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Determination of Major Organophosphorus Pesticide Residues in Eggplant using QuEChERS Extraction and Gas Chromatography

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Abstract:- Nowadays, a great concern about pesticide residues and their harmful impact on human health and environment is growing rapidly. Highly toxic chemical pesticides are responsible for direct and significant harm to all living beings and ecosystem. 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. Therefore, 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.

Keywords:- *Eggplant, Pesticide Residue, QuEChERS Extraction, GC-FTD.*

I. INTRODUCTION

Eggplant (*Solanum melongena L.*), a member of nightshade solanacea family, is a popular non-tuberous vegetable crop that is grown well in the subtropics and tropics. Eggplant is also termed as brinjal in Indian subcontinent and aubergine in Europe. It is an inexpensive, important, and popular vegetable in Bangladesh. Its production is nearly 50,000 hectares, the third most important vegetable in terms of production across the country [1]. A part of small resource-poor Bangladeshi farmers earn cash income from the production of eggplant. The eggplant yield is about 475 tons in 2015-2016 and about 150,000 farmers are engaged in its production in Bangladesh [2]. Globally, it is an important cash crop vegetable among poor farmers and little income consumers. The highest producer of eggplant is China (29.5 million tons) and the second highest is India (13.5 million tons) [3]. Nutritionally, it has substantial amounts of some vitamins and minerals. Eggplant is the greatest source of phenolic acids, carotenoids, glycoalkalloids with the properties of anti-inflammatory, anti-oxidant, cardioprotective, anti-obesity and anti-diabetic [4]. Two main steroidal glycoalkalloids (α-solamargine and α-solasonine) found in eggplant have an anti parasitic effect and these are used for the treatment of different types of cancers, like gastric cancer [5], leukemia [6], liver and lung cancer [7] and basal cell carcinoma [8]. It has also shown favorable effects on age-related muscular degradation [9], reducing cardiovascular disease [10] and protecting against sunburn-related disorders [11].

Bangladesh produces a little of the total world’s eggplant production because of the manifestation of insect pests and diseases. For the cultivation of crops in Bangladesh, the farmers are using different type of pesticides like organocarbamate, organophosphate and synthetic pyrethroid pesticides in their crop fields [12]. They use chemical pesticides in their vegetable crops inappropriately in daily or each alternate day to protect their crops from pest infestation [13]. The cultivators of the country totally depend on chemical pesticides for crop protection because of scarcity of competent alternatives to pesticides and illiteracy about safe pest management. From some studies, it was observed that incompetent labeling and deficient knowledge of farmers, chemical pesticides are extensively misused in Bangladesh [14]. Excessive and haphazard use of chemical pesticides in the crop field, enhance the cost of production and increase some environmental and social issue. This also causes the destruction of biological ecosystem and development of resistance in insect pest, pathogens and weeds.

Pesticide use, in both the United States and worldwide, has increased dramatically over the past 30 years and farmers today have access to a diverse chemical arsenal to protect their crops. As a result, food productivity is higher now than at any other time in human history. In spite of the importance of chemical pesticides to protect crops from pest infestation, consumption of foods contaminated by pesticide residues can cause long term health effects [15]. Acute pesticide poisoning is a great problem in Bangladesh. Farmers who use hand sprayers for spraying pesticides suffer from a lot of morbidity effects. Increasing incidences of cancer [16], chronic kidney diseases [17], cardiovascular diseases [18], sterility of males and females [19], endocrine disorders [20], neurological and behavioral disorders [21] have been attributed to chronic pesticide poisoning.

There is a rising demand for organic agricultural products due to the consumers concern about the strong contamination of vegetables from the applied pesticides [22]. Therefore, it is essential to identify the presence of pesticide residues in vegetables so that consumers could be protected. In this regard, this study was initiated to assess the present status of the seven popular Organophosphorus pesticide residues in eggplant and to increase the awareness level of vegetables growers and it's consumers regarding the pesticide residues and their negative impact on consumer's health.

II. MATERIALS AND METHODS

A. Study Location:

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 bazaar, Baipayl bazaar and Sreepur bazaar during June 2019.

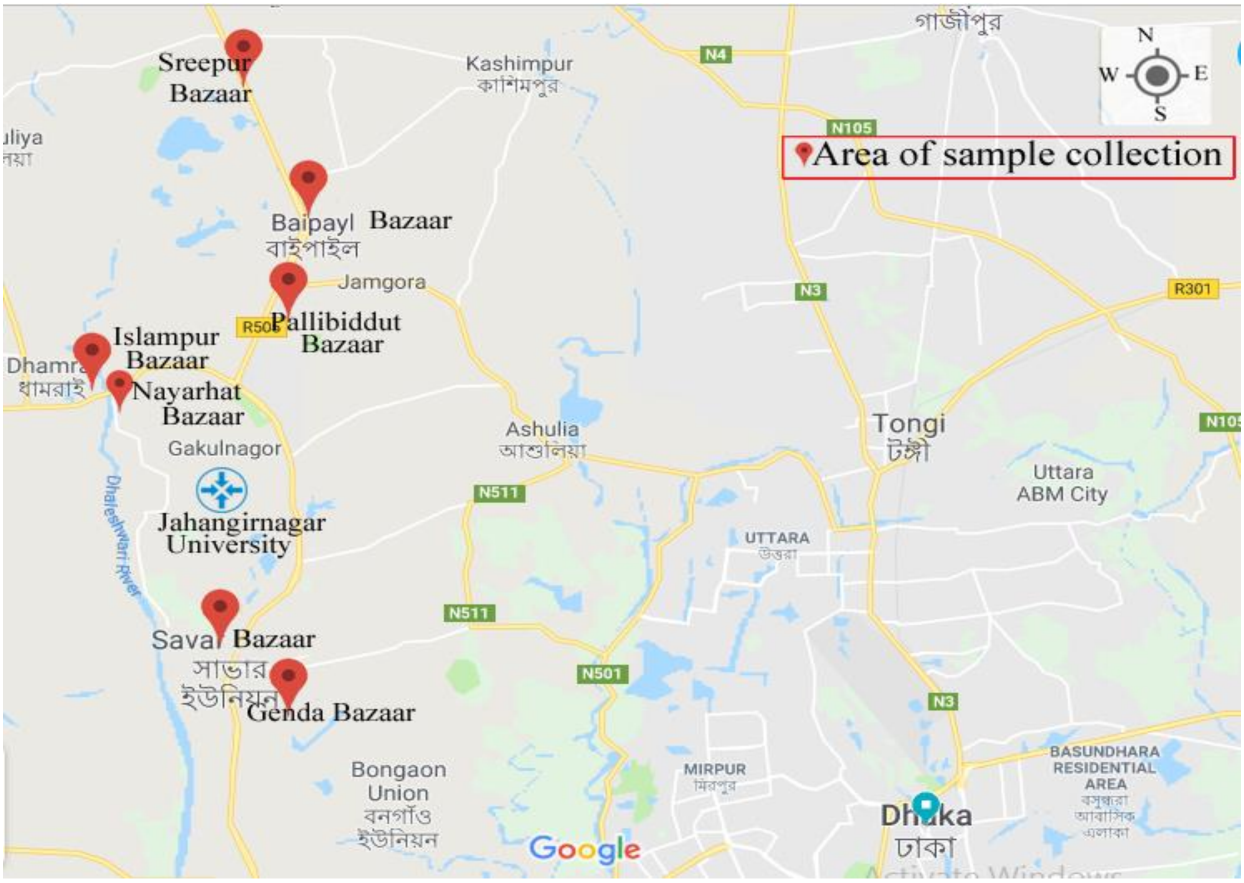

Fig 1:- Map Showing the Places of Sample Collection from the Surrounding Area of Jahangirnagar University.

B. Sample Collection and Preparation:

A total of 78 eggplant 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, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh on the same day of collection. All the samples were chopped and mixed properly in labeled polyethylene zipper bags and stored at -20°C for further analysis.

C. Chemicals and Reagent:

All pure pesticide standards: acephate, dimethoate, fenitrothion, chlorpyrifos, quinalphos, diazinon and malathion (>99.6%) (Table 1) were bought from Sigma-Aldrich Laborchemikalien (St Louis, MO, USA) via Bangladesh Scientific Pvt. Ltd. HPLC grade methanol, acetone, acetonitrile, analytical grade NaCl, anhydrous $MgSO_4$ and Primary Secondary Amine (PSA) were collected from Bangladesh Scientific Pvt. Ltd.

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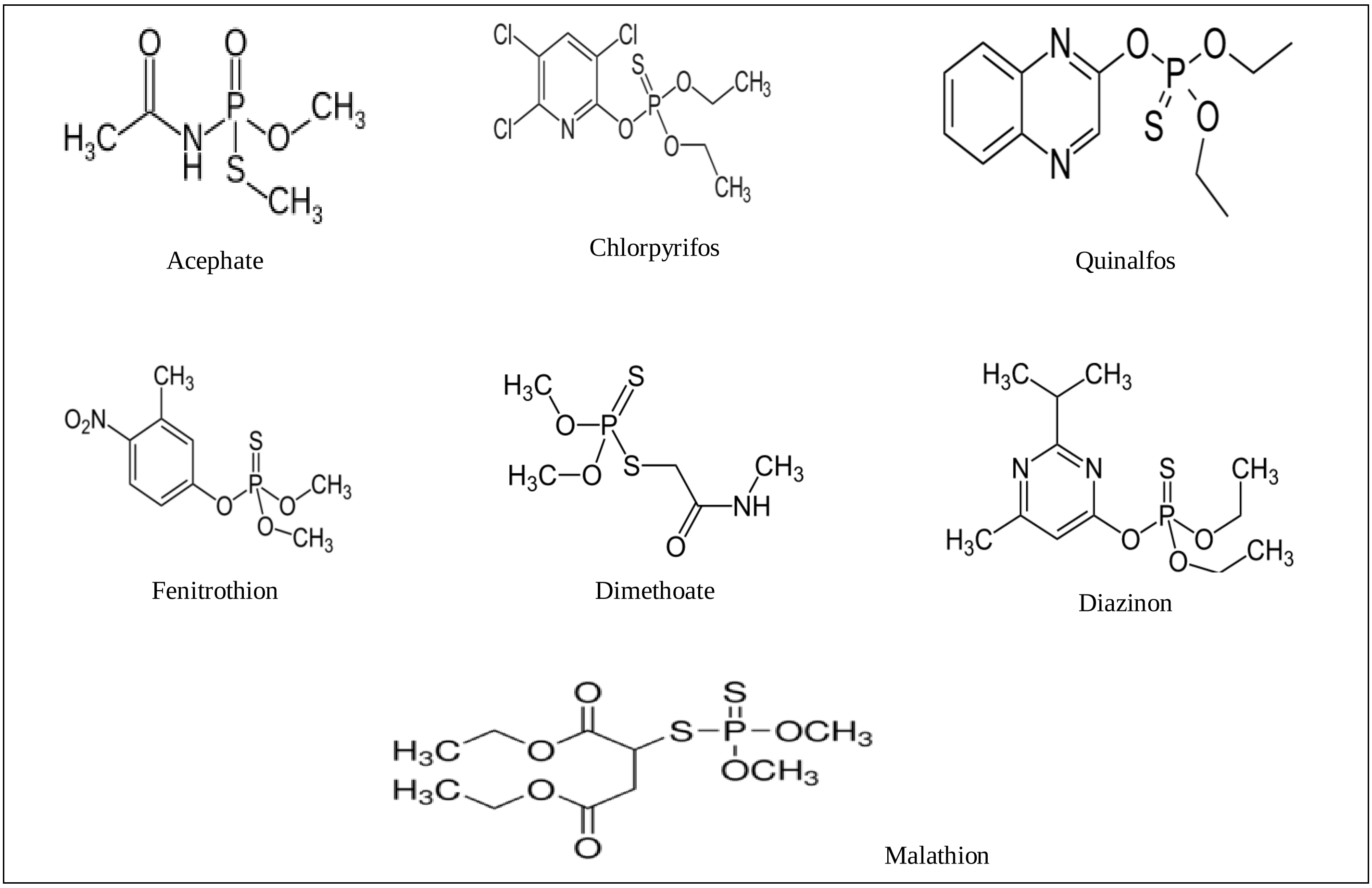

Fig 2:- Chemical Structures of the Pesticides Used in the Present Investigation.

D. Preparation of Standard Solution:

All of the standard stock solutions were made separately in acetone at 1000 ppm and stored until use at -20°C. 50 ppm mixed standards stock solution 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 ppm were prepared from this 50 ppm mixed standard stock. Finally all the working standards solution of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 ppm were prepared by using this mixed intermediate-ate stock solution. All the standard sock and working solutions were stored at -20°C.

E. Extraction and Clean Up:

Different extraction and clean-up methods are used for different food matrices. In the present study, a modified QuEChERS extraction technique developed by Prodhan *et al*. [23] was used to extract the collected eggplant samples. 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 centrifuge 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 $MgSO_4$ and 1 g of NaCl). Then it was shaken for one minute and followed by subsequently centrifuged at 5000 rpm for five minutes. Then 3 mL supernatant was taken in 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.

F. Identification and Quantification of Pesticide Residue in Samples:

The extracts of eggplant samples were analyzed following the method described by Prodhan *et al*. [24] 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 2). The instrumental conditions are described in Tables 2 and 3.

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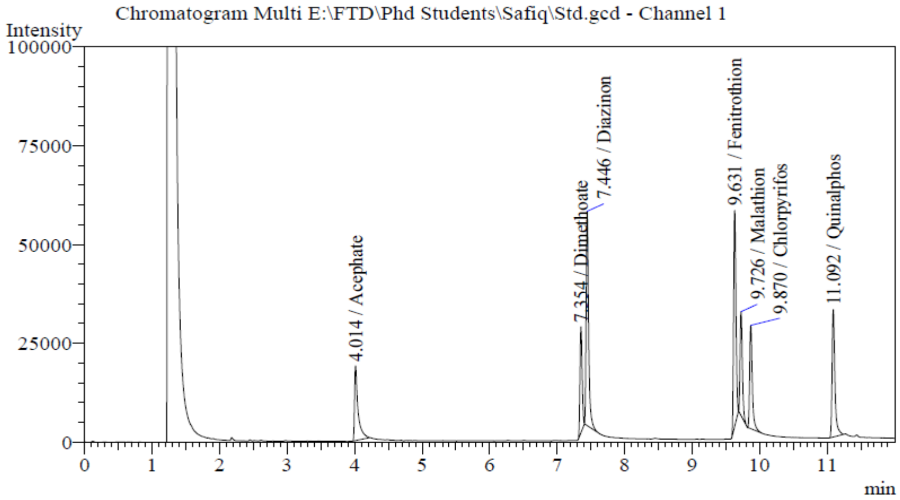

Fig 3:- Typical Chromatograms of Seven Organophosphorus Insecticide Standards Run By GC-FTD

Instruments	Conditions
Injection port SPL	Injection mode: split; temperature: 250^{0}C; flow control rate: linear velocity; split ratio: 30:0
Detected channel 1 FTD	Temperature: 280^{0}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 1:- The Instrument Parameters for GC-FTD

Column oven	Rate	Temperature (^{0}C)	Hold time (min)
Initial temperature	-	150	1
150^{0}C	10	220	2

Table 2:- Condition for Column Oven Temperature for FTD

G. Preparation of Calibration Curve

Different concentration level of standard solutions of each pesticide group were prepared and injected according to previously developed methods parameters prior to the injection of the sample extracts. The samples were calibrated against the five-pointed (50 µg/L, 100 µg/L, 200 µg/L, 300 µg/L and 500 µg/L) calibration curve of the standard pesticide solution (retention time, peak area, etc.). Every peak was distinguished by its time of retention. The GC software automatically displayed sample results in mg/kg.

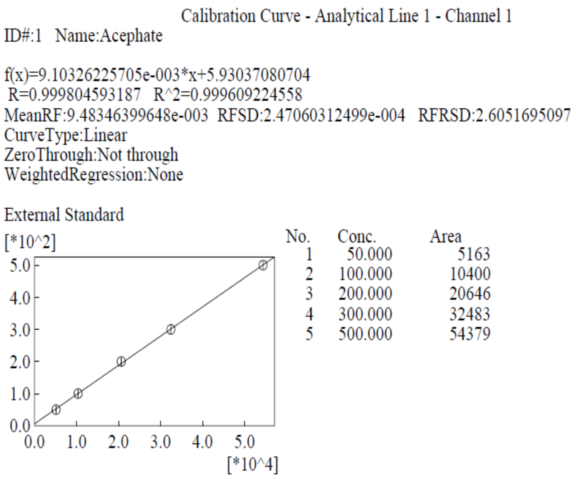

Fig 4:- Calibration Curve Prepared for Acephate.

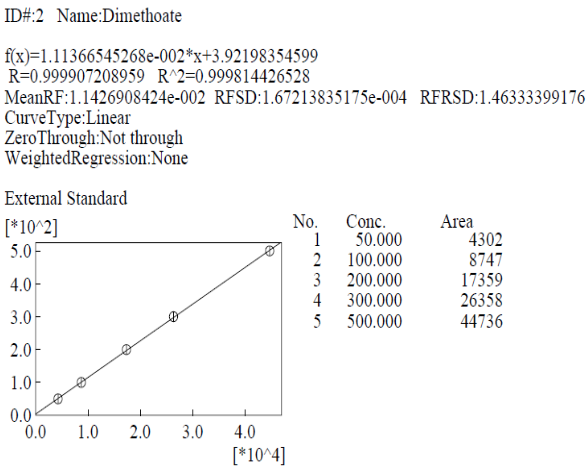

Fig 5:- Calibration Curve Prepared for Dimethoate.

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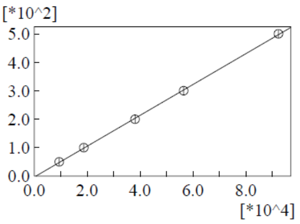
ID#:3 Name:Diazinone

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

External Standard



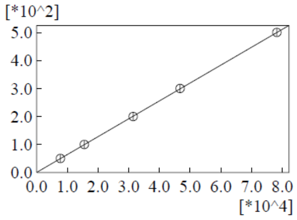
No.	Conc.	Area
1	50.000	9456
2	100.000	18743
3	200.000	38152
4	300.000	56503
5	500.000	92350

Fig 6:- Calibration Curve Prepared for Diazinon.

ID#:4 Name:Fenitrothion

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

External Standard



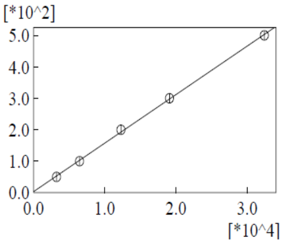
No.	Conc.	Area
1	50.000	7761
2	100.000	15544
3	200.000	31454
4	300.000	46756
5	500.000	78258

Fig 7:- Calibration Curve Prepared for Fenitrothion.

ID#:5 Name:Malathion

f(x)=1.54176807947e-002*x+3.48014008403
R=0.999653865922 R^2=0.999307851654
MeanRF:1.56784704407e-002 RFSD:3.63162883445e-004 RFRSD:2.31631577083
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

External Standard



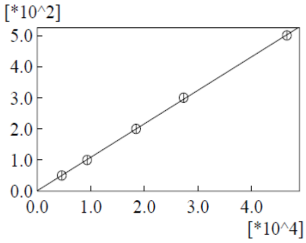
No.	Conc.	Area
1	50.000	3231
2	100.000	6462
3	200.000	12273
4	300.000	19087
5	500.000	32408

Fig 8:- Calibration Curve Prepared for Malathion.

ID#:6 Name:Chlorpyrifos

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

External Standard



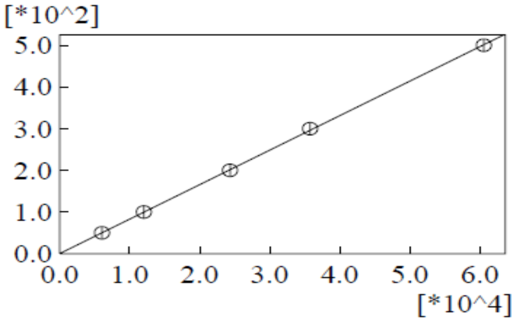
No.	Conc.	Area
1	50.000	4586
2	100.000	9333
3	200.000	18455
4	300.000	27322
5	500.000	46597

Fig 9:- Calibration Curve Prepared for Chlorpyrifos.

ID#:7 Name:Quinalphos

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

External Standard



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

Fig 10:- Calibration Curve Prepared for Quinalphos.

III. RESULTS AND DISCUSSION

A total of seventy eight eggplant samples from different retail markets located at the surrounding area of Jahangirnagar University, Savar, Dhaka, Bangladesh were collected to identify and quantify the presence of seven organophosphorus pesticide residues. The concentrated extracts of eggplant samples were analyzed by GC-2010 (Shimadzu) with Flame Thermionic Detector (FTD) with the preset parameters. The results of the tested samples contaminated with suspected pesticide residues are shown in Table 3.

Sl. No.	Area of sample collection	No. of analyzed samples	No. of contami-nated samples	No. of samples exceeding MRL	Detected pesticide	Residue level (mg/kg)	MRLs (mg/kg)
1.	Genda bazaar	15	2	0	Chlorpyrifos	0.049	0.5
					Diazinon	0.059	0.5
2.	Savar bazaar	10	1	0	Diazinon	0.045	0.5
3.	Nayarhat bazaar	10	1	0	Chlorpyrifos	0.043	0.5
4.	Islampur bazaar	10	0	0	----	ND	----
5.	Pallibiddut bazaar	10	1	0	Diazinon	0.051	0.5
6.	Baipayl bazaar	13	3	2	Chlorpyrifos	0.047	0.5
					Dimethoate	0.058	0.02
					Dimethoate	0.048	0.02
7.	Sreepur bazaar	10	1	0	Diazinon	0.049	0.5

Table 3:- The level of Different Pesticide Residues Obtained in the Analyzed Eggplant Samples (N=78)

- MRL for chlorpyrifos in eggplant is set by EU
- MRL for diazinon and dimethoate in eggplant is set by FAO/WHO

Table 3 shows that, out of seventy eight samples of eggplant, eight samples (11.5%) were contaminated by pesticide residues. Two of them were exceeded the MRL set by FAO/WHO/EU (EC, 2015). Another sixty nine samples (88.5%) were free from the residues of the sought pesticides. Among the contaminated samples, four contained diazinon at a concentration limit from 0.045 to 0.059 mg/kg. Three contained chlorpyrifos at a concentration limit from 0.043 to 0.049 mg/kg and two contained dimethoate at a concentration limit from 0.048 to 0.058 mg/kg. The contaminated samples contained 4-6 times higher residues than the MRL of the respective pesticides.

These results can be compared with another study on the pesticide residues in eggplant conducted by Aktar *et al.* [25]. They showed that out of fifty samples of eggplant collected from Mymensingh, eleven (22%) were contaminated by chlorpyrifos, diazinon, dimethoate and quinalphos of which five contained residue above the EU-MRL. Alam *et al.* [26] conducted another study on the pesticide residues in eggplant collected from Narayanganj where 50% samples contained pesticides and the levels of all the samples were above the EU-MRL. According to Hossain *et al.* [27], fifteen samples (100%) of tomato, brinjal and lady's finger collected from Savar bazaar contained pesticide residues above the corresponding MRLs. Islam *et al.* [28] analyzed 42 samples of three vegetables (brinjal, cauliflower and country bean) collected from Narsingdi district, Bandgladesh, and found that fifteen (15) samples (above 35%) were contaminated by the residues of the sought pesticides.

Prodhan *et al.* [23] collected seventy two eggplant fruit samples from various markets in Thessaloniki, Greece and analyzed to determine the pesticide residues. It was observed that 34 (47 %) of the total analyzed samples were contaminated by pesticide residues, of which, only one had residue above the EU-MRLs. Prodhan *et al.* [29] conducted another study in Greece on eggplant. They have detected eleven insecticides in eggplant fruits samples. Out of the142 analyzed samples, 67 (47%) were found to have pesticide residues and the rest of the samples (53%) were free from pesticide residues. Prodhan *et al.* [30] conducted an investigation to detect the pre harvest interval (PHI) for quinalphos, malathion and diazinon in Eggplant where it was shown that the determined PHI were 10 DAS (days after spray), 7 DAS and 7 DAS for quinalphos, malathion and diazinon respectively. Another investigation was undertaken by Prodhan et al., (31) to quantify the residue loss of quinalphos, diazinon and fenitrothion from eggplant and malathion from yard long bean through washing and steam cooking procedures. It was 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.

This study reveals that the use of pesticide to protect vegetable infestation is increasing and this trend would be detrimental to human beings as well as environment. The regular scrutinizing of pesticide residues in vegetables may be made and develop awareness among people and responsible authorities.

IV. CONCLUSION

Recently, there is a increasing concern about pesticide residues in vegetables and their impact on environment and all living beings. Highly toxic chemical pesticides using in the crop fields are causing direct and substantial harm to human beings, animals and ecosystem. But the usage of chemical pesticides against the pest infestation is essential for farmers and surplus and haphazard use of pesticides has resulted in pest resurgence. This study shows the presence of pesticide residues in the vegetables 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 like cancer, kidney failure, heart attack etc. The benefit of the chemical pesticide used for the more agricultural productivity must be calculated against the possible health hazard due to the pesticide contaminated foods. Therefore, the pesticide residues in vegetables are becoming a major food safety concern for the growers, consumers and the governments.

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