



## Phytochemical screenings, physicochemical properties and effects of pesticides on vegetables

Mohammad Shoeb\*, Sharmin Sultana, Nasrin Jahan, Nilufar Nahar and Waziha Farha

Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh

\*For correspondence: shoeb71@yahoo.com

**Abstract:** Vegetables contain minerals, vitamins, and other beneficiary constituents for a balanced diet. The nutritional value of vegetables may be affected by the application of pesticides which are indiscriminately used in Bangladesh to increase crop production. To study the effects of pesticides on the nutritional values of vegetables, bitter gourd (*Momordica charantia* L.), tomato (*Solanum lycopersicum* L.), chili (*Capsicum annum* L.), eggplant (*Solanum melongena*), pumpkin (*Cucurbita pepo*) and bottle gourd (*Lagearia sicreraria*) were collected from vegetable growing field of Nurundi, Jamalpur, Bangladesh. Two insecticides, cypermethrin, and emamectin benzoate, and one fungicide mancozeb were sprayed in the vegetable field. The water content of pesticides treated and untreated samples were very similar within the range of 85-94%, whereas ash content of treated samples decreased in the case of cypermethrin and emamectin benzoate but increased in the case of mancozeb. The vegetable samples were screened for minor and major phytochemicals. Phytochemical screening revealed the presence of tannins, glycosides, saponins, proteins, and carbohydrates in untreated and pesticide-treated vegetables except for glycosides which were missing in treated vegetables. Percent carbohydrates in the pesticide-treated and untreated samples were not significantly changed. The level of Ca, Cu, Fe, Zn, Mg, Mn and K were in the range of 0.95-4.1, 0.009-0.79, 0.05-1.14, 0.01-0.13, 0.10-3.40, 0.003-0.15 and 15.67-26.49 mg/g, respectively. The results obtained are an affirmation of the use of these vegetables (treated with pesticides) and thus these vegetables may serve as sources of safe food for consumption.

শাকসবজিতে সুস্বাদু ডায়েটের জন্য খনিজ, ভিটামিন এবং অন্যান্য উপকারী উপাদান রয়েছে। ফসলের উৎপাদন বাড়াতে বাংলাদেশে নির্বিচারে ব্যবহৃত কতিপয় কীটনাশক প্রয়োগের ফলে শাকসবজির পুষ্টিগত মান প্রভাবিত হতে পারে। শাকসবজির পুষ্টিমানে কীটনাশকের প্রভাব অধ্যয়ন করতে, জামালপুরের নুরুন্দীর সবজিক্ষেত থেকে করলা (*Momordica charantia* L.), টমেটো (*Solanum lycopersicum* L.), মরিচ (*Capsicum annum* L.), বেগুন (*Solanum melongena*), কুমড়া (*Cucurbita pepo*) এবং লাউ (*Lagearia sicreraria*) সংগ্রহ করা হয়েছিল। উদ্ভিদের জমিতে দুটি কীটনাশক, সাইপারমেথ্রিন এবং ইমামেক্টিন বেনজোয়েট এবং একটি ছত্রাকনাশক ম্যানকোজেব স্প্রে করা হয়েছিল। কীটনাশকযুক্ত এবং কীটনাশকবিহীন নমুনাগুলির water content ৮৫-৯৪% এর মধ্যেই বিদ্যমান, যেখানে কীটনাশকযুক্ত নমুনাগুলির ash



content সাইপারমেথ্রিন এবং ইমামেবিক্টিন বেঞ্জয়েটের ক্ষেত্রে হ্রাস পেয়েছে তবে মানকোজেবের ক্ষেত্রে বৃদ্ধি পেয়েছে। সজির নমুনাগুলি মূখ্য এবং গৌণ ফাইটোকেমিক্যালগুলির জন্য স্ক্রিনিং করা হয়েছিল। ফাইটোকেমিক্যাল স্ক্রিনিংয়ের মাধ্যমে কীটনাশকযুক্ত সজিতে শুধুই গ্লাইকোসাইড ব্যতিত কীটনাশকযুক্ত এবং কীটনাশকবিহীন উভয় প্রকার সজিতেই ট্যানিন, গ্লাইকোসাইডস, স্যাপোনিন, প্রোটিন এবং কার্বোহাইড্রেটের উপস্থিতি প্রমাণিত হয়েছে। সকল নমুনায় কার্বোহাইড্রেটের শতাংশে কোনো উল্লেখযোগ্য পরিবর্তন লক্ষণীয় হয়নি। Ca, Cu, Fe, Zn, Mg, Mn, এবং K এর লেভেল যথাক্রমে 0.95-4.1, 0.009-0.79, 0.05-1.14, 0.01-0.13, 0.10-3.40, 0.003-0.15 এবং 15.67-26.49 mg/g। প্রাপ্ত ফলাফল যেমন কীটনাশকযুক্ত এই সজিগুলো ব্যবহারের নিশ্চয়তা দেয় এবং তেমনি এই সজিগুলি নিরাপদ খাদ্যের উৎস হিসেবেও বিবেচ্য হতে পারে।

**Keywords:** Ash content, pesticides, phytochemical screening, vegetables, water content,

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## INTRODUCTION

Vegetables constitute essential components of the diet such as protein, vitamins, iron, calcium, and other nutrients which are usually necessary for healthy living [1,2]. Increased awareness of the usefulness of vegetable inclusions in human food has enhanced their consumption as a part of the daily diet [3]. The nutritional potential of the vegetables is usually attributed to the high content of vital vitamins as well as micronutrients [4]. Vegetables are highly recommended because of the relatively high nutritional value and their consumption gives diversity to daily food intake, adding flavor and taste to the diet [5,6].

The vegetables are mainly low-fat, high in dietary fibers; enriched with minerals and vitamins, possessing a very high nutritional density [7]. Dietary fiber has been recognized as a good source for preventing and treating constipation in humans. Furthermore, it is an important component to reduce the risk of certain diseases, including cancer, cardiovascular disease, and diabetes [8]. Today, vegetables are available in Bangladesh throughout the year, but especially are plentiful during winter and spring. A wide range of pesticides is used to protect vegetables from heavy infestation by pests around the world including Bangladesh to ensure high yield to meet the increasing food demand, which is becoming a consumer's food safety concern [7, 9].

The global economy has been expanding significantly in the last half-century both industrial and agricultural sectors, resulting in high yield following usage of agrochemicals that enter the ecosystem and finally contributed toxins to human beings [10]. With all of these concern, this work was designed to assess the physicochemical properties, phytochemical screening and effects of pesticides on the pumpkin (*Cucurbita pepo*), eggplant (*Solanum melongena*), bottle gourd (*Lagenaria sicreraria*), bitter gourd (*Momordica charantia* L.), tomato (*Solanum lycopersicum* L.) and chili (*Capsicum annum* L.).



## MATERIAL AND METHODS

*Instrumentation:* An analytical balance (Adventurer TM, OHAUS), a vortex machine (Kebo Lab Reax-2000), a digestion unit (Velp Scientifica DKL heating digestion, Velp Scientifica UDK 129) and a distillation unit were used for experimental purpose. For drying of samples, an oven (Salvis, Inventory No, G-1020) was used. The metal analysis was carried out by an Atomic Absorption Spectrometer (Perkin Elmer, AAnalyst 200). An oven (Binder, Germany) was also used to keep contact temperature at 105 oC. A furnace (Carbolite, England) was also used to determine ash content. Shimadzu UV 160A spectrophotometer was used to determine the absorption maxima ( $\lambda_{max}$ ) of different solutions. The different concentrations of the samples were taken in a (1 cm x 1 cm) quartz cell and recorded the  $\lambda_{max}$  of different fractions one after another.

*Materials & Chemicals:* All the solvents, reagents, and chemicals used in the experimental works were of analytical or reagent grade and were procured from Sigma, E. Merck, or BDH. Concentrated nitric acid, ferric chloride, copper sulfate, sodium hydroxide were analytical grade.

**Cypermethrin** [(RS)- $\alpha$ -cyano-3-phenoxybenzyl(1RS, 3RS; 1RS, 3RS)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate], Chemical Abstract Name: cyano-(3-phenoxyphenyl)methyl- 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate (CAS number: 52315-07-8) is a digestive and contact insecticide effective against a wide range of pests, particularly leaf- and fruit eating Lepidoptera and Coleoptera in cotton, fruit, vegetables, vines, tobacco and other crops. Cypermethrin is widely used by farmers to control insect pests of vegetables [7, 11].

**Emamectin** is the 4''-deoxy-4''-methyl amino derivative of abamectin, a 16-membered macrocyclic lactone produced by the fermentation of the soil actinomycete *Streptomyces avermitilis* [12, 13]. It is generally prepared as the salt with benzoic acid, emamectin benzoate, which is a white or faintly yellow powder [14]. Emamectin is widely used in the US and Canada as an insecticide because of its chloride channel activation properties [15].

**Mancozeb** is a dithiocarbamate non-systemic agricultural fungicide with a multi-site, protective action on contact. It is a combination of two other dithiocarbamates: maneb and zineb [16]. The mixture controls many fungal diseases in a wide range of field crops, fruits, nuts, vegetables, and ornamentals. It is marketed as Dithane, Manzeb, Nemispot, and Manzane.

*Sample Collection:* All vegetables *i.e.*, pumpkin, eggplant, bottle gourd, bitter gourd, tomato, and chili were collected from Nurundi, one of the largest vegetable growing areas of Jamalpur district on 4th February 2016. Samples collected from the field before the application of pesticides and were termed as untreated (control). Later, three pesticides namely cypermethrin, avermectin benzoate, and mancozeb were applied to these vegetable samples and collected 2 hours after application and termed as pesticide-treated samples. Except for bottle gourd, all three pesticides were applied individually for each sample. In bottle gourd combination of three pesticides was used. To protect the samples from microbial growth, the samples were then preserved in the refrigerator at -20 °C for further analysis.

*Drying of samples:* All the samples were blended first. Then they were dried at 60 oC in an electric oven for about 24 hours. After that, the samples were stored in a desiccator. A few of the sample was used for protein analysis and the rest of them for analysis of element.



*Water Extraction:* At first all the samples were chopped and about 40 g of each sample was taken in a 250 mL beaker. Then 100 mL of deionized water was added and heated until the volume of the water was reduced to half.

*Water content:* Fresh vegetable samples (2 g) were placed in the crucible and heated at 105 °C until a constant weight was obtained. The water content of each sample was calculated as a loss in weight from the original sample and expressed as a percentage of water content.

*Ash Content:* The total ash content of substance is the percentage of inorganic residue remaining after the organic matter has been ignited. Pulverized vegetable sample (2 g) was placed in a crucible and ignited in a muffle furnace at 750 °C for 4 hours until a constant weight was obtained.

*Test for Tannins and Phenols:* Brown precipitate was formed when a few drops of 1% FeCl<sub>3</sub> solution was added to the aqueous extract of the sample.

*Test for Alkaloids:* A few drops of dragendorff reagent were added to the 2 mL of aqueous extract. The appearance of red color indicated the presence of alkaloids.

*Test for Proteins:* 2 mL aqueous extract was taken in a test tube. Then 5-6 drops of 5% NaOH and 5-7 drops of 1% Cu(SO<sub>4</sub>)<sub>2</sub> was added. The appearance of the violet color indicated the presence of proteins.

*Test for carbohydrate:* 500 µL aqueous extract was taken in a test tube. 50 µL 80% phenol was added to it and mixed thoroughly by a vortex for 30 sec. and 3 mL of 98% H<sub>2</sub>SO<sub>4</sub> was added. The development of the reddish-brown color indicated the presence of carbohydrates.

*Test for Saponins:* 1 g of sample was taken in a test tube. Then 10 mL of deionized water was added. This was heated to boil. Frothing persistence indicated the presence of saponins.

*Test for Glycosides:* 2 mL of aqueous extract was taken in a test tube. 1 mL of glacial acetic acid added. Then a few drops of FeCl<sub>3</sub> were added. After that, a few drops of H<sub>2</sub>SO<sub>4</sub> was added. Green-blue precipitate indicated the presence of glycosides.

*Total Carbohydrate Determination:* The total carbohydrate of the vegetable samples was accomplished by the modified Molisch test. It is a well-known method for the determination of carbohydrates in the food product. In this method, 80% aqueous phenol and concentrated sulfuric acid (98%) were used.

*Protein Determination:* The nitrogen was determined by the Macro Kjeldahl method [17].

*Sugar determination:* Analytical grade glucose (10 mg) was weighed and taken in a 10 mL volumetric flask. After preparing the standard solution, various dilutions were made by pipetting a known volume of the standard solution. For each run 500 µL standard solution was taken and 50 µL 80% phenol was added and vortex for 30 sec. Then 3 mL 98% H<sub>2</sub>SO<sub>4</sub> was added. The absorbance of the reddish-brown colored solution was taken in the range of 400 nm to 800 nm for the determination of the  $\lambda_{max}$ . Approximately 1.5 g of each vegetable sample was taken in a test tube and 10 mL water was added to it. Then it was kept at room temperature for 24 hours. Then the extract was filtered. This aqueous extract was diluted. 20 µL extract was taken in a total of 1000 µL volume of water. Then, for each run 500 µ solution was used.

The absorbance of the solution was taken at determined  $\lambda_{max}$  (488 nm).



*Digestion:* About 0.5 g of each sample was taken in a digestion tube. Then 10 mL of concentrated nitric acid (65%) was added. After that, they were predigested for half an hour. Then they were digested at 200 oC in a microwave digestion system. After digestion, the solution was diluted with 25 mL deionized water. These samples were kept in r

Table 1: List of analyzed samples and pesticides

English name	Scientific name	Pesticide name	Trade name	Dosage rate/ ha
Bitter gourd	Momordica charantia	Profenofos (40%) + Cypermethrin (2.5%) (Insecticide)	Shobicron 425 EC	2 mL/L water
Chili	Capsicum annum	Avermectin benzoate (Insecticide)	Proclaim	1 g/L of water
Tomato	Solanum lycopersicum	Mancozeb (64%) + Metalaxyl (8%) (Fungicide)	Ridomil Gold MZ 68 WG	2 g/L of water
Eggplant	Solanum melongina	Cypermethrin	Sobicron	2 mL/L water
Pumpkin	Cucurbita pepo	Emamectin benzoate	Proclaim	1 g/L water
Bottle gourd	Lagearia sicreraria	Mancozeb	Ridomil	2 g/L water

## RESULTS AND DISCUSSION

*Water content and ash content:* Water contents of fresh vegetable samples were determined by heating in an oven at 105 oC until a constant weight was obtained. The value of water and ash content in treated and untreated vegetable samples are given in Table 2. Water contents of the control eggplant, pumpkin, and bottle gourd samples were 93%, 94%, and 94%, respectively. When they were treated with emamectin, cypermethrin, and mancozeb, eggplant had the values 92%, 91%, and 92%, respectively. Emamectin benzoate, cypermethrin, and mancozeb treated pumpkin gave water contents 93%, 91%, and 93%, respectively. Mixed bottle gourd had 93% water content. The value of water contents in treated and untreated vegetable samples is also shown in (Figure 1) which indicated that the water level is the highest in the control bottle gourd. Mixed bottle gourd also occupied higher value. Among all three vegetable samples, bottle gourd has a higher value than pumpkin and eggplant has a lower value of water. Water content values in treated eggplant are similar to untreated. The same trained are followed by pumpkin and bottle gourd. Figure 1 also shows untreated tomato had the highest water content (93%) which was significantly higher than the water content of untreated bitter



gourd (85%) and chili (90%). The water content value in treated and untreated samples was almost the same and no significant change was observed. The high water content of these vegetables (more than 80%) suggests that those vegetables should not be stored for a longer period without proper storage system as being susceptible to infection by a micro-organism (18).

*Mean Ash contents (%) in vegetable samples:* The ash content was determined by weighing 5 g of fresh vegetable sample *i.e.*, bitter gourd, chili, tomato into a porcelain crucible which was incinerated at 750 °C in an ash muffle furnace until ash was obtained.

Table 2: Water and ash content of treated and untreated vegetable samples

Samples		Water content (%)	Ash content (%)	
Untreated (Control)	Eggplant	93	0.47	
	Pumpkin	94	0.27	
	Bottle gourd	94	0.67	
	Bitter gourd	85	4.66	
	Chili	90	0.13	
	Tomato	93	0.90	
Treated	Emamectin benzoate	Eggplant	92	0.43
		Pumpkin	93	0.26
		Bitter gourd	85	4.00
		Chili	89	0.09
		Tomato	92	0.98
	Cypermethrin	Eggplant	91	0.32
		Pumpkin	93	0.26
		Bitter gourd	84	4.36
		Chili	89	0.10
		Tomato	94	1.00
	Mancozeb	Eggplant	92	0.57
		Pumpkin	94	0.25
		Bitter gourd	86	4.97
		Chili	89	0.21
		Tomato	92	1.10
Mixed three pesticides	Bottle gourd	94	0.87	

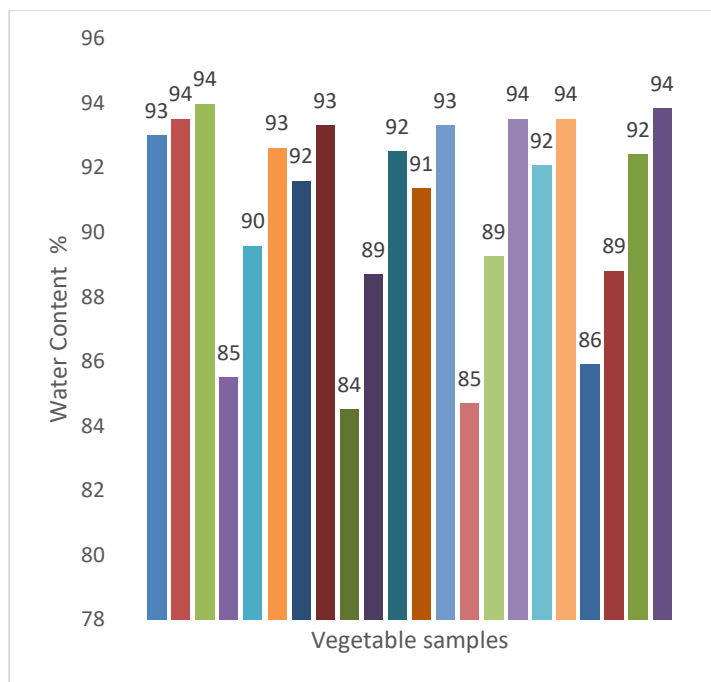


Figure 1: Water contents in treated and untreated vegetable samples

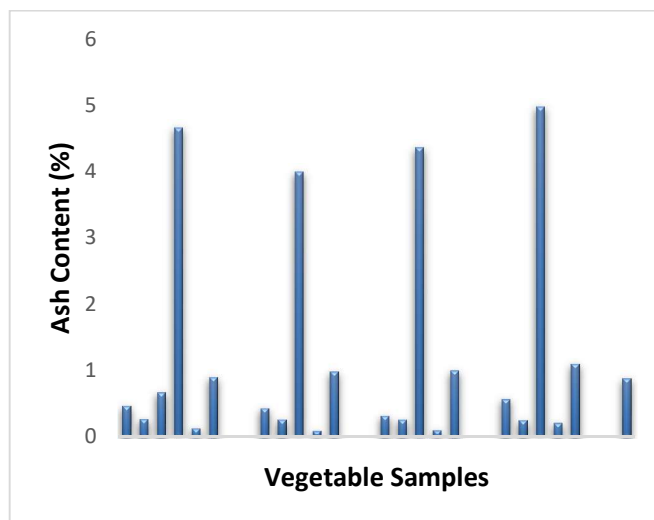


Figure 2: The ash content of vegetable samples

Ash content is also considered as the measure of minerals. A higher amount of ash means a higher value of the mineral. Bitter melon had the highest and Chili had the lowest value of ash content among all analyzed vegetables (Table 2). All these results indicated that ash content did not change significantly after the application of pesticides (Figure 2).



*Phytochemical screening:* Phytochemical screening for the presence of tannins, phenols, alkaloids, proteins, glycosides, carbohydrates, saponins in various extracts of the vegetable samples were performed using standard procedure [19] and results are given in Table 3. It was found that tannins, phenols, glycosides, saponins, proteins, and carbohydrates were present in all the untreated vegetable samples. All the treated and untreated samples gave positive results of tannins & phenols, proteins, carbohydrates, and saponins. In the case of glycosides, only the control sample gave a positive test. Glycosides gave a negative test for all pesticide-treated vegetable samples, however, only cypermethrin-treated pumpkin showed a positive result for glycosides. Other than pumpkin, Glycoside may undergo hydrolysis when cypermethrin was treated on vegetables. The presence of saponins, tannins, phenolic compounds, glycosides, etc. is an indication of medicinal potentials of vegetables. The absence of glycosides in treated samples means that they may be hydrolyzed. The presence of these phytochemical properties showed that they have medicinal properties such as Glycosides are medicinally important constituents due to their action on the heart and tannins play an important role in preventing bacteria from adhering to the walls of the urinary tract, thereby preventing urinary tract infection (UTI). Hence, the present study reveals that these vegetables possess medicinal properties as well.

*Analysis of carbohydrates:* The amount of total percent of carbohydrate in the vegetables samples were determined colorimetrically using modified Molisch test. Absorbance of standard glucose solution at different concentrations was measured by UV-Visible spectrometer at 488 and 489 nm (Table 4).

*Calibration curve for standard glucose solution by UV-Vis spectrometer:* The calibration curve of standard solution was made by plotting absorbance vs. concentration. By using the formula  $y = mx + c$  the concentration was calculated.

Here,

y = Absorbance

x = Concentration

m = Slope of the calibration curve

From the calibration curve, the value of m is 4.438 and c is -0.027. The plot is linear with the best fit obtained with an acceptable regression coefficient ( $>0.983$ ). Absorbance for six (pesticide-treated & pesticide-untreated) vegetable samples by UV-Visible Spectrophotometer was taken to determine the amount of carbohydrate present in the investigated samples from the calibration curve.

*The UV-Visible spectrum of vegetable samples:* The maximum wavelength was determined by taking the sample run in the UV-Visible range. The absorbance of vegetable samples was detected at 488 nm wavelength. The UV-Visible spectrum of eggplant and pumpkin samples are represented in Figure 4. The UV-Visible spectrum showed in Figure 4 describes the peak at 488 nm which is the characteristic peak of glucose. Maximum absorbance was observed for cypermethrin and minimum for mancozeb. Emamectin treated pumpkin showed maximum absorbance and control showed minimum value. Here, Figure 5 shows a characteristic peak for glucose where bitter gourd shows the maximum absorbance when treated with mancozeb and minimum for untreated (control) bitter gourd. Here, Figure 6 shows a characteristic peak for glucose and the highest peak stands for control chili and the minimum peak is for proclaiming treated chili, whereas, it displayed maximum absorbance for tomato when treated with cypermethrin and minimum absorbance for mancozeb treated tomato.



Table 3. Phytochemical screening of pesticide treated and untreated vegetables

Samples		Tannin	Protein	Glycoside	Carbohydrate	Saponin
Eggplant	Untreated	+	+	+	+	+
	Emamectin benzoate	+	+	-	+	+
	Cypermethrin	+	+	-	+	+
	Mancozeb	+	+	-	+	+
Pumpkin	Untreated	+	+	+	+	+
	Emamectin benzoate	+	+	-	+	+
	Cypermethrin	+	+	+	+	+
	Mancozeb	+	+	-	+	+
Bottle gourd	Untreated	+	+	+	+	+
	Mixed Pesticide	+	+	-	+	+
Bitter gourd	Untreated	+	+	+	+	+
	Emamectin benzoate	+	+	-	+	+
	Cypermethrin	+	+	-	+	+
	Mancozeb	+	+	-	+	+
Chili	Untreated	+	+	+	+	+
	Emamectin benzoate	+	+	-	+	+
	Cypermethrin	+	+	-	+	+
	Mancozeb	+	+	-	+	+
Tomato	Untreated	+	+	+	+	+
	Emamectin benzoate	+	+	-	+	+
	Cypermethrin	+	+	-	+	+
	Mancozeb	+	+	-	+	+



Table 4: The value of absorbance for different concentration of glucose

Concentration (mg/mL)	$\lambda_{max}$ (nm)	Absorbance
0.025	488	0.038
0.050		0.207
0.080		0.300
0.100		0.460
0.120		0.500
0.150		0.630
0.015	489	0.057
0.030		0.140
0.050		0.200
0.060		0.245
0.080		0.322
0.010		0.390

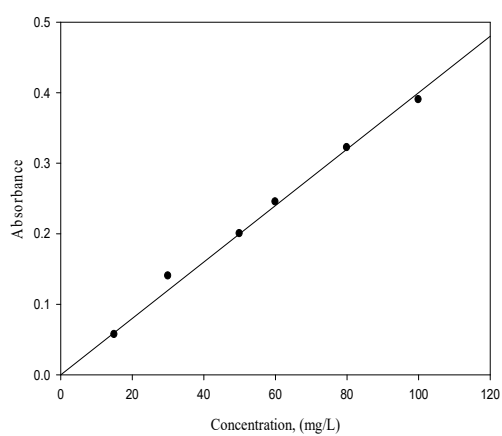
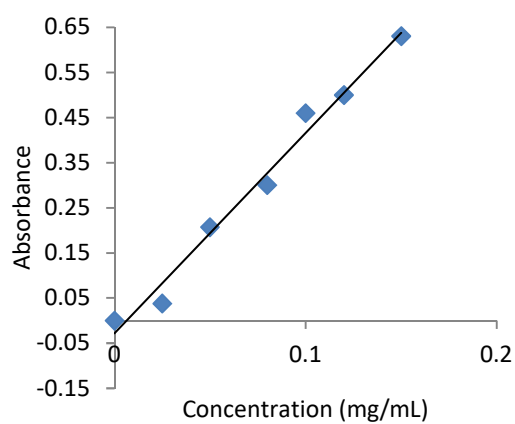


Figure 3: Calibration curve of absorbance (a) 488 nm; (b) 489 nm vs. concentration for standard glucose.

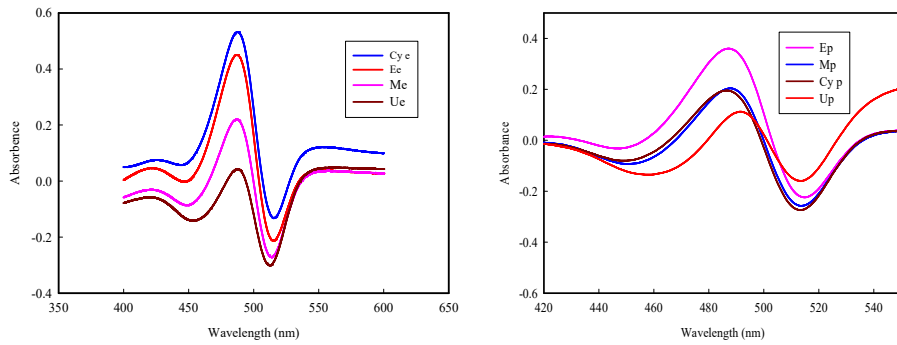


Figure 4: UV-Visible spectrum of pesticide treated and untreated eggplant and pumpkin

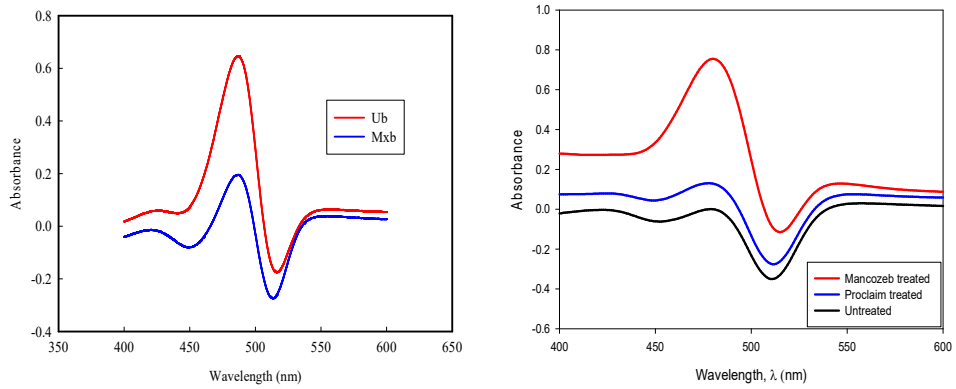


Figure 5: UV-Visible spectrum of pesticide-treated and untreated bottle gourd and Bitter gourd

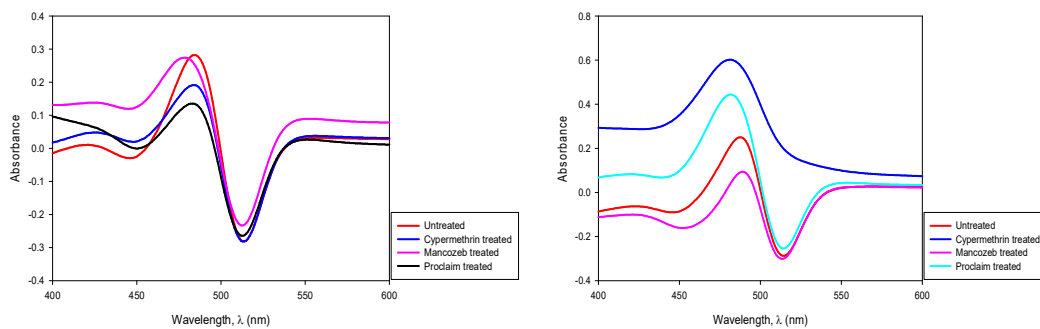


Figure 6: UV-Visible spectrum of pesticide treated and untreated chili & Tomato samples



**Table 5: Absorbance for pesticide-treated & control vegetable samples by UV-Vis Spectrophotometer (488 and 489 nm)**

Samples		$\lambda_{max}$	Absorbance	
Control	Eggplant	488	0.220	
	Pumpkin		0.112	
	Bottle gourd		0.305	
	Bitter gourd	489	0.0020	
	Chili		0.2834	
	Tomato		0.2500	
treated	Emamectin benzoate	488	Eggplant	0.450
			Pumpkin	0.359
		489	Bitter gourd	0.1286
			Chili	0.1380
			Tomato	0.4427
	Cypermethrin	488	Eggplant	0.532
			Pumpkin	0.125
		489	Bitter gourd	Not done
			Chili	0.1929
			Tomato	0.6035
	Mancozeb	488	Eggplant	0.042
			Pumpkin	0.204
		489	Bitter gourd	0.7500
			Chili	0.2738
			Tomato	0.0964
Mixed three pesticides	Bottle gourd	488	0.195	



*Concentration of carbohydrates:* The amount of carbohydrate in vegetable samples were determined from the standard calibration curve. The percentage of carbohydrate in vegetable samples are given in Table 6.

**Table 6: The carbohydrate found for sample by UV-Vis Spectrophotometer**

Samples		Carbohydrate (%)	
Untreated	Eggplant	0.83	
	Pumpkin	0.45	
	Bottle gourd	1.10	
	Bitter gourd	2.90	
	Chili	4.00	
	Tomato	2.65	
Treated	Emamectin benzoate	Eggplant	1.58 #
		Pumpkin	0.28 *
		Bitter gourd	1.95#
		Chili	1.10#
		Tomato	3.24*
	Cypermethrin	Eggplant	1.86 #
		Pumpkin	0.50 #
		Bitter gourd	Not done
		Chili	1.30#
		Tomato	0.08#
	Mancozeb	Eggplant	0.96 #
		Pumpkin	0.76 #
		Bitter gourd	2.50#
		Chili	2.12#
		Tomato	3.10*
Mixed three pesticides	Bottle gourd	0.73*	

The symbol \* for increased and # for decreased value than corresponding untreated vegetable samples



Control eggplant, pumpkin and bottle gourd showed carbohydrate 0.83%, 0.45%, and 1.10%, respectively. Except for emamectin treated pumpkin and mixed bottle gourd all treated samples showed more carbohydrate than corresponding untreated. Besides, mixed bottle gourd showed a lower value of carbohydrate than control. These low carbohydrate levels make them useful to those who are diabetic and individuals who are watching their weight.

*Determination of proteins:* Proteins were determined from the dried vegetable sample by micro Kjeldhal method for 0.5 g dry weight of the sample. The percentage of protein of these samples is given in Table 7.

Table 7: The percentage of protein in vegetable samples

Samples		Protein (%)	
Untreated	Eggplant	13	
	Pumpkin	9	
	Bottle gourd	8	
treated	Emamectin benzoate	Eggplant	10*
		Pumpkin	13#
	Cypermethrin	Eggplant	11 *
		Pumpkin	7 *
	Mancozeb	Eggplant	13*
		Pumpkin	8 *
Mixed three pesticides	Bottle gourd	12#	

The symbol \* for increased and # for decreased value than corresponding untreated vegetable samples

Untreated eggplant, pumpkin, and bottle gourd had protein contents 13%, 9%, and 8%, respectively. The level of protein in cypermethrin and mancozeb treated samples is lower than the control sample. Emamectin treated pumpkin showed a higher amount of protein (13%) than untreated (9%). In the case of bottle gourd with mixed pesticide, the protein value was higher (12%) than control (8%).

The higher level of protein means the presence of a higher amount of nitrogen. The presence of protein is correlated with nitrogen. Their protein contents could be used to supplement the proteins



from staple food. Results (Table 7-8) showed that the amounts of protein were increased in the corresponding treated samples whose carbohydrates had decreased but this changes were not significant. The protein contents in the vegetable samples were determined by the Macro Kjeldahl method. The percentage of protein of the treated and untreated vegetable samples (bitter gourd, chili and tomato) is given in the Table 8.

Table 8: Mean protein content (%) in vegetable samples

Name of vegetables	Protein (%)			
	Untreated	Pesticide treated vegetables		
		Emarmectin benzoate	Mancozeb	Cypermethrin
Bitter gourd	22	21	16	Not done
Chili	13	12	15	10
Tomato	12	10	11	11
Eggplant	13	10	11	13
Pumpkin	9	13	7	8
Bottle gourd	8	Mixed of three pesticides		
		12		

From Table 8 we can see that the highest amount (22%) was in untreated bitter gourd and the lowest amount (8%) in Bottle gourd. The amount of protein in vegetables was decreased while treated with pesticides, except for pumpkin. However, the protein was found to increase in chili & pumpkin, when treated with mancozeb and emamectin benzoate, respectively. The reason for these changes might be due to changes in minor constituents of vegetable samples.

*Elemental analysis:* Mineral such as potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), cobalt (Co), lead (Pb) and nickel (Ni) were determined by atomic absorption spectroscopy for all treated and untreated vegetables. The number of metals were determined from the dried sample. Limit of detection (LOD) of all metals are given in Table 9 (a, b).

**CONCLUSION**

The consequences of the overuse of insecticides lead to ecological imbalance and environmental pollution. Farmers are exposed to pesticides by different routes of exposure such as inhalation, ingestion and dermal contact. Acute and chronic health effects are reported due to exposure of pesticides.

**DECLARATION OF CONFLICT OF INTEREST**

We have no conflict of interest to declare

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Table 9(a): Elemental analysis of pesticide treated & untreated vegetables (Bitter gourd, Chili & Tomato).

Elements	Untreated			Emamectin benzoate			Cypermethrin			Mancozeb		
	Bitter gourd	Chili	Tomato	Bitter gourd	Chili	Tomato	Bitter gourd	Chili	Tomato	Bitter gourd	Chili	Tomato
Zn	0.05	0.022	0.03	0.04	0.01	0.03	0.04	0.03	0.03	0.13	0.04	0.03
K	26.19	23.29	16.02	30.54	17.29	18.89	26.49	15.67	16.49	25.19	21.14	19.27
Mg	3.25	0.83	0.15	3.4	0.10	0.40	3.40	1.23	0.65	3.0	0.98	0.20
Mn	0.02	0.008	0.005	0.01	0.008	0.03	0.03	0.009	0.003	0.15	0.01	0.03
Ca	3.23	1.05	1.97	3.35	0.95	2.38	2.85	1.35	2.13	4.1	2.67	1.78
Cu	0.009	0.008	0.01	0.55	0.29	0.03	0.01	0.02	0.01	0.79	0.18	0.004
Fe	0.085	0.05	0.05	1.21	0.75	0.09	0.06	0.91	0.06	1.38	0.05	0.04
Co	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Pb	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Ni	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL



Table 9(b): Elemental analysis of pesticide treated & untreated vegetables (Eggplant, Pumpkin & Bottle gourd).

Elements mg/g	Untreated			Emamectin Benzoate		Cypermethrin		Mancozeb		Mixed pesticide
	Eggplant	Pumpkin	Bottle gourd	Eggplant	Pumpkin	Eggplant	Pumpkin	Eggplant	Pumpkin	Bottle gourd
Zn	0.03	0.02	0.30	0.02	0.01	0.02	0.03	0.03	0.03	0.34
Mg	0.33	0.25	0.02	0.40	1.50	1.03	6.20	0.33	0.10	2.75
Mn	0.01	0.01	0.01	0.02	0.02	0.02	0.01	0.02	0.01	0.02
K	19.72	11.68	41.29	12.19	20.74	10.27	11.34	11.34	10.92	12.30
Fe	0.05	0.05	0.04	1.30	2.53	2.18	1.83	0.04	0.04	0.62
Cu	0.01	0.01	0.01	0.97	2.50	1.45	1.58	0.01	0.01	0.79
Ca	2.03	3.13	2.68	3.56	6.17	5.43	6.10	2.28	2.30	8.70
Pb	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Ni	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Co	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

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