DETERMINATION OF MULTIPLE PESTICIDE RESIDUES IN COUNTRY BEAN COLLECTED FROM SUPERVISED FIELD TRIAL

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CERTIFICATE

This is to certify that the thesis entitled **“DETERMINATION OF MULTIPLE PESTICIDE RESIDUES IN COUNTRY BEAN COLLECTED FROM SUPERVISED FIELD TRIAL”** submitted to the Department of Entomology, 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 **ENTOMOLOGY**, embodies the result of a piece of bona fide research work carried out by **ROZINA KHANOM**, Registration No. **10-04169** 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.

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Dhaka, Bangladesh **The Author**

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Abstract

The study was conducted to detect and quantify the residue of quinalphos, chloropyrifos, fenitrothion and diazinon in country bean and to compare the detected residues with the Maximum Residue Limit (MRL) using Quick, Easy, Cheap, Effective, Rugged and Safe extraction technique and Gas Chromatography coupled with Flame Thermionized Detector. 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.

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CHAPTER 1

INTRODUCTION

The country bean, *Dolichos lablab* L. is an important winter vegetable crop in Bangladesh. It belongs to the family Fabaceae (Thompson, 1951). Though it is commonly known as country bean in Bangladesh, but it has different names at different regions of the country like Sheem, Uri, Ushi, Deshi Sheem etc. Internationally the crop has also different names e.g. Hyacinth bean, Bonavist bean, Dolichos bean, Indian bean, Egyotian kidney bean, Lima bean, Faba bean etc. (Jadhav *et al*., 1987). It is remarkable that bean crops show a broad range of adaptation to the most varied climatic conditions within a wide range of geographic area that extends from around 50^{o} north latitude to 32^{o} south latitude.

Country bean is a big source of essential vitamins and generally cultivated in Rabi seasons usually around the homestead by trailing its vine either on trees or by providing different kinds of supports. Although beans are assessed as the major group of vegetables grown intensively in Rabi seasons, some varieties of country bean can be grown year round including kharif seasons. The importance of country bean has thus highly significance from growing season point of view (Hossain and Awrangzeb, 1992).

The major vegetables grown in rabi season are various kinds of beans, which appear as one of the most important group of vegetables in our daily food. Country bean plays a significant role in the agro-economy and national health of Bangladesh. The crop has multipurpose functions. Consumers use its young pods and tender beans as vegetables (Purseglove, 1968). As a split pulse (*dhal*) and in various curry preparation, the ripe and dried seeds are used. It is also used as a popular fodder crop. To produce "hay and silage' as animal feed, its foliar portion is used. Country bean has a unique ability to fix atmospheric nitrogen to the soil through rhizobial symbiosis at root zone which enhance soil fertility as a green manure crop. Its mature dried stems are used as fuel and provide more or less an opportunity to combat fuel crisis. It has high digestibility and free from flatulent effects. Its green pods and mature seeds are good source (25%) of protein (on dry basis), vitamins (e.g. vitamin A, vitamin C, riboflavin etc.) and mineral such as magnesium, calcium, phosphorous, potassium, iron, sulfur and sodium (Deka and Sarker, 1990; Newaz, 1992). So it is an important source of cash income as well as alleviating malnutrition and sickness caused as by dietary deficiencies.

In spite of being a prospective crop, high rate of insect pests infestation is responsible to its low yield and poor quality. Farmers face significant yield loss of beans every year due to severe attack of various insect pests in our country. Generally, insect pests cause enormous quantity of yield losses in every season. Though, regular statistical records are not kept, as per conservative estimate the yield loss in country bean due to insect pests is reported to be about 12-30% (Hossain, 1990). Alam (1969) observed that country bean is attacked by nine different insect species and one species of mite. Among these species, four species are regarded as major pests and the rest of them as minor pest. 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 (1979) and Bakar *et al*. (1980) found *M. testulalis* G. is a serious insect pest of leguminous vegetables. Butani and Jotwani (1984) found aphid as the most common pest all over the world and lepidopterous larvae of *M. testulalis* (Geyer) as pests of bean causing damage by boring tender of mature pods. Bean pod borer is able to establish itself on legumes from vegetative to reproductive stage. Aphid is responsible for feeding damage and transmission of plant virus (Kennedy, 1976).

Due to plant pests and diseases 20 to 40 percent of the crop yields are reduced globally (FAO, 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 (McIntyre, 1989; Hajslova and Zrostlikova, 2003; Fenik, *et al.,* 2011). These negative impacts of pesticides are increasing day by day in order to increase the uses of pesticides.

Besides, now a day's food safety is a major concern to the consumers. But the percentage of food containing pesticide residues has increased in the last 10 years (PAN Europe, 2014). In order to ensure the supply of safe food, pesticides should be used following Good Agricultural Practices (GAP). Monitoring of pesticide residues is the essential tool to ensure GAP. To monitor pesticide residues nationally in the commercial produce, reliable multi-residue analytical methods are required. Multi-residue methods, which allow the quantification of residues of different analytes at the same time in a single run, are used advantageously for monitoring purposes.

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, 1990). 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.

Begum (1993) found that the management of insect pests of country bean required 2-3 times spray with several organophosphorus and pyrethroids insecticides. But the farmers, in general, do not follow such need based practice. In this situation for vegetable crops, like country beans, which are harvested and consumed as young pods, the use of integration of some control methods or IPM is more desirable, because the time between insecticide application and harvest of young pods may not be enough for breakdown and detoxification of the applied insecticide residues.

Jessore is one of the dominant country bean growing areas in Bangladesh. It has been documented that a considerable number of farmers of greater Jessore region of Bangladesh spray insecticides on country bean every or every alternate day and thus on an average 80 times sprays are applied in a single season (Kabir *et al*., 1996). However, it is not documented how much active material could be left in edible part of the country bean after washing and cooking. Nevertheless, it may be assumed that the use of commonly available insecticides on the country bean would widen the possibilities of consumers to be in toxicated.

It is obvious that modern insecticides are highly toxic to every organism of the animal kingdom including human beings. Even in a well-managed condition spillover of spray materials on soil, water and other abiotic materials is highly likely to cause pollution. Transformation and volatilization of such materials cause residue load in the environment (Spencer *et al*., 1973). It is evident that the more persistent pesticide, the greater is the danger to the environment. Although it is not well documented but insecticide residues hamper microbial activities in soil, destroy aquatic lives and non-target invertebrates and cause enormous damage to higher vertebrates (Ware and Roan, 1970; Duggan and Duggan, 1973; Edwards, 1974).

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) that should not be exceeded for a food item to be considered safe for consumption (MacIntyre *et al*., 1989). In Bangladesh, since harvesting and selling of vegetables are done without perplexing for the pre-harvest interval, pesticide residue levels in such country bean would undoubtedly be above Maximum Residue Limit. In Bangladesh, most of the country bean 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. The whimsical spray of insecticides and selling of vegetables especially country bean after 1-2 days of spray application are assumed to be a normal practice (Kabir *et al*., 1996). No insecticide is available in Bangladesh who’s with holding period is less than 3-5 days. It has been reported that consumption of low level insecticide residue containing food products over time might cause cancer, teratogenesis, genetic damage and suppression of the immune system (MacIntyre *et al*., 1989).

Pesticide being toxic can become a potential hazard to the manufacturers, the users, the public at large and the environment. Pesticide can produce negative impacts, both socially and economically (Antle and Pingali, 1994). Extensive use of pesticides has resulted in contamination of vital supplies, air, water, and food, the risk to humans may be short term as well as long term depending on the persistence of the pesticide and the exposure period.

Pesticide residue in food has become a consumer safety issue and the consumer has the right to know how much pesticide get incorporated in the food he eats. The detection, identification and quantification of pesticide in the food we eat are a problem of increasing public interest. But still a few references are available on pesticide residues present in vegetables and also the withholding period or pre-harvest interval (PHI) of major vegetables grown in Bangladesh.

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. However, the extent and rate of dissipation depends on the nature of the pesticide, crop, cultural practices and various environmental conditions under which the crop is grown or a treated commodity is stored (Handa *et al.,* 1999). 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. The treated fruits and vegetables are picked/ harvested without taking into account the withholding period.

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

OBJECTIVES

- To quantify the level of selected pesticide residues (mg/kg) in country bean at different days after spray.
- To determine the Pre-harvest interval (PHI) of the selected pesticides in country bean for the supply of safe food.
- To compare the level of selected pesticide residues (mg/kg) present in country bean with the Maximum Residue Limits (MRLs).

CHAPTER 2

REVIEW OF LITERATURE

To up-date our knowledge regarding the current status of research and information on the insecticide residues remain in farm gate and market samples of country bean, the level of 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. The studies on the quantification of detected insecticides residues below or above the Maximum Residue Limit (MRL) of country bean in Bangladesh are rarely reported. With this background, the information collected from different sources have been reviewed and presented below:

Park *et al.* (2016) analyzed a total of 230 pesticide residues in 8496 samples of leafy vegetables (e.g.brassica lee 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).

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

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

Prodhan *et al.* (2016) also found 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.

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 100μg/L. The method limit of detection (LOD) and limit of quantification (LOQ) ranged from 3.0 to 7.6μg/L and from 4.9 to 26μg/L, respectively. The method developed was adequate for the determination of 74 pesticide residues in orange juice.

A research was conducted by Rai *et al.* (2016) 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.

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

Hossain *et al.* (2015) collected and analyzed 10 Brinjal samples from Bogra district and found that one Brinjal sample was contaminated with Diazinon and the level of detected Diazinon was 0.32 mg/Kg. Diazinon was also detected in one Cucumber sample CS-5 (0.18 ppm) among the ten Cucumber samples and one Tomato sample (TS-3 at 0.57 ppm) among five Tomato samples. Chlorpyriphos was being found in one Bringal sample (BS-7 at 0.4 ppm). It was also detected in three Cucumber samples among ten Cucumber samples. The IDs of Chlorpyriphos positive samples were CS-2 (0.05 ppm), CS-7 (0.02 ppm).

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

Satpathy *et al.* (2014) conducted a research on the ''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 Quick Easy Cheap

Effective Rugged and Safe (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 use a mixture of 82 pesticides amenable to gas chromatography (GC) was quantitatively recovered from spiked grapes and pomegranate and determined using gas chromatography tandem mass spectrometry (GC-MS/MS). The method they employed involved initial extraction in a water/ethyl acetate system, an extraction/partitioning step after the addition of salt, and a cleanup step utilizing dispersive solid-phase extraction (d-SPE); which ensured that it was a rapid, simple and cost-effective procedure. Their method setup was streamlined with the new software approach of Compound Based Scanning (CBS). 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 attained adequate pesticide quantification and identity confirmation, even at the lowest concentration levels, considering the high signal-to-noise ratios, the very good accuracies and precisions, as well as the good matches between the observed ion ratios. They found the mean recoveries mostly ranged between 70 and 110 % (91% on average), and RSD were generally below 12% (7.3% on average). The use of analyte Protestants during GC analysis was demonstrated to provide a good alternative to the use of matrix-matched standards to minimize matrix-effect related errors. 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 the ‘‘Detection and quantification of pesticide residues in selected vegetables of Bangladesh’’ in intensive vegetable growing area in the Narsingdi district of Bangladesh regarding pesticides used by farmers on three major vegetables like eggplant, cauliflower, and country bean. On the basis of questionnaires, under their supervision 23 farmers were interviewed and they noted that fourteen pesticides belonging to different groups were found to be commonly used on the selected vegetables by the respondent farmers to control the major pests. 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 collected 42 samples from fields and markets and operate multiple pesticide residue analysis by 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) called for a research entitled ‘‘Organophosphorus pesticide residues in vegetables and soil samples from alau dam and gongulong agricultural areas, Borno State, Nigeria’’. They observed concentrations of organophosphorus pesticide residues (dichlorvos, diazinon, chlorpyrifos, and fenitrothion) in some vegetables (spinach,lettuce, cabbage, tomato and onion) in Borno State, Nigeria. 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 in the Alau Dam and Gongulong agricultural areas were observed in the leaf of tomato, while the lowest concentrations were observed in the root of spinach. The concentrations of all the organophosphorus pesticides in the vegetables and soil samples from the two agricultural areas were observed to be at alarming levels, much higher than the maximum residue limits (MRLs) and set acceptable daily intake values (ADIs) for vegetables Cordex 2009.

Islam *et al.* (2013) carried out a research work entitled “Analysis of Pesticide Residue in Vegetables Collected from Local Market by Using GC Technique” where they detect and quantify the presence of pesticide residues in Cucumber, Spinach, and Brinjal available in local market of Mymensingh sadar upazila. 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 imidachloprid residues. But sample from BAU Sesh More 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.

Milhome *et al*. (2013) conducted 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 were 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.

A research was carried out by Hossain *et al*. (2013) 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.

Neetu (2013) studied the magnitude of contamination of DDT pesticides in vegetables, pulses and cereals which were brought for sales to the consumer in the local markets of Sahibabad and Ghaziabad, 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.

Corteas *et al.* (2013) developed a method for the determination in vegetables of organophosphorus pesticides. 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) conducted 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 0.052ppm, respectively which is far above their respective MRLS set by FAO i.e. 0.05, 0.5, 0.05, 0.5, 0.4 and 0.02ppm. 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) carried out a research on the "Evaluation of QuEChERS Method for 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.

A research was conducted by Kanda *et al.* (2012) of GC analysis on 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.

Dasika *et al.* (2012) conducted a research work 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.

Tibor Cserhati and Maria Szogyi (2012) carried out a research on the ‘‘Chromatographic Determination of Pesticides in Food and Food Products’’. They operate chromatographic analysis of pesticides present in foods and food products which were collected and evaluates the results critically. They presented the employment of preconcentration and prepurification technologies, gas chromatography using ECD, NPD, MS and MS/MS detection methods, liquid chromatographic methodologies such as thin-layer chromatography, high performance liquid chromatographic methods. They mainly conducted their research on insecticides, herbicides, acaricides, organophosphorous and organochlorine compounds.

Chauhan (2012) studied the estimation of pesticide residues of five commonly used pesticides on vegetables, namely endosulfan, carbendazim, chlorpyrifos, cypermethrin and imidacloprid was done 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.

Camino-Sánchez *et al.* (2011) used the QuEChERS extraction method for sample preparation and detection was done by GC-MS-MS for the quantification of 121 pesticide residues in samples of tomato, pepper, lettuce, cucumber, eggplant, zucchini, melon, watermelon and apple acquired from Spain.

Fernandes *et al.* (2011) quantified pesticide residues in vegetables with the several extraction procedures (liquid extraction, single drop microextraction, microwave-assisted extraction, pressurized liquid extraction, supercritical fluid extraction, solid-phase extraction, solid-phase microextraction, matrix solid-phase dispersion, and stir bar sorptive extraction). A combination of techniques reported the use of new extraction methods and chromatography to provide better quantitative recoveries at low levels. The use of mass spectrometric detectors in combination with liquid and gas chromatography has played a vital role to solve many problems related to food safety.

Hrouzkova and Matisova (2011) carried out a research on "Fast gas chromatography and its use in pesticide residues analysis" linuron, folpet, chlordecone, prochloraz, cypermethrin and deltamethrin have the LOD values > 100 pg.ml-1 and LOQ values > 300 pg.ml-1. LODs and LOQs obtained in EI mode are at the level of ng.ml-1. For all analytes except linuron, dicofol and prochloraz, the LOQs were below 10 µg.kg-1, which is the MRL required for the pesticide residues in baby-food.

Parveen *et al*. (2011) conducted a study on the "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) conducted an experiment on the ''Monitoring of pesticide residues in some Egyptian herbs, fruits and vegetables''. They have collected 132 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%. 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) carried out a research 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.

Prodhan *et al*. (2010) conducted a study on the determination of pre harvest interval for Quinalphos, Malathion, Cypermethrin and Diazinon in major vegetables. Six 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 and 12 days after spray. In all the cases, the left over residue of tested insecticides were detected up to 10 DAS except Cypermethrin in tomato which was 7 DAS and the quantities were above MRL up to 7 DAS for Quinalphos in cabbage, Malathion in cauliflower, 5 DAS for Quinalphos in yard long bean, Cypermethrin in yard long bean, Diazinon in brinjal and 3 DAS for Cypermethrin in tomato. No residue was detected from any of the tested samples collected at 12 DAS except Cypermethrin in tomato which was 10 DAS. The PHI may be selected as 10 DAS for quinalphos in cabbage and Malathion in cauliflower; 7 DAS for Quinalphos and Cypermethrin in yard long bean and Diazinon in brinjal; 5 DAS for Cypermethrin in tomato.

Rahman *et al*. (2010) conducted a study on the multi insecticide residue analysis in environmental sample collected from different regions of Bangladesh. A total of 94 samples of vegetables (brinjal, hyacinth bean, cauliflower and yard long bean) were collected from farmers field and market of different regions like Barisal, Bogra, Chittagong, Comilla, Dhaka, Dinajpur, Gazipur, Jessore, Khagrachuri, Narsingdi, Rajshahi and Rangpur and carried out the Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur. Results revealed that among 94 samples, 46 had insecticide residues, of them 18 had above MRL. Among 38 analyzed brinjal samples, 18 samples contained residue of Cypermethrin, Diazinon and Quinalphos; and only 8 had above MRL. Cypermethrin is the frequent one which was found in 14 samples. Of 24 analyzed hyacinth bean samples, 15 had the residue of Cypermethrin, Diazinon, Quinalphos, Fenotrothion and Malathion; and 7 had above MRL. Among the 16 analyzed cauliflower samples, 5 samples contained residue of Cypermethrin, Diazinon, Quinalphos and Malathion; and only 2 had above MRL. Of 16 yard long bean samples, 8 samples contained residue of Cypermethrin, Diazinon, Quinalphos and Malathion; and only 2 had above MRL.

Schreiber and Wittrig (2010) conducted an experiment 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 was 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.

Charan and Sharma (2010) collected 182 samples of six vegetables 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.

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

Afful *et al.* (2010) carried out a study on "Gas Chromatographic Methodology for the Determination of Some Halogenated Pesticides" where gas chromatography (GC) methodology has been validated for the determination of some halogenated pesticides. Complete separation of the pesticide prepared in ethyl acetate was achieved on Rtx - 1 column with dimension, 30m x 0.25mm x 0.25:m. The GC equipped with electron capture detector was run using column temperature programmed from 80ºC (2 min) to 200ºC (15 min) at the rate of 4ºC/min giving a total analysis time of 47 min. The detector and injector were respectively at temperatures of 300 and 225ºC. The method was validated with respect to precision in terms of reproducibility of retention times and peak heights, linearity and minimum detectable quantity of the pesticides. Under the operated GC conditions, diuron eluted first while heptachlor epoxide was the last to elute. The chromatographic detector was more sensitive to endosulfan and endosulfan with Minimum Detectable Quantity (MDQ) of 0.002 ng. The detector was however, less sensitive to captan with MDQ of 0.08 ng. Margins of errors associated with the precision of the method in terms of reproducibility of 11 retention times yielded standard deviation in the range of 0.026-0.063.

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

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

Islam *et al.* (2009) analyzed several pesticide residues in country bean by high performance liquid chromatography. Country bean sprayed with 4 different pesticides (diazinon, malathion, chlorpyrifos and cypermethrin) at recommended dose and double of recommended dose were analyzed for their residual contents. Samples were collected at same day after application of pesticide. Commercial samples of country beans were collected from different markets of Dhaka city. Reversed-phase HPLC system with UV detection was used for the separation, identification and quantification of all this analytes using acetonitrile-water (70:30, v/v) as mobile phase.

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

Kabir *et al.* (2008) conducted a study on the determination of residue of Diazinon and Carbosulfan in brinjal and Quinalphos in bean and comparison between the detected residue levels with maximum residue level (MRL) set by FAO in 1970. They reported that Diazinon residue was found upto 6 days after spray (DAS) and upto 3 DAS. The level of residue was above the MRL. Carbosulfan residue was detected till 7 DAS and the detected quantity of residue was above MRL upto 3 DAS. Left over residue of Quinalphos in bean sample was detected upto 6 DAS and upto 4 DAS the level of residue was above the MRL.

Nguyen *et al.* (2008) undertook a research on the “Multi-residue Determination of 156 Pesticide in Watermelon by Dispersive Solid Phase Extraction and Gas Chromatography/Mass spectrometry” for a simultaneous determination of 156 pesticides in watermelon collected from market. They adopted gas chromatography with electron impact mass spectrometric detection in the selected ion monitoring mode. They found the limit of quantifications (LOQs) for most compound was below 0.005mg/kg.

Ochiai *et al.* (2008) has been developed a multi-residue method to determine 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) GCMS. Pre-extraction with methanol and dilution with water prior to SBSE (60 min) were performed. Dilution of methanol extract for SBSE was examined to obtain high sensitivity and to compensate the effect of adsorption to the glass wall of extraction vessel and to sample matrix for the compounds with high log Ko/w values (e.g. pyrethroid). The methanol extracts were diluted twofold and fivefold, and were simultaneously SBSE-enriched. The two stir bars were placed in a single glass thermal desorption liner and were simultaneously desorbed. The versatility of the method was exhibited by its good linearity (4-100 µg/kg, r2 0.9900) for 66 pesticides and limit of detection (LOD: < 5 µg/kg) for most of the analytes.

Butler *et al.* (2008) conducted a study to determine pesticide residue in vegetables by a new sample preparation method, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), and published recently as AOAC Method 2007.01.1 The sample preparation is shortened by using a single step buffered acetonitrile (MeCN) extraction and liquid-liquid partitioning from water in the sample by salting out with sodium acetate and magnesium sulfate (MgSO4).1 This technical note describes the application of the QuEChERS sample preparation procedure to analysis of pesticide residues in a lettuce matrix using gas chromatography/mass spectrometry (GC/MS) on the Thermo Scientific TRACE GC Ultr and Thermo Scientific DSQ single quadrupole mass spectrometer. Thermo Scientific Quan Lab Forms 2.5 software was used for data review and reporting. The MeCN extract is solvent exchanged to hexane/acetone for splitless injection with detection by electron ionization and selected ion monitoring (SIM). A calibration curve was constructed in iceberg lettuce and then the precision and accuracy of the analytical method were tested by preparing matrix spikes at 5 ng/g and 50 ng/g.

Fenoll *et al.* (2007) was developed an analytical multi-residue method for the simultaneous determination of various classes of pesticides in vegetables, pepper and tomato. Final determination was made by gas chromatography with nitrogen–phosphorus detection.

Ferrer *et al.* (2005) has been developed a new multi-residue methodology using liquid chromatography–time-of-flight mass spectrometry (LC TOF-MS) for the quantitative (routine) analysis of 15 pesticide residues. The analytical performance of the method was evaluated for different types of fruit and vegetables; pepper, broccoli, tomato, orange, lemon, apple and melon. The accurate mass measurements were compared in different matrices at significantly different concentration levels (from 0.01 to 0.5 mg/kg) obtaining accuracy errors lower than 2 ppm, which is well within the accepted limits for elemental confirmation. Instrumental limits of detection (LOD) were between 0.0005 and 0.03 mg/kg depending on the commodity and pesticide studied, all being within European Union regulations for food monitoring program. Finally, the methodology was applied to the analysis of two samples from an inter-laboratory exercise.

Ortelli *et al.* (2004) hold a research on the "Multi-residue analysis of 74 pesticides in fruits and vegetables by liquid chromatography-electrospray-tandem mass spectrometry" where they adopted liquid chromatography, electrospray ionization and tandem mass spectrometry for 2500 samples and they found that more than 30% of sample contained multiple pesticide residues, 12 different pesticide in grape sample but all concentrations found were below MRLs.

Pesticide residue detection methods from food matrices mainly involve two preparation steps prior to the identification and quantification of pesticides: Extraction of target analytes from the bulk of the matrices and partitioning of the residues in an immiscible solvent and or clean up of the analytes from the matrix co-.extractives. Complex samples like meat and meat products need two step clean-up which combines different chromatographic techniques (Rimkus *et al.,* 1996).

To control pests, chemical substances have been used by human from the beginning of agriculture. Now they are extensively applied on fruits, vegetables and other crops on a massive scale. The hazards of these pesticides in the form of toxic residues may possibly reduce if they used in accordance with Good Agriculture Practice. Through regular monitoring procedures, the data about the contamination levels of noxious residues occurring in foodstuffs could be obtained. Monitoring surveys regarding pesticide residues are not only helpful into providing the data, but serve to point out whether or not the ethics of good agriculture practice are being followed as well. In numerous countries of the globe analytical laboratories have ascertained to examine the levels of pesticide residues in vegetables, fruits and supplementary foodstuffs (Dogheim *et al.,* 1999; Dogheim *et al.,* 2001). Nowadays, consumers as well as legislators mutually have shown curiosity greater than before in the protection of food stuffs from residues of pesticides.

Colume *et al.* (2001) observed that Maximum Residue Levels (MRLs) are not exceeded if pesticides are applied according to appropriate agricultural techniques, but unconscious applications may lead to harmful remnants containing environmental pollution and possible health risks. Reductions frequently made in Maximum Remnant Levels (MRLs) accepted by the international institutions like EU and EPA and determination of levels by urgently creating purposive multi-residue methods are dramatical changes.

Gamon *et al.* (2001) determined the Pesticide residues in fruit and vegetables by gas chromatography/tandem mass spectrometry (GC/MS/MS). Electron impact (EI)/MS/MS and chemical ionization (CI)/MS/MS were developed for 80 compounds, including organochlorine, organophosphorus, organonitrogen, and pyrethroids, providing unambiguous spectral confirmation for these complex matrixes. Residues were extracted from samples with acetone followed by a mixture of dichloromethane petroleum ether. Two injections per sample were required for analysis of the entire pesticide list by EI/MS/MS and CI/MS/MS. Initial steps involving cleanup and concentration of extracts were eliminated. The excellent selectivity and good linearity allowed quantification and identification of low levels of pesticides in the most difficult matrixes. The method has been used for routine analysis of many vegetables.

Kumar and Hosmani (2001d) conducted a research work on “Magnitude of the residue of carbofuran and 3-hydroxy carbofuran in/on 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 at maximum GAP rate of 2 kg AI./ha. 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. The grain was then separated from the straw by beaten on a wooden plank and analyzed. Carbofuran residue was 0.16 mg/kg.

Gamon *et al.* (2001) carried out a research on “Multiresidue Determination of Pesticides in Fruit and Vegetables by Gas Chromatography” where they determined the Pesticide residues in fruit and vegetables by gas chromatography/tandem mass spectrometry (GC/MS/MS). Electron impact (EI)/MS/MS and chemical ionization (CI)/MS/MS were 15 developed for 80 compounds, including organochlorine, organophosphorus, organonitrogen, and pyrethroids, providing unambiguous spectral confirmation for these complex matrixes. Residues were extracted from samples with acetone followed by a mixture of dichloromethane petroleum ether. Two injections per sample were required for analysis of the entire pesticide list by EI/MS/MS and CI/MS/MS. Initial steps involving cleanup and concentration of extracts were eliminated. The excellent selectivity and good linearity allowed quantification and identification of low levels of pesticides in the most difficult matrixes.

The most common techniques in modern multi-residue target pesticide analysis are gas chromatography, liquid chromatography coupled to mass spectrometry (GC-MS, LC-MS) and/or tandem mass spectrometry (GC-MS/MS, LC-MS/MS) with triple quadrupole mass analysers. The numerous methods available for pesticide analysis show the importance of this application and rapid pace of developments in analytical chemistry. For example, Aguera *et al.* (2000) described a method (Splitless large-volume GC-MS injection for the analysis of organophosphorus and organochlorine pesticides in vegetables using a miniaturised ethyl acetate extraction) for the measurement of only ten organophosphorus and organochlorine pesticides by GC-MS, but over the past decade, the number of pesticides typically included in methods has increased dramatically. The sample preparation techniques have also advanced to complement the analytical techniques depending on the types of analytes and matrices monitored.

Lehotay (2000) investigated 22 diverse pesticide residues in green bean, and carrot extracts by bench top gas chromatography. The targeted pesticides, some of which were incurred in the samples, included chlorpyrifos, azinphos-methyl, parathion-methyl, diazinon, terbufos, p,p'- 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). The analytical DSI method entailed the following steps: (i) blend 30 g sample with 60 mL acetonitrile for 1 min in a centrifuge bottle; (2) add 6 g NaCl and blend 30 s; (3) centrifuge for 1-2 min; (4) add 5 mL upper layer to 1 g anhydrous MgSO4 in a vial; and (5) analyze 11 NL extract, using DSI/GC/MS-MS. Sample cleanup is not needed because GC/MS-MS is exceptionally selective for the targeted analytes, and nonvolatile extracted matrix components do not contaminate the injector or the GC/MS-MS system. Average recoveries of the pesticides were 103 s 7% with relative standard deviations of 14 t 5% on average average and limits of detection were <2 ng/g for nearly all pesticides studied.

Dogheim *et al.* (1999) monitored some Egyptian fruits and vegetables for the residues of organophosphorous, dithiocarbamtes and few synthetic pyrethroid pesticides commonly used in Egypt, in addition to those organochlorines pesticides which had been termed as persistent and prohibited to use on foodstuffs several years ago. From 8 local markets, total of 397 fruit and vegetable samples were collected and analyzed for 52 pesticides. Out of total 397 samples, there were 42.8% were found positive and contained residues at detectable levels, out of which 1.76% found above to the MRLs. Residues of organochlorine pesticides were not found in most of the samples. Cauliflower, guava and 16 onion samples were found free from any pesticide residues otherwise among all samples, 65 grape samples contained 11 pesticide residues, 22 samples of strawberry contained 10 pesticide residues and 62 samples of tomato contained 13 different pesticide residues. The most frequently detected pesticide was dithiocarbamtes. Out of 98 samples residues of dithiocarbamtes were found in 70.4% and only one sample of grape contained residues above to the MRLs. Samples of eggplant and carrot were found contaminated by the trace amounts of residues of pp'-DDT and pp'-DDE pesticides.

FAO/WHO (1999) reported that metabolism of the Malathion in animal and plants are qualitatively similar. Malathion is hydrolyzed to mono and di-carboxylic acids and these metabolites are further degraded and incorporated into animal and plants constituents. A major quantitative difference is that no parent compound for primary metabolite was detected in animal tissues, eggs or milks, whereas in plants Malathion was the main residue with up to 12.8% of the TRR representing its monocarboxylic metabolite. In fifteen trials on potatoes in Michigan, California, Idaho, New York and Nebraska at 2x1.74 kg a.i/ha (GAP was 0.8- 3.3kg a.i/ha), Malathion residues were 0 day <0.01 (14) and 0.02 mg/kg.

Salwa *et al.* (1999) undertook a research to monitor pesticide residues in Egyptian fruits and vegetables during 1995. Organophosphorus, dithiocarbamates and some synthetic pyrethroids pesticidcs, which were commonly used in Egypt for pest control, were monitored, as well as persistent organochiorines, which had been prohibited from use several years ago. Fruit and vegetable samples (397) were collected from 8 local markets and examined for 52 active ingredients. 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 free from pesticides residues. Samples of carrot, and eggplant contained trace amounts of p, p'-DDT and p, p'-DDE residues. But in general, residues of DDT and HCH have disappeared almost completely from vegetables and fruits. Use of these pesticides in Egypt was completely prohibited by law in 1987.

Ahuja *et al*. (1998) reported that cauliflowers, cabbages, tomatoes, brinjal, okras, beans and cucumbers were monitored for residues of GCH and its isomers, Endosulfan, Dimethoate, Monocrotophos, Quinalphos, Fenvalerate, and Cypermethrin. The residues of alpha, beta 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 beans were found to persist over the prescribed maximum residue limit values

During a research study, residues of organochlorine and organophosphorous pesticides have been determined in food collected from Egyptian local markets (Dogheim *et al.,* 1996). They examined some citrus fruits, potatoes and fish for the presence of organochlorine and organophosphorous pesticide residues which were gathered from the local markets of Egypt. Compliant with Maximum Residue Limits (MRLs) the residues of γ- hexachlorocyclohexane were found above in eight (8) samples of potatoes and in two (2) samples DDT also found exceeded the limits. The presence of fenitrothion in potatoes with the highest residue levels (3.8 ppm) might be as a result of its repeated pre and post harvest use. All organochlorine pesticides were found below to their MRLs.

Dethe *et al*. (1995) conducted a study on the residues of commonly used pesticides in/on vegetables in India. Detectable levels of residues were observed in 33.3% of country bean (Endosulfan, Dimethoate and Monocrotophos), 73.3% of eggplant (Endosulfan, Cypermethrin, Fenvalerate, Quinalphos, Dimethoate and Monocrotophos), 14.3% of okra (Endosulfan), 88.9% of cabbage (Endosulfan, Fenvalerate and Dimethoate) and 100% of cauliflower (Endosulfan, Fenvalerate, Cypermethrin, Dimethoate and Monocrotophos). However, the levels of pesticide residues were lower than the maximum residue limit (MRL) prescribed.

The world health Organization (WHO) and national government authorities such as United States Department of Agriculture, the Department of Health and Human Services, the National Cancer Institute and the American Cancer Society strongly recommend that people should eat plenty of vegetables, fruits, and grain products. Scientists believe that consuming such a diet will help lower the risk of heart disease, obesity, and some cancers. Fortunately, while health experts are urging us to increase our consumption of fruits, vegetables and grains than the food production system are contaminating day by day by residual effect of insecticides (Codex, 1994).

FAO/WHO (1993) reported that the trials were conducted in Canada (4), France (1) and the USA (23) using EC, WP and GR formulations. In the US trails a GR or EC pre-planting application at 4.4 Kg a.i/ha was followed by five foliar sprays at weekly intervals with WP or EC formulations at the rate of 0.55Kg a.i/ha, the other trials were with granules at 2.25 or 10 kg a.i/ha one month after planting. No residues of Diazinon in potatoes were detectable (<0.01 mg/kg) in any of the harvested samples except in three trials where residues of 0.01 mg/kg were found.

FAO/WHO (1993) reported that seventeen supervised trials from Switzerland and the USA using EC, WP and GR formulations. In the US trials a GR or EC formulations was used pre-planting at 4.4 kg a.i/ha followed by three or four foliar sprays at weekly intervals with WP or EC formulations at 0.56 kg a.i/ha. The Swiss trial was with granules at 8.7 kg a.1/ha at the 4-6 leaf stage. Residue of Diazinon in Radish root ranged from <0.01 to 0.08 mg/kg 7.33 days after the last application.

According to Singh and Kalra (1992) eggplant fruits, leaves and soil samples were collected at 0, 1, 2, 5 and 10 days after treatment to determine the Cypermethrin residues by Gas Chromatography. The analytical methodology used involved acetone-hexane extraction, silica gel column cleanup and 63 Ni gas liquid chromatographic estimation. The minimum limits of cis- Cypermethrin and trans-Cypermethrin were 0.008 and 0.006 mg/kg. Initial deposit of Cypermethrin on fruit in 1982 was observed 0.73 mg/kg after eighty sprays at 50g a.i./ha application rate, which declined to 0.61 mg/kg one day after treatment and then became 0.08 mg/kg after 10 days. At double the application rate of insecticides resulted in correspondingly increased levels of residues. During 1983 also almost simila rate of dissipation was observed. Half-life value on eggplant fruits varied between 2.2 and 2.9 days for Cypermethrin. Eggplant leaves when sprayed at low dosage received about 2 to 6 time’s higher level of insecticides than the fruits. Cypermethrin resulted in mean initial deposit of 1.69 and 1.16 mg/kg in 1982 and after eighth spray at 50g a.i./ha application rate, which dissipated by 68% after 10 days. At 100g a.i./ha dosage, the mean initial deposit was observed to be 3.18 and 2.98 mg/kg in 1982 and 1983 after eighth spray which declined to 0.68 mg/kg after 10 days of treatment. In soil, at the minimum effective dosage, the range of mean initial deposit was observed to be 0.28- 0.35 mg/kg in 1982 and 1983. Doubling the application rate resulted in higher recovery of insecticides.

Half- life values of Cypermethrin in soil were 5.2 days. The dissipation of trans-isomer was found to be slightly faster than the cis- isomer in eggplant fruits and leaves. These isomers were lost almost at the same rate from soil.

Frank *et al.* (1990) studied that organophosphorus, synthetic pyrethroid and N-methyl carbamate insecticides and dithiocarbamate, dicarboximide and organochlorine fungicides. The estimation was done in 433 composite vegetable samples representing 16 commodities collected between 1986 and 1988 from farm deliveries to the market place Ontario, Canada. Commodities tested included eggplant, bean, asparagus, carrots celery, cole crops, cucumbers, lettuce, onions, peppers, potatoes, radishes and tomatoes. In 64% of samples, no pesticide residues were identified to the limits of detection which ranged from 0.005 to 0.05 mg/kg. These involved Diazinon and Parathion on celery and Chlarothalonil on peppers. Whereas some commodities had no detectable residues.

Abdallab *et al.* (1990) reported that pesticide residues in food are a potential hazard, which has received much attention during the past 20 years. Extensive regulatory agencies have been created in developed countries to deal with pesticide residues in food. In many developing countries acceptable quantities of pesticide residues in food (tolerances) have not been established, however the guidelines developed by Food and Agriculture Organization and the World Health Organization (FAO/WHO) are generally followed. Because of the very small quantities of pesticide, which are permitted in food, elaborate analytical procedures are required. Some pesticide are relatively stable and since a considerable amount of the applied pesticide frequently ends up in the soil and in some cases bioaccumulation can occur to an extent, which causes damage to fish or birds.

The detection and quantification of most commonly used insecticides and many herbicides and fungicides can be undertaken in an analytical laboratory having a minimum of Gas Chromatograph (GC) with choice of appropriate detectors such as Electron Capture Detector (ECD), Flame Photometric Detector (FPD), Flame Ionization Detector (FID) and Flame Thermionic Detector (FTD). Numerous minor but essential items for extraction and clean-up would be necessary back up apparatus. These include extractors, fractional distillation, adsorption chromatography, clean-up column chromatography, Gel permeation chromatography, Thin-layer chromatography, Rotary vacuum evaporators. Kuderna Danish concentrator, vacuum manifold etc. HPLC becomes a necessary major equipment item in residue analysis especially for analysis of carbamate (carbofuran, carbaryl, aldicarb etc.). Also, methods can be advantageously leveled to use GC and HPLC for other groups of compounds such as Pyrethroids. It needs to be emphasized that nuclear techniques involving Liquid Scintillation Counter (LSC), Biological Material Oxidizer (BMO) with scintillation cocktails and Linear Analyzer Provide unique systems for radio-tracer studies with radio-labeled pesticides. The uses of radio labeled pesticides, preferably C14-compounds, help in methodology validation and recovery tests. Such isotope aided studies contribute unanimously in studying environmental fate and behavior of pesticides. The present day research on development of controlled-release formulations of pesticides had become possible through such nuclear approaches. To address all relevant issues of monitoring and research on uses of pesticides in food and environment continuous development of laboratory infrastructure is essential. In view of the vast sum of money spent on pesticides and the danger foreign exchange earnings if food for export is condemned and for the safety of man and the environment, this sum of money spent on development of residue analysis infrastructure may be regarded as trivial. It may be stressed that adequately trained personnel and principle of Good Laboratory Practices (GLP) are vitally important for pesticide residue analysis laboratory which can only be gradually developed through hard work and sincere but continuous efforts. Moreover, provision for adequate supplies of residue grade solvents; specialized glass wares are routinely required for such analytical work (FAO, 1990).

Singh and Kalra (1989) stated that a study was carried out in Ludhiana, India to detemine the residues of Permethrin aplied for the control of brinjal shoot and fruit borer on fruit and leaves of eggplant and in the soil and to determine the waiting period. The compound was sprayed at a rate of 50g a.i/ha at fortnightly intervals. Initial deposits on fruits ranged from 1.3 to 0.7 mg/kg and MRL 1, 2, 3 and 10 days after spraying was 0.34, 0.2, 0.11 and 0.07 mg/kg respectively. The half-life of the insecticides on fruits ranged from 2.1 to 3.0 days. The mean initial deposit on eggplant leaves was 2.24 mg/kg; residues had dissipated by 70-80% after 10 days. Permethrin residue in soil after 8 sprays was 0.27- 0.28 mg/kg. The half- life value of Permethrin in the soil was about 3.2 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.

Devi and Manjunath (1985) found that Quinaiphos is a less persistent insecticide and dissipated on the fruits more quickly than Fenvalerate and Carbaryl. In the current study, the left over residue of Quinaiphos in the YLB sample had been detected upto 6 DAS, of which upto 4 DAS, the quantity of residue were above MRL. At the 0 DAS, the residue was 0.678 ppm and they were 0.552 ppm, 0.419 ppm, 0.306 ppm, and 0.2 14 ppm at 1 DAS, 2 DAS, 3 DAS, and 4 DAS, respectively. All these quantities were above MRL. The quantity decreased down to 0.164 ppm at 5 DAS and 0.087 ppm at 6 DAS. But Devi and Manjunath (1985) found the residue of Quinalphos (0.56 ppm) in brinjal above the tolerance level upto 10 DAS.

Awasthi *et al*. (1984) reported that residue levels of Phorate and Quinalphos were initially higher at soil depths of 0- 7.5cm and 15.0- 22.5cm than at 7.5- 15.0cm, but 60 days after application residue levels increased with soil depth as the insecticides move down the profile. Aldicarb and Carbofuran levels were highest at 0- 7.5cm depth throughout the 60 day experiment while Aldicarb was not detected below 15cm depth. Insecticides were taken up into foliage and fruits of okra, but were not detected after 45 (carbamates) or 50 (organophosphates) days.

Talekar *et al*. (1977) reported that the soil residues of DDT and Dieldrin declined 20 and 25% in the fall and winter months and there was no accumulation of residue with further application of these chemicals during spring and summer. Two organophosphates (Fonofos and Phorate) and one carbamate (Carbofuran) degraded rapidly resulting in only 8, 0.4 and 32% recoveries respectively at the end of the fall and winter seasons. Their breakdown was further accelerated during the hot, rainy, spring and summer months when their recoveries were lower. When grown in treated silt loam soil, sweet potatoes and white potatoes absorbed the residues of these chemicals and their metabolites at different levels.

CHAPTER 3

MATERIALS AND MERHODS

The study was undertaken in the experimental field of Entomology Division of Bangladesh Agricultural Research Institute (BARI). The study was performed during October 2015 to September 2016 in the experimental field and in the Pesticide Analytical Laboratory of Entomology Division of BARI

3.1 Field Experiment

A supervised field trial was conducted in the experimental field of Entomology Division of Bangladesh Agricultural Research Institute (BARI). All the activities like seeding, transplanting and all other cultural operations were undertaken and performed by the field staffs of Entomology division of BARI. Four pesticides, chlorpyrifos, quinalphos, fenitrothion and diazinon were selected for this experiment. When the fruits were at marketable size, chlorpyrifos, quinalphos, fenitrothion and diazinon were sprayed in the field by knapsac sprayer. The application rate of all of the selected pesticides was 1.5 mL/L of water. The pesticides were tank-mixed and applied to the plants according to normal commercial practices following GAP.

3.2 Sampling and sample preservation

Samples of country bean were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 days after pesticide application from the experimental field of Entomology Division of Bangladesh Agricultural Research Institute (BARI). The samples were collected in clean transparent air tight polyethylene bag and each bag was properly labeled with sample number and the date of collection. Samples were collected in individual polyethylene bag to avoid cross contamination.

All collected samples were carried to the Pesticide Analytical Laboratory, Entomology Division of Bangladesh Agricultural Research Institute (BARI), on the same sampling day. The samples were cut into small pieces and mixed properly and were stored at -20^{0}C until homogenization.

3.3 Analysis of multiple insecticide residues in country bean

Pesticide residue analysis methods mainly involve two steps. Extraction and clean-up of the target analytes from the matrix and determination of the target analytes from the matrix.

3.4 Extraction and clean-up

Nowadays the QuEChERS technique, is widely used for the extraction and clean-up of the extracts of fruit and vegetable matrices (Schenck & Hobbs 2004; Cajka *et al.,* 2005; Lehotay *et al.,* 2005; Liu *et al*., 2005; Aysal *et al*., 2007; Banerjee *et al.,* 2007; Bolanos *et al.,* 2007; Lehotay *et al.,* 2007; Mol *et al.,* 2007; Paya *et al*., 2007; Wang *et al.,* 2007; Banerjee *et al.,* 2008; Fernandez Moreno *et al.,* 2008; Hayama and Takada, 2008; Ji *et al.,* 2008; Kirchner *et al.,* 2008; Kmellar *et al.,* 2008; Kovalczuk *et al*., 2008; Kruve *et al.,* 2008; Mezcua *et al.,* 2008; Nguyen *et al.,* 2008; Romero-Gonzalez *et al*., 2008; Schenck *et al*., 2008; Walorczyk 2008; Banerjee *et al.,* 2009; Cunha *et al.,* 2009; Huskova *et al*., 2009; Li *et al.,* 2009; Schenck *et al.,* 2009; Zhang *et al.,* 2009; Gilbert-Lopez *et al.,* 2010; Lehotay, 2010; Chandrasekaran, 2012; Dasika *et al.,* 2012; Paramasivam & Chadrasekaran, 2012; Singh *et al.,* 2012; Mantzos *et al.,* 2013; Prodhan *et al.,* 2015). This technique was modified by several authors (AOAC Official methods, 2007.1; the European Committee for Standardization (CSN) Standard Method, CSN EN 15662, 2008). This technique is gaining popularity day by day compared to the other existing techniques as it has a lot of advantages. The most importants are the high analyte recoveries, the low organic solvent consumption, and the low cost per sample.

3.5 Determination of the target analytes from the matrix

Determination of the selected pesticides (Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon) was performed by Gas Chromatography (GC) with Flame Thermo Ionized Detector (FTD).

3.6 Chemicals Used in Analysis.

The standard of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon 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.

3.7 Analytical Apparatus Required

- Centrifuge machine, Model: Sigma 3k 30, Germany (Plate 1)
- Electric balance, Model: AY- 220, Shimadzu Corporation, Japan (Plate 2).
- Orbital shaker, Model: Rexmed, Sweden (Plate 3)
- Vortex mixer, Model: Maxi max ii, USA (Plate 4)
- Homogenizer (Plate 5)
- GC-2010, Shimadzu corporation, Japan (Plate 6)

Plate 1. Centrifuge Machine

Plate 2. Electric Balance

Plate 3. Orbital Shaker

Plate 4. Vortex Mixer

Plate 5. Homogenizer

Plate 6. 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 etc.

3.8 Preparation of pesticide standard solution

Pesticide standard stock solutions of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon, 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 methanol were prepared by transferring the appropriate amount from 10 mg/L intermediate mixed standard solution into ten separate 10-mL volumetric flasks. All the standard solutions were kept in a freezer at -20°C until use.

3.9 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 (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 $MgSO_4$ 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 HPLC vial for injection.

3.10 Detection and Quantification of pesticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 coupled with FTD (Flame Therm ionized Detector). The capillary column used in FTD was ATTM-1, length 30m, Inner Diameter (ID) 0.25mm and film thickness 0.25µm. Helium was used as carrier and make up gas in FTD. The instrument parameters for detecting Organophosphorus insecticides were as follows.

Table 1. 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 2. Conditions for column oven

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

Prior to injection of the sample extract, standard solutions of different concentrations were prepared and injected with the above instrument parameters. The samples were calibrated (retention time, peak area etc.) against five pointed calibration curve of standard solution of concerned pesticide. Each peak was characterized by its retention time. Sample results were expressed in mg/kg automatically by the GC software. A typical chromatogram using all of the selected insecticides injected in GC-FTD is presented in Figure 1.

3.11 Determination of Pre Harvest Interval

At first the level of residues in all of the collected samples for tested four insecticides was determined and the PHI was calculated by the following procedures. At first, the sampling day which was next following MRL was selected. That selected day was chosen as PHI, since the level of residue on that day was below MRL.

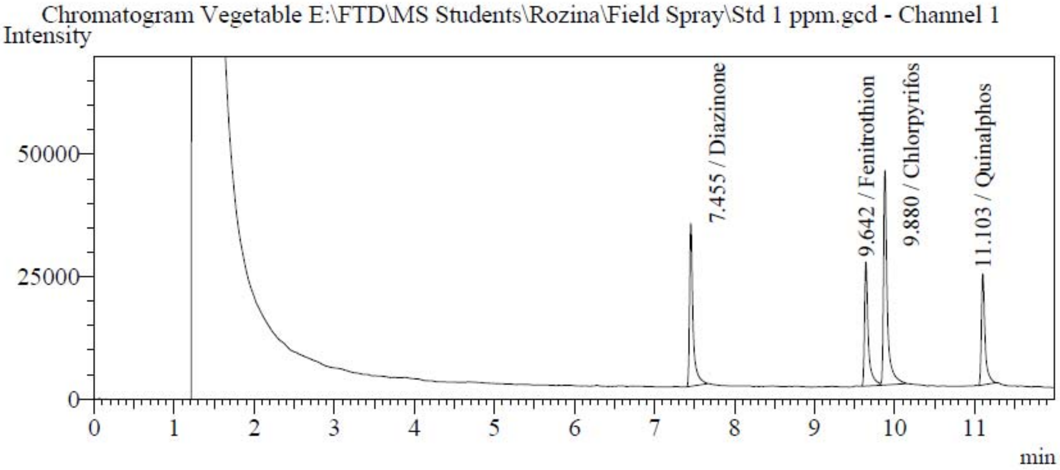
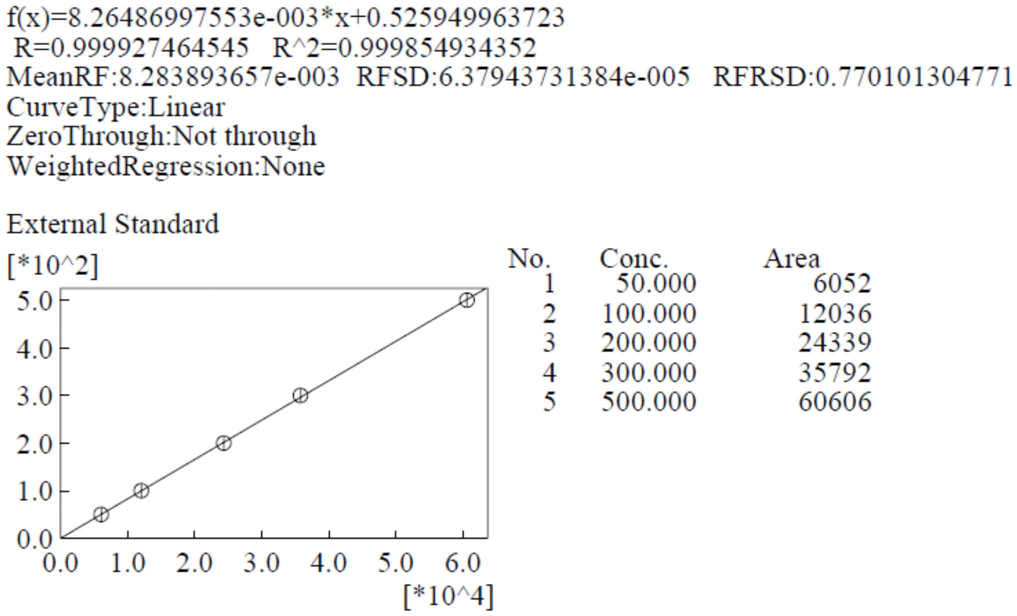


Figure 1. Chromatogram of mixed pesticide standard solutions (1 mg/L) of Diazinon, Fenitrothion, Chlorpyrifos and Quinalphos.

3.12 Preparation of Calibration Curve

Calibration curves were prepared by the use of four selected insecticide standards and analyzed in triplicate. Calibration curves were made by plotting the mean peak areas of the selected pesticides versus the respective concentrations. Linearity was evaluated by calculating the correlation coefficients, intercepts and slopes of the respective regression line at five concentration levels ranging from 0.05 to 0.5 mg/kg. Calibration curves for all the selected insecticides are shown below:

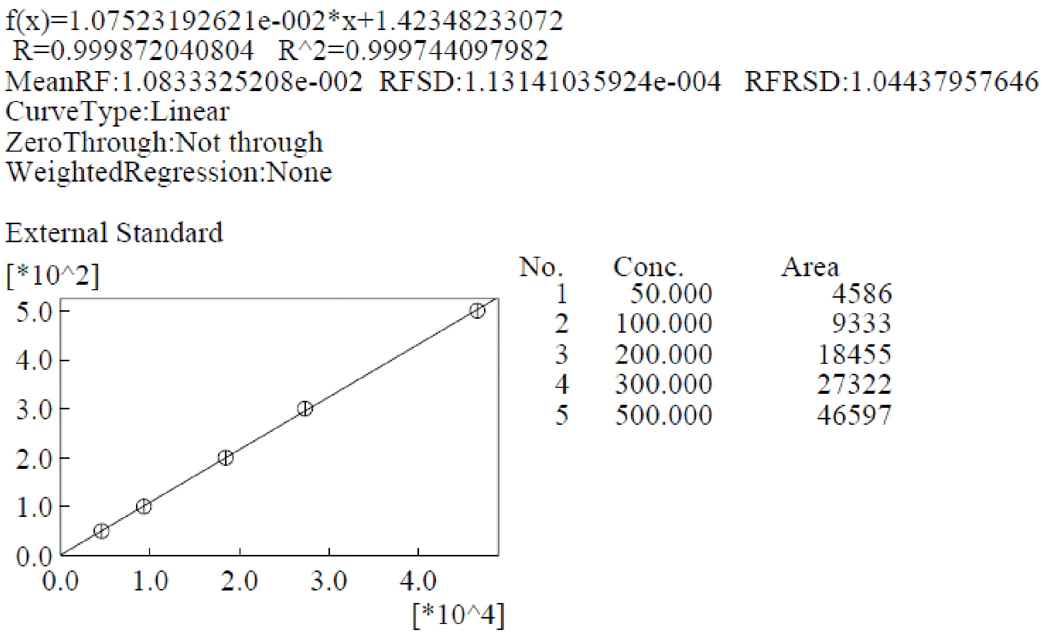


```
f(x)=8.26486997553e-003*x+0.525949963723
 R=0.999927464545   R^2=0.999854934352
MeanRF:8.283893657e-003  RFSD:6.37943731384e-005   RFRSD:0.770101304771
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard
```

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

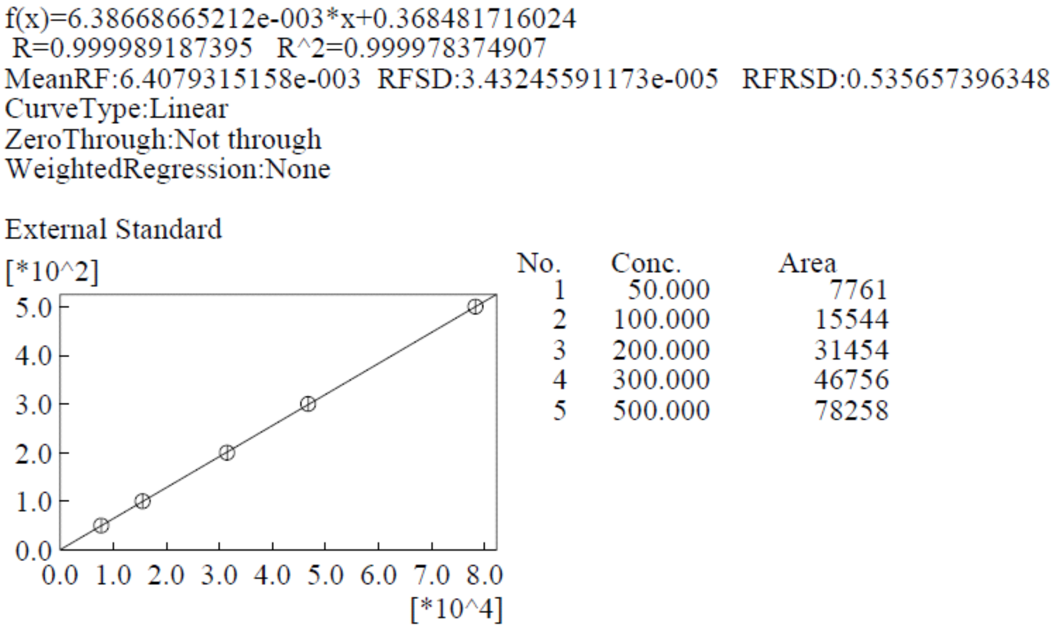
Figure 2. Calibration curve prepared for Quinalphos made with different concentrations ranging from 50µg/L to 500µg/L.



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

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

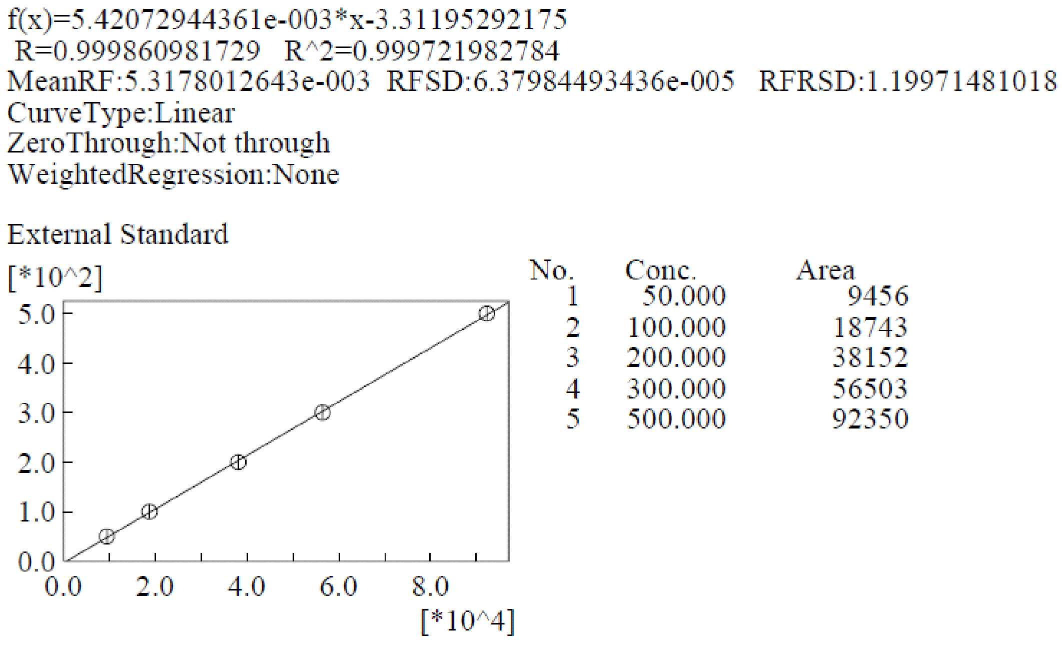
Figure 3. Calibration curve prepared for Chlorpyrifos made with different concentrations ranging from 50µg/L to 500µg/L.



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

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

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



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

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

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

CHAPTER 4

RESULTS AND DISCUSSION

The experiment was conducted on the experimental field of Entomology Division of Bangladesh Agricultural Research Institute (BARI) and in the Pesticide Analytical Laboratory, Entomology Division of BARI. The analysis was performed by Gas Chromatography (GC) with FTD detector. The chromatogram of the selected insecticides found from the injected extract of country bean samples at different days after spray are shown in Figure 6 to 16.

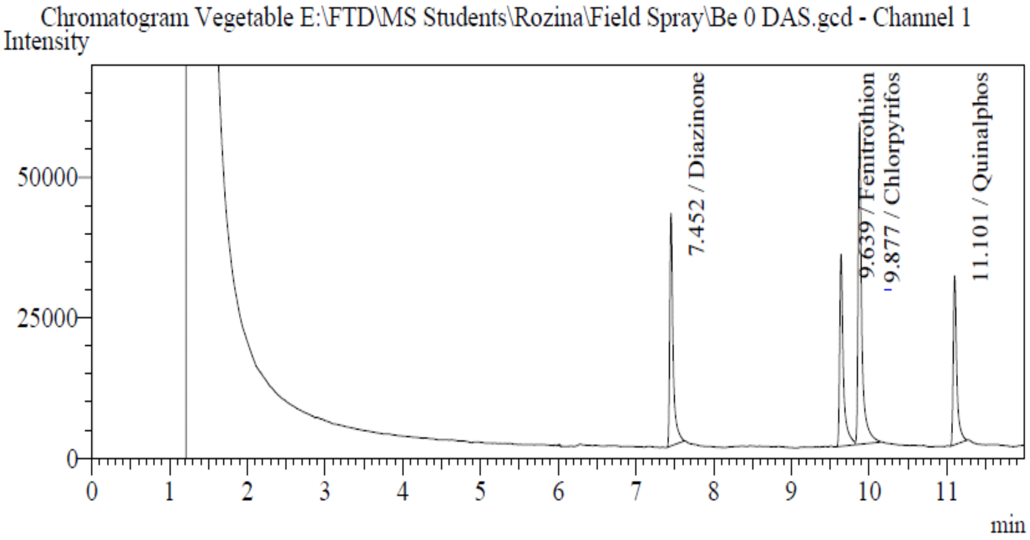


Figure 6. Chromatogram of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon obtained from country bean extract at 0 DAS.

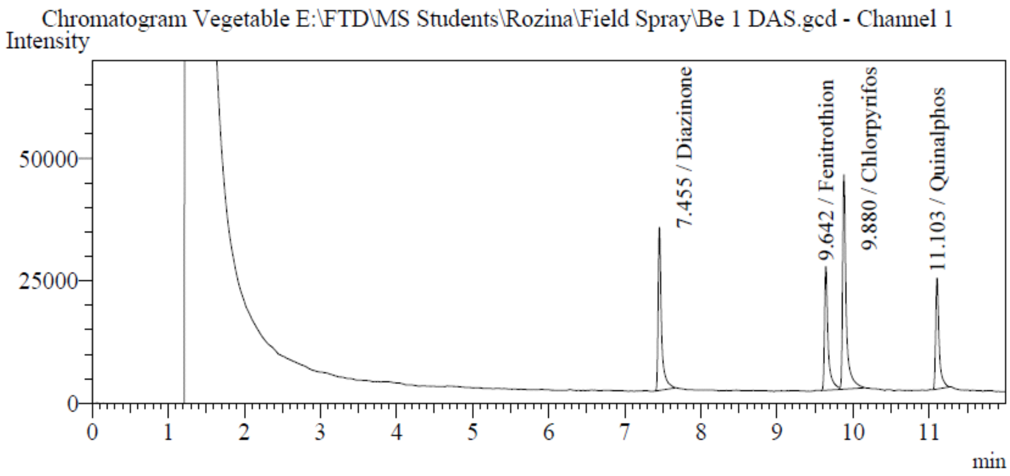


Figure 7. Chromatogram of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon obtained from country bean extract at 1 DAS.

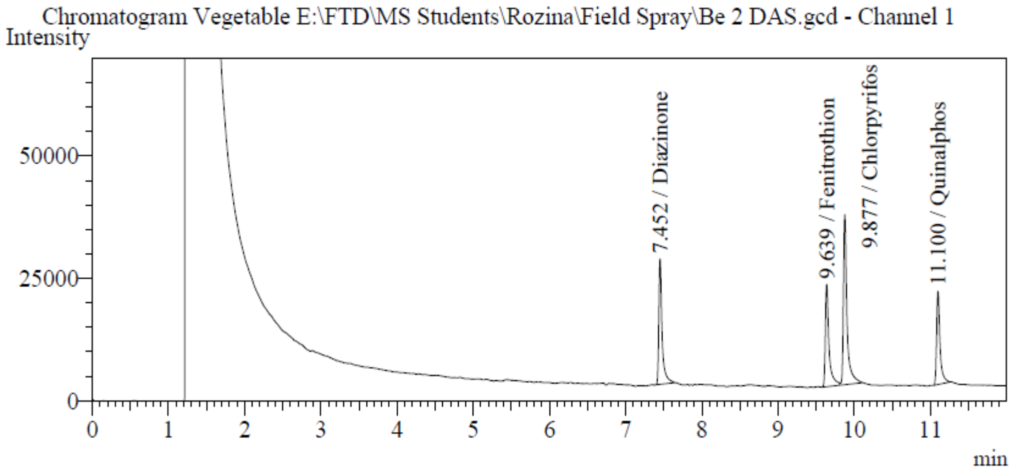


Figure 8. Chromatogram of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon obtained from country bean extract at 2 DAS.

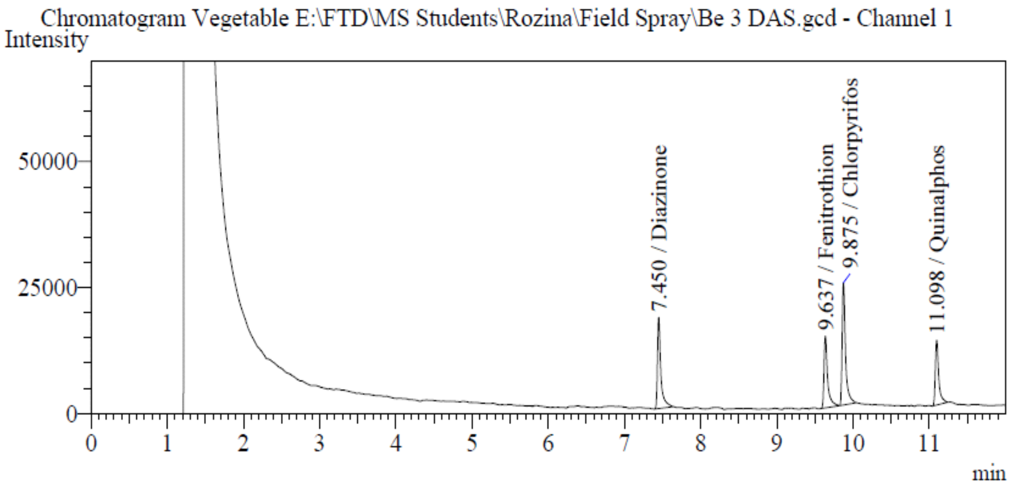


Figure 9. Chromatogram of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon obtained from country bean extract at 3 DAS.

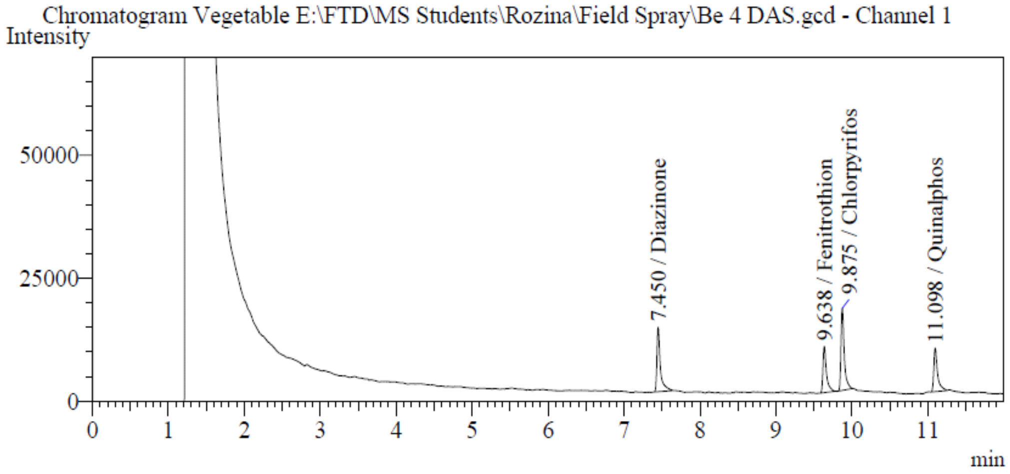


Figure 10. Chromatogram of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon obtained from country bean extract at 4 DAS.

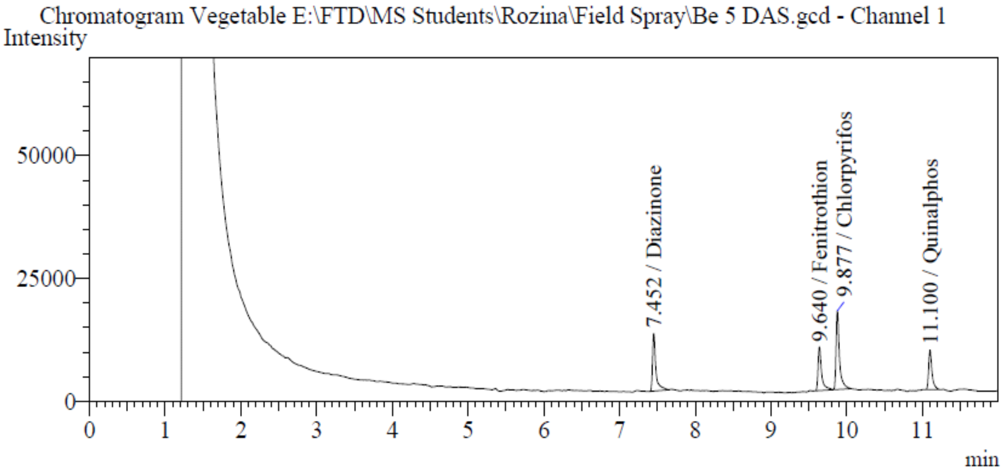


Figure 11. Chromatogram of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon obtained from country bean extract at 5 DAS.

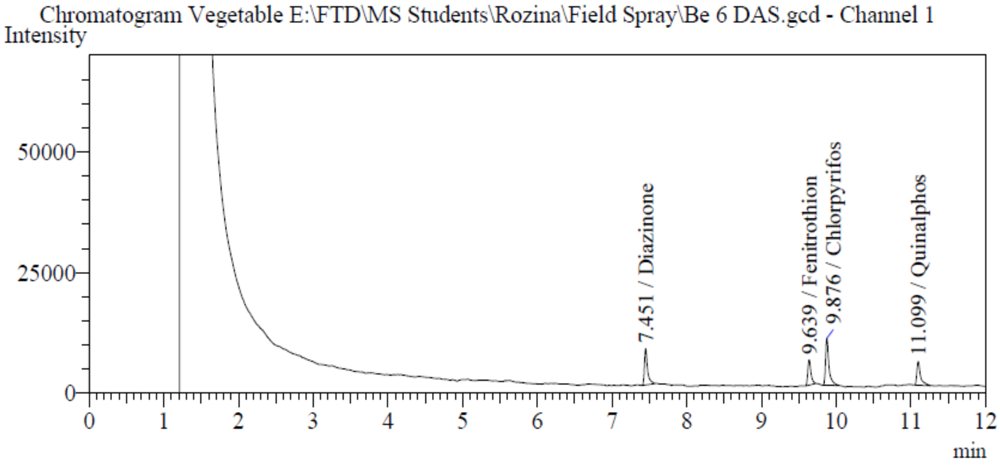


Figure 12. Chromatogram of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon obtained from country bean extract at 6 DAS.

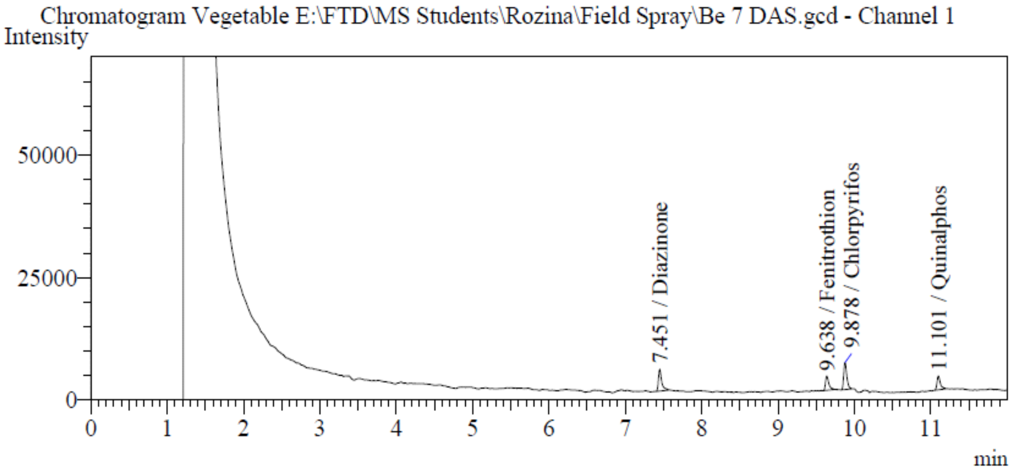


Figure 13. Chromatogram of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon obtained from country bean extract at 7 DAS.

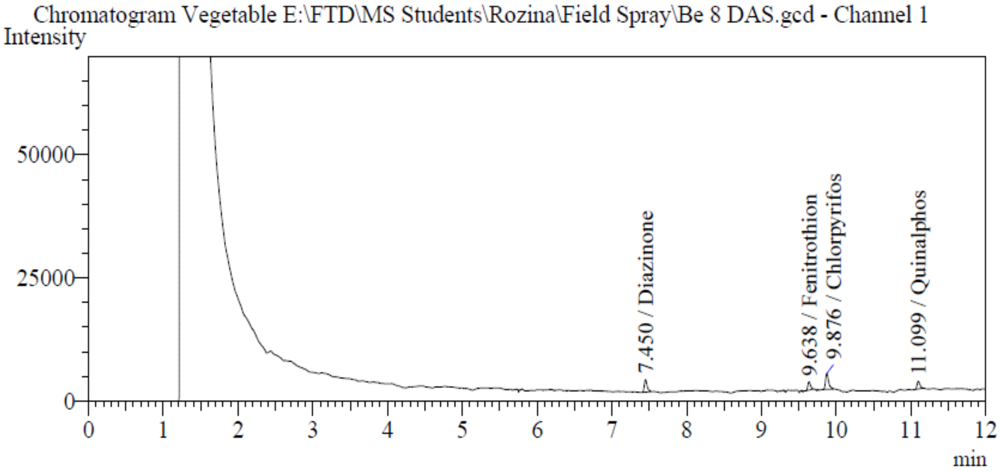


Figure 14. Chromatogram of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon obtained from country bean extract at 8 DAS.

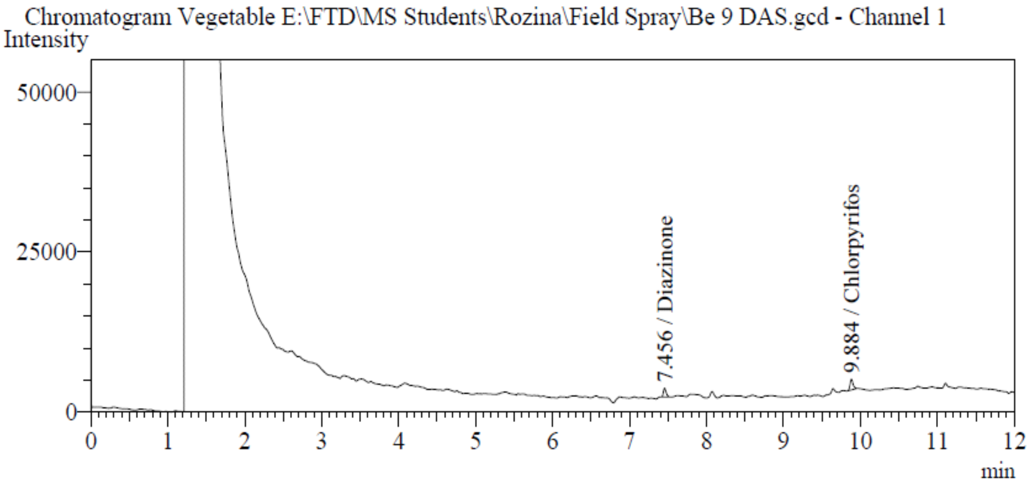


Figure 15. Chromatogram of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon obtained from country bean extract at 9 DAS.

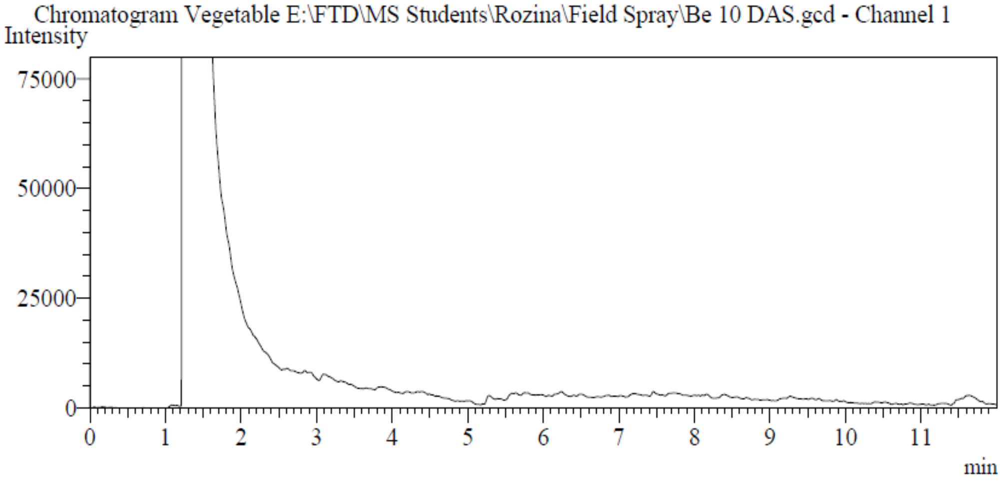


Figure 16. Chromatogram of Quinalphos, Chlorpyrifos, Fenitrothion and Diazinon obtained from country bean extract at 10 DAS.

4.1 Analytical Results

The analytical results of quinalphos, fenitrothion, chlorpyriphos and diazinon residue in country bean samples are summarized in the Table 3-6.

4.1.1 Quinalphos

The country bean sample containing quinalphos residue was analyzed using Gas Chromatography coupled with FTD. The results obtained from this analysis have been summarized in Table 3

Table 3.Level of quinalphos residue (mg/kg) estimated from the samples of country bean

Days after spraying	Total volume prepared	Injected volume (µL)	Level of Residue (mg/kg)	MRL (mg/kg)
0	10	1	0.562	0.01
1	10	1	0.426	
2	10	1	0.352	
3	10	1	0.240	
4	10	1	0.181	
5	10	1	0.146	
6	10	1	0.101	
7	10	1	0.041	
8	10	1	0.02	
9	10	1	0.009	
10	10	1	ND	
12	10	1	ND	

Residue of quinalphos in country bean was detected up to 9 DAS and the level of residues were above MRL up to 8 DAS. The detected residues were 0.562 mg/kg, 0.426 mg/kg, 0.352 mg/kg, 0.240 mg/kg, 0.181 mg/kg, 0.146 mg/kg, 0.101 mg/kg, 0.041 mg/kg and 0.02 mg/kg at 0, 1, 2, 3, 4, 5, 6, 7 and 8 DAS, respectively. Sample of 9 DAS contained 0.009 mg/kg, which were below the EU-MRL (0.01) set by European Union (European commission, 2005). No residue was detected at 10 and 12 DAS. So, the PHI of quinalphos for country bean can be selected at 9 DAS.

4.1.2 Chloropyrifos

The country bean sample containing chloropyrifos residue was analyzed using Gas Chromatography coupled with FTD. The results obtained from this analysis have been summarized in Table 4.

Table 4. Level of chlorpyrifos residue (mg/kg) estimated from the samples of country bean

Days after spraying	Total volume prepared	Injected volume (µL)	Level of Residue (mg/kg)	MRL (mg/kg)
0	10	1	0.532	0.05
1	10	1	0.409	
2	10	1	0.324	
3	10	1	0.213	
4	10	1	0.143	
5	10	1	0.118	
6	10	1	0.087	
7	10	1	0.044	
8	10	1	0.027	
9	10	1	ND	
10	10	1	ND	
12	10	1	ND	

Residue of chloropyrifos in country bean was detected up to 8 DAS and the level of residues were above MRL up to 6 DAS and these were 0.532 mg/kg, 0.409 mg/kg, 0.324 mg/kg, 0.213 mg/kg, 0.143 mg/kg, 0.118 mg/kg and 0.087 mg/kg at 0, 1, 2, 3, 4, 5and 6 DAS, respectively. Sample of 7 and 8 DAS contained 0.044 mg/kg and 0.027 mg/kg chloropyrifos residue which were below EU-MRL (European commission 2005) set by European Union (0.05). No residue was detected at 9, 10 and 12 DAS. So, the PHI of chloropyrifos for country bean can be selected at 7 DAS.

4.1.3 Fenitrothion

The country bean sample containing fenitrothion residue was analyzed using Gas Chromatography coupled with FTD. The results obtained from this analysis have been summarized in Table 5.

Table 5. Level of fenitrothion residue (mg/kg) estimated from the samples of country bean

Days after spraying	Total volume prepared	Injected volume (µL)	Level of Residue (mg/kg)	MRL (mg/kg)
0	10	1	0.488	0.01
1	10	1	0.355	
2	10	1	0.299	
3	10	1	0.201	
4	10	1	0.129	
5	10	1	0.124	
6	10	1	0.065	
7	10	1	0.018	
8	10	1	0.007	
9	10	1	ND	
10	10	1	ND	
12	10	1	ND	

Residue of fenitrothion in country bean was detected up to 8 DAS and the level of residues were above MRL up to 7 DAS and these were 0.488 mg/kg, 0.355 mg/kg, 0.299 mg/kg, 0.201 mg/kg, 0.129 mg/kg, 0.124 mg/kg 0.064 mg/kg and 0.018 mg/kg at 0, 1, 2, 3, 4, 5, 6 and 7 DAS, respectively. Sample of 8 DAS contained 0.007 mg/kg fenitrothion residue which was below EU-MRL set by European Union (0.01). No residue was detected at 9, 10 and 12 DAS. So, the PHI of fenitrothion for country bean can be selected at 8 DAS.

4.1.4 Diazinon

The country bean sample containing diazinon residue was analyzed using Gas Chromatography coupled with FTD. The results obtained from this analysis have been summarized in Table 6.

Table 6. Level of diazinon residue (mg/kg) estimated from the samples of country bean

Days after spraying	Total volume prepared	Injected volume (µL)	Levelof Residue (mg/kg)	MRL (mg/kg)
0	10	1	0.339	0.01
1	10	1	0.279	
2	10	1	0.209	
3	10	1	0.153	
4	10	1	0.115	
5	10	1	0.099	
6	10	1	0.059	
7	10	1	0.031	
8	10	1	0.016	
9	10	1	0.007	
10	10	1	ND	
12	10	1	ND	

Residue of diazinon in country bean was detected up to 9 DAS and the level of residues were above MRL up to 8 DAS and these were 0.339 mg/kg, 0.279 mg/kg, 0.209 mg/kg, 0.153 mg/kg, 0.115 mg/kg, 0.099 mg/kg 0.059 mg/kg , 0.031 mg/kg and 0.016 mg/kg at 0, 1, 2, 3, 4, 5, 6 ,7 and 8 DAS, respectively. Sample of 9 DAS contained 0.007 mg/kg diazinon residue which was below EU-MRL set by European Union (0.01). No residue was detected at 10 and 12 DAS. So, the PHI of diazinon for country bean can be selected at 9 DAS (Table 7).

Table 7. Pre-Harvest Interval (PHI) of Country bean with the selected insecticides

Name of Insecticides	Level of residues ≥ MRL	Pre-Harvest Interval (PHI)
Quinalphos	8 DAS	9 DAS
Chlorpyrifos	6 DAS	7 DAS
Fenitrothion	7 DAS	8 DAS
Diazinon	8 DAS	9 DAS

4.2 TREND OF RESIDUE DEGRADATION

The trend of degradation of detected residue of Diazinon, chlorpyrifos, fenitrothion and Quinalphos in the sample over time is shown in Figure 17 to 20. From the figure it is observed that the degradation rate of all four groups of insecticide is more or less similar because all of the tested insecticides were in organophosphorus insecticides. But the level of detected residues and the degradation rate were not same for all of the selected insecticides at different DAS. The level of detected residues at 0 DAS were 0.562 mg/kg, 0.532 mg/kg, 0.488 mg/kg and 0.339 mg/kg for quinalphos, chlorpyrifos, fenitrothion and diazinon, respectively. The residues were detected upto 9 DAS for Quinalphos and Diazinon and upto 8 DAS for Chlorpyrifos and Fenitrothion.

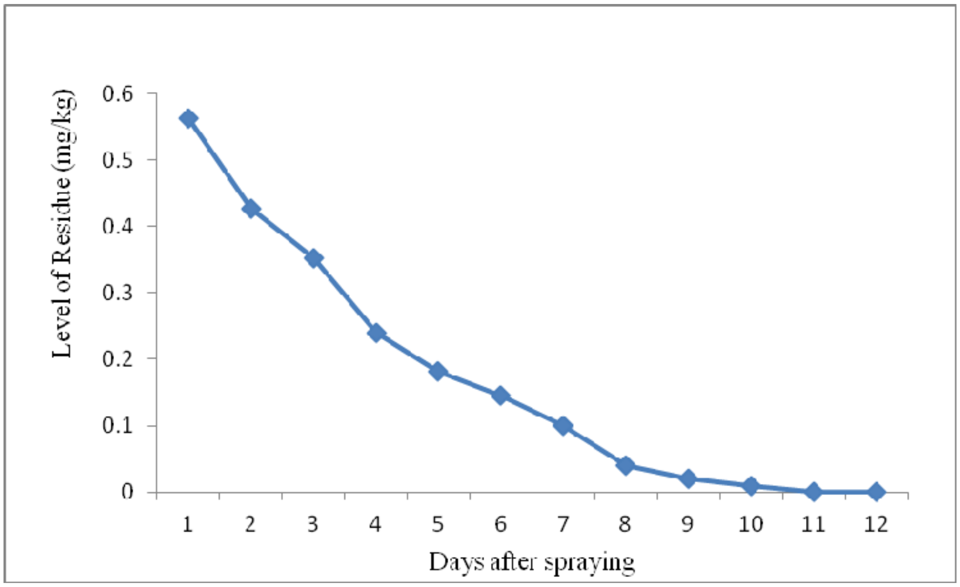


Figure 17. The trend of degradation of detected residue of quinalphos over time

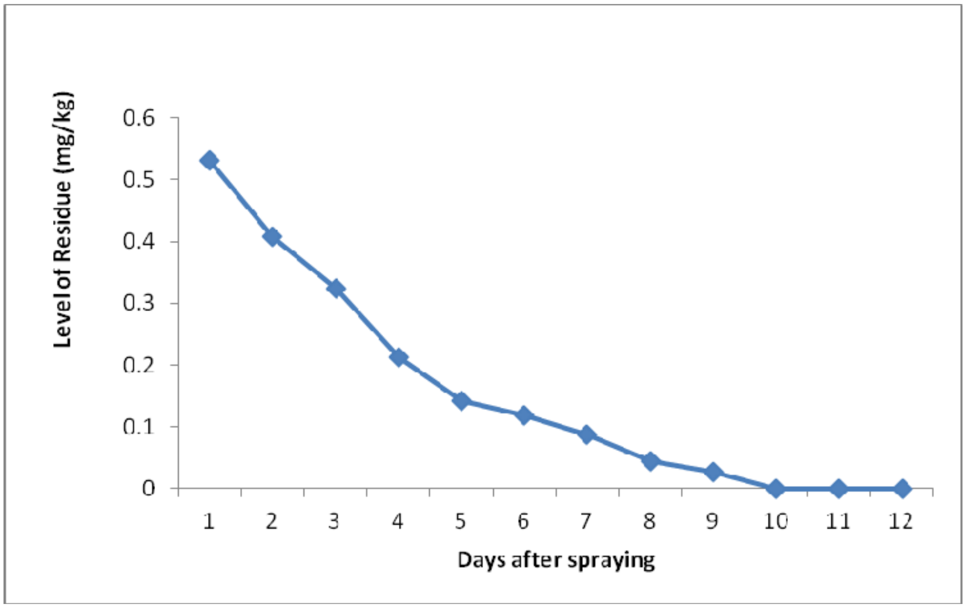


Figure 18. The trend of degradation of detected residue of chlorpyrifos over time

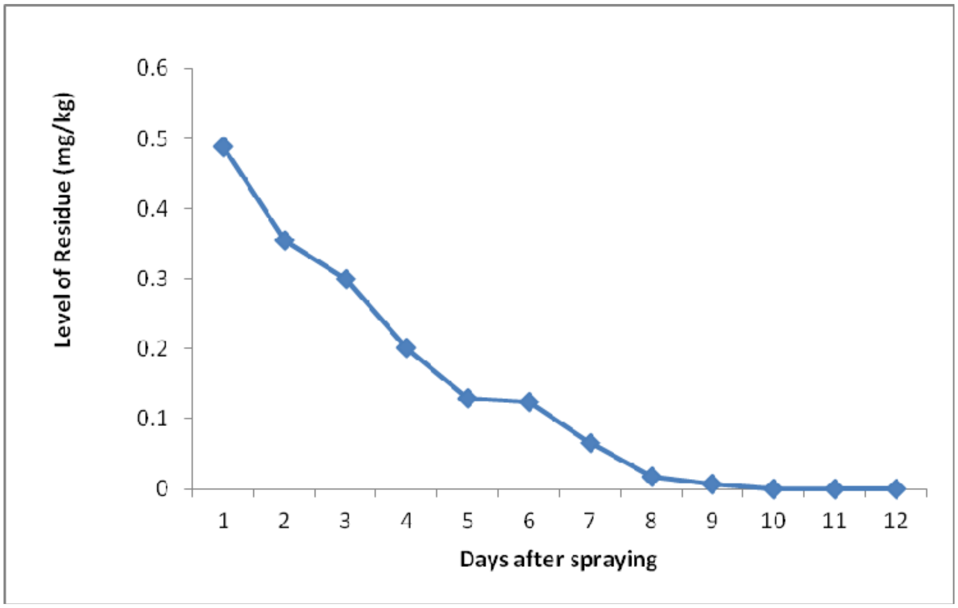


Figure 19. The trend of degradation of detected residue of fenitrothion over time

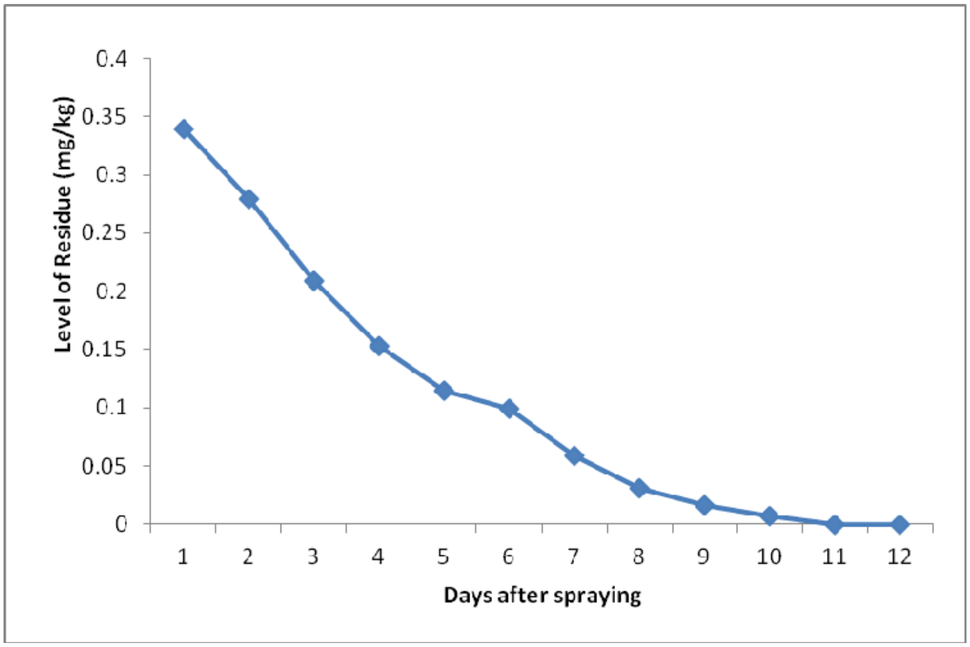


Figure 20. The trend of degradation of detected residue of diazinon over time

CHAPTER 5

SUMMARY AND CONCLUSION

Summary

A study was conducted in the experimental field of Entomology Division of BARI, Bangladesh from October 2015 to September 2016 in order to spray four insecticides in country bean for the residue degradation study of the selected insecticides. The residue analysis was done in Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur using Gas Chromatography coupled with Flame Thermionized Detector (GC- FTD).

This study was carried out to detect and quantify the left over residue of quinalphos, chloropyrifos, fenitrothion and diazinon in country bean and to compare the detected residues of all the selected insecticides with the Maximum Residue Limit (MRL) set by European Union. 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 upto 8 DAS. The quinalphos residue remained 0.009 mg/kg at 9 DAS, which was below the MRL. From 10 DAS, no residue was detected. The residue of chloropyrifos was detected upto 8 DAS, of which the level of residue was above MRL upto 6 DAS. The chlorpyrifos residue remained 0.044 and 0.027 mg/kg at 7 and 8 DAS, respectively which were below the MRL. From 9 DAS, no residue was detected. 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. From 9 DAS, no residue was detected. The residue of diazinon was detected upto 9 DAS, of which the level of residue was above MRL at 8 DAS. The diazinon residue remained 0.007 mg/kg at 9 DAS, which was below the MRL. From 10 DAS, no residue was detected.

Conclusion

Country bean, the delicious winter vegetables in Bangladesh, is more intensively and commercially grown vegetable. In spite of being a prospective crop, farmers in our country face significant yield loss of country beans every year due to severe attack of various insect pests. It is really difficult to manage the insect pests by the farmers using a single method but the farmers of Bangladesh are not habituated with using combination of several control methods. So, the farmers mostly depend on toxic chemical insecticides to control these insect pests. In order to this reason, residues of commonly used insecticides remain in the harvested crops which create a lot of adverse effect in the human health and the environment as well. Based on the results of the above study, the following conclusions were drawn:

- Mostly, the farmers do not follow the label instructions of the insecticide’s bottles, packets and use insecticides at their own choice.
- In most of the cases, farmers are using different insecticides at an alarming rate and frequency. They have little knowledge about the insect pest’s resistance against insecticides and spray insecticides repeatedly.
- Most of the farmers harvest and sell country bean without bothering pre-harvest interval of insecticides, while there is a great chance of consumers to be toxicated by the residues of high toxic insecticides.
- The use pattern of insecticides in country bean led to assume that the country bean for the consumers as well as the environment is getting overloaded with insecticide residue, since insecticides are being used by country bean growers irrationally in some occasions whimsically and indiscriminately.
- Country bean, soil and water sample containing insecticide residue above Maximum Residue Limits (MRLs) indicates that the issue of insecticide residue has a great concern on the aspect of public health and safe environment.

CHAPTER 6

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