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Analysis of the pesticide residues in bitter gourd using modified QuEChERS extraction coupled with Gas Chromatography

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ABSTRACT

Objective: Globally, pesticide residue in vegetables is a great concern to the consumers due to their negative impact on human health and environment. 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. **Methods:** 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 thermoionic detector (GC-FTD). **Results:** 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. **Conclusion:** 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.

Keywords: Bitter gourd, Pesticide residues, QuEChERS Extraction, GC-FTD

1. Introduction

Now a days, food demand is changing away from traditional commodities towards high value food commodities like vegetables, fruits, spices, fishes etc. In this regard, vegetable growing has become an important farming activity from the point of view of dietary fulfillment as well as economics returns (Aktar *et al.,* 2017). Vegetables are any herbaceous plant whose fruit, seeds, roots, tubers, bulbs, leaves etc are consumed raw or cooked as food. These are very important for nutritional, financial, and food security in any country (Anonymous I). Vegetables have been strongly associated with improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some forms of cancer. Vegetables make up a major portion of the diet of humans in many parts of the world and play a significant role in human nutrition, especially as sources of phytonutriceuticals: vitamins (A, B_1, B_6, B_9, C, E), minerals, dietary fiber and phytochemicals (Dias and Ryder, 2011). They contain valuable food ingredients which can be successfully utilized to build up and repair the body. Vegetables are valuable in maintaining alkaline reserve of the body. People who consume more fruit and vegetables as part of a healthy and balanced diet are more likely to reduce the risk of several chronic diseases (Anonymous II). In Bangladesh, present consumption of vegetables is 166.1 gram per capita per day as per Household Income and Expenditure Survey (HIES) 2010 whereas desirable dietary intake for Bangladesh is 200 gram per capita per day (Hortex Foundation, 2013). According to a world vegetable survey, 402 vegetable crops are cultivated worldwide, representing 69 families and 230 genera [Kays, S. J. and Dias, J. S., 1995].

Bangladesh is an agrarian country. Nearly 100 different types of vegetable comprising both local and exotic type are grown in Bangladesh (Anonymous I). According to FAO (2012), vegetable cultivation has increased five times in past 40 years. Mostly grown vegetables in Bangladesh are cabbage, cauliflower, tomato, brinjal, potato, radish, country bean, bottle gourd, pumpkin, bitter gourd, teasle gourd, ribbed gourd, ash gourd, okra, yard long bean, spinach etc (Hasan *et al.,* 2017). All these vegetables do not only meet our daily diet demand but also occupying a more or less significant position in earning foreign currency. There are 67 items of vegetables of Bangladesh like potatoes, tomatoes, cabbages and cauliflowers, lettuce and chicory, carrots turnips, leguminous vegetables, frozen vegetables, dried vegetables, manioc (yams) and others are exported. Bangladesh earned foreign exchange US$ 147.55 million by exporting agricultural commodity in 2013-14 (Hortex Foundation, 2015).

Bitter gourd (*Momordica charantia*) belongs to the family cucurbitaceae, is one of the most popular vegetable in South Asia. The Latin name Momordica means "to bite" referring to the jagged edges of the leaves, which appear as if they have been bitten. It is regarded as one of the world's major vegetable crops and has great economic importance (Krishnendu *et al*., 2016). It has important role as a source of carbohydrate, proteins, vitamins, minerals and other nutrients in human diet which are necessary for maintaining proper health. It is an excellent source of vitamins B1, B2, B3, Vitamin C, magnesium, folic acid, zinc, phosphorus, manganese, and has high dietary fiber (Keding and Krawinkel, 2006). More than 200 medicinal compounds have been isolated from its leaves, stems, pericarp, entire plants, callus tissues and seeds (Katiyar *et al*. 2017). China, India, Africa, and the south-eastern US have traditionally used the leaves and fruits as a medicine as anti-diabetic, anti-ulcerogenic, anti-mutagenic, antioxidant, anti-tumour, anti-lipolytic, analgesic, abortifacient, anti-viral, hypoglycemic, anti-carcinogenic, anti-scabies, anti-fever, wound-healing and immunemodulatory (Aminah and Anna, 2011; Pişkin *et al*., 2012; Joseph *et al*., 2013; Saeed *et al*., 2018; Shubha *et al*., 2018).

However, vegetable plants being green, succulent and juicy, are often and ideal host for insect pest and diseases (Sah *et al***.,** 2018**)**. Vegetables crops on a conservative basis face about 25 - 30 percent losses due to insect pest and diseases (Agnihotri, 1999). Farmers use chemical pesticides to protect their crops from the unexpected insect pest and diseases. Globally, about 5 billion kg of plant protection materials are applied every year, preventing over 40% of potential crop loss from pest infestation (Oerke, 2006). But through over use and misuse there is considerable waste, adding to the cost and contributing to the adverse environmental and health consequences (Abhilash, P.C. and Singh, N., 2009). Pesticides being used in agricultural lands are usually released into the environment and come into human contact directly or indirectly. Moreover, inappropriate application of pesticides affects ecosystem negatively and can be accumulated in the food chain through air, water and soil. Exposure to pesticides causes both acute and chronic health problems (Abhilash, P.C. and Singh, N., 2009). Increasing incidence of cancer (Alewu, B. and Nosiri, C., 2011), chronic kidney diseases (Siddharth, *et al*. 2012), cardiovascular diseases (Zamzila, *et al*. 2011), suppression of the immune system (Corsini, 2008), sterility among males and females (Bretveld, R. W., 2006), endocrine disorders (Mnif, W., 2011), neurological and behavioral disorders especially among children (Bjorling-Poulsen M., *et. al,* 2008) have been attributed to chronic pesticide poisoning. Furthermore, hospitalization and death have been occurred by the result of high occupational, accidental, or intentional exposure to pesticides (Gunnell, D., 2007). So, it is needed to monitor the residues of pesticide in vegetables so that consumers can be protected. In this regard, an investigation was done to monitor the presence of seven popular organophosphorous pesticides residues in bitter gourd collected from seven retail markets located at the adjacent area of Jahangirnagar University, Savar, Dhaka, Bangladesh. Therefore, the quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction method and Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD) was used in this study to monitor the selected pesticides residues in bitter gourd.

2. Materials and Method

2.1. Study Location

The bitter gourd samples were collected from wholesale and retail markets located at the adjacent area of Jahangirnagar University, Savar, Dhaka, Bangladesh namely Genda bazaar, Savar bazaar, Nayarhatbazar, Islampur bazaar, Pallibiddut bazar, Baipayl bazaar and Sreepur bazaar during June 2019.

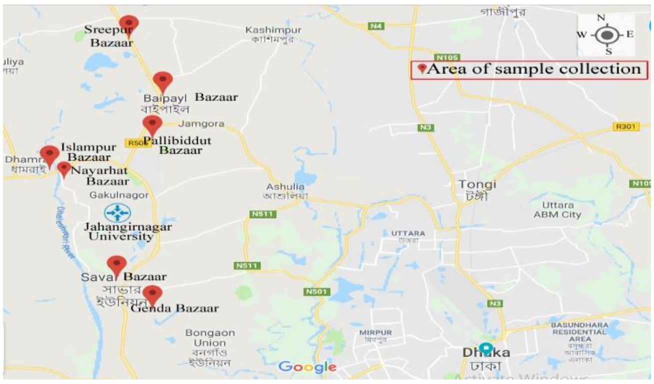

Figure 1: Map showing the places of sample collection from the adjacent area of Jahangirnagar University.

2.2. Sample Collection and Preparation

A total of 65 bitter gourd samples were collected for this investigation. Every sample amount was 1 kg. To avoid any cross contamination each samples have been collected and labeled properly in separate, transparent, clean polyethylene zipper bag. The collected samples were carried to the Pesticide Analytical Laboratory, Pesticide Research & Environmental Toxicology Section of Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur on the day of collection. All the samples were chopped and mixed properly in labeled polyethylene zipper bags and stored at -20℃ for further analysis.

2.3. Chemicals and Reagent

All pure pesticide standards: acephate, dimethoate, fenitrothion, chlorpyrifos, quinalphos, diazinon and malathion (>99.6%) were bought from Sigma-Aldrich Laborchemikalien (St Louis, MO, USA) via Bangladesh Scientific Pvt. Ltd. HPLC grade methanol, acetone, acetonitrile, analytical grade NaCl, anhydrous MgSO4 and Primary Secondary Amine (PSA) were collected from Bangladesh Scientific Pvt. Ltd.

2.4. Preparation of Standard Solution

All of the standard stock solutions were made separately in acetone at 1000 mg/L and stored until use at -20°C. 50 mg/L mixed standard stock solutions were made from all of the individual standard stock solution in 50 mL volumetric flask by adding appropriate amount of stock standards and acetone. Mixed intermediate stock solution of 10 mg/L were prepared from this 50 mg/L mixed standard stock solution. Finally all the working standards solution of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 mg/L were prepared by using this mixed intermediate stock solution. All the standard stock solutions and working solutions were stored at -20°C.

2.5 Extraction and Clean up

In the present study, a modified QuEChERS extraction technique developed by Prodhan *et al*. (2015) was used. In brief, the sliced samples were grounded thoroughly using a homestead fruit blender. Ten gram of this homogenized sample was transferred into a 50 mL teflon centrifuged tube followed by adding of 10 mL of acetonitrile. Then the tube was vortex for one minute followed by adding extraction salt (4 g of anhydrous $MgSO_4$ + 1 g of NaCl). Then shake it for one minute and followed by subsequently centrifuged at 5000 rpm for 5 minutes. Then 3 mL supernatant was transferred to a 15 mL centrifuge tube containing 600 mg $MgSO_4$ anhydrous and 120 mg of PSA. Again the tube was vortex for one minute, and centrifuged at 4000 rpm for five minutes. Finally, 1 mL of supernatant was filtered using a 0.2 µm PTFE filter and then transferred into a clean HPLC vial.

2.6 Detection and Quantification of Pesticide Residue

The concentrated extracts were analyzed following the method described by Prodhan *et al*. (2009) using GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD). The capillary column was AT-1, 30 m long, 0.25 mm ID and 0.25 µm film thick. Helium was used as carrier gas. Target pesticide was identified by the retention times of pure standards (Figure 3). The instrumental conditions are described in Tables 1 and 2.

2.7 Calibration Curve Preparation

Prior to the injection of the sample extract, standard solutions of different concentrations of each pesticide group were prepared and injected according to previously developed methods parameters. The samples were calibrated (retention time, peak area etc.) against the five pointed calibration curve of the standard pesticide solution (Figure: 4-10). Each peak was characterized by its retention time. Sample results were expressed in mg/kg automatically by the GC software.

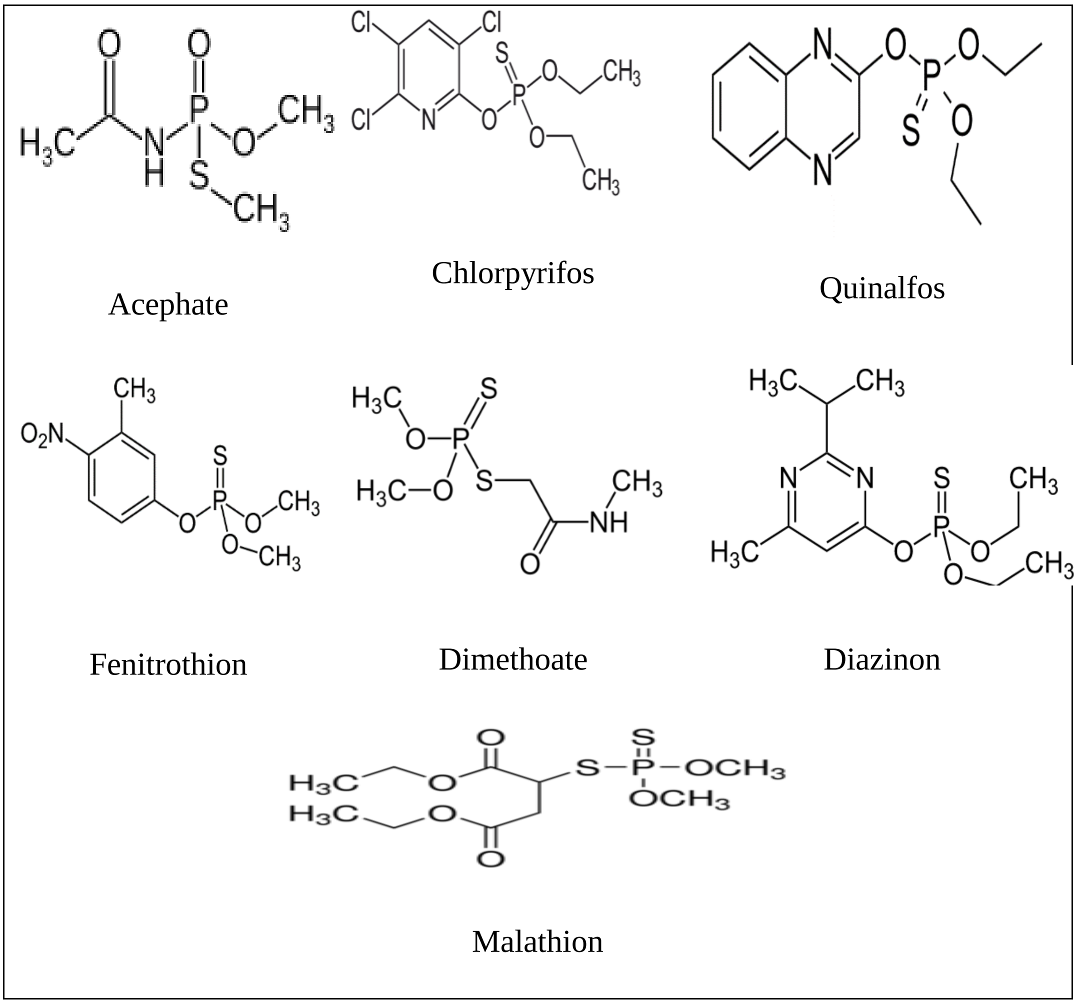

Figure 2: Chemical structures of the pesticides used in the present study

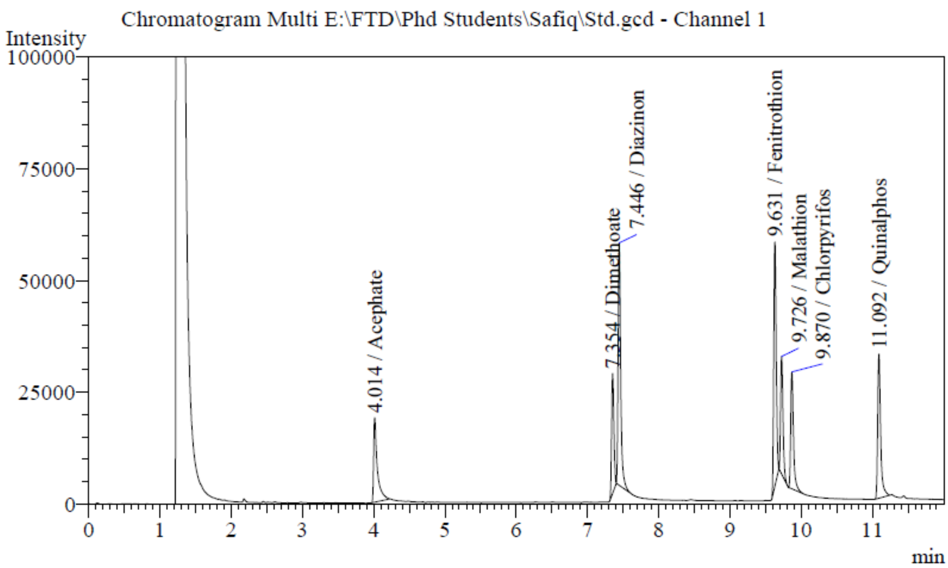

Figure 3: Typical chromatograms of four organophosphorus insecticide standards run by GC-FTD

Table 1: The Instrument parameters for GC-FTD

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

Table 2: Condition for column oven temperature for FTD

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

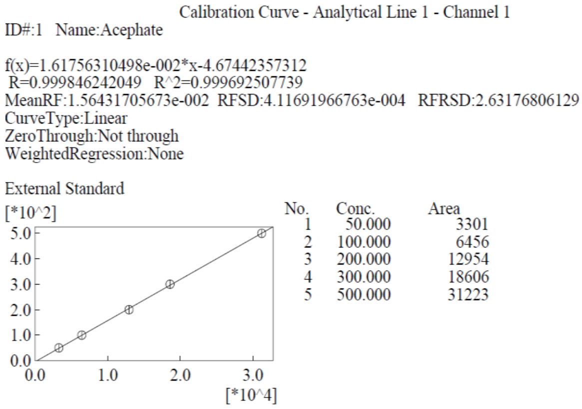

Figure 4: Calibration curve prepared for Acephate made with different concentrations ranging from 50 μg/L to 500 μg/L.

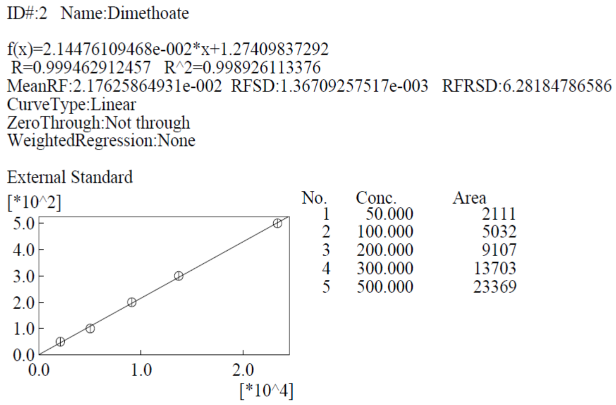

Figure 5. Calibration curve prepared for Dimethoate made with different concentrations ranging from 50 μg/L to 500 μg/L.

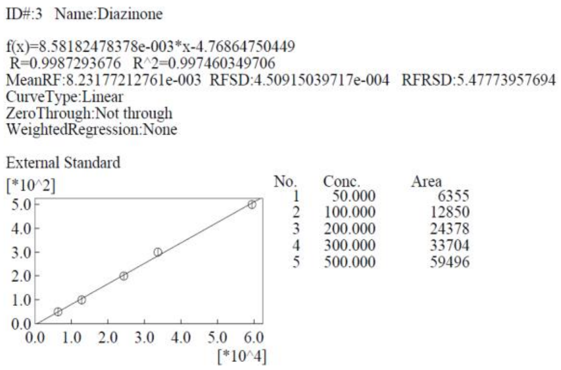

Figure 6. Calibration curve prepared for Diazinone made with different concentrations ranging from 50 μg/L to 500 μg/L.

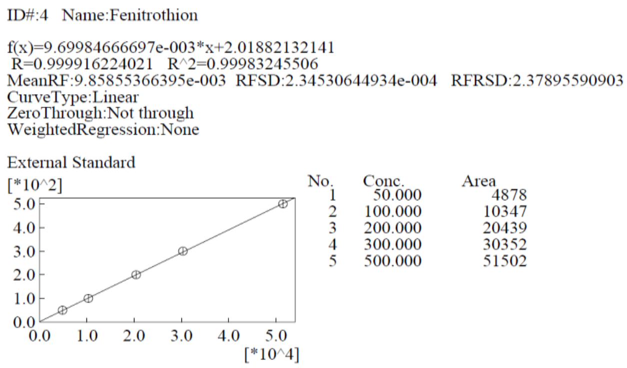

Figure 7. Calibration curve prepared for Fenitrothion made with different concentrations ranging from 50 μg/L to 500 μg/L.

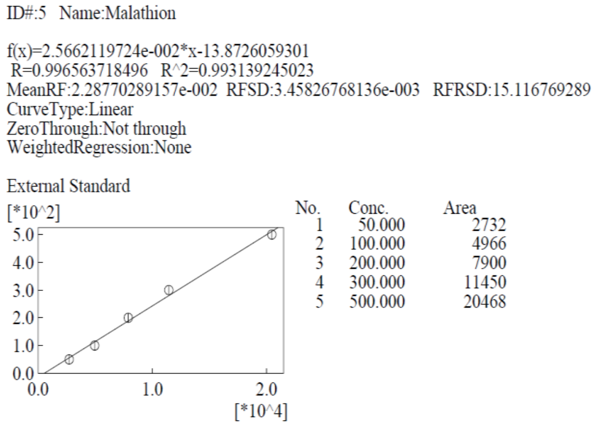

Figure 8. Calibration curve prepared for Malathion made with different concentrations ranging from 50 µg/L to 500 µg/L.

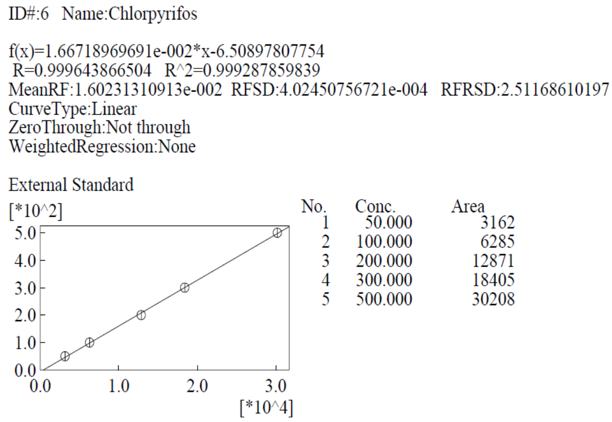

Figure 9. Calibration curve prepared for Chlorpyrifos made with different concentrations ranging from 50 µg/L to 500 µg/L.

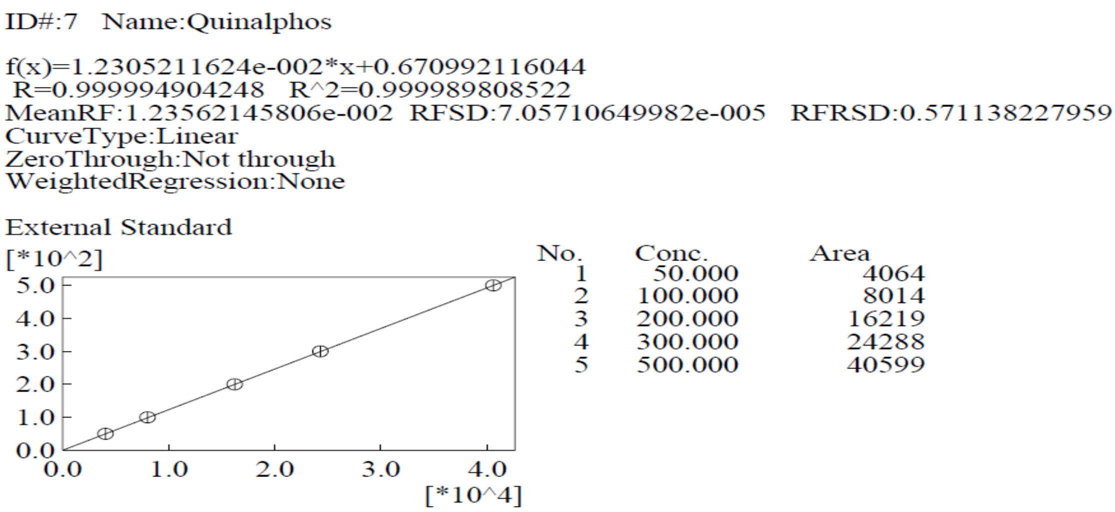

Figure 10. Calibration curve prepared for Quinalphos made with different concentrations ranging from 50 µg/L to 500 µg/L.

3. Results and Discussion

A total of sixty five bitter gourd samples from different retail markets located at the adjacent area of Jahangirnagar University, Savar, Dhaka were collected to identify and quantify the presence of organophosphorus pesticide residues. The concentrated extracts of bitter gourd samples were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the pre-set parameters. The results of the tested samples contaminated with suspected pesticide residues are shown in Table 3.

Table -3 shows that, out of 65 samples of bitter gourd, eight samples (12.3% of the total number of samples) were contaminated by pesticide residues. All of them were exceeded the MRL (0.01mg/kg) set by EU (EC, 2015). Another fifty seven samples (87.7% of the total number of samples) contained no detectable residues of the sought pesticides. Among the contaminated samples, two were contaminated by diazinon at a concentration ranging from 0.0101 to 0.058 mg/kg. Four samples contained chlorpyrifos at a concentration ranging from 0.023 to 0.159 mg/kg and two samples contained dimethoate at a concentration ranging from 0.062 to 0.095 mg/kg. The contaminated samples contained 2-16 times higher residues than the MRL of the respective pesticides.

These results can be compared with another study on the pesticide residues in bitter gourd conducted by Ahmed *et al.* (2016). They showed that out of twenty samples of bitter gourd five were contaminated by chlorpyrifos at a concentration ranging from 0.094 to 0.441 mg/kg that are above the MRL. Latif *et al.* (2011) conducted another study on the pesticide residues in bitter gourd where twenty four samples (96% of the total number of samples) were contaminated by chlorpyrifos from twenty five and five samples (20% of the total number of samples) exceeded MRL with concentration ranging 0.09-0.096 mg/kg. According to Hossain *et al.* (2014), 15 samples (100%) of three common vegetables (tomato, lady's finger and brinjal) collected from Savar bazaar contained acephate, fenitrothion, malathion, parathion, ethion and carbaryl residues, all of the pesticides were found at higher levels than the corresponding MRLs in all vegetable samples. Islam *et al.* (2014) collected 42 samples of brinjal, cauliflower and country bean from fields and markets of Narsingdi district, Bandgladesh, where they found fifteen samples (above 68% of total samples) contained no residues of the sought pesticides. Prodhan *et al.* (2016) conducted a study on the determination of pesticide residues in cabbage, among the 132 analyzed samples, residues were found in 41 samples (31% of the total number of samples), of which, two contained multiple pesticide residues.

Table 3: The level of residues (mg/kg) of different pesticides found in the analyzed bitter gourd samples (N=65)

SL. No.	Area of sample collection	No. of analyzed samples	No. of contami-nated samples	No. of samples exceeding MRL	Name of detected pesticide	Level of residue (mg/kg)	EU MRLs (mg/kg)
1.	Genda bazaar	10	3	3	Chlorpyrifos	0.125	0.01
					Chlorpyrifos	0.159	
					Diazinon	0.101	
2.	Savar bazaar	10	1	1	Chlorpyrifos	0.087	0.01
3.	Nayarhat bazaar	10	0	0	----	ND	0.01
4.	Islampur bazaar	05	0	0	----	ND	0.01
5.	Pallibiddut bazaar	10	1	1	Diazinon	0.058	0.01
6.	Baipayl bazaar	10	2	2	Chlorpyrifos,	0.023	0.01
					Dimethoate	0.062	
7.	Sreepur bazaar	10	1	1	Dimethoate	0.095	0.01

Prodhan *et al*. (2017) conducted another study in Greece on eggplant. They have detected eleven insecticides (thiamethoxam, cypermethrin, deltamethrin, thiacloprid, acetamiprid, azoxystrobin, chlorpyrifos, dimethoate, propamocarb hydrochloride and chlorpyrifos methyl) in eggplant fruits samples. Among the 142 analyzed samples, 67 (47% of the total number of samples) were found to have pesticide residues and the rest of the samples (53% of the total number of samples) were free from pesticide residues.

The results of this study are in a good agreement with Hasan *et al*. (2017). They had been detected two types of insecticides (dimethoate and quinalphos) in country bean samples collected from different market places of Dhaka. Among the 50 analyzed samples of country bean, ten samples (20% of the total number of samples) contained residues of dimethoate and quinalphos, of which five were above the maximum residue limits (MRLs). Most of the contaminated samples (8 samples) contained residue of dimethoate.

From this study and discussion, it is clear that bitter gourd as well as other vegetables were contaminated with various pesticide residues globally that are great concern to the consumer's health and the environment.

4. Conclusion

Bangladesh is an over populated country. To feed the increased population, the agricultural productivity needs to be increased. Food shortage and malnutrition are two major problems in Bangladesh. Worldwide, the main obstacle of vegetables production is insect pest infestation. Therefore, farmers are always applying pesticides in their fields to protect crop yields. So the use of pesticide is now an intrinsic part of agriculture for pest control. Many countries of the world including Bangladesh have been extensively using chemical pesticides to cultivate agricultural crops and a major portion of these pesticides are intercepted by the plant leaves during application. As a result, pesticide residues remain in the vegetable which pose a threat to human body. Consumers, who intake vegetables with high residual contamination in regular basis for long time will be affected by various types of chronic diseases e.g. cancer, kidney failure, heart attack etc. So, the pesticide residues in vegetables are becoming a major food safety concern for the consumers and the governments. Therefore, this investigation on pesticide residues level in bitter gourd at retail market at the adjacent area of Jahangirnagar University will help to increase public awareness and the policy planners to take necessary action to minimize the pesticide residues level in vegetables of Bangladesh.

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