EFFECT OF PRE-HARVEST CYPERMETHRIN SPRAY ON RESIDUAL LEVEL AND BIOCHEMICAL ATTRIBUTES OF STRAWBERRY FRUIT

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This is to certify that the thesis entitled **"EFFECT OF PRE-HARVEST CYPERMETHRIN SPRAY ON RESIDUAL LEVEL AND BIOCHEMICAL ATTRIBUTES OF STRAWBERRY FRUIT "** submitted to the Department of Horticulture, 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 **HORTICULTURE**, embodies the result of a piece of bonafide research work carried out by **SASWATI EDBER**, Registration No. **19-10036** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

June, 2021

Dhaka, Bangladesh

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Dedicated to

My

Beloved Parents

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Abstract

The effect of pre-harvest sprays of pesticides on agricultural products poses a risk to human health. Field studies on residues of pyrethroid pesticide, cypermethrin (ripcord 10E) in ripe strawberry fruits were conducted during November 2020 to February 2021. Residues were quantified at different harvest intervals of 0 (2h), 1, 3, 5, 7, 10 and 12 days after insecticide application. Strawberry fruits had the highest residual level (0.874 mg/kg) at 0 DAS (Days after spraying). The detected residue level was higher than the MRL (maximum residue limit) up to 7 DAS (0.101 mg/kg). Therefore, the Pre-Harvest Interval (PHI) of cypermethrin was detected up to 10 DAS for strawberry. Data indicated that no pesticide residues were detected after 12 DAS. Spraying of insecticide also affect the quality of strawberries. Strawberry juice had a lower content of ascorbic acid at 1-7 DAS and a lower titratable acidity at 0 and 3 DAS. The phenolic content in fruits were increased gradually 1-7 DAS of cypermethrin. As a result, it can be concluded that delaying fruit harvesting after 7 DAS improves fruit quality by lowering residue levels below the MRL.

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LIST OF ABBREVIATIONS

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

CHAPTER I

INTRODUCTION

Strawberry (Fragaria× ananassa Duchesne) is a tasty and nutritious fruit which is popular for its attractive colour, aroma and sweetness. It produces fruits during November to April when most of the fruits are not available which may help to increase the availability of fruits in lean period of Bangladesh. Bangladesh is now growing strawbwrries on around 10000 acre of land every year. Commercial farming of strawberry has been gaining popularity in greater Rajshahi including vast Barind tract (BBS 2022, Feb 19; m.independantbd.com 240141). It is consumed in large quantities either fresh or processed as preserves; fruit juice, ice cream milkshakes, jam, jelly pickles, chocolates, biscuits, cakes and drinks. Strawberry juice extracts be full of high levels of antioxidant which works against superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen free radicals. It also has the total antioxidant capacity for low-density lipoproteins of the fruit extracts (Salami *et al*., 2010). A cup of strawberries provides 55 calories and vitamin C content is more than the recommended human daily requirement (Salami *et al*., 2010).

 In spite of being a prospective crop, high rate of insect pests' infestation is responsible to its low yield and poor quality. Due to the infection of pests and diseases 20 to 40 percent of the crop yield are reduced globally (FAO, 2012). Farmers face significant yield loss of strawberries every year due to severe attack of various insect pests in our country such as *Phytonemus pallidus* Banks (Acari: Tarsonemidae), *Tetranychus urticae* Koch (Acari: Tetranychidae), *Frankliniella occidentalis* Perg. (Thysanoptera, Thripidae), *Lygus* sp., and *Botrytis cinerea* Pers., *Colletotrichum acutatum* (JH Simmonds), *Phytophthora* spp., *Xanthomonas fragariae* (Kennedy and King), *Sphaerotheca pannosa* (Wallr.)Lév., *Verticillium* sp., *Rhizoctonia* sp., *Pythium* sp., and *Fusarium* sp. during the crop cycle and the post-harvest period (Maas,1998). In order to manage this limitation, producers apply several pesticides (Wang *et al*., 2017). Pesticides play a key role to control the insect pests and diseases and hence protect and promote production (Prodhan *et al.,* 2015). The intensive use of these substances in crops can contaminate the soil, air, and ground and surface water sources (Rodríguez *et al*., 2014), as well as generating collateral damage to

beneficial organisms. In addition, pesticide residues in agricultural products pose a risk to human health (Nougadère *et al*., 2011). Adverse effects on human health include blindness, liver diseases, increased cholesterol, neurological toxicity, alterations in the immune and reproductive system, lymphomas, prostate cancer, m ultiple myeloma, Parkinson's disease, infant mortality, and genetic disorders, among others (Chatterjee *et al*., 2013; Sinha *et al*., 2012; Gupta, 2006; Lozowicka, 2015).

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.

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 fruits are done without perplexing for the pre-harvest interval, pesticide residue levels in such strawberry would undoubtedly be above MRL. Most of the strawberry 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 unusual spray of insecticides and selling of fruits 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).

The quality of fruits at harvest was evaluated on physio-chemical parameters such as starch content, total soluble solids (TSS), and acid content. Several biochemical changes occur when a plant is attacked by a pathogen or is subjected to chemical, physical, or biological stress. These alterations could be an increase or decrease in the expression of certain phenolic chemicals, which play an important role in plant resistance/susceptibility (Singh *et al*., 2015). The phenolic chemicals implicated in disease resistance are also found in healthy plant tissue as a type of base resistance, although phenolic compound synthesis and accumulation appears to be enhanced after infection and can thus be viewed as a post-infection response .

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. In Bangladesh indiscriminate use of insecticides is very common phenomenon without following any guidelines. Duration of retention/persistance of its chemical residues in harvesting product varies depending on nature of insecticide (contact/systemic) applied. 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, Cho *et al* 2013, Rai *et al*. 2016, Lozowicka 2015, Sundaram *et al* 2018 and Lopez *et al* 2019). 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). In Bangladesh almost all the researches on pesticidal residues have been executed/concentrated on vegetables and scanty on fruits like cabbage, cauliflower, eggplant, bittergourd, yard long bean. Betel leaf, mango, melon etc. (Islam *et al*. 2014, Hossain *et al.* 2014, Prodhan *et al.* 2016, 2018, 2021, Hasan *et al.* 2017, Islam *et al*. 2019 and Nahar *et al.* 2020) and not on strawberry. However, knowledge of withholding period becomes important even for less persistent insecticides, specifically in fruits and vegetables since these crops are harvested shortly after pesticide application and consume instantly or within very short period. With this view, the present study was initiated with the following objectives:

- To quantify the level of cypermethrin residues in strawberry at different days after spray.

- To determine the effects of pre-harvest foliar spray of cypermethrin on biochemical attributes of strawberry.

CHAPTER II
REVIEW OF LITERATURE

In this chapter challenge has been made to assessment literatures for updating the information regarding the existing status of research and knowledge about the determination of pesticide residues in fruits and vegetables. Available and accessible sources of information have been systematically reviewed and summarized with essential comments as appropriately as possible. In spite of the fact, there have been inadequate source of information, most of the relevant information available in and around Bangladesh was collected and reviewed. It is discovered that most of the information on the aspects searched as mentioned above are mostly available from research station and information of farmers' field condition are scanty. However, a significant number of study-reports on insecticides residues in fruits and vegetables 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 fruits and vegetables in Bangladesh are rarely reported. With this background, the information collected from different sources have been reviewed and presented below:

Zhang *et al.* (2021) conducted a study on the analysis of 284 pesticides in five local fruit cultivars using ultrahigh-performance liquid chromatography-quadrupole time-of-fight mass spectrometry (UPLC-QTOF/MS) in Shanghai, China. The limits of determination and the limits of quantitation of pesticides were 0.6–10 and 2–30 μg/kg, respectively. A total of 44, 10, 10, 18, and 7 pesticides were detected in strawberries, watermelons, melons, peaches, and grapes, respectively. The pesticide levels in 95.0% of the samples were below the maximum residual limits (MRLs) prescribed by China, and in 66.2% of the samples below the EU MRLs. The dietary risk assessment study showed big differences in the chronic and acute exposure risk values among different Chinese consumer groups. Nevertheless, certain measures are needed for both growers and the government in order to decrease the MRL-exceeding rate of pesticide residues and ensure the quality and safety of fruits for consumers.

Wang *et al.* (2021) initiated a study to investigate the dissipation and accumulation of four fungicides (pyraclostrobin, pyrimethanil, procymidone, and cyprodinil) after two typical repeated sprayings (a single fungicide repeated spraying and two fungicides sprayed using an alternate repeated technique) on strawberry. A safety assessment demonstrated that the maximum number of times cyprodinil could be sprayed after single spraying was one; however, this number doubled after alternate spraying. The risk of exceeding the maximum residue limits of the fungicides on greenhouse strawberries decreased; however, the combined dietary risks of fungicides after alternate spraying might be high. Alternate repeated spraying of procymidone and pyrimethanil may be the optimal repeated spraying combination for greenhouse strawberries.

Prodhan *et al.* (2021) has been developed and validated a novel method in order to determine 8 pesticide residues (acephate, diazinon, malathion, fenitrothion, chlorpyrifos, quinalphos, dimethoate and cypermethrin) in betel leaf using quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction in combination to Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD) and Electron Captured Detector (ECD). In this study, the optimization of cleanup materials was done properly and found that 600 mg anhydrous $MgSO_4$, 150 mg activated charcoal powder, and 120 mg PSA (Primary Secondary Amine) was the best combination for proper cleanup of betel leaf matrix. Recoveries for all the selected pesticides at fortification levels of 0.02, 0.1, and 0.3 mg/kg ranged from 86 to 108% with RSDr ≤ 9% and the matrix matched calibration curve showed good linearity ($r^2 ≥ 0.996$). The limit of detection ranged from 0.003 to 0.005 mg/kg and the limit of quantification was 0.02 mg/kg, which was lower than the EU-MRLs. The matrix effects of the selected pesticides were also evaluated in this study and found that cypermethrin had a prominent matrix effect (+124%). The proposed method was applied successfully to analyze 110 fresh betel leaf samples and found that 12 were contaminated with cypermethrin, chlorpyrifos, and dimethoate at a level above EU-MRLs.

Khan *et al.* (2020) conducted a study to assess the contamination and health risk due to the presence of pesticides in fruits and vegetables. A total of six vegetable samples, 3

fruit samples, 7 soil samples, and 6 water samples were collected from three different sampling points. High-performance liquid chromatography using acetonitrile and water solvent system was employed for the quantitative and qualitative analysis. Consumers utilizing these vegetables and fruits are under potential health risks due to the presence of pesticides in soil and water Practical Applications. In this study, an analytical method for detecting pesticides in a variety of environmental matrices including fruits, vegetables, water, and the soil was presented and the health risk associated with the presence of pesticides in a wide range of fruits and vegetables was assessed. It is highly significant because in developing countries agricultural activities contribute majorly toward the total gross domestic product and pesticides are extensively used to control, prevent, devastate, and diminish any harmful pest that destroys crops.

Sarangapani *et al*. (2020) have been conducted a study for atmospheric air plasma discharge for the generation of plasma-activated water (PAW), with the aim of reducing pesticide residues on fresh fruit. For this purpose, a large discharge volume pin-to-plate cold plasma reactor was employed. The pesticide-spiked grapes and strawberries were processed with varying PAW concentrations to study their efficacies for pesticide degradation combined with an evaluation of any induced changes in key nutritional and quality attributes. The results suggest that the reduction of chlorpyrifos was 79% on grapes and 69% on strawberries while that of carbaryl was 86% on grapes and 73% on strawberries, respectively. The degradation of pesticides in PAW is due to the generation of metastable reactive species including nitrates, nitrites, and hydrogen peroxide. The high oxidation potential and acidic environment of this PAW are proposed as important actors for pesticide dissipation. In addition to the effective pesticide reductions obtained, there were no significant changes in the key physical attributes (color and firmness) of the treated samples and only slight changes in the ascorbic acid levels observed for both strawberries and grapes. This study points to the effective potential of PAW for chemical decontamination of fruit while maintaining important quality and nutritional parameters.

Nahar *et al.* (2020) conducted a study to assess the health hazards associated with the residual effect of pesticides in two common vegetables (cauliflower and tomato) collected from five different markets of a northern city of Bangladesh. A total of 80 samples (i.e. 40 of each vegetable) were collected for the analysis of seven major organophosphorus insecticides namely acephate, chlorpyrifos, diazinon, dimethoate, fenitrothion, malathion and quinalphos used in that region. Modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction technique and GC-FTD (Gas Chromatography coupled with Flame Thermionic Detector) used for the analysis of the samples. Results revealed that 11 (6 cauliflower, 5 tomato) analyzed samples contained residues which is about 14% of the total number of samples. Most of the samples contaminated diazinon at a level above EU-MRLs. However, health risk assessment based on ADI, the contaminated samples were safe. Continuous monitoring together with a sample traceability system is suggested to protect consumers' health from the cumulative effects of other contaminated dietary products.

Yazici *et al.* (2020) developed an infrared-based prediction method for easy, fast and non-destructive detection of pesticide residue levels measured in strawberry (*Fragaria × ananassa* Duch, cv. Albion) samples using near-infrared spectroscopy and demonstrating its potential alternative or complementary use instead of traditional pesticide determination methods. Strawberries of Albion variety, which were supplied directly from greenhouses, were used as the study material. A total of 60 batch sample groups, each consisting of eight strawberries, was formed, and each group was treated with a commercial pesticide at different concentrations (26.7% boscalid + 6.7% pyraclostrobin) and varying residual levels were obtained in strawberry batches. The strawberry samples with pesticide residuals were used both to collect near-infrared spectra and to determine reference pesticide levels, applying QuEChERS (quick, easy, cheap, rugged, safe) extraction, followed by liquid chromatographic–mass spectrometric analysis. Partial least squares regression (PLSR) models were developed for boscalid and pyraclostrobin active substances. During model development, the samples were randomly divided into two groups as calibration (n = 48) and validation (n = 12) sets. A calibration model was developed for each active substance, and then the models were validated using

cross-validation and external sets. Performance evaluation of the PLSR models was evaluated based on the residual predictive deviation (RPD) of each model. An RPD of 2.28 was obtained for boscalid, while it was 2.31 for pyraclostrobin. These results indicate that the developed models have reasonable predictive power.

Stensv and Christiansen (2020) were investigated the residue levels of eight different fungicides in strawberries of the cultivar Korona grown in a commercial greenhouse at 4, 7, and 14 days after application at recommended doses by the manufacturers and at half rates. Iprodione, tolylfluanid, and vinclozolin were tested in two experiments, while chinomethionat, chlorothalonil, imazalil, penconazole, and triadimefon were tested once. For chinomethionat, imazalil, iprodione, penconazole, and vinclozolin, the residue levels were below MRL 2 weeks after application. Application of triadimefon in normal rate gave residues below MRL 14 days after application. However, its metabolite, triadimenol, was above MRL at the same time. Tolylfluanid gave very high residue levels, and except from half concentration in the second experiment, all other residue levels were above MRL. Seven days after application, residues in both experiments were approximately 3 times higher than MRL when normal rate of tolylfluanid was applied. For chlorothalonil at the recommended rate, the residue level was above MRL at any sampling time, while half rate gave residues below MRL 14 days after treatment. In view of the present results, tolylfluanid, chlorothalonil, and triadimefon will need longer time from last application to harvest and/or reduced application rates in greenhouse-grown compared to field-grown strawberries. In addition or as an alternative, recommended rates could be lowered.

López V *et al.* (2019) conducted a study to determine and compare pesticide residues in strawberry fruits from two different production systems distributed in the main producing areas of the Cundinamarca Department in Colombia. Eight samples of strawberry crops were collected in four producer municipalities (Guasca, Facatativa, Mosquera and Sibate) to compare different systems (conventional production vs. production based on Integrated Pest Management, IPM). Samples with a concentration of 394 molecules were examined using liquid and gas spectrometry. Fischer's exact test was used to determine the

association between the pesticide type and residue level in the fruits, with more insecticide samples that exceeded the permitted threshold than when using fungicides. Twenty-two different molecules were detected in the analyzed samples, with 37 detection events, of which eight were reported in the IPM production systems and 29 in the conventional producers. The results revealed that nine molecules of insecticides and two of fungicides exceeded the concentrations set by Colombian regulations, The Food and Agriculture Organization (FAO) and but no significant differences were found between the two production systems. The calibration of equipment and applications must be improved in order to avoid over-concentration of pesticides, especially insecticides.

Islam *et al.* (2019) carried out a study for the determination of major organophosphate insecticide residues in cabbage samples from different markets of Dhaka. The study was conducted to analyse 4 organophosphorus pesticide namely chlorpyrifos, diazinon, fenitrothion and quinalphos residues in cabbage. Between September 2016 and March 2017, 50 Cabbage samples were collected from 5 vegetables markets of Dhaka city, namely Rampurakacha bazar, Jatrabarikrishi market, Kawran Bazar, Taltola Bazar and Mohammadpur Krishi Market. The collected samples were extracted and analyzed by QuEChERS based Gas Chromatography coupled with Flame Thermionic Detector (GC-FTD) method. Total 6 samples (12%) contained pesticide residues and interestingly all of them were above the MRLs set by EC. Among the four organophosphorus insecticides tested only Chlorpyrifos and Diazinon were detected above the MRLs. This research represents a snapshot situation of contamination of pesticides in one of the common winter vegetables available in Dhaka City's local markets linked to consumer safety.

Islam *et al.* (2019a) initiated a study for the analysis of pesticide residues in bitter gourd using modified QuEChERS extraction coupled with Gas Chromatography. This study was undertaken to monitor the presence of seven organophosphorous pesticide residues like acephate, dimethoate, fenitrothion, chlorpyrifos, quinalphos, diazinon and malathion in bitter gourd. 65 samples were collected from retail markets located at the adjacent area of Jahangirnagar University, Savar, Dhaka, Bangladesh namely Genda bazaar, Savar bazaar, Nayarhat bazaar, Islampur bazaar, Pallibiddut bazaar, Baipayl bazaar and Sreepur

bazaar. The samples were extracted by modified quick, easy, cheap, effective, rugged and safe (QuEChERS) method and analyzed by gas chromatography coupled with flame thermo ionic detector (GC-FTD). Among the 65 analyzed samples, eight (12.3% of the total number of samples) were contaminated with pesticide residues and all of them contained residues above Maximum Residue Limit (MRL) set by European Commission (EC). Another fifty-seven samples (87.7% of the total number of samples) contained no detectable pesticide residues of the sought pesticides. The findings from this current study showed the common scenario of pesticide residues in daily consumed vegetables of Savar, Dhaka, Bangladesh that pointed to the imminent health hazards. Therefore, it is suggested to control the overuse of pesticide in vegetable field strictly as well as to increase the awareness of the harmful effect of pesticide residues in vegetables for the growers and the consumers as well.

Islam *et al.* (2019b) established a method for the determination of major organophosphorus pesticide residues using modified QuEChERS Extraction and Gas Chromatography. This investigation was undertaken to scrutinize the entity of seven organophosphorus pesticide residues like acephate, dimethoate, fenitrothion, chlorpyrifos, quinalphos, diazinon and malathion in eggplant. Seventy eight eggplant samples were collected from retail markets located at the surrounding area of Jahangirnagar University, Savar, Dhaka, Bangladesh namely Genda bazaar, Savar bazaar, Nayarhat bazaar, Islampur bazaar, Pallibiddut bazar, Baipayl bazaar and Sreepur bazaar. The samples were extracted by modified quick, easy, cheap, effective, rugged and safe (QuEChERS) method and analyzed by Gas Chromatography coupled with Flame Thermionic Detector (GC-FTD). Among the seventy eight analyzed samples, nine (11.5%) were contaminated by pesticide residues. Two of them were exceeded the EU-MRL (EC, 2015). Another sixty nine samples (88.5%) were free from the contamination of the sought pesticides. The findings from this current study showed the subsistence of pesticide residues in daily consumed vegetables of Savar, Dhaka, Bangladesh that pointed to the imminent health hazards. So, public awareness about the pesticides and other related matter should be increased for practicing a pesticide free agriculture as well as gain contaminate free environment.

Sundaram *et al.* (2018) conducted a study to quantitatively and qualitatively analyze the pesticide residues (PR) in selected fruits with and without pre-treatment. Two fruits (grapes and strawberry) were selected randomly from a local market in Coimbatore district. Pesticides were quantified by Gas Chromatograph with and without treatment (washing by dipping in 2% salt solution for 10 min, mechanical peeling and juicing) concurrently. QuEChERS method was used for preparation of samples for analysis of pesticide residue. The samples without pre-treatment showed the presence of Organo Phosphorus (OP) pesticides. The amount of Ethion in the grape sample was 15.99 ppm and MePrimphose was 22.98 ppm, which is above the MRL (i.e. > 0.05), while strawberry was reported to have Ethion (3855.56 ppm). The samples analysed after pre-treatment showed no detectable level of pesticide residue. The study puts forward the importance of organic foods and kitchen garden for elimination of these toxicities to human health.

Prodhan *et al.* (2018) conducted a research work on the variability of pesticide residues in eggplant units collected from a field trial and marketplaces in Greece. In total, 120 samples from a trial field and 142 samples from different marketplaces in Thessaloniki, Greece, were collected to estimate the variability of pesticide residues in eggplant units. They were extracted by the QuEChERS method and the residues were determined by LC–MS/MS. For the field samples, the level of estimated cypermethrin and deltamethrin residues were 0.01-0.349 mg/Kg and 0.01-0.097 mg/Kg, respectively; and the unit-to-unit variability factors (VFs) obtained for cypermethrin and deltamethrin residues were 2.54 and 2.51, respectively. The mean residue levels of both pesticides were higher in the composite samples than in the individual samples. The average VFs for the marketplace samples was 3.89. The eggplant units exposed to pesticides were higher in residues than the non-exposed units.

Prodhan *et al*. (2018a) conducted a study to determine the pre harvest interval (PHI) for quinalphos in Eggplant, Cabbage and Yard long bean; malathion in Eggplant, Yard Long bean and Cauliflower; cypermethrin in Tomato and Yard long bean; and diazinon in

Eggplant and Yard long bean depending on Maximum Residue Limit (MRL) set by FAO/ WHO. Five supervised field trials were conducted and sprayed with the field dose (2 ml/L of water) of each pesticide except cypermethrin (1 ml/L of water). Samples were collected at 0, 1, 3, 5, 7, 10, 12, 15 and 18 days after spray. The collected samples were analyzed using Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD) and Electron Capture Detector (ECD) for the determination of pesticide residues. The level of residues were above MRL up to 10 DAS for quinalphos in Cabbage, 7 DAS in Eggplant, 5 DAS in Yard long bean; for malathion 5 DAS in Yard long bean and Eggplant, 7 DAS in cauliflower; for diazinon 5 DAS in Yard long bean and Eggplant; and 3 DAS for cypermethrin in Yard long bean and Tomato. The determined PHI for quinalphos were 12 DAS in Cabbage and 10 DAS in Eggplant and 7 DAS in Yard long bean; For malathion 7 DAS in Yard long bean and Eggplant and 10 DAS in cauliflower; For diazinon 7 DAS in Yard long bean and Eggplant; For cypermethrin 5 DAS in Yard long bean and Tomato.

Prodhan *et al.* (2018b) conducted a study to quantify the residue loss of Quinalphos, Diazinon and Fenitrothion from eggplant and Malathion from Yard long bean through washing and cooking procedures. Samples were collected from the Research field of Entomology Division of Bangladesh Agricultural Research Institute (BARI). The samples were analyzed using a simple Gas Chromatographic technique. Washing with water reduced 34% Quinalphos, 28% Diazinon and 41% Fenitrothion and heating with water at 100 ^{0}C reduced 95% Quinalphos, 84% Diazinon and 100% Fenitrothion from eggplant. Washing with water reduced 45% Malathion and heating with water at 100 ^{0}C reduced 100% Malathion from Yard long bean. Effect of O_3 sterilizer in reducing pesticide residues from eggplant was also investigated in this study and found that O_3 sterilizer reduced 79.00% Diazinon and 62.50% Quinalphos while washing with only water reduced 60.50% Diazinon and 40.00% Quinalphos from eggplant.

Prodhan *et al.* (2018c) has been developed and validated a simple and efficient multiple organochlorine pesticide residues analytical method using quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction technique and Gas Chromatography coupled

with Electron Capture Detector (ECD) for the determination of 19 organochlorine pesticides (Alpha BHC, Delta BHC, Beta BHC, Gama BHC, Heptachlor, Aldrin, Heptachlor Epoxide, Gama Chlordane, Alpha Chlordane, Alpha Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, Beta Endosulfan, 4,4 DDT, Endrin Aldehyde, Endosulfan sulphate, Methoxychlor, and Endrin Ketone) in shrimp. The method was validated by evaluating the accuracy, precision and linearity limit of detection (LOD) and limit of quantification (LOQ). The average recoveries of the selected pesticides ranged from 84% to 106% with RSDr ≤ 14% in four fortification levels of 0.05, 0.1, 0.2 and 0.3 mg/Kg. The linearity was ≥0.996 for all of the selected pesticides with matrix matched calibration standards. The LOD ranged from 0.003 to 0.009 mg/Kg and the LOQ was 0.05 mg/Kg. This method was applied successfully for the residue analysis of 40 shrimp samples collected from different market places in Bangladesh.

Hasan *et al*. (2017) initiated a study to quantify pesticide residues in country bean collected from different markets of Dhaka city. The collected samples were analyzed using modified QuEChERS Extraction and Gas Chromatography. They have been detected two organophosphorus insecticides (Dimethoate and Quinalphos) in the analyzed country bean samples. Among the 50 analyzed samples of country bean, 10 samples (20%) contained residues of Dimethoate and Quinalphos, of which 5 samples were above the maximum residue limits (MRLs). Most of the contaminated samples (8 samples) contained residue of Dimethoate.

Akter *et al.* (2017) conducted a study for the determination of pesticide residues in eggplant collected from different local markets of Mymensingh Sadar, Mymensingh. The collected samples were extracted using modified QuEChERS Extraction and analyzed with Gas chromatography. This study reflects the overall scenario of pesticide residue contamination in eggplant available in the local markets of MymensinghSadar, Mymensingh. In this study, a simple and efficient multiple pesticide residue analytical method based on QuEChERS extraction and gas chromatography-flame thermionic detector (GC-FTD) was used for the determination of pesticide residues. Among the 50 analyzed samples, 11 (22% of the total number of the samples) contained residues of

diazinon, dimethoate, quinalfos, and chlorpyrifos, of which, 2 had multiple pesticide residues and 5 samples contained residue above the European Union maximum residue limit (EU-MRLs). Chlorpyrifos was detected as the most used pesticide in eggplant in the studied area.

Prodhan *et al.* (2016) has been developed and validated a multiresidue analytical method for the determination of pesticide residues in cauliflower collected from different market places in Thessaloniki, Greece. In this study, the liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for the quantification of pesticide residues at trace levels. Among the 120 analyzed samples, 48 (40% of the total no. of samples)were found to have pesticide residues. The detected pesticides were chlorpyrifos, cypermethrin, deltamethrin and indoxacarb.

Prodhan *et al.* (2016a) has been developed and validated a multiresidue analytical method to determine pesticide residues in cabbage collected from different market places in Thessaloniki, Greece. In this study, the modified QuEChERS extraction in combination to liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for the quantification of pesticide residues at trace levels. 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. Of the detected pesticides, three were insecticides (chlorpyrifos, cypermethrin and deltamethrin) and two were fungicides (fluopicolide and propamocarb hydrochloride).

Rizzetti *et al.* (2016) conducted a research on different extraction procedures along with 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 CH3COONa 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 micro extraction (DLLME) for the quantitative determination of 36 multiclass, 9 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.

Sójka *et al.* (2015) conducted a study to assess dietary risk measured by the % ADI (acceptable daily intake) and MOE (margin of exposure) associated with the presence of pesticide residues for the consumption of strawberry processing by-products containing an amount of ellagitannins equivalent to amount present in 100 g of fresh strawberries. In the study, they investigated the contents of pesticides that are approved for use against strawberry diseases and pests. The total contents of pesticides in strawberry press-cake seeds (SPCS), exhausted strawberry flesh (ESF) and strawberry ellagitannin preparation (SEP) were 2143, 13,464 and 20,225 µg/kg, respectively. To ensure good recovery and high specificity of the analyzed pesticides, the QuEChERS extraction method and liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) or gas chromatography with a single mass detector (GC/MS) were used. Multiple pesticide residues were extracted from plant material by liquid–liquid extraction followed by dispersive solid phase extraction (dSPE) purification. The analyzed products were dominated by fungicides as 96% of the total content of the tested pesticides. In the tested samples, they detected 11 fungicides and 3 insecticides. The dietary risk to consumer health, which depends on the presence of pesticide residues, in %ADI of daily consumption of ellagitannins (as dried extract (SEP), seeds (SPCS) or flesh (ESF))

ranged from 0.2% to 4.1% in a dose that was equivalent to 100 g of strawberries. Although the pesticide residue contents in strawberry by-products are higher than in fresh fruits, the suggested doses of the by-products are lower. Therefore, the dietary risk to consumers from strawberry byproducts is comparable to that from fresh fruits.

 Lozowicka *et al.* (2015) conducted a study that the effects of washing with tap and ozone water, ultrasonic cleaning and boiling on 16 pesticide (ten fungicides and six insecticides) residue levels in raw strawberries were investigated at different processing times (1, 2 and 5 min). An analysis of these pesticides was conducted using gas chromatography with nitrogen-phosphorous and electron capture detection (GC-NPD/ECD). The processing factor (PF) for each pesticide in each processing technique was determined. Washing with ozonated water was demonstrated to be more effective (reduction from 36.1 to 75.1 %) than washing with tap water (reduction from 19.8 to 68.1 %). Boiling decreased the residues of the most compounds, with reductions ranging from 42.8 to 92.9 %. Ultrasonic cleaning lowered residues for all analysed pesticides with removal of up to 91.2 %. The data indicated that ultrasonic cleaning and boiling were the most effective treatments for the reduction of 16 pesticide residues in raw strawberries, resulting in a lower health risk exposure. Calculated PFs for alpha-cypermethrin were used to perform an acute risk assessment of dietary exposure. To investigate the relationship between the levels of 16 pesticides in strawberry samples and their physicochemical properties, a principal component analysis (PCA) was performed.

Prodhan *et al.* (2015) undertook an experiment to determine pesticide residues in 72 fresh eggplant samples collected from different market in Thessaloniki, Greece with Liquid Chromatography-Mass Spectrometry by adopting QuEChERS extraction method. Among the 72 analyzed samples, 34 (47 % of the total number of samples) had pesticide residues, of which, 5 had multiple pesticide residues and 29 had single pesticide residue. Only one sample contained residue above the EU-MRLs (European Union- Maximum Residue Limits).

Prodhan *et al.* (2015a) has been developed and validated a precise and an effective analytical method to determine pesticide residues in melon collected from different market places in Thessaloniki, Greece. In this study, the modified QuEChERS extraction in combination to liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for the quantification of pesticide residues at trace levels. In this study, both insecticides and fungicides have been detected in melon samples. Among the 122 analyzed samples, 32 (26% of the total number of samples) were found to have pesticide residues.

Islam *et al.* (2014) conducted a survey 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, 23 farmers were interviewed and it was 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. In two selected locations of Narsinghdi 8.33 to 45.00 percent farmers were recorded to apply different pesticides every day and in some cases even twice in a day on vegetables. A total of 42 samples were collected from fields and markets and multiple pesticide residue analysis was done by Gas Chromatography (GC) with Flame Thermionized Detector (FTD) and Electron Capture Detector (ECD). Out of 42 samples, 27 had pesticide residue. Among these 27 samples, 14 samples had pesticide residues above the Maximum Residue Limit (MRL). The detected pesticides were Diazinon, Malathion, Quinalphos, Fenitrothion, Cypermethrin, Fenvalerate and Propiconazole.

Hossain *et al.* (2014) conducted a study to determine the pre harvest interval (PHI) for cypermethrin and acephate in Yard long bean depending on Maximum Residue Limit (MRL) set by FAO/ WHO. Two supervised field trials were conducted and sprayed with the field dose (2 ml/L of water for acephate) and for cypermethrin, it was1 ml/L of water. Samples were collected at 0, 1, 3, 5, 7, 10, 12, and 15 days after spray. The collected samples were analyzed using Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD) and Electron Capture Detector (ECD). The level of residues were

detected up to 10 DAS for cypermethrin (0.096 mg/kg), and 7 DAS for acephate (0.435 mg/kg), however, the level of detected residues for both of the pesticides were above MRLs up to 5 DAS. Therefore, The PHI was determined for both of the pesticides were 7 DAS.

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.

Hendawi *et al.* (2013) conducted a study to evaluate the effects of some technological processes on the residual levels of imidacloprid in strawberry fruits and products. According to their half-life (t1/2) values, strawberry fruits can be safely harvested for human consumption 7.4 days after spraying. The removal ratio of imidacloprid residue in strawberries was in the range of 9.9–30.55% by washing with tap water. The average amount of imidacloprid residue in strawberry fruits, juice, and syrup under cold and hot break greatly decreased compared with those in unwashed strawberry fruits. Moreover, the residue of pesticide decreased more in strawberries syrup under hot break than cold break. Imidacloprid residue was concentrated into jam and increased to higher levels than strawberry juice and syrup under cold and hot break. A change in physical and chemical properties of strawberry fruits and products was related mainly to the processing operations.

Panhwar and Sheikh (2013) conducted a research to analyze the effect of traditional food processing on the reduction of pesticide residues in cauliflower through GCµECD 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).

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.

Cserhati and 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.

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.

Bozdogan *et al*. (2011) conducted a study to determine the effects of different spraying methods on spray deposits and drift, pesticide residues and biological efficacy on strawberries. For this purpose, strawberries were sprayed with dicofol by broadcast and band spraying. Broadcast sprayings were applied via hollow cone nozzles (HC) and air-assisted spinning cage nozzles (ASC). Band spraying was applied via flat fan nozzles (FF). Pesticide deposits on leaf surfaces, ground, drift and pesticide residues on strawberries were analyzed with Gas Chromatography/Electron capture detector (GC/ECD). Strawberries were analyzed based on dicofol residues up to 6 days after spraying. The results obtained showed that the highest pesticide deposits on leaf surfaces and also biological efficiency were obtained with FF. The lowest pesticide deposits on ground were obtained by HC and the highest deposit of drift was obtained by ASC. According to Commission Directive 2000/42/EC, the MRL's of dicofol on strawberry is 0.02 mg/kg. In this study, residues on strawberries for all types of nozzles were below the suggested maximum residue level (MRL) of dicofol by Commission Directive 2000/42/EC.

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.

Prodhan *et al*. (2010) has been developed an analytical method for the determination of pesticide residues in fish using Gas Chromatogarphy-Mass Spectrometry (GC-MS). A total of 125 samples of fish covering Rui, Carp, Shrimp, White fish, and Fin fish were collected from Dhaka, Khulna and Chittagong region of Bangladesh and carried to the Pesticide Analytical Laboratory (PAL), Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur. All samples were extracted and prepared for injection using the standard protocols for multi-residue analyses during September, 2007 to April, 2008. The elutes were injected into either GCMS-EI or GCMS-NCI depending on the nature of target insecticides. Results revealed that among 125 samples, 49 had insecticide residues. Out of seven samples from Dhaka, 2 had multiple insecticide residues and 1 had single insecticide residue. The detected carbofuran residues ranged from 0.29 to 1.13 ppm, the residues of diazinon, carbaryl and fenvalerate were 1.38, 0.35 and 0.009 ppm, repectively. Out of 60 samples from Chittagong, 8 had multiple insecticide residues and 15 had single insecticide residue. The detected residues of carbofuran, diazinon, fenvalerate, chlorpyrifos, heptachlor and DDT ranged from 0.17-0.89 ppm, 0.03-2.75 ppm, 0.01-0.03 ppm, 0.005 ppm, 0.19-1.15 ppm and 0.06-0.52 ppm, repectively. Out of 58 samples from Khulna, 9 had multiple insecticide residues and 14 had single insecticide residue. The detected residues of carbofuran, diazinon and DDT ranged from 0.03-1.25 ppm, 0.02-1.03 ppm and 0.04-0.12 ppm respectively. Thus 22 samples contained insecticide residue above ADI (Acceptable Daily Intake) referring to

average body weight of 50 kg/person, of which 02 samples collected from Dhaka, 10 from Chittagong and 10 from Khulna region.

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 a simple technique for the quantification of organophosphorus and organochlorine insecticide residues from fish samples using Gas Chromatograph (GC) couple to Electron Capture Detector (ECD) and Flame Thermionic Detector (FTD). They collected sixty eight samples of fish (Rui, Shrimp & Others) from Dhaka, Khulna and Chittagong and carried to the 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 MRL.

Kabir *et al.* (2008) developed an analytical method for the determination of residue of diazinon and carbosulfan in brinjal and quinalphos in yard long bean under supervised field trial. The present study was undertaken to detect and quantify the left over residue of Diazinon and Carbosulfan in brinjal and Quinalphos in yard long bean and comparison between the detected residue level with maximum residue level (MRL) set by FAO (1970). Three supervised field trials (two for brinjal and one for yard long bean) were carried out sprayed with the field dose (1.5 ml/L of water) of Diazinon, Carbosulfan, and Quinaiphos. Samples were collected daily after spraying till residue were found. In case of Diazinon, left over 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 yard long bean sample was detected upto 6 DAS and upto 4 DAS the level of residue was above the MRL.

Kabir *et al.* (2008a) conducted an experiment to quantify the purity in respect of active ingredient (AI) of some common insecticides used against vegetable insect pests. A series of analyses were made at Pesticide Research Laboratory, Pesticide Research & Environmental Toxicology Section, Entomology Division, BARI, Gazipur using GC-2010 (with FID and ECD detectors) and HPLC- 20A Prominence (with PDA detector). Tested insecticides were 9 brands of Carbofuran 5G and 3G, 2 brands of Carbaryl 85SP, 3 brands of Carbosulfan 20EC, 8 brands of Malathion 57EC, 9 brands of Diazinon 10G and 60EC, 3 brands of Quinalphos 25EC, 3 brands of Dimethoate 40EC, 4 brands of Cypermethrin 10EC and 7 brands of Chloropyrifos 20EC). These collected insecticides were collected from pesticide traders of Jessore and Tongi region of Bangladesh. Results of the present investigation clearly indicated that most of the marketed insecticides were found to have lower AI (≤60%) than that stated on the label. In some cases AI of unknown chemical was being used. Of 9 tested brands of Carbofuran, 3 were found to have 100% purity, 5 had 75-78% AI and 1 had nothing except carrier. Among 2 tested brands of Carbaryl none had >50% purity while all brands of Carbosulfan were found to have 78-98% purity. Eight brands of Malathion were tested, of which only 2 were found as pure, 5 had 80-90% AI and 1 had 60% AI. Of 9 tested brands of Diazinon, only 1 had >90 AI, while 3 had 80%, 4 had 40% and the other 1 had 10%. All 3 tested brands of Quinalphos provided 68-76% AI, while all Dimethoate had only 15-20% AI. Among 4 brands of Cypermethrin, 3 consisted of 100% AI, only 1 had 65%. Seven brands of Chloropyrifos were analyzed, 2 were found to have 100% purity, 3 had 91-97% and 2 had 85-87% AI.

Kabir *et al.* (2007) conducted an experiment at the Regional Sugarcane Research Station, Gazipur in which carbofuran (2 kg AI/ha) was applied in sugarcane field to document the level of carbofuran residue left in soil and plant samples after different days of application (DAA). Plant and soil samples were analyzed by using GCMS-EI.

Carbofuran residues were found even at 90 DAA both in soil and plant. In case of soil, the amount of carbofuran residues were 24.84, 3.32, 2.12, 0.59, 0.035, 0.02 and 0.005 ppm at 0, 3, 7, 15, 30, 60 and 90 DAA, respectively. In case of plant samples, the lower residue (0.0035 ppm) was observed at 0 DAA compared to those at 3 DAA (0.075 ppm), 7 DAA (0.035 ppm) and 15 DAA (0.015 ppm). At 60 and 90 DAA, residues were the same (0.002 ppm) while at 30 DAA it was 0.0025 ppm. The highest level of carbofuran residue (0.075 ppm) in plant samples was found at 3 DAA which is lower than FAO/WHO recommended MRL (0.1mg/kg crop).

CHAPTER III

MATERIALS AND METHODS

3.1 Field Experiment

A. Sher-e-Bangla Agricultural University, Dhaka where supervised field trial on strawberry was conducted in the experimental field and quality analysis was done in laboratory of Department of Horticulture

B. Pesticide Research & Environmental Toxicology Section, Entomology Division of BARI, Gazipur where residual analysis was performed by Gas Chromatography (GC) with ECD .

- Insecticide used

The synthetic pyrethroid pesticide, cypermethrin (Ripcord 10EC) was used at the rate of 1.5 ml/l of water. Application of Ripcord was initiated when the fruits were attained at marketable size.

3.2 Sampling and sample preservation

Samples of strawberry were collected at 0(hr), 1, 3, 5, 7, 10 and 12 days after pesticide application from the experimental field of Horticulture farm, Sher-e-Bangla Agricultural University, Dhaka. 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 the collected samples were carried to the Pesticide Analytical Laboratory, Entomology Division of Bangladesh Agricultural Research Institute (BARI), Gazipur.

Pesticide standard stock solutions of cypermethrin was prepared in acetonitrile at a concentration of 1000 mg/L and stored at -20°C until use. A standard solution of 50 mg/L in acetonitrile 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 acetonitrile. An

intermediate standard solution of 10 mg/L in acetonitrile was prepared from the 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 acetonitrile were prepared by transferring the appropriate amount from 10 mg/L intermediate standard solution into seven separate 10-mL volumetric flasks. All the standard solutions were kept in a freezer at -20°C until use.

3.3 Analysis of pesticide residue in strawberry

Pesticide residue analysis procedures are primarily divided into two parts. Extraction and cleaning of target analytes from the matrix, as well as detection of target analytes from the matrix.

3.3.1 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 M.D.H. *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 (Plale 6) , and it was shaken immediately by the vortex mixer for 1 minute to prevent the formation of magnesium sulfate aggregates (Plale 7). Afterwards, the extract was centrifuged for 5 min at 5000 rpm (Plale 8). An aliquot of 3 mL of the MeCN layer was transferredinto 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. (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 (Plale 6).

3.3.2 Determination of the target analytes from the matrix

Determination of the selected pesticides (cypermethrin) was performed by Gas Chromatography (GC) with Electron Capture Detector (ECD).

3.4 Chemicals Used in Analysis

The standard of cypermethrin was obtained from Sigma-Aldrich Laborchemikalien (St Louis, MO, USA) via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. The purity of the selected pesticide was>99.6% purity. Methanol, gradient grade acetonitrile, sodium chloride (NaCl), anhydrous magnesium sulphate (MgSO4) and Primary Secondary Amine (PSA) were purchased from Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh.

3.5 Analytical Apparatus Required

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

Plate 1: Centrifuge Machine

Plate 2: Electric Balance

Plate 3: Vortex Mixer



Plate 4: Gas Chromatograph (GC)

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

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

3.5.1 Some pictorial view related to sample preparation:
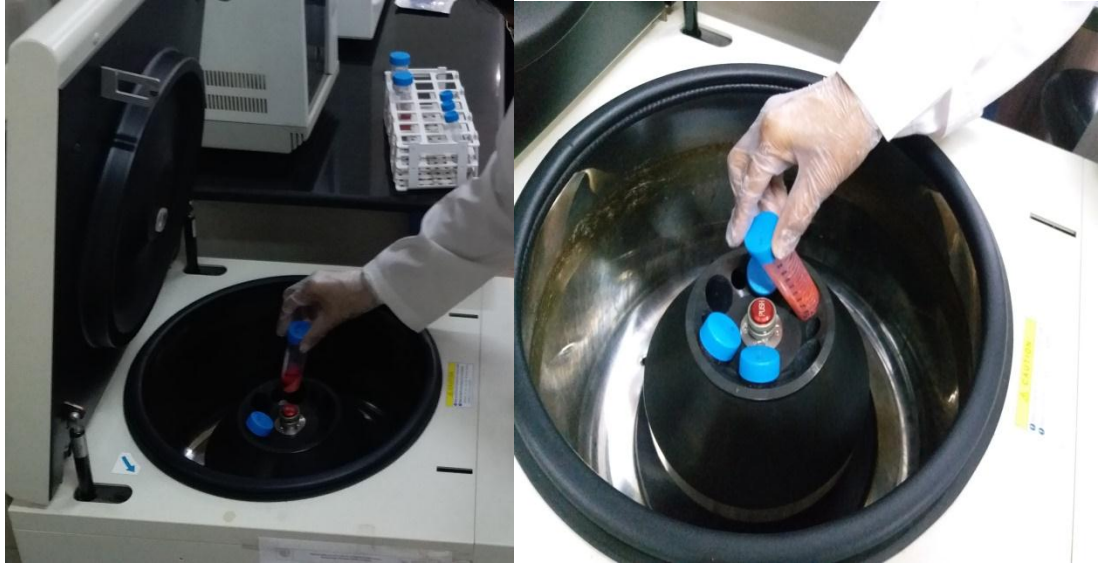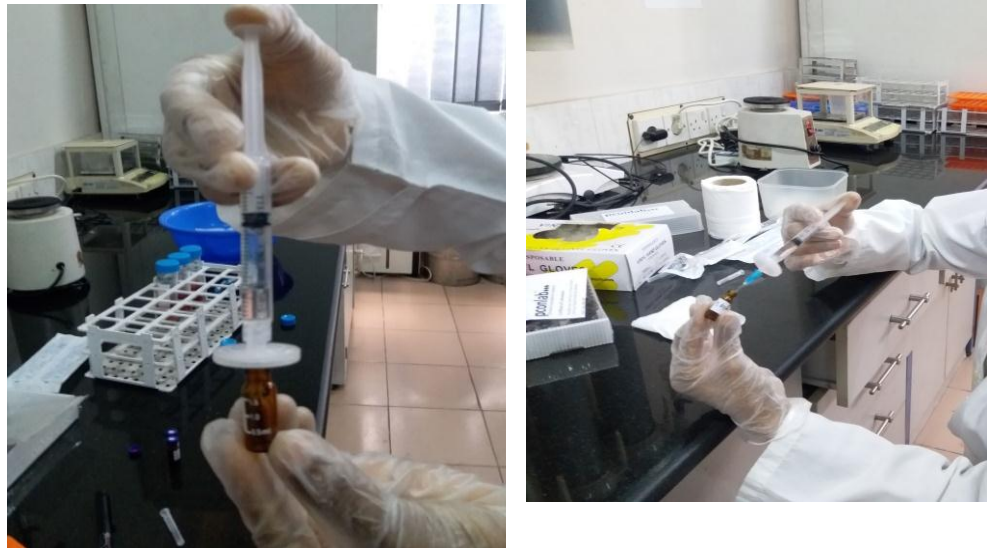

Plate 5: Homogenization of collected sample
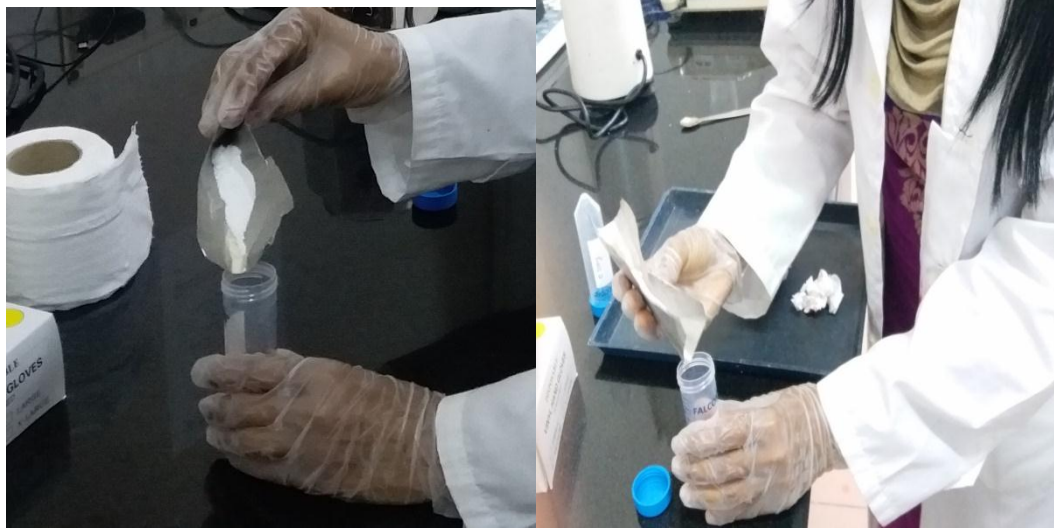
Plate 6: Adding of salt (NaCl and MgSO₄)

**Plate 7: Shaking of sample**

Plate 8: Centrifuging the sample

Plate 9 : Filtration through PTFE Filter

Plate 10. Final sample extract ready for injection

3.6 Detection and Quantification of pesticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu) with Electron Capture Detector (ECD) for the detection of cypermethrin (Plale 10). The capillary column was Rtx-CLPesticides2, with a length of 30 m, ID of 0.32 mm, and film thickness of 0.25 μm. Nitrogen was used as carrier and make up gas for GC-ECD. The identification of suspected pesticide was performed by peak retention times in samples to those of peaks in the pure analytical standards (Figure 1). The instrument conditions are described in Table 1-2.
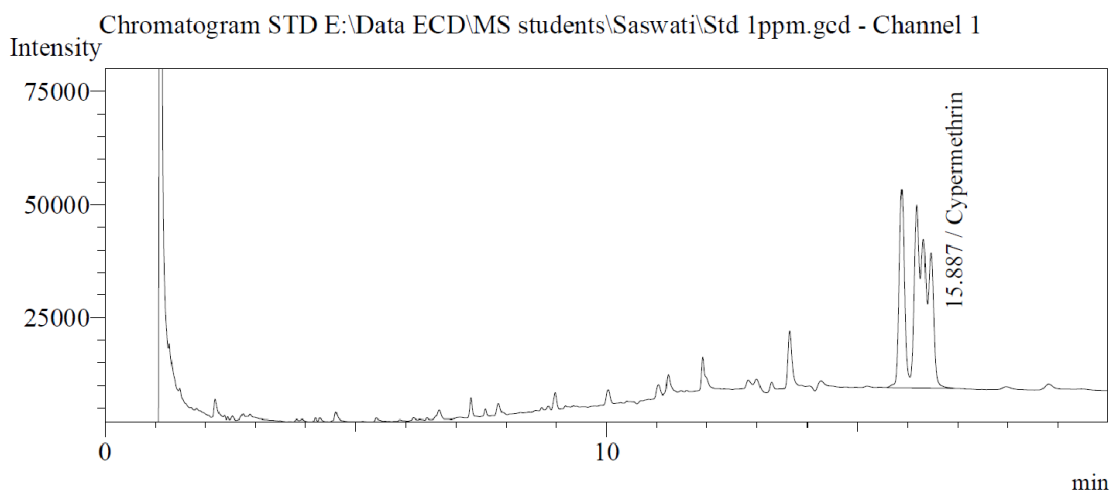
Figure 1. Typical Chromatogram of cypermethrin standard run by GC-ECD

Table 1: The instrument parameters for GC-ECD

Instruments	Conditions
Injection port SPL	Injection mode: split; temperature:280°C; flow control mode: linear velocity; split ratio: 10:0
Detector channel 1 ECD	Temperature:300°C; current: 0.50 Pa; make up flow: 30 mL/min;

Table 2: Conditions for column oven temperature for cypermethrin determination

Column oven	Rate	Temperature ($^{\circ}$C)	Hold time (min)
Initial temperature: 160°C	-	160	1
	10	270	8

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-ECD is presented in figure 1.

3.7 Calibration curve preparation

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

```
                    Calibration Curve - Analytical Line 2 - Channel 1
ID#:1   Name:Cypermethrin

f(x)=9.72861566278e-005*x+9.9950013555e-003
 R=0.999966763424   R^2=0.999933527952
MeanRF:9.88583736873e-005  RFSD:9.19328503897e-007   RFRSD:0.929945000719
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard
                                        No.    Conc.              Area
                                         1     0.020              2020
  2.0                                    2     0.050              5011
                                         3     0.100             10096
                                         4     0.200             20494

  1.0

  0.0
     0.0        1.0        2.0
                [*10^4]
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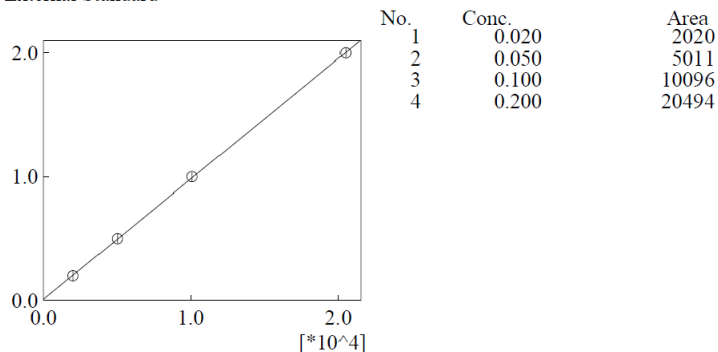
Figure 2: Calibration curve of cypermethrin standard ranging from 0.02 mg/kg to 0.2 mg/kg.

3.8 Determination of Pre Harvest Interval.

At first, the level of residues in all of the collected samples for tested pesticide was determined and the PHI was calculated by the following procedures. Firstly, 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.

3.9 Quality parameters

3.9.1 Total soluble solids content

The TSS content of strawberry was measured by a digital refractometer (MA871; Romania). A drop of strawberry juice was obtained with a dropper and placed on the refractometer prism. The refractometer showed a reading of total soluble solids.

3.9.2 Titratable acidity (TA %)

The mortar and pestle were macerated for the determination of the 5g sample. Then they filtered it and added distilled water, which rendered 100ml of total volume. Then 10ml of stock solution was taken in a conical flask and 2 drops of phenalpthelin were added. The solution was titrated with .1N NaOH. The titrate colour will be rosy pink and the reading was recorded. The titration was done three times.

3.9.3 Ascorbic acid determination

Tee et al. (1988) method was used to calculate the Vitamin C content of papaya. The single fruit was mixed and filtrated with filter paper Whatman No.1. The volume was made up to 100ml with a 5% oxalic acid solution. The titration was done with dye solution 2, 6-dichlorophenol indophenol. The mean observations provided the amount of dye required to oxidize an unknown concentration of a definite amount of L-ascorbic acid solution, using L-ascorbic acid as a known sample. A 5ml solution was taken for titration each time, and the pink colour determined the last point of titration, which remained for 10 seconds. The burette reading was recorded.

3.9.4 Phenolic content

The content of phenols was calculated using the Singleton, Orthofer, and Lamuela-Raventós (1999) process. Fresh fruits (250 mg) were homogenized with methanol (85%). At 10°C, the extract was centrifuged at 3,000 g for 15 min and separated the supernatant. Folinand Ciocalteu's reagent (2 ml) has been added to the supernatant per 2 ml. A sodium carbonate solution was applied to each test tube (7.5%, 2 ml) and after 30–45 min, the absorbance was read against a reagent blank at a wave length of 725 nm. To determine the concentration of total phenols in the unknown sample, a standard curve was generated using gallic acid.

RESULTS AND DISCUSSION

The experiment was conducted in the experimental field of Horticultural Farm, Sher-e-Bangla Agricultural University and the collected strawberry samples were carried to the Pesticide Analytical Laboratory, Pesticide Research & Environmental Toxicology Section, Entomology Division of BARI, Gazipur. The analysis was performed by Gas Chromatography (GC) with ECD detector.

The chromatogram of the selected insecticides found from the injected extract of strawberry samples at different days after spray are shown in figure 3 to 9. Figure 3 shows the chromatogram of cypermethrin residue observed in strawberry collected immediately after spraying (0 days after spraying, hereafter called DAS). Other several chromatograms were also observed but they are not related to cypermethrin. The GC chart exhibits the main peak of cypermethrin residue at R.T 15.857 min (Figure 3).
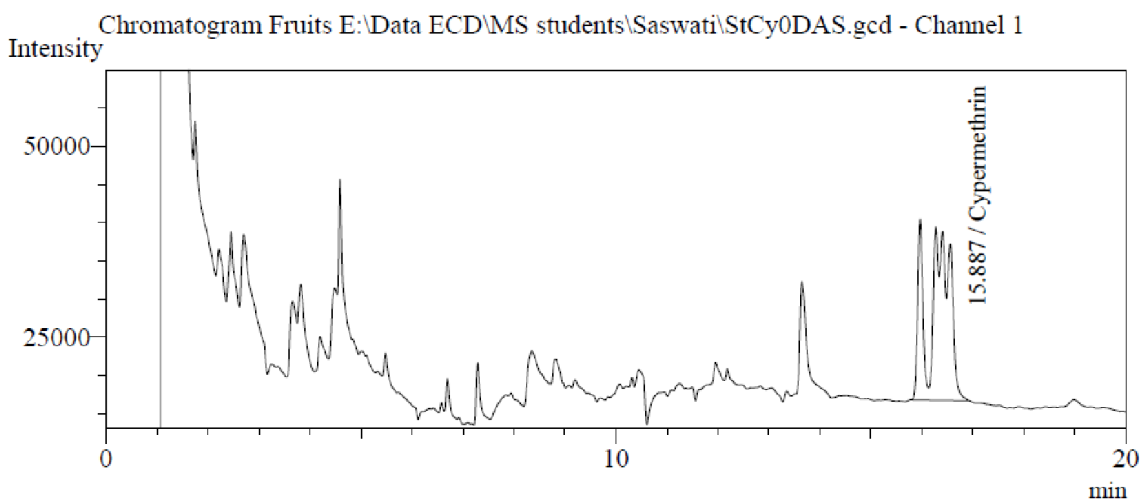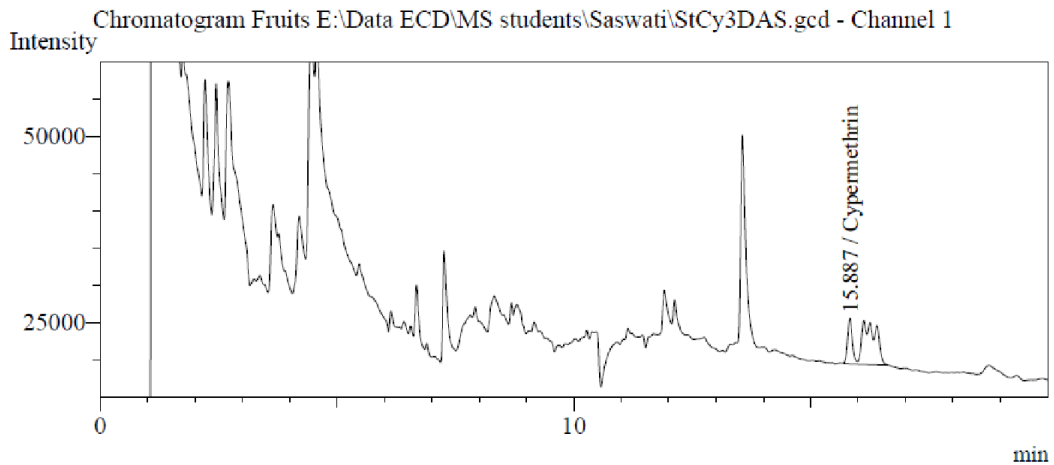

Figure 3: Chromatogram of cypermethrin obtained from strawberry extract at 0 DAS

The height of cypermethrin residue peak found in strawberry declined with days after spraying (Figs. 4-7). Figure 4 shows the chromatogram peak of cypermethrin at 1 DAS. Peak was observed at the same retention time, 15.887 but height is lower than 0 DAS chromatogram peak. Similarly, lower height cypermethrin chromatogram peak was
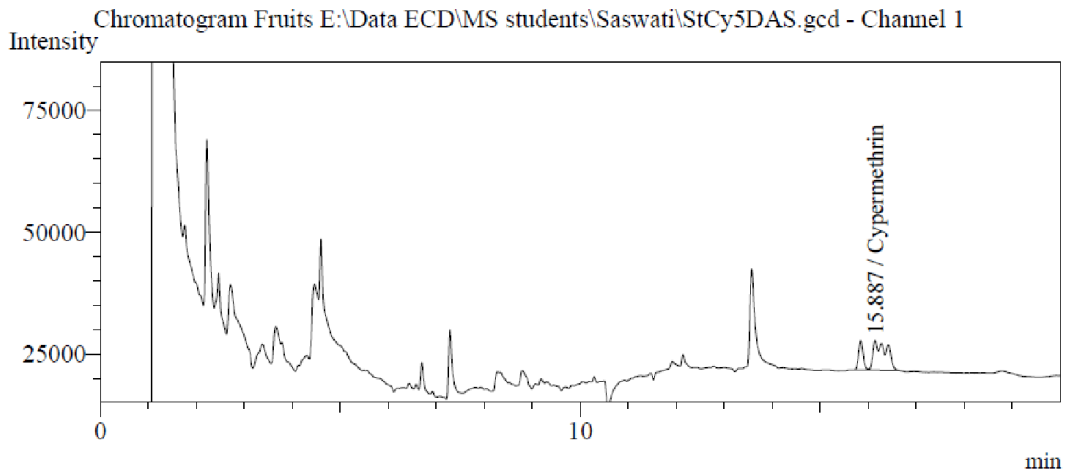
observed at 3 DAS at same retention time (Fig. 4).
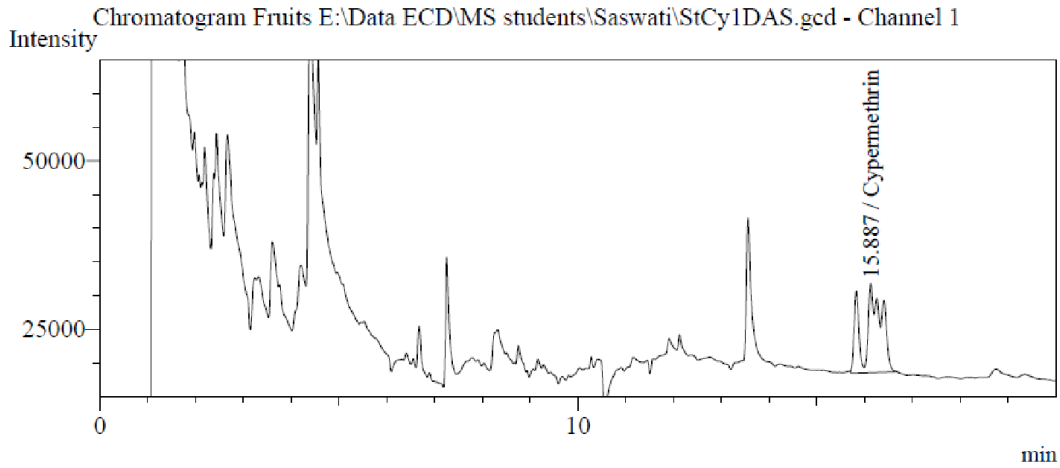
Figure 4: Chromatogram of cypermethrin obtained from strawberry extract at 1 DAS

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Figure 5: Chromatogram of cypermethrin obtained from strawberry extract at 3 DAS

Figure 6 shows the chromatogram peak of cypermethrin residue observed in strawberry samples collected at 5 DAS. The height of this peak was lower than that of 3 and 1 DAS samples. Similarly, reduced peak height was observed when samples were collected at 7 and 10 DAS (Figs. 7 & 8). However, chromatogram peak of cypermethrin residue was not observed in strawberry sample when collected at 12 DAS (Fig. 6). This result

indicates that measurable amount of cypermethrin residues does not exist in strawberry after 10 DAS.
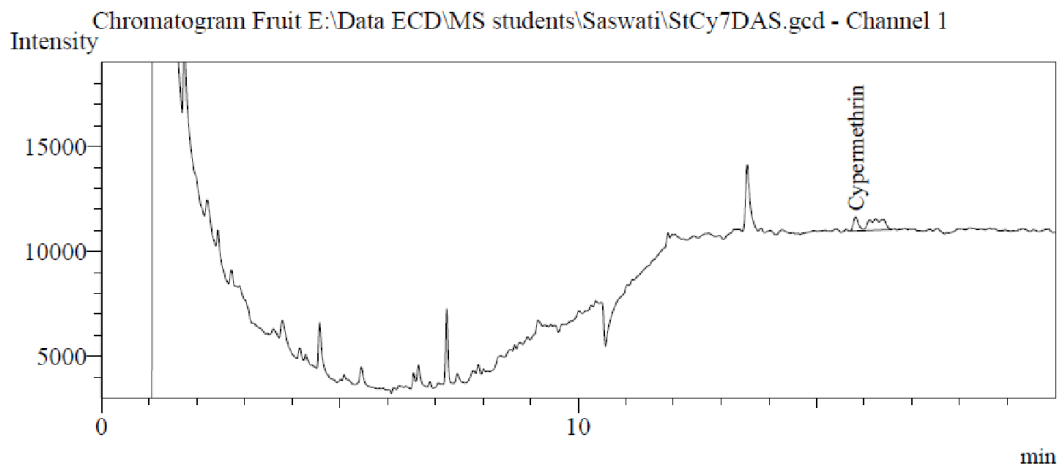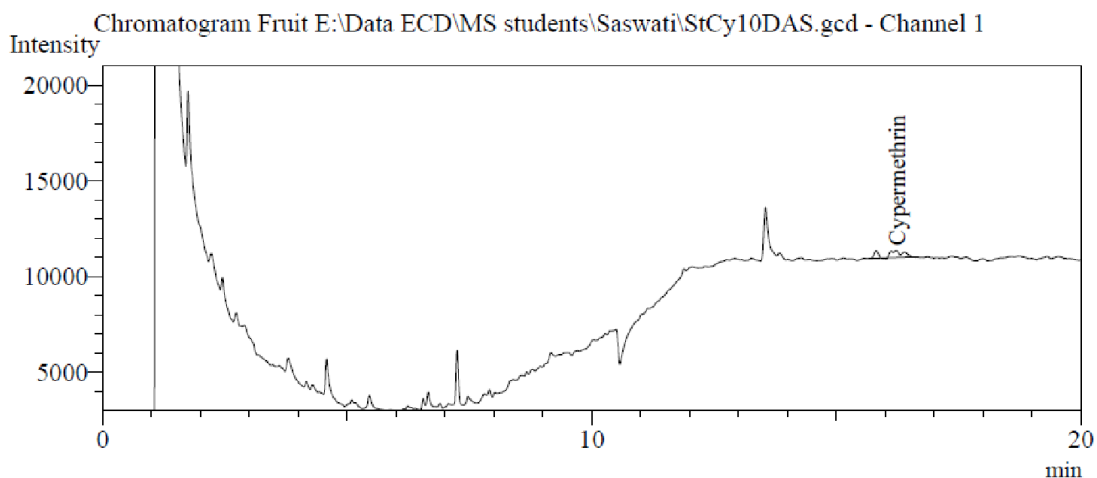

Figure 6: Chromatogram of cypermethrin obtained from strawberry extract at 5 DAS

Figure 7: Chromatogram of cypermethrin obtained from strawberry extract at 7 DAS

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Figure 8: Chromatogram of cypermethrin obtained from strawberry extract at 10 DAS
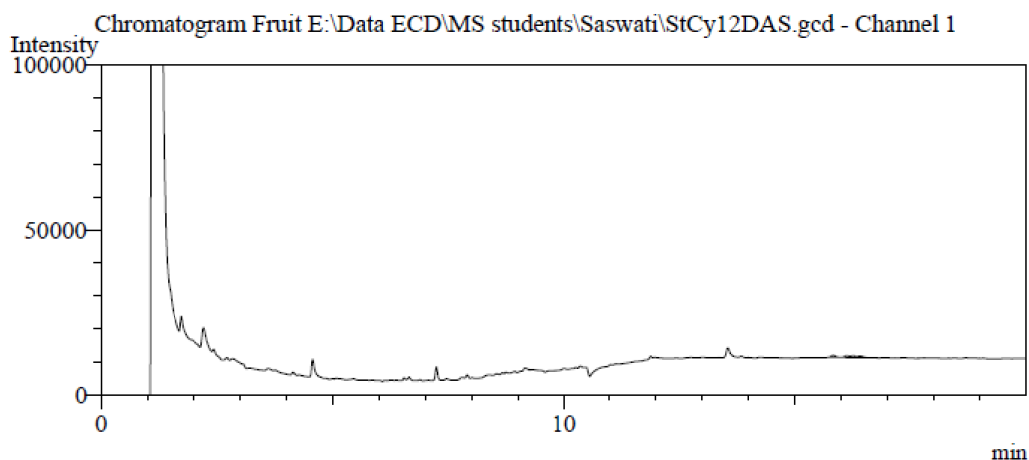
Figure 9: Chromatogram of cypermethrin obtained from strawberry extract at 12 DAS

4.1 Residue of cypermethrin in strawberry

Residues of cypermethrin in fruits of strawberry during a period of 12 days are shown in Table (3). Results revealed that the initial deposits of cypermethrin on fruits of strawberry were 0.874 mg/kg. A moderate degradation of the tested insecticide residues was noticed, one day after application with values of 0.552 mg/kg. The time elapsed after application resulted in more degradation of residues. The initial deposits were gradually decreased

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during the experimental period. It could be noticed that 0.043 mg/kg of cypermeyhrin was detected on strawberry fruits 10 days after application. This indicated that only 10 days were long enough to reduce the residues below the maximum residue limits (0.1 mg/kg) on strawberry according to EU Pesticides database - European Commission. Therefore, strawberry fruits could be marketed with apparent safely for human consumption.

Table 3: Level of residues (mg/kg) of cypermethrin (ripcord 10 EC) found in strawberry samples.

Days after spraying	Level of residue (mg/kg)	EU-MRL (mg/kg)
0 (2 h)	0.874	
1 (d)	0.552	
3 (d)	0.309	0.10
5 (d)	0.182	
7 (d)	0.101	
10 (d)	0.043	
12 (d)	ND	

The PHI of cypermethrin was determined at 10 DAS for strawberry. Farmers in Bangladesh have no idea about pesticide residue levels in food, as well as MRL levels and effect of pesticides.People may consume pesticide-contaminated vegetables and fruits, which might result in serious health concerns (Chowdhury *et al.,* 2012). Hossen (2008) found that cypermethrin remains in tomato samples up to 5 days after harvest, which is similar to the current study's findings

4.2 Trend of residue degradation

The trend of degradation of detected residue of cypermethrin in the sample over time is shown in Figure 8. From the figure it is observed that the cypermethrin residue was degraded gradually, however, the level of detected residues and the degradation rate were not same at different DAS. The level of detected residues at 0 DAS was 0.874 mg/kg. The residues were detected up to 10 DAS.
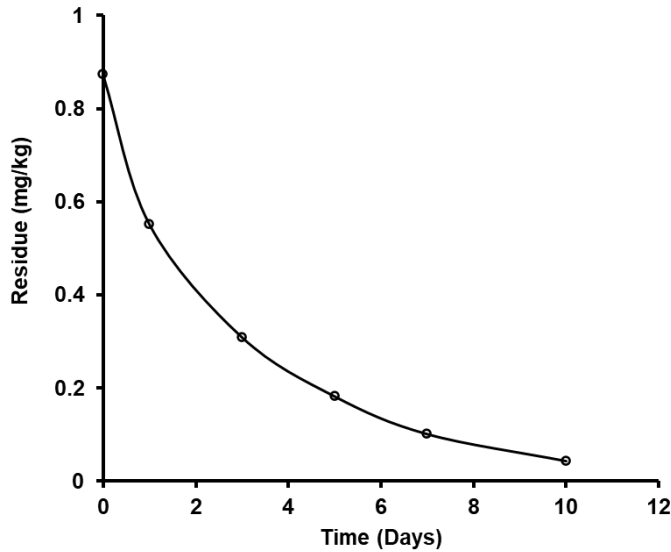
Figure 10: The trend of degradation of detected residue of cypermethrin in strawberry over time.

According to the World Health Organization, developing countries account for 20% of global pesticide use (PAN G 2012).Agriculture is the most pesticide-consuming sector in many South Asian countries, and in the case of India, Pakistan, and Bangladesh, this is due to the fact that around 14% of world agricultural area is used for agricultural activities (AtapattuandKodituwakku 2009). To ensure consumer safety, many developing countries have established Maximum Residue Limits (MRLs) based on Acceptable Daily Intake (ADI) and Potential Daily Intake (PDI) that should not be exceeded for a food item to be consumed safely (Rahman 2007).Cypermethrin residues in yard-long bean were found to be above the MRLs up to 5 DAS (Prodhan et al. 2009).It is assumed that the use of toxic pesticides on vegetables and fruits has increased the risk of consumer in toxication as well as disease transmission (Fatema 2013).Pesticide residues contaminate approximately 50-70% of fresh fruits and vegetables (Karanth 2002).

4.3 Biochemical parameters

4.3.1 Total soluble solid (TSS)

TSS content in fruits of strawberry during a period of 12 days was shown in figure (11). Results revealed that the initial TSS on fruits of strawberry was 5.7 °Brix. A moderate degradation of the TSS was noticed one day after application with values of 5.4 °Brix.

The lower TSS content was found in fruits 0-7 DAS of cypermethrin and the TSS value started to increase at 10 DAS (Figure 11). The decreased TSS and ascorbic acid content of fruit decreased the quality in fruits (Lata *et al*., 2018).
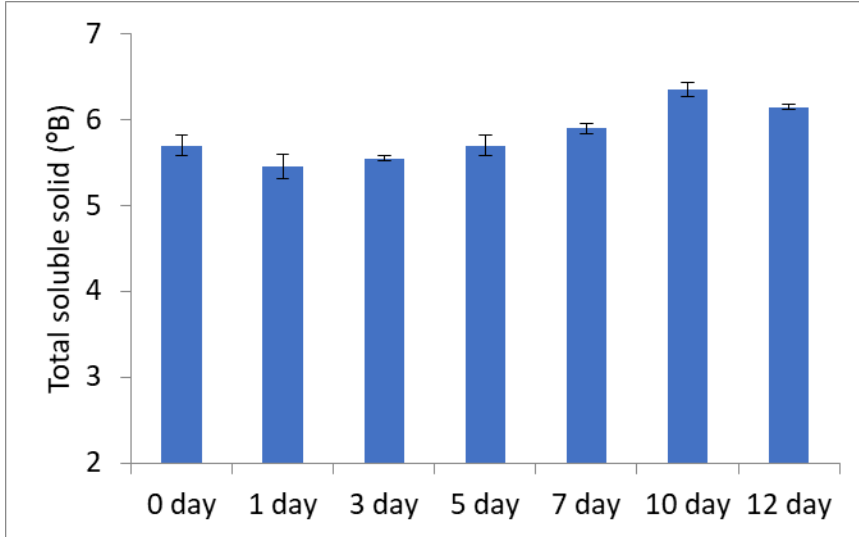
Figure 11: Average content of total soluble solid (TSS) (^{o}B) of strawberry extract at different days after pesticide spraying. Standard error bar are shown on each column.

4.3.2 Titratable acidity (%)

The trend of increased of titratable acidity in the sample over time is shown in Figure 12. From the figure it is observed that titratable acidity was increased gradually. The higher titratable acidity was found in fruits 1-7 DAS of cypermethrin and the value started to increase at 7 DAS.A moderate increased of the titratable acidity was noticed one day after application with values of 0.32%. However, the increasing levels were not same at different DAS. The increasing level of titratable acidity was higher at 7 DAS (0.63%).Relatively high acid content is required for good strawberry flavor (Kader, 1990).
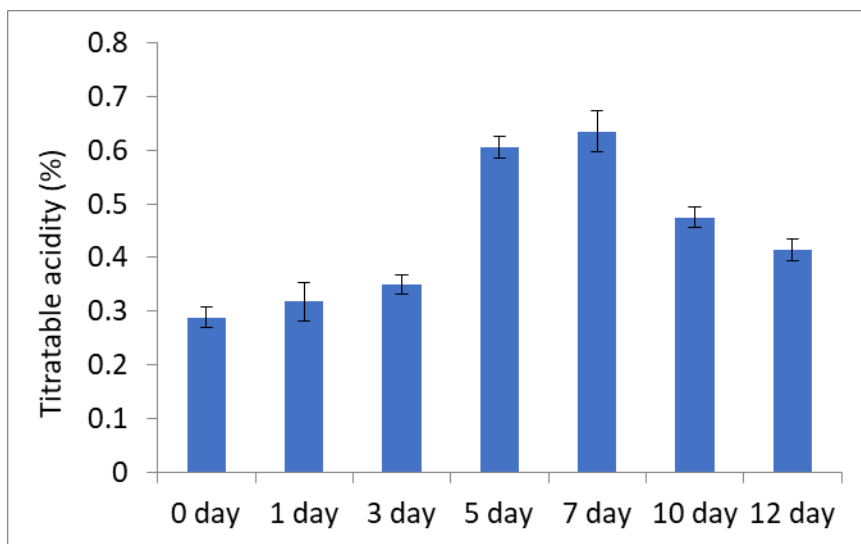
Figure 12: Average content of titratable acidity (%) of strawberry extract at different days after pesticide spraying. Standard error bar are shown on each column

4.3.3 Ascorbic acid content

Figure 13: Average content of ascorbic acid (mg/100g) (C) of strawberry extract at different days after pesticide spraying. Standard error bar are shown on each column

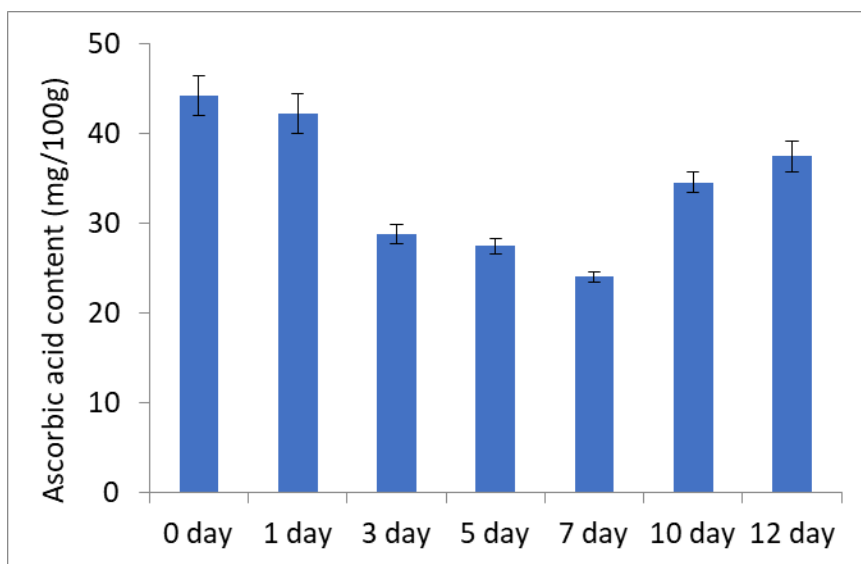Ascorbic acid content in fruits of strawberry during a period of 12 days was shown in figure 13. Results revealed that the initial ascorbic acid content in fruits of strawberry was 44.16 mg/100g. A moderate degradation of the ascorbic acid was noticed, one day after application with values of 42mg/100g. The time elapsed after application resulted in

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more degradation of ascorbic acid content. The initial amount was gradually decreased up to 7 DAS. Pesticide residues significantly decreased the levels of ascorbic acid in pepper fruits (Shalaby 2017).

4.3.4 Phenolic content

From the data represented in figure 14, it was found that phenolic content in fruits were increased gradually 1-7 DAS of cypermethrin. The increasing levels were not same at different DAS. A moderate increased of phenolic content was noticed one day after application with values of 510 mg/100g. The increasing level of phenolic content was higher at 7 DAS (1034 mg/100g). Phenols are secondary metabolites found in plants and within the framework of plant physiology serving to defend stresses, e.g., pathogen attacks (Slatnar et al., 2016). The increased content of phenols in pesticide treated fruits indicates more infection of fruits (Borum 2017).
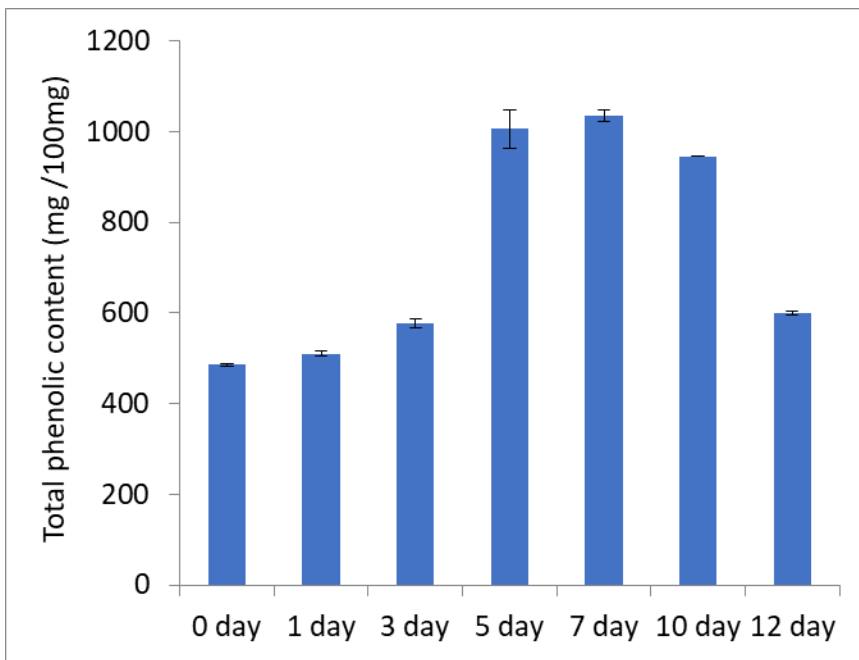

Figure 14: Average content of phenolic content (mg/100g FW) (D) of strawberry extract at different days after pesticide spraying. Standard error bar are shown on each column

CHAPTER V

SUMMARY AND CONCLUSION

A field trial was conducted in the Horticulture farm, Sher-e-Bangla Agricultural University, Dhaka. All the activities like seeding, transplanting and all other cultural operations were undertaken and performed by the field staffs of Horticulture Department of SAU. The synthetic pyrethroid pesticide, cypermethrin (ripcord 10EC) was selected for this experiment. When the fruits were at marketable size, ripcord 10EC was sprayed in the field by knapsac sprayer. 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 level of cypermethrin (ripcord 10EC) residues (mg/kg) in strawberry at different days after spray and to compare whether the level of detected cypermethrin residues (mg/kg) were above the Maximum Residue Limit (MRL) or not, with the effects of pre-harvest foliar spray of cypermethrin on biochemical attributes of strawberry.The application rate was 1.5 mL/L of water. The pesticides were tank-mixed and applied to the plants following GAP. Samples were collected at 2 hrs, 1, 3, 5, 7, 10 and 12 days after spray. The level of residue of cypermethrin was 0.874 mg/kg at 2 hrs and gradually the level of residues were decreased and at 7 DAS (0.101 mg/kg), it was above the EU-MRLs (0.10 mg/kg) and at 10 DAS (0.043 mg/kg), it went to below EU-MRLs (0.10 mg/kg) in strawberry and at 12 DAS, no residue of cypermethrin was detected in the analyzed strawberry sample. Strawberry juice had considerably lower concentrations of total phenols and titratable acidity at 2 hrs, 1, and 3 DAS, and a higher concentration of ascorbic acid at 0 and 1 DAS, which indicates less infection. Pesticides on strawberries reduced pest infection and preserved fruit quality, but residues higher than the maximum residue limit of up to 7 DAS were found.

In this study, Strawberry fruits had the maximum residual level (0.874 mg/kg) at 0 DAS, whereas no pesticide residues were identified after 12 DAS. The observed

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residue level was higher than the MRL (maximum residue limit) up to 7 DAS (0.101 mg/kg). It can be concluded that fruit harvesting after spraying (7 DAS) improves fruit quality by dropping residue levels below the MRL.

CHAPTER VI
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