ANALYSIS OF PESTICIDE RESIDUES IN FRUITS AVAILABLE IN MYMENSINGH LOCAL MARKET

MS Thesis

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Mymensingh

December, 2013

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A Thesis

Submitted to
Bangladesh Agricultural University, Mymensingh
In partial fulfillment of the requirements for the degree of
Master of Science

in

Environmental Science

By

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The Author

ABSTRACT

This experiment was carried out at the environmental science laboratory of Bangladesh Agricultural University, Mymensingh. Gas Chromatography (GC) was used to determine the pesticide residues in fruits in Mymensingh local markets. GC is specific to chemical and so efficient to determine the pesticide residues in very small amount of sample by the compare of retention time (t_R) and peak area with the standard solution. For conducting research work three selected fruits (Mango, Apple and Banana) were collected from 3 selected sites *(BAU Sesh More, BAU KR market and Notun Bazar, Mymensingh town)* of Mymensingh sadar upazila. Standard chemicals were analyzed to confirm their retention times and area of eluted peaks. Calibration curves were made for each standard samples using the data analyzed in different concentrations. By comparing the retention times of standard chemicals and extracted samples, confirmation of residual effects of examined chemicals were studied repeatedly. Among the studied 9 samples, only 3 samples responded to form peaks. Formalin residues was found in only one apple sample which was collected from *BAU Sesh More market*. The quantity of the formalin residue was about 10 ppm. On the other hand, out of 3 banana samples, 2 of them showed presence of ethopen residues. But sample from *BAU Sesh More* eluted a small area contained peak which was very minute level (less than 0.1 ppm). Banana sample from *Mymensingh Notun Bazar market* showed a remarkable peak which was approximately 32 ppm level of ethopen residue. This research result revealed on theoverall scenario of chemical contamination as well as residue in available fruits in our local market.

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CHAPTER 1
INTRODUCTION

Pesticide residues refer any specified substance in food, agricultural commodities or animal feed resulting from the use of pesticides. It includes any derivatives of a pesticide, such as conversion products, metabolites, reaction product, and impurities considered to be of toxicological significance.

Pesticides (insecticides, fungicides, etc.) are used globally for the protection of food, fibre, human health and comfort. However, their excessive use/misuse especially in the developing countries, their volatility, long-distance transports eventually result in widespread environmental contamination. In addition, many older, non-patented, more toxic, environmentally persistent and inexpensive chemicals are used extensively in developing nations, creating serious acute health problems and local and global environmental impacts. Remarkable progress has been made in the development of effective pesticides. In fact, a very small fraction of all applied pesticides is directly involved in the pesticidal mechanism. This implies that most of the applied pesticides find their way as ‘residue’ in the environment into the terrestrial and aquatic food chains where they undergo concentration and exert potential long term adverse health effects.

Residue analysis provides a measure of the nature and level of any chemical contamination within the environment and of its persistence. It is often difficult to correlate pesticide residues in the environment with effects on fauna and ecological processes. They can, show whether an animal or site has been exposed to chemicals and identify the potential for future problem.

All pesticides are subject to degradation and metabolism once released into the environment. The rates of degradation and dissipation vary greatly from pesticide to pesticide and situation to situation. The object of residue analysis is to indicate the residues present at the time of sampling and every precaution must be taken to ensure that the sample arriving at the laboratory.

Fruits are important components of the human diet since they provide essential nutrients that are required for most of the reactions occurring in the body. A high intake of fruits (five or more servings per day) has been encouraged not only to prevent consequences due to vitamin deficiency but also to reduce the incidence of major diseases such as cancer, cardiovascular diseases and obesity. Like other crops, fruits are attacked by pests and diseases during production and storage leading to damages that reduce the quality and the yield. In order to reduce the loss and maintain the quality of fruits harvest, pesticides are used together with other pest management techniques during cropping to destroy pests and prevent diseases. The use of pesticides have increased because they have rapid action, decrease toxins produced by food infecting organisms and are less labour intensive than other pest control methods. However, the use of pesticides during production often leads to the presence of pesticide residues in fruits after harvest. 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 foetal development as well as their capacity to cause cancer and asthma. Some of the pesticides are persistent and therefore remain in the body causing long term exposure. The concern has led to governments setting up monitoring systems in order to

assess the safety situation and make informed decisions when passing legislation.

Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). GC may help in identifying a compound and used to prepare pure compound from a mixture. It is a chemical analysis instrument for separating chemicals in a complex sample. It uses a flow-through narrow tube known as the column, through which different chemical constituents of a sample pass in a gas stream (carrier gas, mobile phase) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the stationary phase. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (retention time) (Pavia *et al.*, 2006).

In a GC analysis, a known volume of gaseous or liquid analyte is injected into the "entrance" (head) of the column, usually using a microsyringe (or, solid phase microextraction fibers, or a gas source switching system). As the carrier gas sweeps the analyte molecules through the column, this motion is inhibited by the adsorption of the analyte molecules either onto the column walls or onto packing materials in the column. The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and on the stationary phase

materials. Since each type of molecule has a different rate of progression, the various components of the analyte mixture are separated as they progress along the column and reach the end of the column at different times (retention time). A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Generally, substances are identified (qualitatively) by the order in which they emerge (elute) from the column and by the retention time of the analyte in the column (Pavia *et al.*, 2006).

After the separation of the compounds, Flame Ionization Detector (FID) is used to identify each of them and determine their retention time. The effluent from the column is mixed with carrier gases (e.g, hydrogen, Nitrogen) and ignited. Organic compounds burning in the flame produce ions and electrons that can conduct electricity through the flame. A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame. The current resulting from the pyrolysis of any organic compounds is measured. The FID is a useful general detector for the analysis of organic compounds.It has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but it destroys the injected sample (Anonymous, 2005). After detection, a signal is sent to the recording device.The time between sample injection and an analyte peak reaching a detector at the end of the column is termed the retention time (t_R). Each analyte in a sample will have a different retention time. The time taken for the mobile phase to pass through the column is called t_M. The area under the each peak may be expressed in terms of concentration of the specific

compound by running some calibration standards at known concentration (Anonymous, 2005).

Different scientists (Salwa *et al*., 1999; Butler *et al*., 2008; Fillion *et al*., 2000; Fernandes *et al*., 2011; Gamon *et al*., 2001; Paranthamam *et al*., 2012; Hussain *et al*., 2002) analysed pesticide residues in fruits and vegitables in India, England, China, and other developing country but limited pesticide residues analysis conducted by GC for available fruits in Bangladesh. in case of pesticide residues analysis GC is so efficient to determine the pesticide residues in very small amount of sample by the compare of retention time (t_R) and peak area with the standared solution.

A little work has been done for the determination of pesticide residues in fruit in our country but developed countries have set-up many analytical laboratories to monitor the pesticide residue level in fruits (Hossain *et al*., 2013). That's why the research is makes a plan of work with the following objectives**:-**

i. To explore the existing condition of available fruits in local market

ii. To compare the pesticide residues among the studied samples

iii. To quantify pesticide residues in samples.

CHAPTER 2

REVIEW OF LITERATURE

Pesticide use in agriculture in the second half of last century has produced certain benefits, including a decrease in the percentage of household income spent on food and an increase in food quality. However, pesticide use also has created concern regarding its effect on the environment and the potentially toxic or carcinogenic residues remaining in the food chain. In recent years, pesticides residues are available in water, fruits, vegetable, farm commodities and aquifers. In the present investigation major emphasis was given on the pesticide residues in fruits, while reviewing the subject. Nevertheless, indirect evidence was provided from pesticides in general to support some assertions.

2.1 Problems caused by pesticides

Increasing amount of pesticide using also creates a general and potential danger like use of other toxic materials. Three main problems determine the limits in continuous use of pesticides (Deveci and Ekmekyapar, 2008) **:-**

a. Organisms become resistant against pesticides in time.

b. Some pesticides do not undergo biodegradation easily, but remains resisting in the environment they are implemented or carried.

c. They also harm some living things other than those targeted.

Carbamate group pesticides are the group which should be preferred in terms of environmental pollution since they have a low level of persistence. The most resistant ones against decomposition processes and undesired ones in

terms of environmental pollution are chlorine hydrocarbons and inorganic pesticides. On the other hand, chlorine hydrocarbon pesticides have the characteristic of accumulating in adipose tissue of mammalians. By this way, they may cause more toxic effects in receiving living group by accumulating from one living to another. The residues of pesticides especially on vegetables and possible risks of them on human health has become the prior subject of pesticide researchers who evaluate vegetable quality recently (Colume *et al.*, 2001; Padron-Sanz *et al.*, 2005).

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 (Colume *et al.*, 2001).

Pesticides reach surface water resources with different ways. For example, they contaminate through their application in water to fight with water plants and water insects, through carriage of soils, plants and organisms containing pesticide residues to water resources with different ways, through discharge of pesticide production industry wastes into water resources, through washing of pesticide boxes and tools and equipments used in insecticide application, and through sedimentation of pesticide residues carried due to atmosphere pollution as a result of powder or liquid pesticide applications into water resources. While some part of pesticide molecules reached to

surface water resources through these ways dissolves in the water, other part remains suspended, and remaining part accumulates in the sediment. Then, pesticide is released from the sediment continuously (Huang & Iskandar, 2000).

World public opinion has reached a highly sensitive position against allergen, mutagen and cancerogenic effects created by pesticide residues on soil, water and foods depending on extinction events occurred in bird species feeding with accumulated pesticide residue. Forbiddance of production and consumption of pesticides causing cancer has been recommended by World Health Organization (WHO) and International Cancer Research Institutions, some has been forbidden and production of some other has been decreased. Some among them are DDT, endosulphan, fenitrothion, fenthion, malathion, parathion and trifluralin (Hura, 1999).

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 (Abdallab *et al.,* 1990).

The widespread and sometimes improper use of pesticides has lead to resistant strains of insects, plant diseases, weeds, and rodents, which can no longer be controlled by certain pesticides. New pesticides must be developed to control these resistant pests. The increased cost of pesticide development (currently estimated at US$ 15 million to US$ 20 million per pesticide over a six to ten year period) has resulted in fewer new pesticides being developed in recent years (Goring, 1977).

2.2 Pesticide residue in fruit

Hossain *et al.* (2013) used gas chromatography with a photo diode array detector (HPLC-PDA) was used 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.

The QuEChERS method was crefited to easily clean up and prepare food samples for multi-class, multi-residue pesticide analysis. Usher & Majors (2013) describes the use of the original, non-buffered QuEChERS method to prepare apple samples for residue analysis by gas chromatography/mass

spectrometry (GC/MS). Fifteen pesticides of different classes were studied. The experiments were done using Agilent Bond Elut QuEChERS extraction kit for 10-g samples and dispersive kits for 1-mL sample volumes. The analysis was done by GC/MS using selective ion monitoring (SIM) mode. The limit of quantitation for the pesticides studied was 10 ng/g in apple using this method. At 200 ng/g, the recoveries ranged from 89% to 102%, and at 10 ng/g, the recoveries ranged from 72% to 103%. The relative standard deviations associated with these recoveries were less than 11 % in all cases.

Zhao *et al*. (2013) reported on the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) AOAC sample preparation approach for extraction and cleanup of 17 GC-amenable pesticide residues from multiple classes, in apple. The method employed involves initial extraction in a buffered aqueous/acetonitrile system, an extraction/ partitioning step after the addition of salt, and a cleanup step utilizing dispersive solid phase extraction (dispersive SPE). The two different dispersive SPE clean-up approaches used either a 1 mL or 8 mL sample volume and were evaluated in parallel after sample extraction. The target pesticides in the apple extracts were then analyzed by gas chromatography/mass spectrometry (GC/MS) operating in selective ion monitoring (SIM) mode. The method was validated in terms of recovery and reproducibility. The limit of quantitation (LOG) for most pesticides is 10 ng/g; however, the pesticide Folpet has an LOG of 50 ng/g in apple. This application employing Bond Elut QuEChERS kits produced results well below the maximum residue limits (MRLs) for all the pesticides screened. The spiked levels for the

recovery experiments were 10, 50, and 200 ng/g. Recoveries ranged between 70 and 136% (92.5% on average), with RSD below 15% (5.0% on average).

Paranthaman *et al.* (2012) investigated the occurrence of endosulfan, carbendazim, chloropyripos in 10 banana samples in southern area of Tamilnadu, India (hill banana, karpuravalli, monthan, nendran, ney poovan, pachanadan, poovan, rasthali, red banana, robusta). In 7 samples, Carbendazim was found at concentrations ranging from 0.002-0.11 mg kg^{-1}. The seven samples contained carbendazim that not exceeded the FAO/WHO codex alimentarius standards for MRLs (Maximum Residue Limit) values of carbendazim pesticide on banana (whole) is 1.0 mg kg^{-1}. Based on the HPLC results carbendazim is finding in Hill banana (0.007 mg kg^{-1}), Monthan (0.019 mg kg^{-1}), Nendran (0.002 mg kg^{-1}), Pachanadan (0.007 mg kg^{-1}), Poovan (0.016 mg kg^{-1}), Rasthali (0.017 mg kg^{-1}) and Robusta (0.11 mg kg-1) and carbendazim is not finding Karpuravalli, Ney poovan and Red banana. Endosulfan, Chloropyrifos and Carbendazim in Robusta Banana sample are identified by matching their retention times and characteristic ion.

Fernandes *et al*. (2011) reported that pesticide residues in fruits and fruit juices for 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.

Guan *et al.* (2009) presented a novel solid-phase extraction technique called disposable pipette extraction (DPX). The solid-phase sorbent contained in the DPX tip is loose, which permits mixing of solutions to provide unsurpassed extraction efficiency and short equilibration times. DPX extractions are automated using the GERSTEL MultiPurpose Sampler (MPS), enabling efficient, high-throughput sample preparation. The GERSTEL DPX-Q and the DPX-Qg with graphitized carbon black, represent the only commercially available automated QuEChERS application for multi-residue analysis of pesticides.

Yamagami *et al.* (2009) 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 enabled to determine pesticides at low μg/kg in tomato, cucumber, green soybeans, spinach, grape and green tea.

Butler *et al*. (2008) reported on determination of pesticides in vegetables using a new sample preparation method, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). 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 ($MgSO_4$). It 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). 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.

Ochiai *et al*. (2008) determined 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, 2 >0.9900) for 66 pesticides and limit of detection (LOD: < 5 μg/kg) for most of the analytes.

The national monitoring programs for pesticides residues are the key means of ensuring compliance with regulations. They also create a database to help assess the levels of pesticide residues and the levels of residue intake. The information is invaluable in assessing human exposure to pesticide residues through the diet and assists in the country's formulation of a pesticide strategy. Monitoring of pesticide residues also provides a check on compliance with good agricultural practice in the use of pesticides. Many countries in the world have established analytical laboratories to evaluate the actual contamination of food by pesticides and they check routinely the most common fruits and other food products received from agricultural origin consumed daily bases (Dogheim *et al.*, 2002). In recent years, both legislators and consumers have shown increased interest in the safety of food products. Events such as the appearance of pesticide residues in fruits and vegetables have impelled governments to set-up monitoring programs.

Hussain *et al.* (2002) studied to access the residue of commonly used pesticides viz Cypermethrin, Methamedophos, Monocrotophos, Cyfluthrin, Dialdrin and Methyl Parathian respectively in three varieties of Mango being

collected from the grower fields in Multan division. The samples were treated with organic solvent Cyclohexane and ethylacetate (1:1), cleaned on Gel Permeation Chromatograph (GPC) and analyzed on auto system Gas Chromatograph (GC) with electron capture detector (ECD).

Gamon *et al*. (2001) determined the pesticide residues in fruit and vegetables by gas chromatography/tandem mass spectrometry (GC/MS/MS). Electron impact (EI) and chemical ionization (CI) 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.

Methods were developed that reliable and rapidly detect as many pesticides as possible in the most cost-effective manner. A rapid and efficient multiresidue method for analysis of 251 pesticides in fruit and vegetables by gas chromatography- mass-selective detection (GC-MSD) and for 10 pesticides by liquid chromatography (LC) with fluorescence detection was described by Fillion *et al.* (2000).

Direct Sample Introduction (DSI) or dirty sample injection was investigated in the determination of 22 diverse pesticide residues in mixed apple, green bean, and carrot extracts by benchtop gas chromatography/tandem mass spectrometry (DSUGC/MS-MS) (Lehotay, 2000) The targeted pesticides, some of which were incurred in the samples, included chlorpyrifos, azinphos-methyl, parathion-methyl, diazinon, terbufos, endosulfan sulfate, carbofuran, carbaryl, propargite, bifenthrin, dacthal, trifluralin, metalaxyl, pendimethalin, atrazine, piperonyl butoxide, diphenylamine, vinclozolin, chlorothalonil, quintozene, and tetrahydrophthelimide (the breakdown product of captan). Average recoveries of the pesticides were 103± 7% with relative standard deviations of 14± 5% on average, and limits of detection were <2 ng/g for nearly all pesticides studied.

Salwa *et al*. (1999) work out a plan 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.

Wylie and Quimby (1998) developed a gas chromatographic (GC) method that can be used to screen for 567 pesticides and suspected endocrine disrupters. The method relies on a technique called retention time locking (RTL). RTL is a procedure that allows the chromatographer to reproduce analyte retention times independent of GC system, column length, or detector so long as columns with the same stationary phase, nominal phase ratio, and diameter are used. The chromatographer first locks the GC method so that all retention times match those listed in a 567-compound pesticide and endocrine disrupter retention time table. With retention time locking, pesticides have the same retention time on all GC systems; this makes GC-MS confirmation much easier because the analyte's retention time is already known.

GC Internal standard analysis method was established with 9 chlorinated catechol compounds and the techniques is schematically presented (Sattar, 1994) and the examples of chromatographic peaks were reported. This was the basic, applied and fundamental contribution and pioneer research in the environmental sciences of Bangladesh.

GC external standard (Sattar, 1985c) and GC internal standard (Sattar, 1985d) analysis methods (procedure) of phenoxyherbicides were schematically described and the examples of GC chromatograms were presented. Different solvent mixtures and clean up procedures were applied where sensitivity recorded to ppb levels. This technique was widely recommended to detect the residues of the compounds from soils, crops and food materials (Sattar and Passivirta, 1990).

Agriculture uses of pyrethroid insecticides have increased rapidly since the development of the first pyrethroid perrnethrin.The food and Agriculture Organization and the World Health Organization have recommended residue limits for some pyrethroid include in agricultural and animals products. Gas chromatographic (GC) methods were reported for muliiresidue determination of pyrethroid insecticides in fruits, vegetables and grains (Bolygo *et al.*, 1983).

Sattar (1983b, 1987, 1986) described the GC external standard analysis method (procedure) of five organochlorine and four DDT-type organochlorine pesticide and their identical chloromatograms are listed against respective peaks of organochlorine pesticides and DDT-types pesticides. Different solvent systems were developed with residue recovery upto 100%. The method widely used for the detection of residue in soil lives (Sattar, 1983b; Sattar 1985e; Sattar 1990).

Sattar and Passivirta (1980), Passivirta and Sattar (1983), Sattar (1981a) and Sattar (1980) schematicaly presented the detailed internal standard GC

analysis method (procedure) of MCPA (4-chloro-2-methyl phenoxyacetic acid) together its two metabolites 4-chlo-o-cresol and 5-chloro-3-methyl cathechol and the examples of chromatograms are reported as proof records. This was the universal applied fundamental contribution for the detection of the residues of MCPA and/or its metabolites in soils, crops and food materials (Sattar, 1982; Sattar, 1983a; Sattar, 1981b). Different solvent/solvent systems were developed including three cleanup procedures (Column, water, toluene-shaking and TLC) where shaking one mostly used. This internal standard procedure of Sattar and Passivirta (1980) historically invented (1^{st} times in the history of mankind and 1^{st} time in the history of environmental/analytical chemistry of the world) the two metabolites of MCPA by GC, GC-MS, and NMR applications from foods and vegetables (Passivirta and Sattar, 1983) and soils (Sattar, 1985b) where by applying of only MCPA compound, the recovery recorded upto three compounds like MCPA with its 2 metabolites.

Sattar *et al.* (1977); Sattar and Passivirta (1979a) described the detailed external standard procedure and GC analysis methods of MCPA (4-chloro-2-methyl phenoxyacetic acid) and MCPA together with its two metabolites 4-chloro-o-cresol and 5-chloro-3methyl catechol (Sattar and Passivirta, 1979b; Sattar and Passivirta, 1979c; Sattar, 1985a) are schernatically described. The method covered different solvent systems for extraction and three clean up procedures like TLC column and water-toluene shaking. This was the excellent basic, applied and fundamental contribution and still largely using in pesticide, agriculture, environmental

and analytical chemistry for minimizing residues and hazards and building of peace.

2.3 Concluding remarks

So far limited systematic research has been performed in Bangladesh on monitoring pesticide residues in fruits. The present study was initiated to work on these lines and status of pesticidal residues in fruits of available local markets.

CHAPTER 3

MATERIALS AND METHODS

3.1 Site selection

For conducting research work on chemical contamination of available fruits in nearest markets of Mymensingh sadar upazila, Mymensingh, researcher selected 3 sites: a) *BAU sesh mure market*, b) *BAU KR market*, c) *Notun bazaar, Mymensingh town*.

3.2 Sample collection

Three selected fruits (Mango, Apple and Banana) were collected from 3 sites randomly on July 14/2013. Total 9 samples of selected fruits were collected from 3 sites. Aluminum foil was used just after sample collection to evaporate the existing condition of the sample.

3.3 Extraction of samples

The collected samples were immediately taken into the laboratory of the department of environmental science, Bangladesh Agricultural University, Mymensingh and cut into small pieces with proper labeling for future identification. The labeled samples (Table 3.1) were kept in deep freezer (-20^0C). About 2 gm of each sample (frozen part) was grinded by hand grinder then allowed in 5 ml Hx (n-Hexane) containing test tube for 15 minutes. To avoid the evaporation of solvents and volatile chemicals from the samples, the mouth of the test tube was tightly closed by cork. After 15 minutes, each

part was mixed well by 2 minutes hand shaking and keeps 5 minutes for stabling the mixture. The supernatant solution (Hx with extracted compound) was collected by small glass pipette carefully and collected the solution in labeled small vial. The vial will be keep for further analytical analysis. The method of sample extraction (liquid extraction technique) was followed by Fernandes *et al.* (2011) and Usher & Majors (2013) with modification.

Table 3.1: Different samples collected from selected sites of the study area.

Sample ID	Sample name	Location
FM1	Mango	*BAU sesh more*
FM2		*BAU KR market*
FM3		*Notun Bazar, Mymensingh town*
FB1	Banana	*BAU sesh more*
FB2		*BAU KR market*
FB3		*Notun Bazar, Mymensingh town*
FA1	Apple	*BAU sesh more*
FA2		*BAU KR market*
FA3		*Notun Bazar, Mymensingh town*

3.4 Gas Chromatographic Analysis

The analytical analysis was carried out with Gas Chromatograph (GC) [GC-2014] Shimadzu Corporation, Kyoto, Japan. DFID detector was used in this experiment. The overall GC condition are given below:

GC condition		
	GC/DFID	**Stability/Reproducibility**
Column:	RT-Mseive 5A, serial number: 1104596. **Length:** 30 m, **Inner diameter:** 0.53 mm, **Film thickness:** 50.00 um.	**Column flow:** 6.63 mL/min Maximum temperature: 300°C.
Oven:	50°C (3 min), to 200°C at 10°C /min, to 300°C at 15°C/min and hold finally 5 min.	**Column oven:** 50°C
Carrier:	Hydrogen (30 mL/min), Air (142.1 mL/min)	Hydrogen (30 ml/min),
Detection:	DFID, 300°C, **Sampling rate:** 40 msec.	DFID, 300°C
Injection:	1µL, Direct injection, 200°C	Split
Linear velocity	60.7 cm/sec	**Pressure:** 66.8 kPa
Purge Flow	3.0 mL/min	**Split ratio:** 20

3.5 Standard Chemicals

The formaldehyde solution (GR-grade) (EM Science, Gibbs-town, NJ) consisting of approximately 36.5–38% formaldehyde; 10–15% methanol; 47–53.5% water; and trace amounts of chloride, sulfate, and heavy metals was used as the source of formaldehyde. Ethophen 48% solution was used as

another standard solution, which used for fruit ripening artificially. The standard solution of ethopen and formalin was collected from Z.H. Scientific and Chemicals Mart, Dhaka, Bangladesh. Injection of each standard solution was injected several times (at least 5 times repetition) for the confirmation of the respective retention time.

3.6 Data comparison

The different concentration of both standard solutions were injected in GC system under above mentioned condition and made calibration curve using known concentration. Calibration curve was made by MS Excel program using scattered diagram followed by regression line. By comparing the retention time and area of the standard solution and studied samples, researcher was matched both and confirmed / calculated the GC eluted compound.

CHAPTER 4
RESULTS AND DISCUSSION

The experimental works conducted by using only n-Hexane for extraction purposes. For this reason, there are not remarkable gas chromatography (GC) chromatograms from all samples after analysis. It may be due to use of single solvent extraction procedure. But due to lack of time, standard chemical availabilities this research conducted based on only liquid extraction (using n-Hexane). Fernandes *et al.* (2011) reported that pesticide residues in fruits and fruit juices for 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. But this research only done by n-Hexane solvent for extraction. So that there were so many limitations will be consider before explanation.

4.1 Calibration of standard formalin solution

The different concentrations of standard solutions (formalin) were eluded at 9.96 minutes (Fig. 4.1). During making a calibration curve with the standard solutions areas of peaks for every concentration were calculated which were shown in table 4.1. Single peak for different concentrations of formalin was observed (Fig. 4.1; left part) and a normal standard straight line was drawn using formalin content and peak areas (Fig. 4.1; right part).

Table 4.1: Scheme used for creation of five level calibrations of standard formalin solution.

Calibration level	Concentration of formalin (ppm)	Area (000000)
1	0	0.00
2	2	2.00
3	5	4.20
4	7	6.50
5	10	9.70

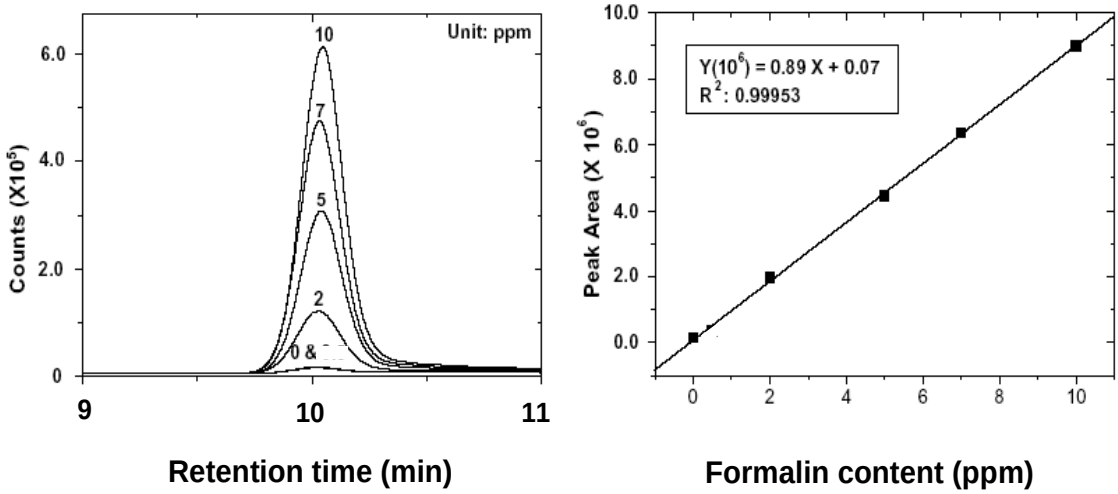


Fig. 4.1: Single ion monitoring (SIM) and calibration curve of different concentrations of standard formalin.

4.2 Calibration of standard ethopen solution

The different concentrations of standard ethopen solutions were observed at 9.45 minutes and calibration curves prepared by using those data (Fig. 4.2). During making a calibration curve with the standard solutions areas of peaks for every concentration were calculated which were shown in table 4.2. The

calibration curve showed the relationship between concentration of ethopen and peak area (10^6).

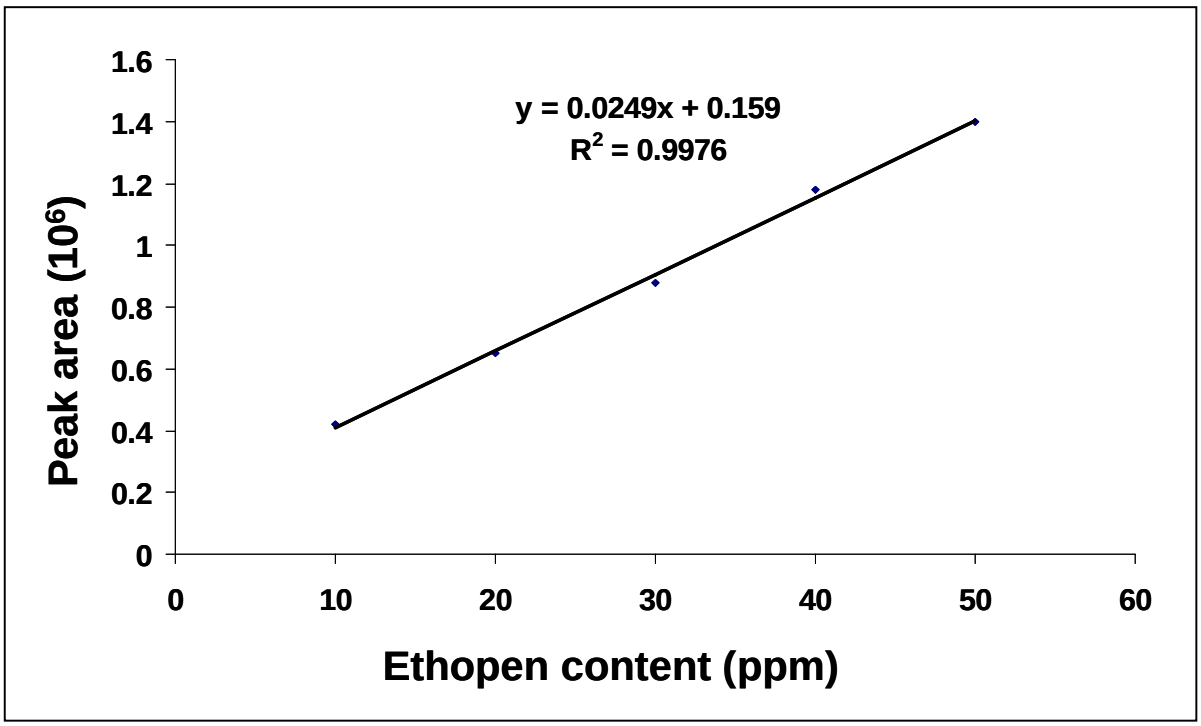


Fig. 4.2: Calibration curve of different concentration of ethopen.

Table 4.2: Scheme used for creation of a five level calibration.

Calibration level	Concentration of ethopen in ppm	Area ('000000)
1	10	0.42
2	20	0.65
3	30	0.88
4	40	1.18
5	50	1.40

The peak area of the specific amount of a chemical varied from the nature and elution and characteristic of chemicals studied. In Fig. 4.1 and Fig. 4.2,

there were enormous peak area differences found in respect of specific amount of standard chemicals. Some of the researchers (Usher and Major, 2013; Zhao *et al.*, 2013; Paranthaman *et al.*, 2012; Yamagami *et al.*, 2009; Butler *et al.*, 2008) suggested the similar variation among the different chemicals occurred in their peak areas in GC analysis.

4.3 GC analysis of standard formalin solution

In GC analysis, standard formalin solution was showed the response at 9.96 minutes, which was repeated several times (at least 5 times). When the eluted retention time was fixed at 9.96 minutes then researcher confirmed about the peak for standard formalin, which was shown in figure 4.3.

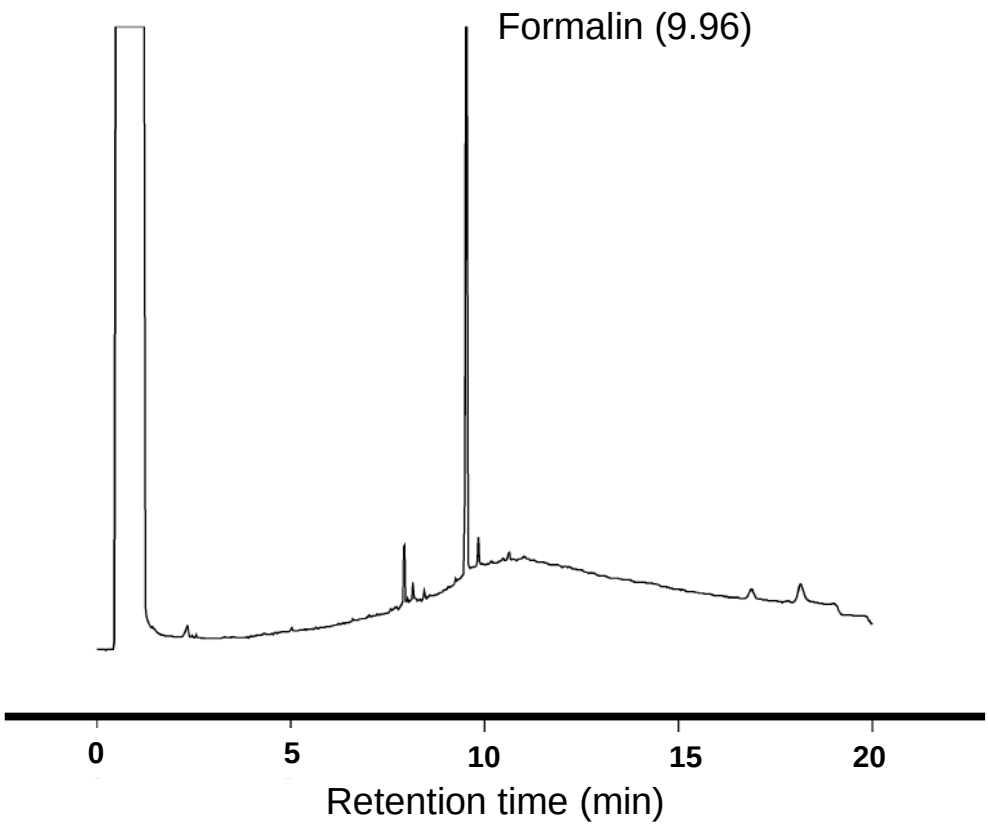


Fig. 4.3: Chromatograms eluted by GC for standard solution. One major eluted peak (at 9.96 min) showed formalin elution.

4.4 GC analysis of apple samples

When the extracted samples were injected in the analytical tools (GC) then most of the samples did not show such reasonable peaks. There was no such response observed due to absence of such analytical compounds in the samples or any other difficulties occurred during the process from sampling to analysis. But, one extracted sample (code name: FA1) eluted many peaks within 6 to 12 minutes but the areas were negligible (Fig. 4.4a). The dilution of the sample may cause the less response of analysis. That's why more concentrated sample (FA1) injected again to elucidate similar types of peaks with great response and remarkable area. Then the elucidated peaks and their retention times were compared with standard formalin data, where 9.97 minute elucidated compound found (Fig. 4.4b). This sample was injected repeated 3 times more to confirm the similarity of the elucidated peaks as well as to confirm the retention time. Results revealed the peak responded at 9.97 minutes which was due to the presence of formalin in that sample.

In apple sample FA1 (collected from *BAU Sesh More*) the residues of formaline was found in the concentration of 10 ppm. In apple sample FA2 and FA3 (collected from *BAU KR market* and *Notun Bazar market*) the residues of formaline were not found. Usher and Majors (2013) found the limit of quantitation for the pesticides residues was 10 ng/g in apple. At 200 ng/g, the recoveries ranged from 89% to 102%, and at 10 ng/g, the recoveries ranged from 72% to 103%. Zhao *et al.* (2013) found the limit of quantitation (LOG) for most pesticides is 10 ng/g; however, the pesticide folpet has an LOG of 50 ng/g in apple. The spiked levels for the recovery experiments were 10, 50, and 200 ng/g. Recoveries ranged between 70 and 136% (92.5% on average), with RSD below 15% (5.0% on average).

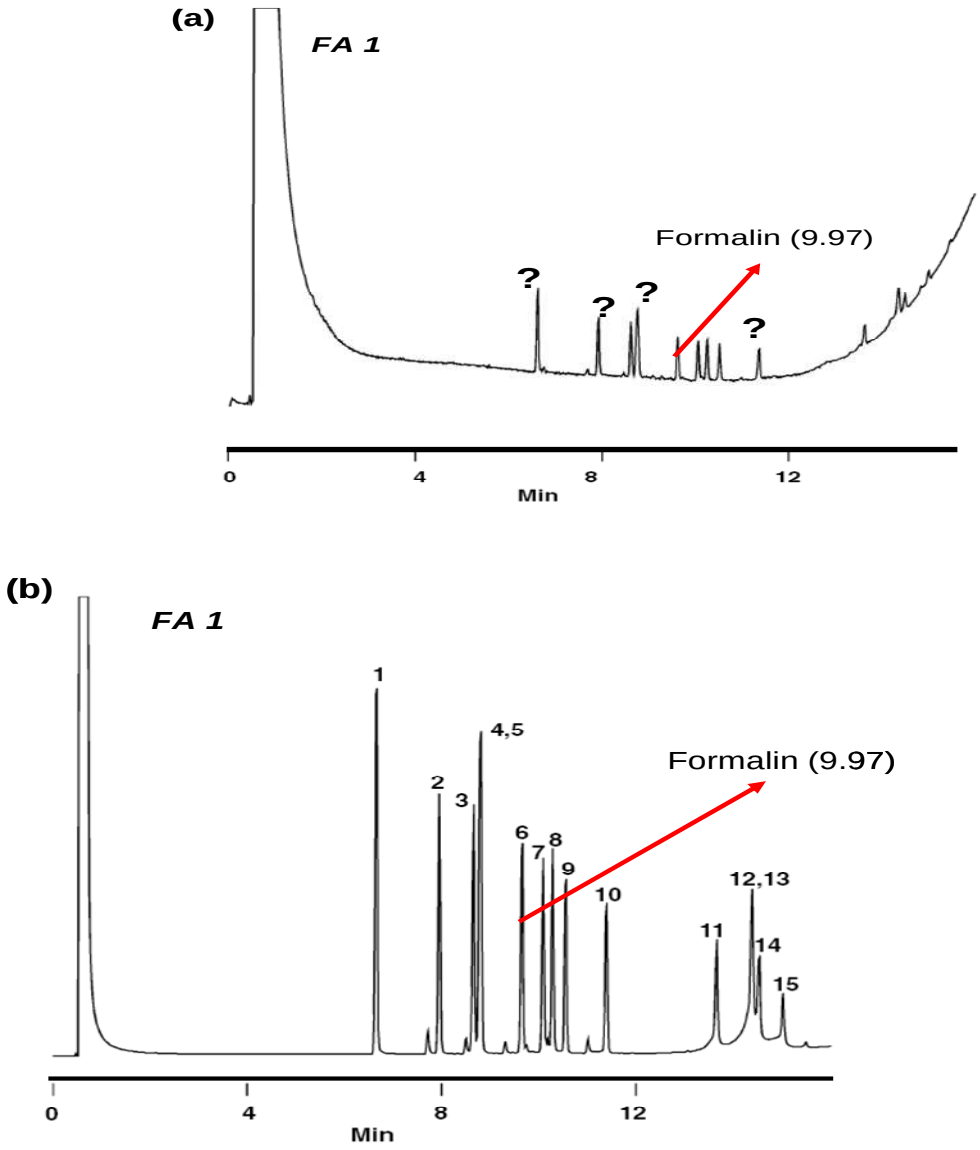
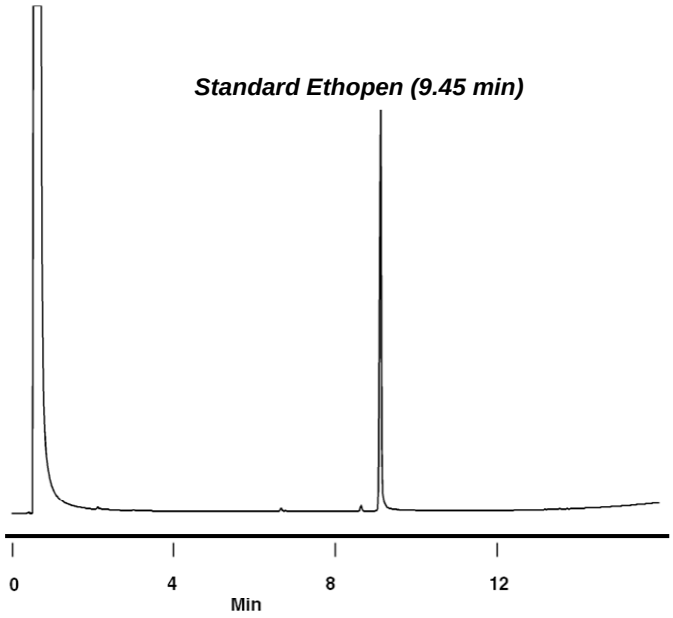


Fig. 4.4: Eluted chromatograms for apple sample (FA1) with many peaks; (a). diluted sample showing peaks, Question marks (?) indicate the unknown elutions / peaks of the crude mixture of the banana sample. (b). more concentrated sample showing clear comparison between peaks. The retention time (9.97 min) marked based on eluted peak from standard formalin solution as figure 4.3. Numbers at the top of the peaks indicate the unknown compounds of the crude mixture of the apple sample.

By comparing with the area of known standard chemicals of a specific retention time and an unknown concentrated peak, the quantity was determined. The calibration curve of the standard known sample was helpful to determine the unknown concentration. By plotting the area of eluted peak the quantity of analyzed compound was calculated easily. After plotting the peak area (FA1, 9500000) of a specific retention time in the respective standard solution curve (calibration curve), about 10 ppm concentration of formalin recorded (peak shown in Fig. 4.4b).

4.5 GC analysis of standard ethopen solution

In GC analysis, standard ethopen solution was showed the response at 9.45 minutes, which was repeated several times (at least 5 times). When the eluted retention time was fixed at 9.45 minutes then researcher confirmed about the peak for standard ethopen, which was shown in figure 4.5.



Retention time (min)

Fig. 4.5: Chromatograms eluted by GC for standard solution. One major eluted peak (at 9.43 min) showed ethopen elution.

4.6 GC analysis of banana samples

In another sample of fruit (FB1) showed a minute response like ethopen at 9.43 minutes which may be identical with previously obtained results (Fig. 4.5). But there are many unknown compounds elucidated in both analysis (Fig. 4.4, 4.5), which may be the compounds containing in that fruits or other foreign substance (pesticides, other metabolites etc.). Since the researcher used crude extract during the study so that many natural compound or unknown compounds was appeared after gas chromatography analysis. Another sample of fruits (FB3) showed distinguished peaks at 9.43 minutes which was also identifiable with ethopen standard solution (Fig. 4.6). There were many unknown peaks observed in that fruit sample analysis.

In banana sample FB1 (collected from *BAU Sesh More*) the residues of ethopen is found in very minute level of contamination occurred. In banana sample FB2 (collected from *BAU KR market*) the residues of ethopen was not found. In banana sample FB3 (collected from *Notun Bazar market*) the residues of ethopen was found in the concentration of 32 ppm. Paranthaman *et al.* (2012) investigated the occurrence of endosulfan, carbendazim, chloropyripos in 10 banana samples in southern area of Tamilnadu, India (hill banana, karpuravalli, monthan, nendran, ney poovan, pachanadan, poovan, rasthali, red banana, robusta). In 7 samples, Carbendazim was found at concentrations ranging from 0.002-0.11 mg kg^{-1}. Based on the HPLC results carbendazim is finding in Hill banana (0.007 mg kg^{-1}), Monthan (0.019 mg kg^{-1}), Nendran (0.002 mg kg^{-1}), Pachanadan (0.007 mg kg^{-1}), Poovan (0.016 mg kg^{-1}), Rasthali (0.017 mg kg^{-1}) and Robusta (0.1mg kg^{-1}) and carbendazim is not finding Karpuravalli, Ney poovan and Red banana.

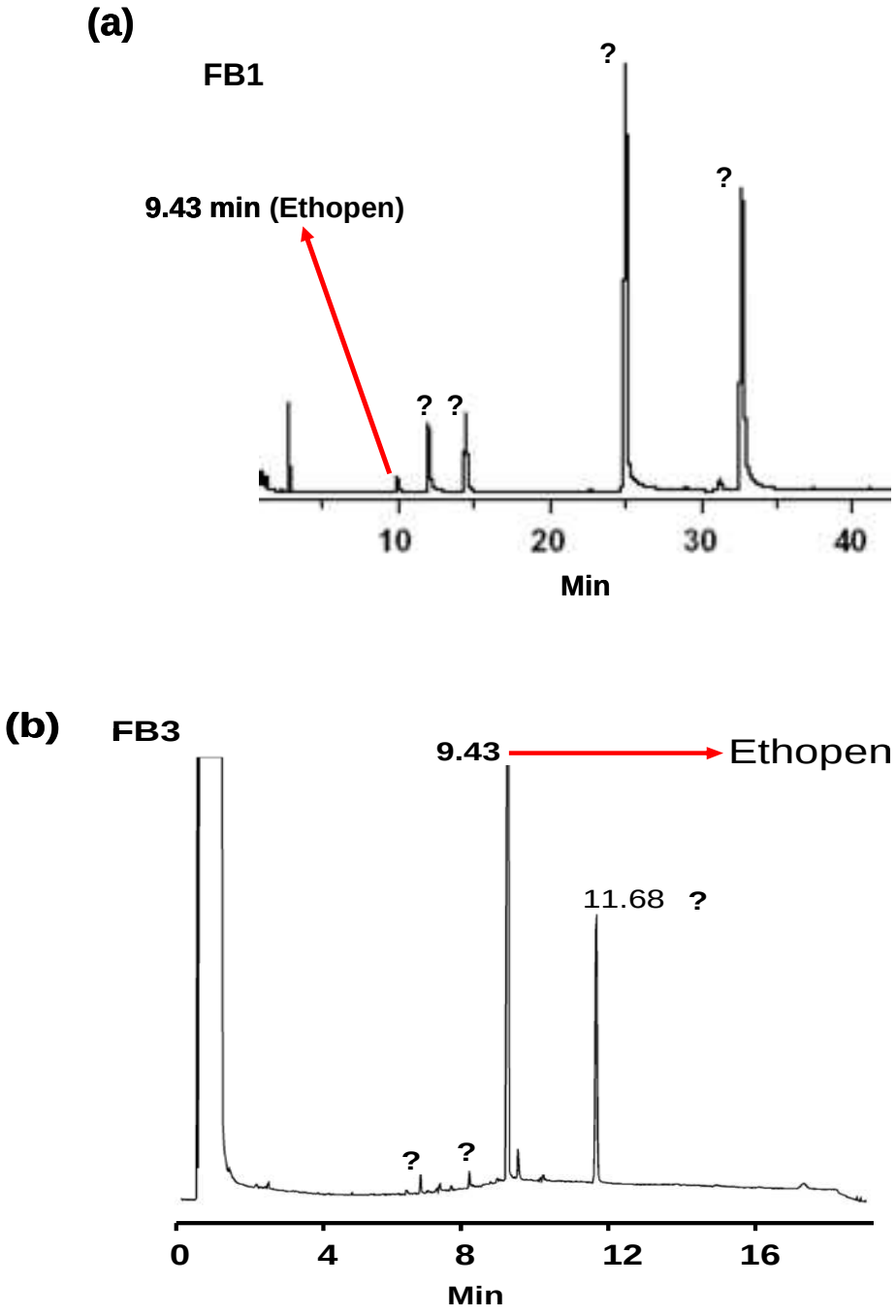


Fig. 4.6: Eluted chromatograms for banana sample (FB1 and FB3) with 6 peaks (a) and 2 major and 3 minor peaks (b), Question marks (?) indicate the unknown elutions/ peaks of the crude mixture of the banana sample.

When the banana samples were investigated then very minute level of ethopen contamination occurred in one sample (FB1, Fig. 4.6a) but a remarkable amount (32 ppm) was recorded in another sample (FB3, Fig. 4.6b), where as the area was 910000.

4.7 GC analysis of mango samples

In mango sample FM1, FM2 and FM3 (collected from *BAU Sesh More*, *BAU KR market* and *Notun Bazar market*) the residues of formaline were not found. Hussain *et al*. (2002) determined the pesticide residues in mango samples collected from the fields of farmers in Multan division. In this, study three varieties of mango samples, sample I (Dusehri), sample II (Chaunsa) and sample III (Sindhri) were analyzed for 6 pesticides namely Cypermethrin, Methamedophos, Monocrotophos, Cyfluthrin, Dialdrin and Methyl Parathian. It was observed that all the samples were contaminated with pesticides.

The above data show that fruits sample collected from different sites are contaminated with above mentioned chemicals but some samples have no detectable residues. Out of 9 samples collected from different market, 6 samples (67%) are free of pesticide residues and 3 (33%) samples contain pesticide residues. The rate of contamination of different chemicals were varied from 0 to 86% (Salwa *et al.,* 1999). That research group reported most commonly detected residues in fruits, like- dithiocarbamates (15.1% of 397 samples), dimethoate (6.8%), tetradifon (4.5%), Malathion (3.3%), Chlorothalonil (2.0%). Salwa et al. (1999) also reported that 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 pesticides residues. This research results revealed about 33.3% samples were contaminated with chemicals (formalin, ethopen), which were used for post-harvest activities.

But some samples have no detectable residues for different possible reason such as study area variation, differences of extraction procedure, variation of sampling portion of fruits, variation of GC analytical condition. Many peaks were eluted from studied samples which were not identified due to limitation of availability of standard solution. Further research would be necessary to identify unknown eluted peaks in the studies materials.

CHAPTER 5
CONCLUSION, SUMMARY AND RECOMMENDATIONS

Fruits provide essential nutrients for human body. Food is the basic necessity of life and foods are contaminated with toxic pesticides associated with severe effects on the human health. Like other crops, fruits are attacked by pests and diseases during production and storage leading to damages that reduce the quality and the yield. In order to reduce the loss and maintain the quality of fruits harvest, pesticides are used together with other pest management techniques during cropping to destroy pests and prevent diseases. Some of chemicals are spray on fruits after harvesting for the purposes of ripening and to overcome decomposition of fruits but sometimes chemical residues remain in the fruits which is harmful to human health. In fact, a very small fraction of all applied pesticides is directly involved in the pesticidal mechanism. The presence of pesticide residues is a concern for consumers.

The experiment conducted to indicate the residues present or absent at the time of sampling from different study areas. Three sites (*BAU Sesh More market, BAU KR market* and *Mymensingh Notun Bazar market*) were selected under Mymensingh sadar upazila for collection of study materials. There were 3 fruit samples (Apple, banana, mango) were collected from selected study area without biasness. Normal Hexane solution was used to extract for each collected sample. The extracted samples were analysed by Gas chromatography (GC). GC is so efficient to determine the pesticide residues in very small amount of sample by the compare of retention time

(t_R) and peak area with the standared solution. The standard solutions of formalin and ethopen were used. Gas Chromatography (GC) with DFID (Diode Flame Ionization Detector) was used to determine chemicals residues. The analytical instrumentation was maintained in same condition for all samples and standard chemicals.

The different concentration of both standard solutions were injected 5 times for the confirmation of the respective retention time which was important to identify unknown chemical.The calibration curve was made using concentration and peak area. By comparing the retention time and area of the standard solution and studied samples were matched both and calculated the amount eluted compound.

Results revealed only 3 samples (out of 9 samples) responded to chemical elusions. Several peaks were eluted when extracted sample had been injected in GC. Retention time of eluted peak was compare to standard solution peak to identify chemical. One peak was found in apple sample of *BAU Sesh More market* which was same as formalin peak. The quantity of the formalin residue of that sample was about 10 ppm using peak area in standard calibration curve . On the other hand, out of 3 banana samples, 2 of them showed presence of chemical residues. One peak was identifided in banana sample of *BAU Sesh More and Mymensingh Notun Bazar market* which was same as ethopen peak. But sample from *BAU Sesh More* eluted a small area contained peak which was very minute amount (less than 0.1 ppm). Banana sample from *Mymensingh Notun Bazar market* showed a remarkable peak which was approximately 32 ppm level of ethopen residue. Chemical

residues were not found in other 6 sample due to different possible reason such as study area variation, differences of extraction procedure, variation of sampling portion of fruits, variation of GC analytical conditions and limitation of availability of standard solution.

Pesticide residues in fruits are a potential hazard, which has received much attention during the past 20 years. Because of very small amount of chemical contamination or pesticide residues, which are permitted in food, elaborate analytical procedures are required. This result would be played important role in fundamental contribution and chemical contamination research in the environmental sciences of Bangladesh.

RECOMMENDATIONS:

Pesticide contamination of foodstuff has become a worldwide concern. Researchr would like to share some of recommendation to overcome the presence of chemical residues in local market products.

1. Local authority should be monitored the residual effect of fruits in local market.
2. Government should be formulated a policy that will restrict the use and availability of chemicals.
3. The common people should be awared about pesticide residual effect.
4. Different improved research systems have to establish to detect the pesticide residues in fruits and their health hazard effect.
5. Regulation and monitoring should be prompt at various levels.

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