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Quantitative Analysis of Selected Pesticide Residues in Brinjal Using Gas Chromatography

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ABSTRACT

Pesticide residual analyses of selected pesticides (Carbofuran, Cypermethrin, Mancozeb and Chlorpyrifos) in brinjal fruits are explained by using Gas Chromatographic system. There were two varieties (BARI-8 and UTTARA) of brinjal, cultivated at Department of Environmental Science Field, Bangladesh Agricultural University, Mymensingh and the harvesting time was selected as 0 day, 3rd day and 7th day from the pesticide spray. After harvesting 54 brinjal fruit samples had been dried and packed properly. In GC analysis, calibration curve was drawn for every concentration of standard solution with areas of peak as well as a major eluted peak for one and every pesticides solution was obtained. Results revealed that the contents of pesticide residues were higher in 0-day as compared to 3rd day and 7th day for both of the varieties. In contrast, the incidences of pesticides in fruits of all the samples in 0 day for both varieties exceeded the MRL with the highest significant for all pesticide residues. On the 7 days interval the pesticide residues for all selected pesticide under the MRL of both varieties. UTTARA brinjal fruits had more trend to reduce the pesticide residue within day variation than the BARI-8 variety. The reduction trend (Reduction Factor) ranged from 0.164 ppm to 0.230 ppm for both varieties which had a positive response to the pesticide concentration reduction over day variation. This study also suggested that on considering the consumer safety, brinjal should not be harvested less than 3-5 days as well as it may be consumed at approximately 7 days later from the spray. 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 : Pesticide, Residues, Brinjal Fruit, GC, Post-Harvest Interval.

I. INTRODUCTION

Agriculture is the principal economic anchor of Bangladesh, which contributes about 33% to the country's gross domestic product (GDP) and 80% of people depend on agriculture for their livings [1,2]. Agriculture plays an important role to protect food scarcity in this densely populated country. Rice, wheat, jute, potato, sugarcane, vegetables and tea are the main crops of this country [3]. Brinjal, tomato, cabbage, bitter gourd, snake gourd, pointed gourd, okra, hyacinth, bean and yard long bean are grown in Bangladesh as vegetables and these are nutritious, valuable and very popular to consumers [4,5]. The importance of brinjal is well known. It helps to avert colon cancer, decreases cholesterol level, helps in the type 2 diabetes management, is very rich in antioxidant, and also helps to control weight [6]. To ensure the food demand, control of insects, pests and diseases plays a key role. Until today, pesticides play a vital role for the control of insects, pests and diseases, but pesticides create several adverse effects on human health, agro-ecosystem and the environment resulting from indiscriminateness, overuse, and misuse of pesticides [7]. Like many other developing countries, pesticides are used extensively in Bangladesh to increase the crop yield per acre [8-10]. As agricultural production is being increased every year to meet the growing demand of the people, uses of pesticides also being rose up. In Bangladesh context, the vegetable growers have been using the pesticides frequently to have the higher yield. The widespread use of pesticides may contaminate the environment as well as foods, which may create health problem [3,11,12]. The most alarming concern is that pesticide use is very indiscriminate in Bangladesh [13]. There are areas where pesticides are used in excessive quantities [14]. Application of different pesticides is increasing day by day in Bangladesh and this trend would be detrimental to mankind as well as environment [15]. Therefore, the presence of these pesticide residues in food commodities has always been a matter of serious concern especially when these commodities are consumed fresh [16-18].

Brinjal is an important vegetable and very much sensitive to pest attack and disease infestation from seedling to fruiting stage [4, 19]. The main pests that attack plants are Brinjal Fruit Borer, Stem borer, Spider Mite, Aphid, Jassid, Whitefly and Roots-cutworm. For the control of these pests, the farmers are used different types of pesticides. The farmer community uses the major pesticides such as Acephate, Chlorpyriphos, Dichlorovos, Carbofuran and Imidacloprid. It has been reported that in brinjal, bitter gourd and beans, the attack of insect pests are severe and farmers' sprayed pesticides quite frequently even every day [4,14]. Farmers believe that for better yield the pesticide application is very important parameter to be conducted during crop production; by following this they tremendously apply the pesticides over crops even on fruiting. This repeated application of pesticides on crops particularly at fruiting stage and no adoption of safe waiting period leads for accumulation of pesticide residues in fruits and vegetables [20].

Most screening procedures in use today are capable of finding only a fraction of the pesticides that are registered around the world. Gas Chromatography (GC) provides the most comprehensive and reliable screening method available for pesticides, metabolites, and suspected endocrine disrupters. It has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but it destroys the injected sample [21]. Many scientists [22-24] analyzed pesticide residues in fruit and vegetables in India, Spain, China, Japan and other developed country. But limited pesticide residual analysis conducted by GC for available vegetables in Bangladesh [10,25,26]. Islam et al. [10, 11] were conducted research works on pesticide residues in various vegetables and fruits which from nearby local markets. Most of the scientists e.g. Hossain et al., [15]; Chandra et al. [27]; Fakhruddin et al., [28] worked out to determine pesticide residues in different ways and their maximum work was executed to comparison between verities or depending on various factors. But this study is undertaken to determine the pesticide residues in brinjal from the experimental field. The important thing is that the market product always not enough to determine the exact values but cultivated vegetables can be capable of providing exact values because it maintained by supervision. This study is conducted to determine the residual effect of pesticides over day variation from the day of pesticide application till a week and also shown a comparison between residual effect and Maximum Residue Limit (MRL).

II. METHODS AND MATERIAL

Sampling and Experimentation

Fifty-four brinjal fruits samples (18 samples per sampling day) were collected during 0 day, 3rd day and 7th day after pesticide spraying from 18 plots (9 plots BARI-8 and 9 plots UTTARA variety) of brinjal yields on considering the fruits color, size and freshness. The samples dried and packed separately with properly labeled polythene bags and brought to the Department of Environmental Science Laboratory, Bangladesh Agricultural University, Mymensingh and after few days all samples were transferred to Bangladesh Agricultural Research Institute (BARI), Gazipur for the experiment. The collected samples were extracted (Liquid extraction technique following by Usher and Majors [21] with changing extraction time) properly and cut into small pieces with proper labeling for future identification and kept in deep freezer (-20 °C). About 2 gm of each sample (frozen part) was grinded by hand grinder then allowed in 5 mL Hx (n-Hexane) in test tube for 15 minutes and tightly closed by cork to avoid the evaporation of solvents and volatile chemicals. After 15 minutes, each part was mixed well by shaking. After that it was kept 5 minutes for stabling the mixture. The supernatant solution (Hx with extracted compound) was taken in a vial by small glass pipette carefully and kept for the experiment.

Calibration Curve

Individual stock standard solutions (1 mg/mL) were prepared in ethyl acetate and stored in the dark at −20 °C. Prior to their use, they were kept for 1h at ambient temperature. A mixed stock standard solution of pesticides was prepared in ethyl acetate at 15 μg/mL with respect to each pesticide. Two different types of calibration curve were studied: matrix-matched calibration (MC) and solvent calibration (SC). Matrix-matched calibration solutions were prepared by mixing known volumes of the pesticide working solutions and the volume with extracts of blank samples. Calibration curves at 5 levels of 10, 20, 30, 40 and 50 ppm triplicate were prepared by addition of 10 μL, 20 μL, 30 μL, 40 μL and 50 μL of mixed standard stock solution, respectively, to 15 gm portions of blank brinjal samples in each case. A stock solution of triphenylmethane (TPM) in ethyl acetate at concentration of 1 mg/mL was used as internal standard and an aliquot of 10 μL of TPM solution in ethyl acetate was added to the brinjal sample.

Sample Preparation

An aliquot of 10 μL of internal standard solution (1000 mg/L) was added to 15 g of blended blank brinjal sample in a 50 ml falcon tube and after being left for 1h at ambient temperature in dark, 15 mL acetonitrile (containing acetic acid 1% and sodium acetate tri hydrate (0.45gm) was added. The mixture was mixed at high speed with vortex mixer for 1 min. 4 gm of activated anhydrous MgSO4 was added to the mixture, and mixing was continued for an additional 60 s. The mixture was centrifuged for 5 min at 3000 rpm at -5 °C. The supernatant was transferred to a 15 mL falcon tube containing 2 gm $MgSO_4$, 300 mg PSA and 100 mg florisil. After shaking for 1 min and centrifugation for 5 min at 3000 rpm at -5 °C, 4 mL of supernatant was transferred to a 5 mL vial and evaporated to dryness under a gentle stream of nitrogen gas. The residue was reconstituted by toluene to obtain 1 mL solution, and after shaking for 3 minutes, 2 μL of the solution was injected into gas chromatograph.

Gas Chromatographic (GC) Analysis

The analysis was conducted with Gas Chromatograph (GC) [GC-2014] Shimadzu Corporation, Kyoto, Japan. FID (Flame Ionization Detector) was used in this experiment. The whole GC system is tabulated below (Table 1). Injection of each standard solution was repeated several times (at least 5 times) for the confirmation of the respective retention time. The different concentration of standard solutions was injected in GC system under above mentioned condition and made calibration curve. GC eluted compound was calculated by comparing the retention time with specific peak area of the standard solution and studied samples. After acquisition of the total chromatogram for the mixed stock standard solutions in scan mode, peaks were identified by their retention time. The most abundant compound that showed no evidence of chromatographic interference and had the highest signal-to-noise ratio was selected for quantification purposes.

Table 1: Parameters of gas chromatography (GC).

Parameters	GC/FID	Stability/Reproducibility
Column	RT-Missive 5A, serial number: 1104596. Length: 30 m Inner diameter: 0.53 mm Film thickness: 50.00 um.	Column flow: 6.63 mL/min Maximum temperature: 300°C.
Oven	50°C (3 min), to 200°C at 10°C /min, to 300°C at 15°C/min and hold finally 5 min. (For Mancozeb and Cypermethrin)	Column oven: 50 °C (for Mancozeb); 60 °C (for Chlorpyrifos); 60 °C (for Carbofuran); 50 °C (for Cypermethrin).
	60°C (5 min), to 180°C at 10°C /min, to 280°C at 10°C/min and hold finally 8 min. (For Chlorpyrifos and Carbofuran)	
Carrier	Hydrogen (30 mL/min); Air (142.1 mL/min)	Hydrogen (30 ml/min)
Detection	DFID, 300 °C, Sampling rate: 40 msec	DFID, 300°C
Injection	1µL, Direct injection, 200°C	Split
Linear velocity	60.7 cm/sec	Pressure: 66.8 kPa
Purge Flow	3.0 mL/min	Split ratio: 20

Quantitation

The concentrations of pesticides were determined by interpolation of the relative peak areas for each pesticide to internal standard peak area in the sample on the calibration curve. In order to compensate for losses during sample processing and instrumental analysis, internal standard (TPM) was used.

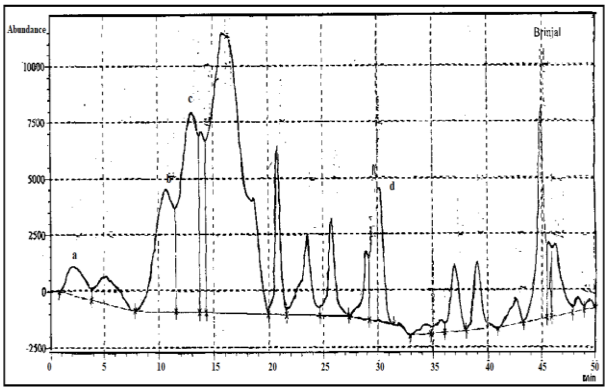

Figure 1: A representative chromatogram obtained for four pesticides and internal standard a) chlorpyrifos; b) mancozeb; c) carbofuran; d) cypermethrin in brinjal samples.

Calibration Curve of Pesticide Solutions

From the figure 2 – 5, the straight-line calibration curves were made by using the peak areas and concentration of standard pesticide solutions where carbofuran (12.3 min), chlorpyrifos (1.5 min), mancozeb (11.68 min) and cypermethrin (33.7 min) solution of different concentration were eluted. These curves were made for measuring the different concentration of pesticide in brinjal fruits where areas of peak for every concentration were calculated.

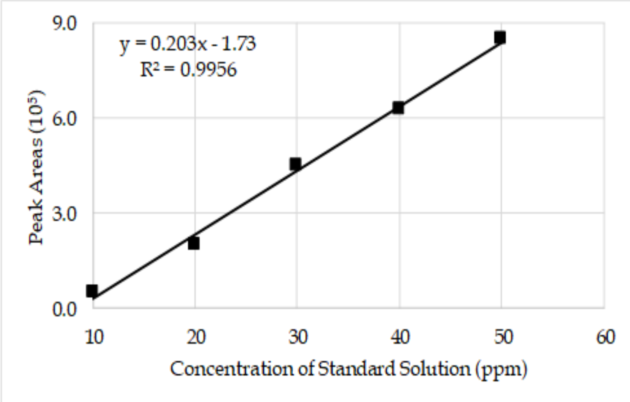

Figure 2: Curve for various concentration of standard carbofuran solution.

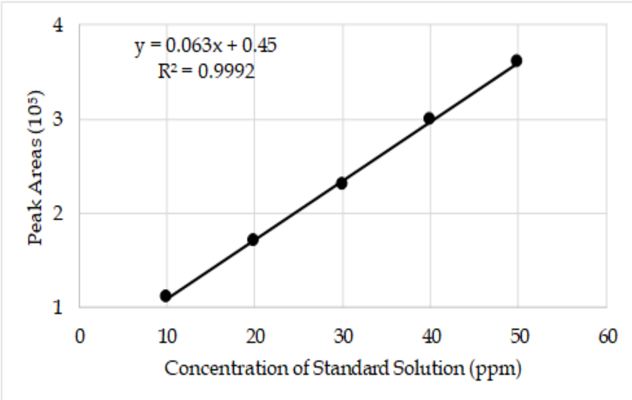

Figure 3: Curve for various concentration of standard chlorpyrifos solution.

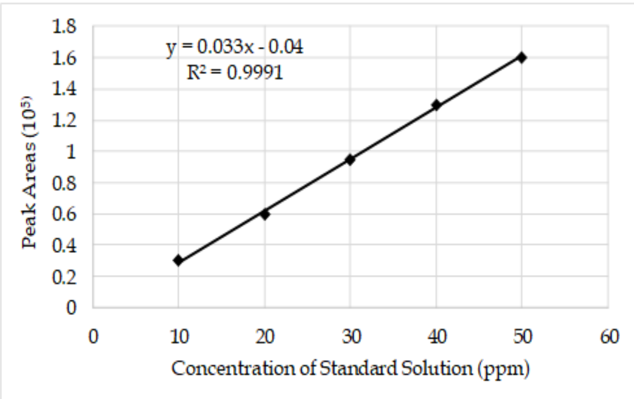

Figure 4: Curve for various concentration of standard mancozeb solution.

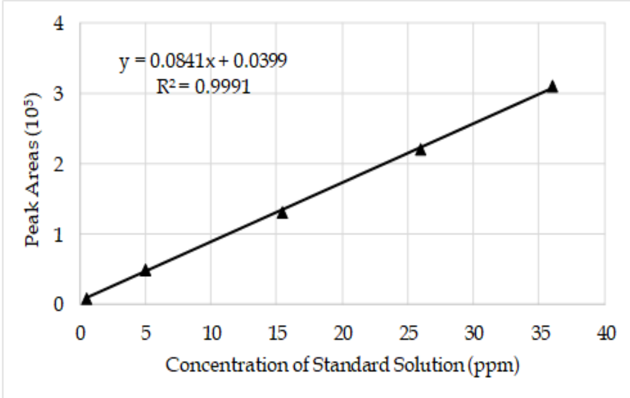

Figure 5: Curve for various concentration of standard cypermethrin solution.

The curve of peak areas and concentration of this present study was agreed to the previous study of Islam *et al.* [10,11] and Afia *et al.* [29] where their drawing curves are quite similar on depending the concentration and peak areas. Islam *et al.* [10,11] showed the GC calibration curve and suggested that peak area of the specific amount of a chemical dependent upon the nature, elution and characteristic of chemicals studied. There was enormous peak area differences found in respect of specific amount of standard chemicals. Few researchers [10,11, 30-32] suggested the similar variation among the different chemicals occurred in their peak areas in GC analysis.

Chromatograms of Pesticide Solution

Figure 6 – 9 are showed that the carbofuran, chlorpyrifos, mancozeb and cypermethrin solution response at 12.3, 1.5, 11.5 and 33.7 minutes in GC system and for confirmation, the system repeated for several times.

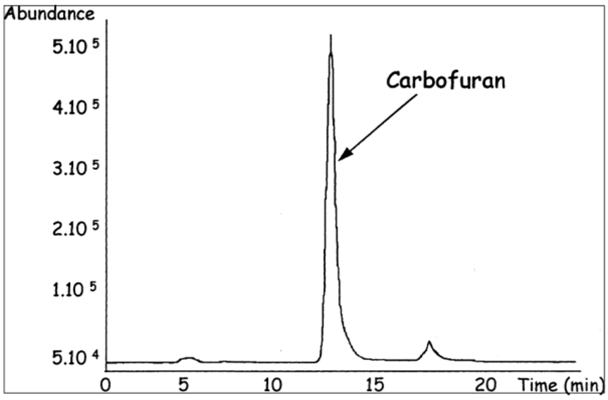

Figure 6: Eluted peak for carbofuran solutions.

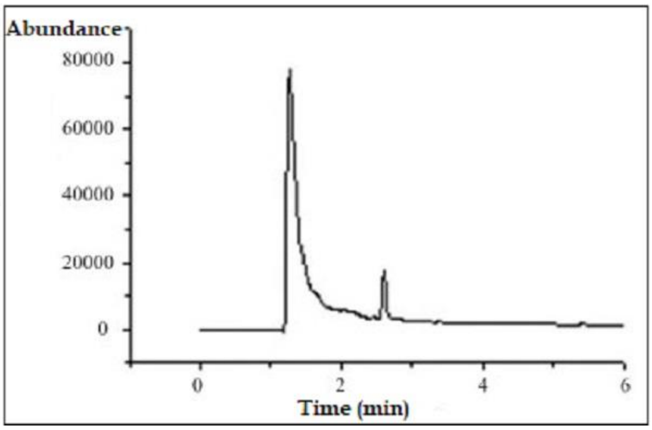

Figure 7: GC Eluted a major peak showed chlorpyrifos solutions.

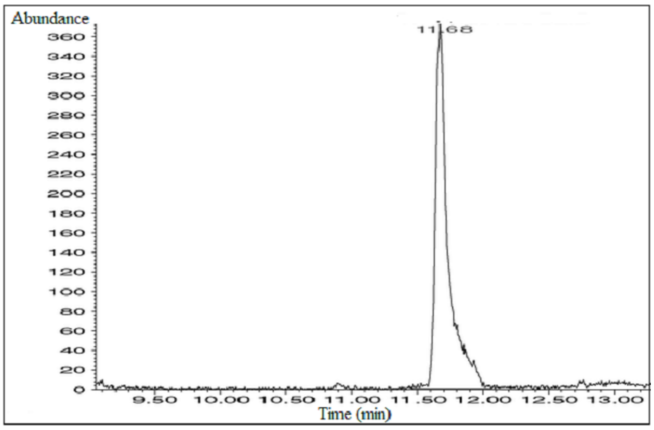

Figure 8: A Major eluted peak for mancozeb solution.

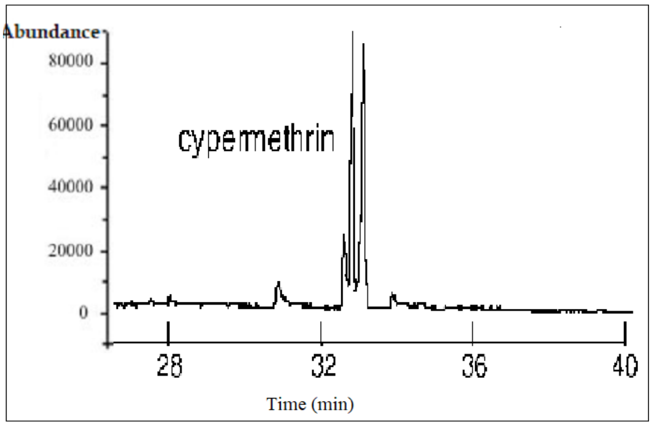

Figure 9: GC eluted peak for cypermethrin solution.

Islam *et al.* [10] found the compound of imidacloprid solution at 19.20 minutes of GC system and Afia *et al.* [29] found the compound of chlorpyrifos at 9.870 minutes that have a good agreement to this study. Islam *et al.* [10] also showed the retention time for mancozeb solution was 14.52 minutes that similar to this present study. The eluted peak and retention time actual and no disagreement.

Residues of Pesticide in Brinjal

Comparison of Pesticide Residue between BARI-8 and UTTARA

At the day of pesticide spray, the concentration was so high and then followed by 3 days and 7 days interval the concentration decreasing randomly for all pesticides (Figure 8 and 9). The pesticide decreasing over time variation was agreed to the previous observation of Li *et al.* [33] where they reported about the cypermethrin, carbofuran and diazinon pesticide reduction trend with passing the time. Hiralal *et al.* [34] indicated that the residues of pesticed had controlled to maintain the 4-7 days interval of spray. So, at a certain stage the concentration of pesticide was reached to the safe consume level or MRL limitation. The brinjal variety of UTTARA was also up taken pesticide concentration and over time variation it's also showed the positive response to the concentration level. UTTARA fruits gave up concentration of pesticide more than the 0 day. At the stage of 7 days interval of harvesting, the fruits showed a lower level of pesticide concentration which was the similar decision of Chowdhury *et al.* [35]. So, it's a clear observation that, after time interval the concentration would be disappeared.

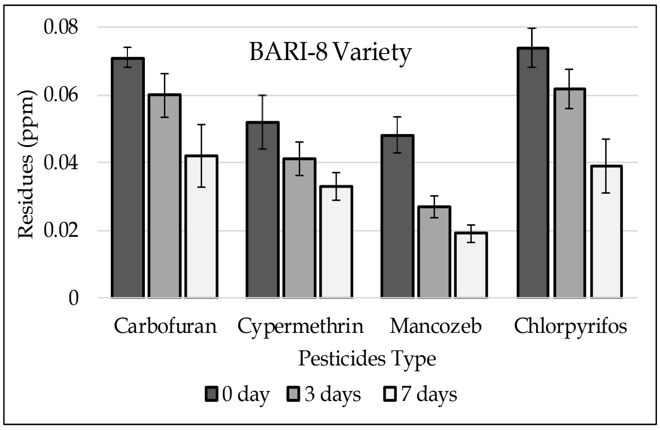

Figure 10: Pesticide residues in BARI-8 with day variation.

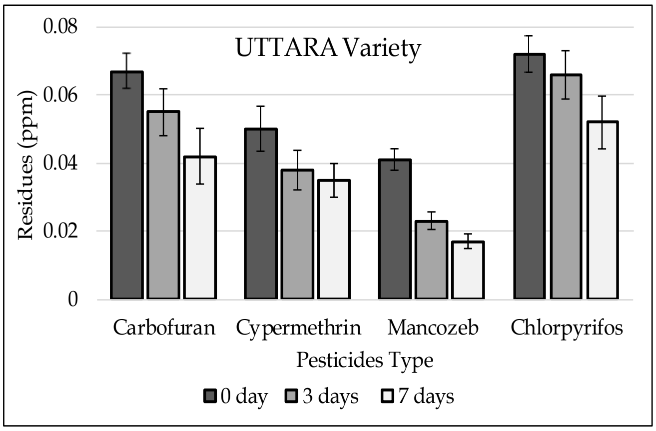

Figure 11: Pesticide Residues in UTTARA with day variation.

Few researcher [14, 36, 37] suggested that by following the recommendation dose and maintained day interval harvesting had a positive response on the residue reduction of pesticide. This study was undertaken for the identification of residue reduction system where a good feedback was obtained as well as that had a similar observation of previous researcher.

Reduction Factors (R_f) of Pesticide Residues

The chromatograph experiment of brinjal fruits for both varieties showed reduction factor (R_f) varying from 0.164 to 0.230 ppm (Table 2). Depending on the day variation, a significant change was observed and which was agreed to the observation of Iqbal *et al.* [38] where, the reduction factor varied from 0.050 to 0.231 ppm and the sample amount was 5 for Carbofuran, Chlorpyriphos, Imidacloprid, Dichlorovos and Acephate pesticides.

Table 2: Reduction factors (R_f) for BARI-8 and UTTARA on depending day variation.

Reduction Factor per 3 days Variation (ppm)				
Variety	Carbofuran	Cypermethrin	Mancozeb	Chlorpyrifos
BARI-8	0.195	0.198	0.230	0.210
UTTARA	0.185	0.164	0.225	0.168

The highest reduction rate for BARI-8 was obtained 0.230 ppm in Mancozeb concentration. On the other hand, 0.225 ppm of highest reduction rate was observed in UTTARA fruits for Mancozeb also. Sardana *et al.* [39] conducted a study and indicated about a reduction factor for their results analysis and depending on the residue reduction system. Table 2 revealed that a continuous reduction rate for all pesticides and varieties. From the table, it's a clear observation that the reduction rate of pesticides residues was decreasing with a similar reduction value.

Average Residual Comparison of Both Varieties with Time Variation

Average residues of both brinjal varieties were calculated during the chromatograph. By the day variation, most of the pesticide residues had a trend to reduce the pesticide concentration. Figure 12 revealed that the average value for all day variation of both BARI-8 and UTTARA had a reduction trend to the lower limit of pesticide residue.

The highest average pesticide residue (0.058 ppm for BARI-8 and 0.063 ppm for UTTARA) was observed for both brinjal varieties at 0 day of pesticide spray and lowest concentration (0.031 ppm for BARI-8 and 0.027 ppm for UTTARA) observed in the 7 days intervals which trend was almost similar to the observation of Jyot et al. [40] and Ahuja et al. [41] who observed the trend of reduction within 4 to 7-days variation. From the previous research study, most of the results revealed that the residues of pesticide reduced individually or averagely with time interval. The trend of average residues reduction may be reached a lower limit within more day's variation.

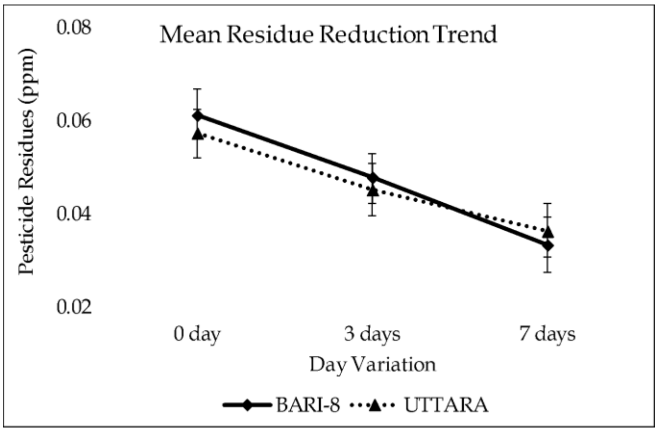

Figure 12 : Average pesticide residue reduction trend with day variation of both BARI-8 and UTTARA.

Comparison of the Residues with MRL for Both Brinjal Varieties

An experimentation of two varieties of brinjal fruits (BARI-8 and UTTARA) had applied four specific pesticides and that responded a significant change in residues on depending the day variation. All of the varieties received the same dose of pesticide where BARI-8 up took pesticide concentration more than the UTTARA variety. Table 3 revealed that on the 0 day of harvesting results for both varieties (0.071, 0.052, 0.048, 0.074 ppm and 0.067, 0.050, 0.041, 0.072 ppm for BARI-8 and UTTARA respectively of four pesticides) exceeded the MRL with a highest significant for all pesticide residues. On the 3 days interval of spray results showed only chlorpyrifos (0.062 ppm) for BARI-8 below the MRL as well as carbofuran (0.055 ppm) and chlorpyrifos (0.066 ppm) for UTTARA within the MRL. Besides, on the 7 days interval the pesticide residues (0.042, 0.033, 0.019, 0.039 ppm and 0.042, 0.035, 0.017, 0.052 ppm for BARI-8 and UTTARA respectively) for all selected pesticide under the MRL of both varieties. UTTARA brinjal fruits had more trend to reduce the pesticide residue within day variation than the BARI-8 variety. Ahuja *et al.* [41] and Kaur *et al.* [42] observed the pesticide residues for lambda – cyhalothrin, cypermethrin, decamethrin pesticide of the aubergine fruits and brinjal within 10 days variation but these pesticide residues and that reached the MRL within 10 days variation from the spray. Another study was conducted by Iqbal *et al.* [38] and they revealed that on the 7 days interval the concentration of Carbofuran, Chlorpyriphos, Imidacloprid, Dichlorovos and Acephate residues of 0.026, 0.035, 0.039, 0.034 and 0.028 ppm respectively in brinjal reached below the MRL but the 0-day residues were 0.084, 0.095, 0.097, 0.070 and 0.071 ppm. The significant results

Table 3 : Comparison of the residues with MRL for BARI-8 and UTTARA.

Variety	Time Variation	Carbofuran (ppm)	Cypermethrin (ppm)	Mancozeb (ppm)	Chlorpyrifos (ppm)
BARI-8	0 day	0.071	0.052	0.048	0.074
	3 days	0.060	0.041	0.027	0.062
	7 days	0.042	0.033	0.019	0.039
UTTARA	0 day	0.067	0.050	0.041	0.072
	3 days	0.055	0.038	0.023	0.066
	7 days	0.042	0.035	0.017	0.052
MRL		**0.05**	**0.03**	**0.01**	**0.06**

were obtained when comparing to the MRLs. Most of the pesticide concentration violated the MRLs except some pesticides below the MRLs. Although four selected pesticides in brinjal fruits are so dangerous to exposed, 7 days interval from the spray will safe to consume.

III. CONCLUSIONS

The obtained results from the experiment of both in the brinjal varieties, all of the samples were showed the response to the residue reduction. The experiment was conducted depending on the 0, 3- and 7-days variation and the residues had a trend to reduce. The GC system had the responded for the eluted peak as well as depending on the peak areas and standard solution concentration, straight-line curves were drawn. All of the samples were contained pesticides which detectable. Overall the study, BARI-8 variety had a strong significance to more pesticide absorption than the UTTARA variation. The interaction of pesticides between BARI-8 and UTTARA had a significant response to the time variation. After 7-days interval, all of the residues were minimized and reached below the MRL. Considering the consumer safety in Bangladesh, brinjal as well as other vegetables should not be eaten at least 3-5 days interval from the pesticide spray. The whole study revealed a decision that the reduction rate or factor played an important role for pesticide residue trend for all pesticide and varieties. It's a risk to consume the pesticide contaminated vegetables within the short-day variation of pesticide spray. The cultivators of the vegetables should follow the recommended dose of pesticide and harvest them depending on the recommended day variation for specific vegetables. So, it's easy to say at least that the public awareness and following the recommended dose can be effective to reduce the risk of pesticide related diseases as well as the environmental contamination.

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