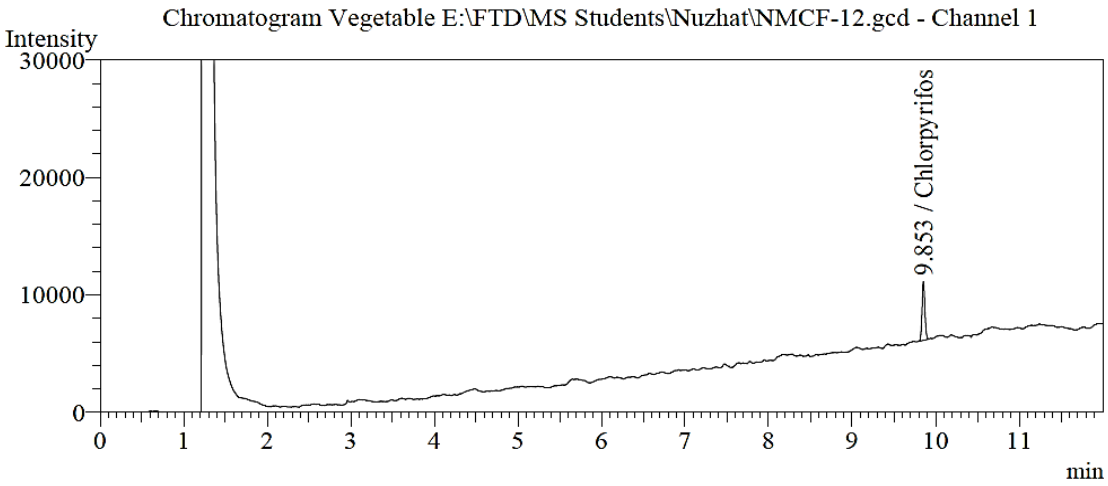


Figure 16. Chromatogram of chlorpyrifos found in one of the cauliflower marketed sample ($NMCF_{12}$) showing retention time.

Table 7. The level of residues (mg/kg) of different pesticides found in the analyzed cauliflower samples from Narsingdi

Area of collection	Sample code	Name of detected pesticide	Level of residue	MRL
Shibpur	NMCF 01	ND		
	NMCF 02	ND		
	NMCF 03	ND		
C&B bazar	NMCF 04	Dimethoate	1.266	0.02*
	NMCF 05	ND		
	NMCF 06	ND		
Belabo	NMCF 07	ND		
	NMCF 08	ND		
	NMCF 09	ND		
Marjal	NMCF 10	ND		
	NMCF 11	ND		
	NMCF 12	Chloropyrifos	0.120	0.05*
Velanagar	NMCF 13	ND		
	NMCF 14	ND		
	NMCF 15	ND		

Fifteen samples of cauliflower bean collected from 5 different markets of Narsingdi city (Shibpur, C& B, Belabo, Marjal and Velanagar) were analyzed to find out the presence of left over residue of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos). Out of 15 samples, 2 samples (around 13% of total samples) contained pesticide residues and other 13 samples detected no pesticide residue.

Among 15 samples of cauliflower collected from Narsingdi region one sample collected from CNB bazar (NMCF 04) contained dimethoate pesticide with 0.1.266 mg/kg residue which was above MRL. Another sample from Marjal Bazar (NMCF 12) contained with 0.120mg/kg residue and that is also above MRL.

4.3 Pesticide residue in yard long bean

4.3.1. Gazipur region

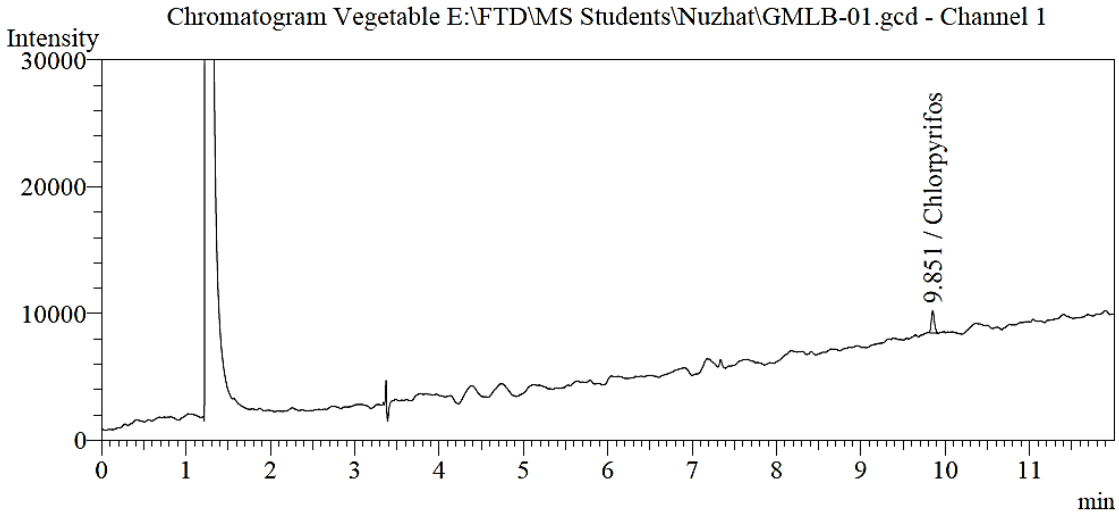


Figure 17. Chromatogram of chlorpyrifos found in one of the yard long bean marketed sample ($GMLB_{01}$) showing retention time

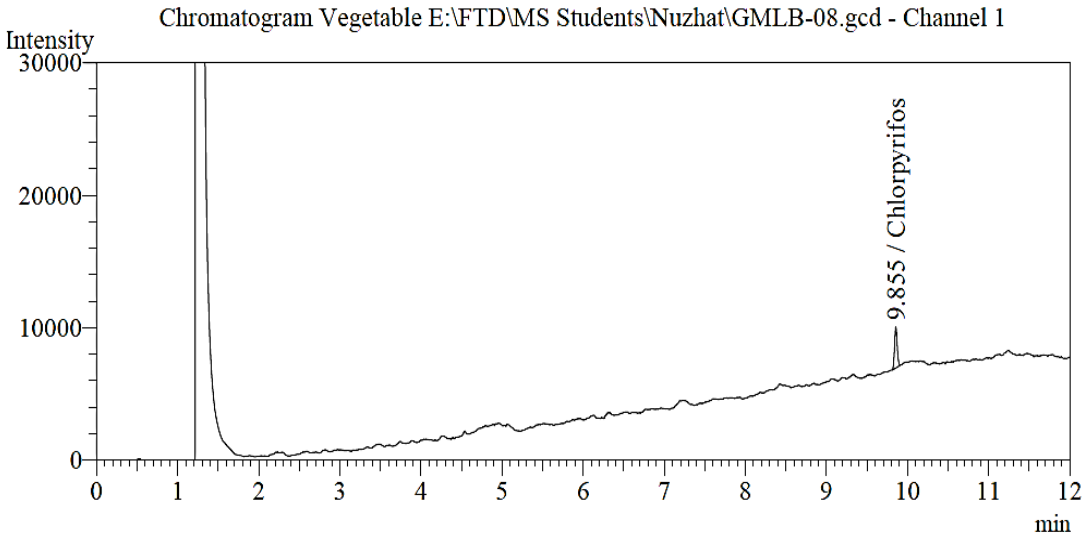


Figure 18. Chromatogram of chlorpyrifos found in one of the yard long bean marketed sample (GMLB$_{08}$) showing retention time

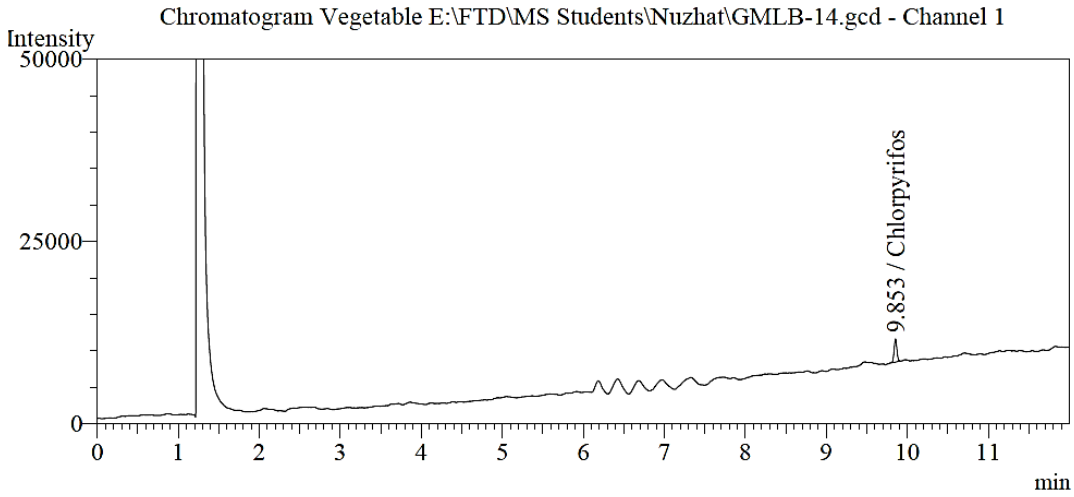


Figure 19. Chromatogram of chlorpyrifos found in one of the yard long bean marketed sample (GMLB14) showing retention time.

Table 8. The level of residues (mg/kg) of different pesticides found in the analyzed Yard long bean samples collected from Gazipur

Area of collection	Sample code	Name of detected pesticide	Level of residue	MRL
Joydebpur	GMLB 01	Chloropyrifos	0.062	0.01*
	GMLB 02	ND		
	GMLB 03	ND		
Harinal	GMLB 04	ND		
	GMLB 05	ND		
	GMLB 06	ND		
BADC	GMLB 07	ND		
	GMLB 08	Chloropyrifos	0.100	0.01*
	GMLB 09	ND		
Pubail	GMLB 10	ND		
	GMLB 11	ND		
	GMLB 12	ND		
Luxmipura	GMLB13	ND		
	GMLB 14	Chloropyrifos	0.106	0.01*
	GMLB 15	ND		

Fifteen samples of yard long bean collected from 5 different markets of Gazipur city(Joydebpur, Harinal, BADC,Luxmipura and Pubail) were analyzed to find out the presence of left over residue of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos. Out of 15 samples, 3 samples (13% of the total number of samples) contained pesticide residues and 12 samples (of the total number of samples) contained no detectable residues out of the sought pesticides.

Among 15 samples of yard long bean collected from Gazipur region, one sample collected from Joydebpur bazar (sample code GMLB 01) contained chloropyrifos pesticide with 0.062 mg residue and that was above its MRL. Another sample collected from BADC bazar (GMBe 08) contained chloropyrifos with 0.100 mg/kg residue and that was also above MRL. Sample collected from luxmipura (GMLB 14) also contained chloropyrifos with 0.106 mg/kg residue and that was above MRL.

4.3.2. Narsingdi region

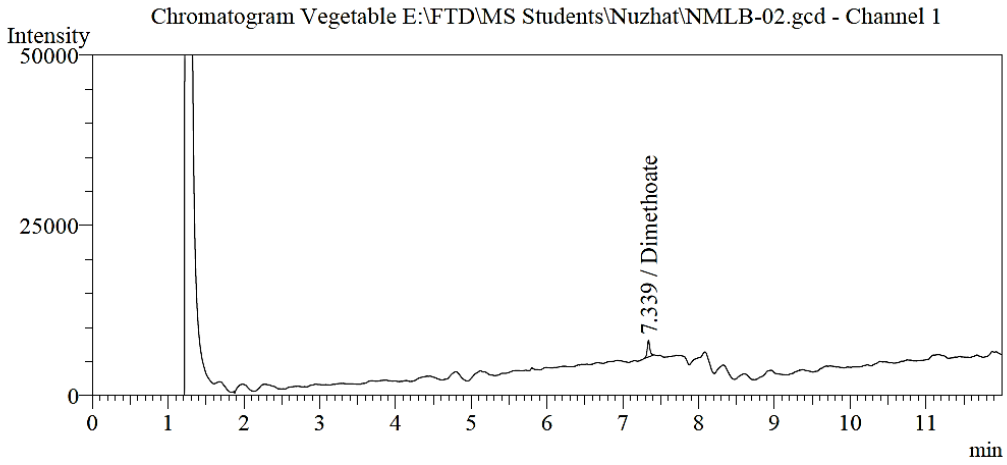


Figure 20 . Chromatogram of dimethoate found in one of the Yard long bean marketed sample (NMLB02) showing retention time

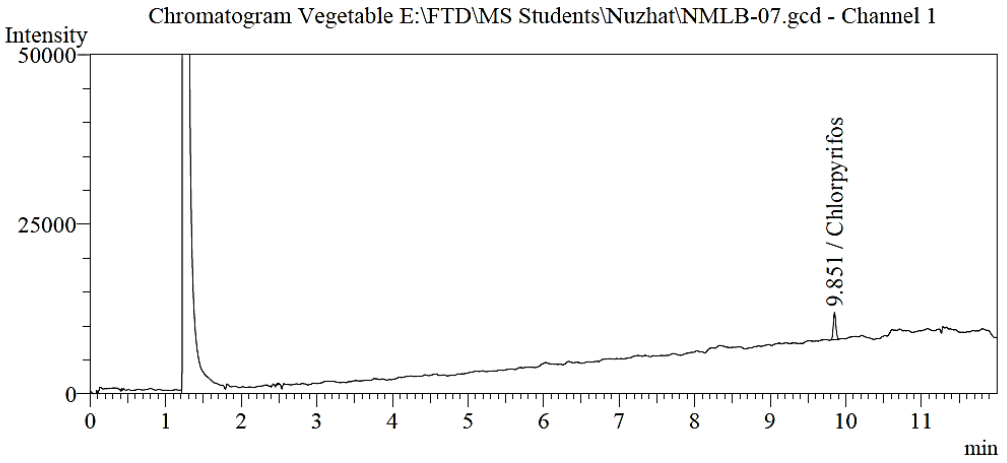


Figure 21. Chromatogram of chlorpyrifos found in one of the Yard long bean marketed sample (NMLB07) showing retention time

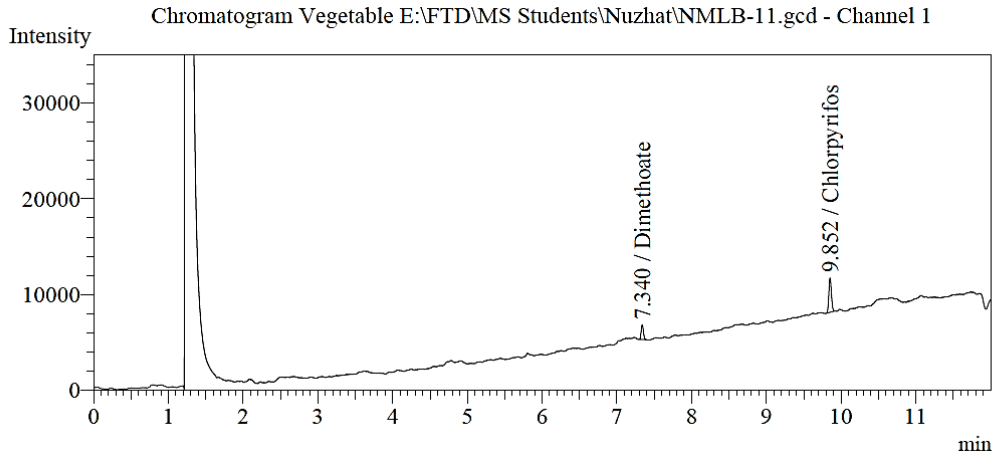


Figure 22. Chromatogram of chlorpyrifos and dimethoate found in one of the Yard long bean marketed sample (NMLB11) showing retention time

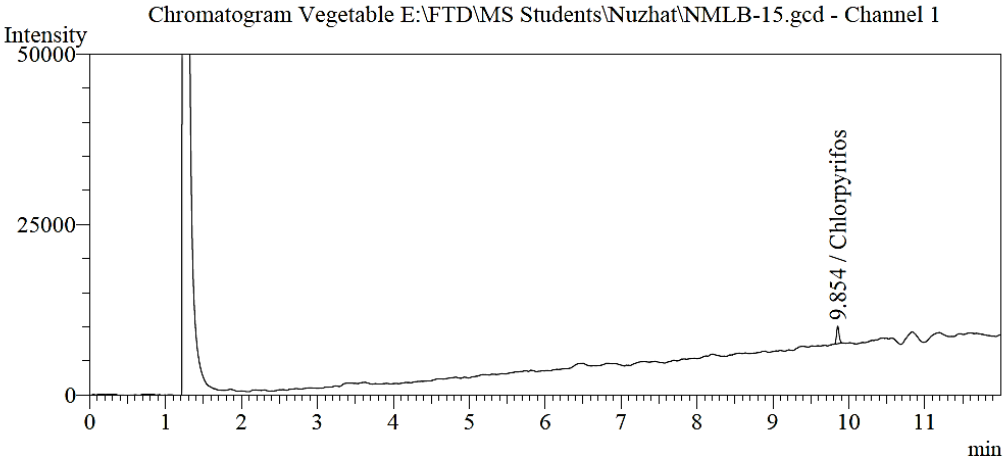


Figure 23. Chromatogram of chlorpyrifos found in one of the Yard long bean marketed sample (GMLB15) showing retention time

Table 9. The level of residues (mg/kg) of different pesticides found in the analyzed Yard long bean samples collected from Narsingdi

Area of collection	Sample code	Name of Detected pesticide	Level of residue	MRL
Shibpur	NMLB 01	ND		
	NMLB 02	Dimethoate	0.098	0.02*
	NMLB 03	ND		
C&B bazar	NMLB 04	ND		
	NMLB 05	ND		
	NMLB 06	ND		
Belabo	NMLB 07	Chloropyrifos	0.134	0.01*
	NMLB08	ND		
	NMLB 09	ND		
Marjal	NMLB 10	ND		
	NMLB 11	Dimethoate	0.053	0.02*
		Chloropyrifos	0.119	0.01*
	NMLB 12	ND		
Velanagar	NMLB 13	ND		
	NMLB 14	ND		
	NMLB 15	Chloropyrifos	0.086	0.01*

Fifteen samples of yard long bean collected from 5 different markets of Narsingdi city (Shibpur, C & B, Belabo, Marjal and Velanagar) were analyzed to find out the presence of left over residue of seven pesticides (acephate, diazinon, dimethoate, malathion, fenitrothion, chlorpyrifos and quinalphos). Out of 15 samples, 4 samples (around 13% of total samples) contained pesticide residues and other 13 samples detected no pesticide residue.

Among 15 samples of yard long bean collected from Narsingdi region one sample collected from Shibpur Bazar (NMLB 02) contained dimethoate pesticide with 0.098 mg/kg residue which was above MRL. Another sample from Belabo (NMLB 07) contained chloropyrifos with 0.134mg/kg residue and that was also above MRL. Sample collected from Marjal (NMLB 11) contained dimethoate and chloropyrifos pesticide with 0.053 and 0.119 mg/kg respectively and that was also above MRL. Another sample from velanagar (NMLB 15) contained chloropyrifos with 0.086mg/kg residue which was above MRL.

Islam *et al.*, 2014 collected 42 samples of eggplant, cauliflower and country bean from fields and markets of Narshingdi district, Bangladesh, where they found 15 samples (above 68% of the total number of samples) contained no pesticide residues. Prodhan *et al.*, 2015 collected 72 samples of eggplant from different market places in Thessaloniki, Greece and found that 38 samples did not contain any pesticide residues.

Prodhan *et al.* (2012) analyzed insecticide residues in 94 samples of brinjal, hyacinth bean, cauliflower and yard long bean which were collected from farmers field and market of different regions like Barisal, Bogra, Chittagong, Comilla, Dhaka, Dinajpur, Gazipur, Jessore, Khagrachari, Narsingdi, Rajshahi and Rangpur using GC-ECD and GC-FTD depending on the nature of insecticides. Results revealed that among 94 samples, 46 had insecticide residues and of them 18 had above MRL. Among 38 analyzed brinjal samples, 18 samples contained residue of cypermethrin, diazinon and quinalphos; and only 8 had above MRL. They found cypermethrin was frequent one which was found in 14 samples. Of 24 analyzed hyacinth bean samples, 15 had the residue of cypermethrin, diazinon, quinalphos, fenitrothion and Malathion; and 7 had above MRL. Among the 16 analyzed cauliflower samples, 5 samples contained residue of cypermethrin, diazinon, quinalphos and Malathion; and only 2 had above

MRL. Among 16 samples of yard long bean, 8 samples contained residue of cypermethrin, diazinon, quinalphos and Malathion; and only 2 had above MRL.

Rakib *et al*. (2017) conducted to analyze pesticide residues in country bean collected from different areas of Dhaka city from January 2016 to September 2016. 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) contain residue of Dimethoate.

Khanom (2017) conducted a study to detect and quantify the residue of quinalphos, chloropyrifos, fenitrothion and diazinon in country bean. A supervised field trial was undertaken sprayed with the field dose (1.5 ml/L of water) of all of the selected insecticides. Samples were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 days after spray. The residue of quinalphos was detected upto 9 DAS, of which the level of residue was above MRL at 8 DAS. The quinalphos residue remained 0.009 mg/kg at 9 DAS, which was below MRL. Therefore, the Pre-Harvest Interval (PHI) for quinalphos was set at 9 DAS and no residue was detected at 10 DAS. The residue of chloropyrifos was detected upto 8 DAS, of which the level of residue was above MRL at 6 DAS. The chlorpyrifos residue remained 0.044 mg/kg at 7 DAS, which was below MRL. So, the pre-harvest interval (PHI) for chlorpyrifos was set at 7 DAS and no residue was detected at 9 DAS. The residue of fenitrothion was detected upto 8 DAS, of which the level of residue was above MRL at 7 DAS. The fenitrothion residue remained 0.007 mg/kg at 8 DAS, which was below MRL. So, the pre-harvest interval (PHI) for fenitrothion was set at 8 DAS and no residue was detected at 9 DAS. The residue of diazinon was detected upto 9 DAS, of which the level of residue was above MRL at 8 DAS. The residue of diazinon remained 0.007 mg/kg at 9 DAS, which was below MRL. So, the Pre-Harvest Interval (PHI) for diazinon was set at 9 DAS and no residue was detected at 10 DAS.

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

CHAPTER V

CONCLUSION

Being an overpopulated country, food shortage and malnutrition are major problems of Bangladesh. The main obstacle of vegetables production in our country is insect pest infestation. As a result use of different pesticides and other chemicals are becoming a common agricultural practice by the farmers, and a major portion of these pesticides are intercepted by the plant leaves during application. Thus, pesticide residues remain in the vegetable which pose a threat to human body. Food safety become utmost important for all involved value chain. Consumers have to be assured that they are not exposed to unacceptable pesticide residue levels. Therefore, it is a prime need to detect and quantify pesticide residues in the food commodities. The purpose of this study was intended to identify and quantify the pesticide residue level present in the vegetables available in various local markets of Gazipur and Narsingdi regions.

Fifteen samples of country bean, cauliflower and yard long bean for each vegetable collected from each 5 different markets of Gazipur and Narsingdi district were analyzed to find out the presence of left over residue of seven pesticides (acephate, diazinon, dimethoate, Malathion, fenitrothion, chlorpyrifos and quinalphos). Out of 15 samples of country bean, 2 samples contained pesticide residues with above MRL in Gazipur, whereas it was 3 samples above MRL in Narsingdi and no detectable residues of the sought pesticides were identified from remaining samples. Again, among the same number of analyzed samples of cauliflower, 2 samples from each location contained pesticide residues with above MRL and rest of the samples had no detectable pesticide residues. In case of yard long bean, out of 15 samples from each surveyed area, pesticide residues with above MRL was identified and quantified in 3 samples from each region. No pesticide residue was found from remnant samples. The findings of this present study will provide an insight towards devising a potential strategy of food safety for regulatory authorities.

CHAPTER VI

REFERENCES

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