



## Pesticide Residue Analysis from Winter Vegetables Collected from Six Markets of Rajshahi Bangladesh

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### Abstract

Pesticide residues in vegetables have become a major concern associated with food safety issues. Five types of winter vegetables like bean, brinjal, tomato, cauliflower and cabbage were collected from six markets of Rajshahi District during January, 2019 in Bangladesh. Residues of commonly used pesticides (Acephate, Dimethoate, Diazinon, Fenothion, Malathion, Chlorpyrifos and Quinalphos) in winter vegetable samples were determined using Gas Chromatography-Flame Thermionic Detector (GC-FTD) procedures. A quick, easy, cheap, effective, rugged and safe (QuEChERS) method with acetate buffering (AOAC Official Method 2007.01) was used for sample preparation. Out of 30 samples, pesticide residues were found only in one brinjal and two tomato samples. The result showed that tomato samples collected from Shaheb bazar and Kharkhari bazar of Rajshahi City contained 0.047 mg/kg and 0.139 mg/kg dimethoate residue, respectively. Brinjal sample collected from Shalbagan bazar, Rajshahi, contained 0.052 mg/kg dimethoate residues. The detected pesticide residue dimethoate of these three samples was higher than maximum residue level (0.01 mg/kg).

**Key words:** Food safety, GC-MS, MRL, Pesticide residue, Vegetable

### Introduction

The contamination of food by chemicals is a public health concern worldwide, and pesticides are a chemical hazard associated with food contamination (Prüss-Ustün *et al.*, 2011). Pesticide refers to any substance or mixture of substances in the food of humans or animals, including any specified derivatives such as degradation and conversion products, metabolites, reaction products, and impurities, of toxicological significance (McNaught and Wilkinson, 2000). Pesticide residues cause both short- and long-term toxic effects that are hazardous to health, especially at higher levels that can lead to toxicity. Headaches, nausea, irritation, vomiting, diarrhea, abdominal pain, and hypersensitivity are repeatedly reported impacts of acute pesticide exposure. Additionally, chronic pesticide exposure increases the risk of reproductive defects, neurodegenerative disorders, organ damage (kidney/liver), mutagenic and carcinogenic transformation, and endocrine disruption (Hayes and Laws, 1991; Bungush and Anwar, 2000 and Eskenazi *et al.*, 1999). Children are more susceptible due to their small body size, immature immune systems, and rapid growth cycles, especially in the brain and nervous systems (Berrada *et al.*, 2010).

Pesticide exposure is increasing in Bangladesh due to the acreage of irrigated agriculture. Approximately 80 types of registered pesticides with 170 different trade names are commonly used in agriculture and public health sectors in Bangladesh (Rahman and Khan, 2005). Of these types, organophosphates comprise 60.4%, carbamates comprise 28.60%, organochlorines comprise 7.60%, and others comprise 3.4% of the total pesticides used (Khan, 2011). The problem is compounded when the indiscriminate use of pesticides

by farmers is made worse due to illiteracy and low levels of awareness of the hazardous effect of pesticides to human health (Khan, 2011; Chowdhury *et al.*, 2012).

Bangladesh has an irresistible agricultural economy. Diversification of vegetable crops and increasing commercialization can support the development of the agriculture sector in several ways. But, in case of vegetables, data show the output growth in Bangladesh mainly through area expansion, without much improvement in yield. One potential drawback associated with a shift toward more intensive vegetable production is the common reliance of most vegetable producers on heavy application of pesticide (Hossain *et al.*, 2000). Among the vegetables grown in Bangladesh, brinjal, tomato, cabbage, bittergourd, snake gourd, pointed gourd, okra, hyacinth bean and yard long bean are nutritious, valuable and very popular to consumers. 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 (Anonymous, 2001; Ahmed *et al.*, 2005). Vegetable are major part of diet contributing nutrients, vitamins and minerals. Vegetable grown in Bangladesh are vulnerable to be contaminated by different pesticides. Contamination of foods with toxic chemicals poses a serious threat to public health, especially in a country like Bangladesh where due to poor health literacy and the level of awareness is very low. Pesticides have been associated with a wide spectrum of human health hazards ranging from short-term impacts such as headache and nausea to chronic impacts like cancer, reproductive harm and endocrine disruption (Chowdhury *et al.*, 2014). Extensive deliberate use of pesticides has resulted in

contamination of vital supplies, air, water and food. The risk to humans may be short-term as well as long term depending on the persistence of the pesticide and the exposure period. Pesticide residue in food makes deleterious effect on human health such as headaches and nausea to chronic impacts like cancer, reproductive harm and endocrine disruption (Berrada *et al.*, 2010). Now pesticide residue in food has become a consumer's safety issue and the consumers have the right to know how much pesticide get incorporated in the food they eat. However, very little references area available on the presence of pesticides in vegetables in Bangladesh (Khaton *et al.*, 2004). Under such circumstances, the present study was undertaken to detect and quantify the amount of leftover residue of pesticide in different vegetable samples collected from different local bazars of Rajshahi district.

### Materials and Methods

The pesticide residue analysis from winter vegetable samples was conducted in Pesticide Analytical Laboratory under the Pesticide Research & Environmental Toxicology Section of Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur.

### Study location

All the vegetable samples were collected from wholesale and retail markets located at the adjacent area of Rajshahi City. The market includes Shaheb bazar, Shalbagan bazaar, Binodpur bazaar, Kharkhari bazaar, Katakali bazaar and Baneshwar bazaar during January 2019 (Table 1).

**Table 1.** Information of collected vegetable samples

Sl No	Name of Bazar	Sample collection date	Name of the vegetable samples
1.	Shaheb Bazar	21.01.2019	Bean
2.	Shalbagan Bazar	21.01.2019	Brinjal
3.	Katakali Bazar	21.01.2019	Tomato
4.	Kharkhari Bazar	21.01.2019	Cauliflower
5.	Binodpur Bazar	22.01.2019	Cabbage
6.	Baneshwar Bazar	22.01.2019	

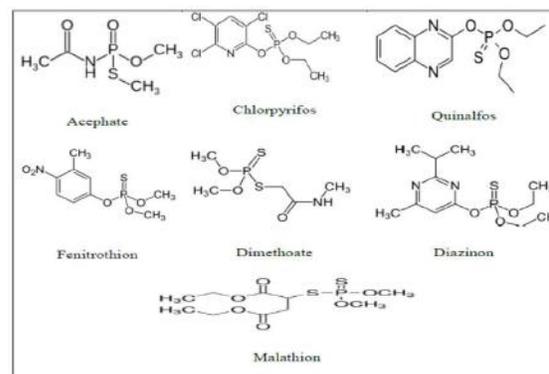
### Sample collection and preparation

A total of 30 winter vegetable samples were collected for this investigation. The weight of every sample was 500 g. Samples were collected according to regulation made in the "Guidelines for the control of pesticide residues in foods" (Anonymous, 1996), which incorporate the EU directive (Anonymous, 1979) and Codex recommendations (Anonymous, 1993) regarding sampling. Collected samples were kept in "Chilled box" and carried to the laboratory within the

quickest time and stored in refrigerator (-20°C) until analysis. The collected samples were carried to the Pesticide Analytical Laboratory of 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°C for further analysis.

### Chemicals and reagent

All pesticide standards (Acephate, Dimethoate, Diazinon, Fenothion, Malathion, Chlorpyrifos and Quinalphos), purity >99.6% were bought from Sigma-Aldrich (St Louis, MO, USA) via Bangladesh Scientific Pvt. Ltd. HPLC grade methanol, acetone, acetonitrile, analytical grade NaCl, anhydrous MgSO<sub>4</sub> and Primary Secondary Amine (PSA) were collected from Bangladesh Scientific Pvt. Ltd. Chemical structures of standard pesticides are shown in Figure 1.



**Fig. 1.** Chemical structures of the pesticides used in the present study

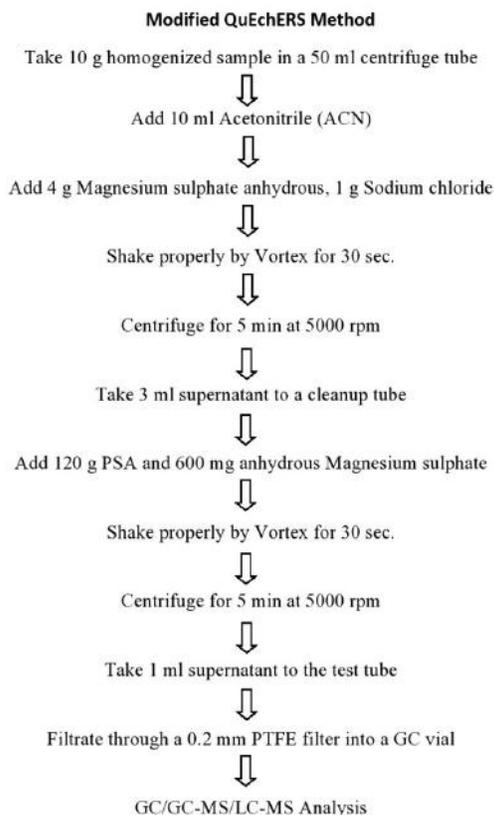
### Preparation of standard solution

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.

### Extraction and clean up

During extraction and clean-up, a modified QuEChERS extraction technique developed by Prophan *et al.* (2015) was followed. The sliced winter vegetable samples were grounded thoroughly using a homestead fruit blender. About 10 gm of homogenized sample was transferred into a 50 ml teflon centrifuged tube followed by adding of 10 ml of acetonitrile. The tube was vortex for 30 second followed by adding

extraction salt (4g of anhydrous MgSO<sub>4</sub> + 1 g of NaCl) and shake it for one minute and followed by subsequently centrifuged at 5000 rpm for 5 minutes. From the upper level, 3 ml supernatant was transferred to a 15 ml centrifuge tube containing 600 mg MgSO<sub>4</sub> anhydrous and 120 mg of PSA. The tube was vortex for 30 second, and centrifuged at 5000 rpm for five minutes. At last, 1 ml of supernatant was filtered using a 0.2 mm PTFE filter and then transferred into a clean HPLC vial. The flowchart of extraction of pesticide is shown in Figure 2.



**Fig. 2.** The flowchart of extraction of pesticide by modified QuEchERS method

**Detection and quantification of pesticide residue**

Residue in sample (mg/kg):

$$= \frac{\text{Conc. obtained in injected volume (mg/kg)} \times \text{Quantity of final volume (L)}}{\text{Amount of sample taken (kg)}}$$

**Results and Discussion**

Pesticide residue from 30 winter samples of bean, brinjal, tomato, cauliflower and cabbage of six different bazars of Rajshahi were analyzed in the laboratory of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh. Pesticide residues were detected in one brinjal and two tomato samples (Table 4).

The pesticide residue from extracts of winter vegetables was 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 1). The instrumental conditions are shown in Tables 2 and 3.

**Table 2.** 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 <sub>2</sub> flow: 1.5 ml/min; stop time: 10 min; make up flow: 30 ml/min; air flow: 145 ml/min

**Table 3.** Condition for column oven temperature for FTD

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

Prior to the injection of the sample extract, standard solutions of different concentrations of each pesticide were prepared and injected with selected instrument parameters. The samples were calibrated (retention time, peak area, etc.) against three to four 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 which represented the concentration of the final volume injected. From this value, the actual amount of pesticide residue present in the sample was determined by using the following formula:

Figure 3 shows the chromatograms of standards of organophosphorus insecticides. Figure 4. Shows the chromatograms of dimethoate obtained from the extract of tomato collected from Shaheb Bazar of Rajshahi City, Figure 5 shows the chromatograms of dimethoate obtained from the extract of brinjal collected from Shalbagan Bazar of Rajshahi city, figure 6 shows the chromatograms of dimethoate obtained from the extract of tomato collected from Kharkhari Bazar of Rajshahi City.

**Table 4.** Analysis of vegetable samples for determining pesticide residues

Collected Location	Vegetable Name	Name of Insecticides						
		Acephate	Dimethoate	Diazinon	Fenotrothion	Malathion	Chlorpyrifos	Quinalphos
		residue (mg/kg)						
Shaheb Bazar	Bean	ND	ND	ND	ND	ND	ND	ND
	Brinjal	ND	ND	ND	ND	ND	ND	ND
	Tomato	ND	<b>0.047</b>	ND	ND	ND	ND	ND
	Cauliflower	ND	ND	ND	ND	ND	ND	ND
	Cabbage	ND	ND	ND	ND	ND	ND	ND
Shalbagan Bazar	Bean	ND	ND	ND	ND	ND	ND	ND
	Brinjal	ND	<b>0.052</b>	ND	ND	ND	ND	ND
	Tomato	ND	ND	ND	ND	ND	ND	ND
	Cauliflower	ND	ND	ND	ND	ND	ND	ND
	Cabbage	ND	ND	ND	ND	ND	ND	ND
Katakali Bazar	Bean	ND	ND	ND	ND	ND	ND	ND
	Brinjal	ND	ND	ND	ND	ND	ND	ND
	Tomato	ND	ND	ND	ND	ND	ND	ND
	Cauliflower	ND	ND	ND	ND	ND	ND	ND
	Cabbage	ND	ND	ND	ND	ND	ND	ND
Kharkhari Bazar	Bean	ND	ND	ND	ND	ND	ND	ND
	Brinjal	ND	ND	ND	ND	ND	ND	ND
	Tomato	ND	<b>0.139</b>	ND	ND	ND	ND	ND
	Cauliflower	ND	ND	ND	ND	ND	ND	ND
	Cabbage	ND	ND	ND	ND	ND	ND	ND
Binodpur Bazar	Bean	ND	ND	ND	ND	ND	ND	ND
	Brinjal	ND	ND	ND	ND	ND	ND	ND
	Tomato	ND	ND	ND	ND	ND	ND	ND
	Cauliflower	ND	ND	ND	ND	ND	ND	ND
	Cabbage	ND	ND	ND	ND	ND	ND	ND
Baneswar Bazar	Bean	ND	ND	ND	ND	ND	ND	ND
	Brinjal	ND	ND	ND	ND	ND	ND	ND
	Tomato	ND	ND	ND	ND	ND	ND	ND
	Cauliflower	ND	ND	ND	ND	ND	ND	ND
	Cabbage	ND	ND	ND	ND	ND	ND	ND

ND= Not detected

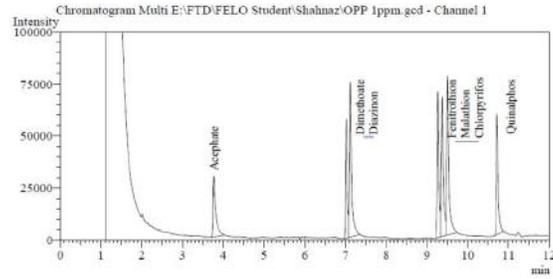


Fig. 3. Chromatograms of standards of organophosphorus insecticides run by GC-FTD

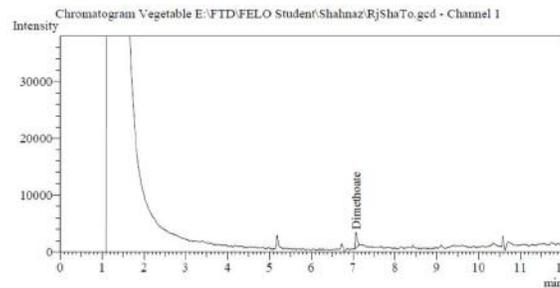


Fig. 4. Chromatograms of dimethoate obtained from the extract of tomato collected from Shaheb Bazaar of Rajshahi City

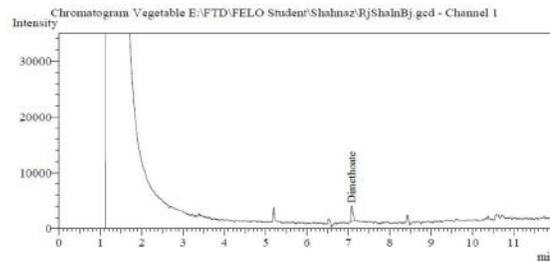


Fig. 5. Chromatograms of dimethoate obtained from the extract of brinjal collected from Shalbagan Bazar of Rajshahi City

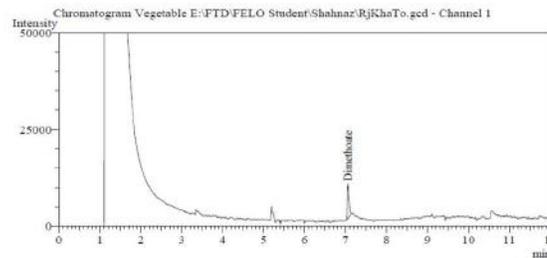


Fig. 6. Chromatograms of dimethoate obtained from the extract of tomato collected from Kharkhari Bazar of Rajshahi City

The result showed that tomatoes collected from Shaheb bazar and Kharkhari bazaar contained 0.047 and 0.139 mg/kg dimethoate, respectively which is higher than maximum residue level (0.01 mg/kg). Among brinjal samples of different markets, only one sample collected from Shalbagan bazaar contained 0.052 mg/kg dimethoate which is also higher than MRL. Other samples of six different markets did not contain any residue of pesticides. However, early-winter vegetable samples of the same six bazars were found to contain more pesticide than winter vegetable samples. During early winter period, 7 samples out of 30 samples (about 23% samples) are found to contain residual pesticide. Bean samples of almost all bazars have been found to contain dimethoate pesticide. Bean samples collected from Shaheb bazar, Rajshahi, contained 0.051 mg/kg diazinon and 0.044 mg/kg chlorpyrifos residues. Cauliflower samples collected from Shaheb bazar, Rajshahi, during early winter also contained 1.423 mg/kg chlorpyrifos residue which is much higher than MRL value (MRL 0.05 mg/kg). Pesticide residues were found to absent totally in three winter vegetable samples (bean, cabbage and cauliflower) because farmers applied low dose of pesticide in this period due to low numbers of pest infestation in winter period. El-Saeid and Selim (2012) found residues of organophosphorus, organochlorine, pyrethroid and carbamate from market vegetables (viz., beans, eggplant, cauliflower, tomato, pepper, carrot, cucumber, squash, potato, onions and okra) which showed above the MRL in 15.89% of the total tested samples. About 26% vegetables (viz., brinjal, hyacinth bean, cabbage, cauliflower and red amaranth) samples from five major vegetables growing regions of Bogra, Jessore, Narsingdi, Jamalpur and Rajshahi of Bangladesh were contaminated with dimethoate, chlorpyrifos and quinalphos residues, of which 24% of the contaminated samples contained residue above MRL (Rahman *et al.*, 2014).

The mean concentration of carbaryl residue 0.10 mg/kg in tomato and 0.09 mg/kg found in brinjal in Al-Qassim region of Saudi Arabia (Osman *et al.*, 2011). Tomato sample collected from Narsingdi sadar wet market contained 8.50 µg/kg Quinalphos residue, which is lower than Maximum Residue Levels (10 µg/kg). There were no pesticide residues in other samples of Narsingdi sadar wet market. Brinjal sample of Mithapukur bazar, Rangpur wet market contained 0.65 µg/kg Quinalphos residue, which is higher than Maximum Residue Levels (10 µg/kg). These results are in accordance with those of Yu *et al.*, 2016 who monitored organophosphorus pesticides (OPs) in leafy vegetables in Changchun, China. Tomato, cucumber, green chili and country bean of Mithapukur bazar were negative to pesticide residues. Bempah *et al.*, 2012 analyzed in laboratory and found 0.007-0.019 mg/kg dimethoate residue in Ghanaian tomatoes and 0.001-0.002 mg/kg in Indian eggplants (Kumari 2008). Osman *et al.*, (2011) recorded the mean concentration of carbaryl residue as 0.10 mg/kg in tomato and 0.09

mg/kg in brinjal in Al-Qassim region of Saudi Arabia. Carbaryl residue was detected in tomato sample as 1.52 mg/kg. Some pesticides are converted to their metabolite and hence, remain below detection limit, though some previous studies have recorded carbamate residues in different vegetables in different region of the world (Latif *et al.*, 2011). Some factors may contribute to the high occurrence of pesticide residues detected in the vegetable samples from the wet markets. Bangladesh normally has an unplanned control mechanism, and rampant pesticide overdosing is practiced in field level to increase crop productivity (Rahman and Alam, 2007). Most of the farmers have lack of perception and knowledge about the nature of chemical pesticides and their effects on health by consuming pesticides residues. Therefore, peoples may consume vegetables and fruits with high level of pesticide residues that ultimately leads to severe health problems (Chowdhury *et al.*, 2012). Human exposure to pesticides through contaminated food leads to a spectrum of adverse health effects that depend on the nature of the pesticide and on the amount and duration of exposure (Gupta, 2014). Symptoms of exposure to organophosphates such as Dimethoate, Ethoprophos, Diazinon, Malathion, Quinalphos, Cypermethrin, parathion, and phosphamidon cause health problems like miosis, urination, diarrhea, diaphoresis, lacrimation, excitation, and salivation (Moore, 2009). Psychological and behavioral effects of organophosphorus pesticide like anxiety, depression, coma and convulsions are acute exposure, while chronic exposure of pesticide residues leads to cognitive and emotional deficits. As organophosphates act directly on the nervous system, severe organophosphate exposure is clinically manifested by marked miosis and loss of the pupillary light reflex, fasciculations, flaccid paralysis, pulmonary rales, respiratory distress, and cyanosis with less than 10% of the normal value of serum cholinesterase (Kumar *et al.*, 2010). Exposure to carbofuran leads to overstimulation of the nervous system due to direct inhibition of acetylcholinesterase. Symptoms of carbofuran overexposure in humans include headache, weakness, abdominal cramping, nausea, blurred vision, convulsions, tremor, and coma (Tenenbaum, 2008). Pesticide residues in food and crops are a direct result of the application of pesticides to crops growing in the field, and to a lesser extent from pesticide residues remaining in the soil (Puri, 2014). Besides, due to lack of awareness and proper knowledge of the farmers, the misuse of pesticides has become a serious problem in agricultural production (Abhilash and Singh, 2009). Hence, immediate interventions are needed to stop any malpractice during production and marketing of vegetable. Consumption of good quality, nutritious and safe vegetables can only be assured if quality and safety can be ensured at the production and postproduction stages (Ferdousi and Rahman, 2016). The use of toxic pesticides is increasing alarmingly in Bangladesh and found that pesticides use increased by 328 percent during the last 10 years, posing a serious hazards on human health due to its long-term residual

effect (BRRI, 2010). According to Chowdhury *et al.*, 2012, this problem becomes worse when farmers apply indiscriminate dose of pesticides in the vegetable fields due to illiteracy and low levels of awareness of the hazardous effect of pesticides to human health.

### Conclusion

From pesticide residue analysis of five winter vegetables, it can be concluded that farmers are applying low dose of pesticides in the field during winter season compare to early winter period. In winter vegetable samples, pesticide residue was detected only in 3 samples out of 30 winter vegetable samples. Only organophosphate pesticide dimethoate was identified in these three samples. The residue concentration was higher than the MRL (0.01 mg/kg). However, lack of awareness and proper knowledge of farmers may create misuse of pesticides which can cause serious problem in vegetable production. This study revealed that pesticide residue in brinjal and tomato samples was higher than Maximum Residue Levels. Long term accumulation of pesticides residues in human body via dietary intake of vegetable and other food commodities is an alarming problem. However, the impact of pesticide residues can be minimized by preventive measures such as rational use of pesticides, washing and proper processing of vegetables, practicing organic farming, use of natural pesticides and bio-pesticides, and strict implementation and amendment of pesticide related laws. The adoption of effective legislation for properly regulating pesticide use and increasing awareness and technical know-how in the farming community should be incorporated. To minimize the pesticide residue level, farmers are suggested to wash the vegetables after harvest from the field, and consumers are advised to wash the vegetables several times with water before cooking.

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