Cypermethrin pesticide contamination in some vegetables available in Kathmandu market

Gita Pandey [*] and Vinay Kumar Jha [*]

[*]Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Abstract: This study was conducted in order to assess the pesticide (Cypermethrin) residue in some vegetables and examine the related human health risk. The purpose of this study was to investigate pesticide residue in vegetables from the Kathmandu market of Nepal. Total of 16 samples were collected from March 2018 to Nov 2019. The samples were analyzed to determine the concentration of pesticide residue. Cypermethrin is the most widely used pyrethroid pesticide to control many pests and is also most frequently detected pyrethroid in vegetables and fruits in Nepal. It interacts with the sodium channels in nerve cells through which sodium enters the cell in order to transmit a nerve signal. Symptoms of poisoning include abnormal facial sensations, dizziness, headache, nausea, anorexia and fatigue, vomiting and increased stomach secretion. Analysis was performed by gas-chromatography equipped with mass-spectrometry. In 13 of the tested samples, no residues were found while 3 samples contained pesticide residue 47.091, 118.334 and 306.103 ppb, respectively. Sample with residue is above maximum residue limit that is 200 ppb may pose health hazards to the consumers. It may be due to lack of awareness of farmer about the application dose, method of application and withholding period.

Keywords*:* Awareness; Cypermethrin; GC-Mass spectroscopy; Health hazard; Pyrethroid Pesticide residue.

Introduction

Nepal is agricultural based country and its economy highly depends on agriculture. Twenty nine percent area of Nepal is classified as agricultural land while only 1.5 percent of total land is permanent cropland. Rice, maize, millet, wheat barley and buckwheat are the major crops grown in Nepal[1].

The rapid population growth has resulted increasing demand for food globally and in order to fulfill it the agricultural productivity needs to be increased. It has been found that many countries have been extensively using chemical pesticides to increase the agricultural efficiency to fulfill the growing demand. About 900 chemical pesticides are used worldwide, legally and illegally, in various food products and for the management of crops and soil.

The cauliflower, cabbage, bean, tomato, potato are main vegetable crops in Nepal. In spite of being a prospective crop, high rate of insect pest infestation is responsible to its low yield and poor quality.

Farmers are facing significant yield loss of vegetable every year due to severe attack of various insect pests in our country. Generally, insect pests cause enormous quantity of yield losses in every season.

Kathmandu is the place where the vegetable are import from various other districts and also from India in huge amount. Pesticides have been used legally or illegally in extensive quantities at the different place of Nepal for various purposes.

A study on pesticide residues in Nepalese vegetables and potential health risks showed that out of the 23 tested analytes, 14 different residues (approximately 61% of the tested analytes) were detected. About 97% of the tested vegetable samples contained at least one analyte. The observed concentration of chloropyrifos in eggplant,

***Author for correspondence**:* Vinay Kumar Jha, Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal.
Email: vinayj2@yahoo.com ; https://orcid.org/0000-0001-6375-8482
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tomato and chilli samples ranged from 1.19 to 45.3 µg/kg (41% of the samples), 1.07 to 1772 µg/kg (94% of the samples) and 1.29 to 491 µg/kg (81% of the samples), respectively. Likewise, the concentration of carbendazim in eggplant, tomato and chilli samples ranged from 1.21 to 154 µg/kg (78% of the samples), 1.45 to 337 µg/kg (100% of the samples) and 1.11 to 95 µg/kg (81% of the samples), respectively. The analysis of the vegetables showed the presence of different metabolites with the following concentrations in tomato samples: omethoate (ranged 6.93-27.9 µg/kg, 6% of the samples) and N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine (1.47 µg/kg, 3% of the samples). Likewise, the concentrations of 3,5,6-trichloro-2-pyridinol in eggplant samples and N-(2,6-dimethylphenyl)-N-(methoxyacetyl) in chilli samples ranged from 4.53 µg/kg to 30 µg/kg (22% of the samples) and 1.32 to 2.32 µg/kg (7% of the samples), respectively. Of all the observed concentrations of pesticides, the mean concentration of carbendazim in tomato samples was significantly higher ($p<0.05$) than the concentration of this compound in chilli and eggplant samples[2].

In a study of use of pesticides and its residue on vegetable crops in Nepal showed that 58 percent of the vegetable samples analyzed contained no detectable level (NDL) of the monitored pesticides, 38 percent of the samples resulted in trace level of the pesticide residue or level below the maximum residual limit (MRL), while four percent of the samples showed above MRL. The analysis detected the highest concentration of cypermethrin in tomato and brinjal. Likewise, concentration of deltamethrin was the highest in cowpea followed by cauliflower, tomato and brinjal (eggplant). The concentration of Carbandazim and Mancozeb were also the highest in tomato followed by bottle guard and chilli[3].

A study on the pesticide residues in some vegetables grown in Kavrepalanchok and Bhaktapur districts revealed that dichlorovos residue (in ppm) was present in cauliflower (0.0031), cabbage (0.0046), broad bean (0.0038), elephant ear plant (0.004), garlic green (0.01880, lettuce (0.012), spinach (0.008), spring onion (0.0050), turnip (0.03) and zucchini (0.0028). Similarly, methyl parathion residue (in ppm) was present in cauliflower (0.0038), broad bean (0.0054), chilly (0.025) and elephant ear plant (0.113)[4].

There are numerous reports of excessive pesticide residues in food in our country. Annual Bulletins from the Central Food Research Laboratory (CFRL) routinely detected residues from their sampling program. From 1981 to 1986, the residues of organochlorine and organophosphate insecticides were a more serious problem in cereal grains, legumes and vegetables but have declined in recent times. The government established Rapid Bioassay for Pesticide Residue Laboratory at the Kalimati fruits and vegetables market in 18 June, 2014 to reduce increasing use of chemical fertilizers and pesticides in fruits and vegetables. The laboratory also aimed at raising awareness among farmers regarding the use of pesticides. Farmers are now aware of the impact of haphazard use of pesticides and chemical fertilizers in vegetables and fruits on human health. It is one of the reasons behind significant drop in pesticide residues in vegetables; the pesticide residue in fruits and vegetables was very high in Fiscal Year 2014/2015. Such residue started falling significantly from the period of started testing vegetables by taking samples from the Kalimati market every day and has found very less harmful chemicals during similar tests conducted in other cities as well[5].

At present, detailed information is not available about the level of pesticide residues remain in different vegetable samples available in Kathmandu. But application of pesticides for the control of insect pests and diseases in Nepal is very high for increasing crops yield. Moreover, there are no substantial works have yet been done for the determination of pesticide residues levels at this area in order to investigate their harmful effects as a result of the irresponsible and indiscriminate use of pesticides in farmer's fields.

Pesticide residues in locally grown vegetables tomato, cabbage and capsicum in Puttalum, Dambulla and Nuwara Eliya districts in Sri Lanka was done by Gas Chromatography with Mass Spectrometry after multi residue extraction procedure ([Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method]. Out of

45 vegetables samples, 15 samples were detected with pesticide residues Chlopyrifos, Prothiofos or Tebuconazole. However, pesticide residual values were less than MRL for all the tested pesticides. Tebuconazole was the mostly detected pesticide residue with 0.128 and 0.052 ppm in tomato and cabbage collected from Matale and Puttlum districts respectively[6].

A study for pesticide residues in a total of 182 samples of six vegetables collected from different agricultural fields of central Aravalli region, India was carried out. In the study, pesticide residues were analyzed using GC-ECD and GC-NPD systems equipped with capillary columns by using a multiple residue method. About 40.11% of total analyzed samples were contaminated with different pesticide residues, among which 35.62% exceeded the MRL values[7].

A study of three insecticides (Chlorpyrifos, Cypermethrin and Deltamethrin) and two fungicides (fluopicolide and propamocarb hydrochloride) in cabbage samples of Thessaloniki, Greece was carried. Among the 132 analyzed samples, 41 (31% of the total No. of samples) had pesticide residues, of which, 2 had multiple pesticide residues and 39 had single pesticide residues were recorded[8].

A study on the variability of pesticide residues in eggplant units collected from a field trial and marketplaces was carried in Greece. In total, 120 samples from a trial field and 142 samples from different marketplaces were collected to estimate the variability of pesticide residues in eggplant units. The level of estimated Cypermethrin and Deltamethrin residues were 0.01-0.349 and 0.01-0.097 mg/kg, respectively; and the unit-to-unit variability factors (VFs) obtained for Cypermethrin and Deltamethrin residues were 2.54 and 2.51, respectively. The mean residue levels of both pesticides were higher in the composite samples than in the individual samples. The average VFs for the marketplace samples was 3.89. The eggplant units exposed to pesticides were higher in residues than the non-exposed units[9].

A recent study showed the presence of residual *cis*-alpha-cypermethrin in between 4.62 to 13.58 ppm while that of *trans*-alpha-cypermethrin in between11.12 to 23.82 ppm in locally produced tomato of Kenya[10].

In a report 0.052 to 4.567 mg/kg of residual cypermethrin was found in yard-long bean while 0.059 to 2.986 mg/kg of same pesticide was found in tomato in Banladesh[11].

A study was carried out to detect and estimate the residue of cypermethrin in brinjal, tomato and cauliflower. The quantities of cypermethrin residue found were 0.538 mg/kg in cauliflower, 0.508 mg/kg in brinjal and 0.695 mg/kg in tomato[12].

Pesticide residue is becoming a major food safety concern for the consumers and governments. In order to remove residual effect of pesticides, accurate dose and the harvest time after spray of each pesticide which should be recommended to the farmer, so that the amount of residual pesticides in vegetables might be lower than the acceptable range.

Since after the introduction of pesticides in Nepal in 1952 to protect plants from agricultural pests, its use has been increasing rapidly throughout years for the purpose of improving crop yields, controlling and eradicating vector borne disease, pests, disease control in agriculture and forest crops. The trend of pesticide use is increasing in Nepal by about 10-20% per year. Most common insecticides in Nepal are Organochlorine (chemical group: endosulfan), organophosphate (Dichlorovos, Malathion, Dimethoate, chloropyrifos), synthetic pyrethroids (cypermethrin) and carbamates (carbofuran). Studies have shown that more than 90% of the total pesticides are used in vegetable farming. In Nepal, organochlorine was more popular previously while organophosphate are more popular now-a-days[13].

Government of Nepal has developed policy for judicious use of pesticides and safety regulations but unfortunately not yet materialized at the farmer's level due to lack of studies on other alternatives of pest management, such as biological, botanicals, and safe chemicals including indigenous knowledge of farmer's practices, as basic component of Integrated Pest Management[3].

Cypermethrin is a synthetic pyrethroid used as an insect-

-icide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes. It behaves as a fast-acting neurotoxin in insects. It is easily degraded on soil and plants but can be effective for weeks when applied to indoor inert surfaces. Exposure to sunlight, water and oxygen will accelerate its decomposition. Cypermethrin is highly toxic to fish, bees and aquatic insects, according to the National Pesticides Telecommunications Network (NPTN). It is found in many house-hold ant and cockroach killers, including Raid, Ortho, Combat and ant chalk. Cypermethrin is used in agriculture to control ectoparasites which infest cattle, sheep, and poultry[14].

In this study, the quantitative analysis of the presence of Cypermethrin pesticide in some common vegetables available in Kathmandu market was done with GC-MS technique.

Materials and Methods

Materials

A total 16 different vegetable samples (6 bean, 2 cabbage, 5 cauliflower and 3 tomato samples) were collected from Asan, Banasthali, Balaju, Balkhu, Kirtipur and Suryabinayak of Kathmandu valley and kept in clean transparent polythene bag to avoid cross contamination and carried to the Pesticide Analytical Laboratory, Department of Food Technology & Quality Control, Government of Nepal, Babarmahal, Kathmandu for pesticide residue analysis by GC-MS technique during November 2018 to March 2019. The whole unit of each sample was cut into small pieces and mixed properly. Clean air tight polythene bags were used to store chopped samples in refrigerator at – 20 °C.

Methanol, acetone, gradient grade acetonitrile, sodium chloride (NaCl), anhydrous magnesium Sulphate ($MgSO_4$) and Primary Secondary Amine (PSA) of Merck group were used for the analysis process.

Methods

Rapid bioassay pesticide residue analysis

Sample vegetables of 1 g each was chopped into fine pieces and kept into two test tubes A and B, then 1 and 2 mL 95% ethanol were added to test tube A and B respectively. Bromine water 100 µL was added test tube B then shaken and drained the sample extract to new test tube at 3 min sharp. Additional 20 min was required for test tube B for evaporate excess bromine.

Three mL of phosphate buffer solution of pH 8, 20 µL AChE (Acetycholinesterase) solution and 20 µL sample extract were added in the cuvette. Solution was then mixed for 5 Sec. and 100 µL DTNB (5,5'dithio-bis-nitrobenzoic acid) and 20 µL ATCI (Acetylthiocholine iodine) solutions were added. It was again mixed for 5 Sec. to start enzyme reaction. The absorbance change reading by spectrophotometer at 412 nm was taken. The percent inhibition was calculated as follows:

$$\%\ \text{inhibition} = \frac{\text{Abs. change (Normal)} - \text{Abs. change (Sample)}}{\text{Abs. change (Normal)}} \times 100 \quad \cdots \quad (1)$$

Sample processing for GC-MS

A representative 10 g portion of samples was weighted in a 50 mL polypropylene centrifuge tube and 10 mL of acetonitrile (MeCN) was added into it. The centrifuge tube was closed properly and shaken vigorously for 30 second by the use of a vortex mixer. Then, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added into the centrifuge tube. It was shaken immediately by the vortex mixer for 1 minute to prevent the formation of magnesium sulfate aggregates. Afterwards, the extract was centrifuged for 5 min. at 5000 rpm. An aliquot of 3 mL of the MeCN layer was transferred into a 15 mL micro centrifuge tube containing 600 mg anhydrous $MgSO_4$ and 120 mg Primary Secondary Amine (PSA). It was thoroughly mixed by vortex for 30 second and centrifuged for 5 min. at 4000 rpm. After centrifuge, a 1 mL supernatant was filtered and taken in a clean GC vial for injection.

The condition of GC instrument

The column oven temp was 100 °C; injection temperature 250 °C and injection mode was split. The flow control mode was linear velocity and pressure was 73 kPa. Total flow 9 mL/min and column flow 1 mL/min and split ratio 5. High pressure injection, carrier gas saver and splitter hold was off. There was no external wait and equilibrium time 3 min.

Results and Discussion

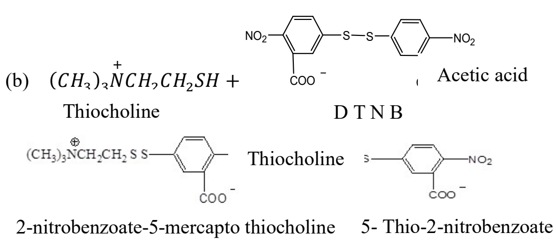
Rapid Bioassay Pesticide Residue (RBPR) Analysis

The RBPR technology is developed by Taiwan Agriculture Research Institute in 1985 to check the residue of organophosphate and carbamates groups of pesticides and fungicides residue in certain level through enzymatic reaction:

Acetyl cholinesterase (AchE) test is to screen the residues of acetyl cholinesterase-inhibiting in fruits and vegetable. This test gives only inhibition percentage from which the result is withdrawn below 35% is edible, 35- 45% need quarantine for few days and warning and more than 45% considered as not edible in toxicological sense. The RBPR/TARI program pack is recorded the AchE reaction and calculated the inhibition percentage through UV-Vis spectrophotometer. The increasing absorbance for sample at 412 nm is recorded and compared to the insecticide free blank (normal) AchE reaction[2].

$(a)\ (CH_3)_3NCH_2CH_2COCH_2 + H_2O \xrightarrow{AChE} CH_3COOH$

Acetylthiocholine $+ (CH_3)_3\overset{+}{N}CH_2CH_2SH$



The nine-month rapid bioassay pesticide residue analysis data of different vegetable samples provided from the Kalimati Fruits and Vegetable market has shown that among the total 1506 vegetable samples, 1503 samples were having green signal (i.e. lower inhibition % or below 35% of inhibition) which was consider to be the safe for eat. Similarly, 2 vegetable samples were found in the range of inhibition of 35-45 % (quarantine) that was needed to wait for some days to consume and rest 1 sample had above 45% (non-edible). These 2 yellows and 1 red sample were the bean and found contaminated in the same month i.e. in Poush (due to the oversized Table, it is not shown here).

From above data analysis; bean sample was selected for the quantitative analysis. Similarly other previous data analysis helped to select other vegetable products for quantitative analysis. The selected vegetables for quantitative analysis in this study were beans, cauliflower, cabbage and tomato.

Analysis of Cypermethrin Residue in Vegetable Samples from GC-MS

Calibration of Standard Sample Cypermethrin

The different concentration of standard sample of Cypermethrin was directly proportional to mean area of peak. When the concentration of standard solution increases, the mean area of peak also increases which is shown in Table 2.

Table 2: Concentration and area of standard sample of Cypermethrin.

S. No.	Concentration (ppb)	Mean Area
1	0	0
2	50	865
3	100	3404
4	200	9624
5	400	23258

The concentration of Cypermethrin was calculated using calibration curve between mean area of peak and different concentrations of standard samples Cypermethrin and is shown in Figure 1.

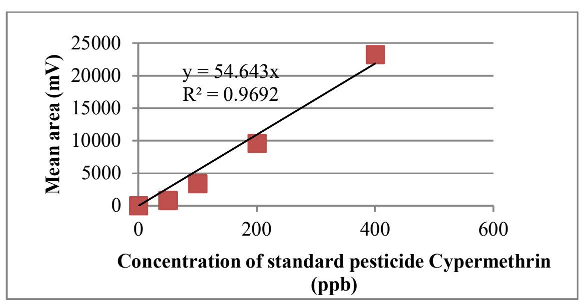


Figure 1: Calibration curve of the standard sample of Cypermethrin.

A total of 16 vegetable samples (bean, cabbage, cauliflower and tomato) were analyzed by GC-2010 with Flame Thermionic Detector (FTD) with the pre-set parameters. The cauliflower sample taken from the Suryabinayak, Bhaktapur; bean sample from Banasthali and tomato sample from Kirtipur were contaminated with Cypermethrin with concentrations 47.091; 118.334 and 306.103 ppb with retention times 31.538; 31.484 and 31.560 min. respectively. The chromatograms of all the three samples are shown in Figure 2:

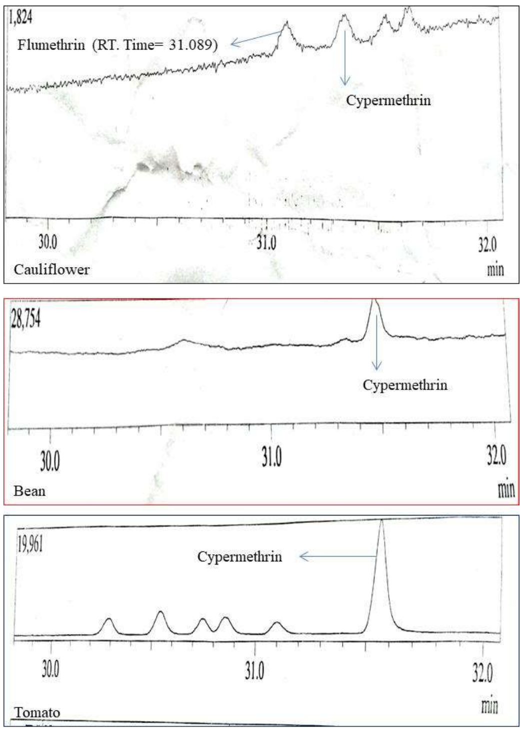


Figure 2: Chromatograms of cauliflower, bean and tomato samples.

The samples which were contaminated with Cypermethrin pesticide is given in Table 3.

Table 3: Cypermethrin residue estimation of Vegetable samples collected from different market of Kathmandu.

S. No.	Vegetable name	Place	Sample name	Pesticide residue value in (ppb)
1	Cauliflower	Balkhu	C1	ND
2	Cauliflower	Banasthali	C2	ND
3	Cauliflower	Kirtipur	C3	ND
4	**Cauliflower**	**Suryabinayak**	**C4**	**47.091**
5	Cauliflower	Kalimati	C5	ND
6	Cabbage	Suryabinayak	S1	ND
7	Cabbage	Kirtipur	S2	ND
8	**Bean**	**Banasthali**	**B1**	**118.334**
9	Bean	Balaju	B2	ND
10	Bean	Kirtipur	B3	ND
11	Bean	Suryabinayak	B4	ND
12	Bean	Assan	B5	ND
13	Bean	Kalimati	B6	ND
14	**Tomato**	**Kirtipur**	**T1**	**306.103**
15	Tomato	Banasthali	T2	ND
16	Tomato	Assan	T3	ND

ND: Not detected

The Table 3 showed that out of 16 samples, 3 samples were contaminated with cypermethrin. Cauliflower contained 47.091 ppb which is less than MRL value that is 1000 ppb. Similarly, another bean sample was contaminated with 118.334 ppb this was also below the MRL value of cypermethrin in bean i.e. 2000 ppb and the next sample tomato also contaminated with 306.103 ppb. This tomato was above the MRL value of cypermethrin in tomato that is 200 ppb. If contamination value is greater than the MRL value, it will cause the various health effects.

The findings of the present study are based on small sample size. A small sample size decreases the probability of finding contaminated samples. However, it has been believed that the present sample size was sufficient to study and get idea about the pesticide (Cypermethrin) use and safety among farmer communities of Nepal.

Conclusion

The rapid bioassay pesticide residue analysis result of Kalimati fruit and vegetable market of 9 months data showed that most of the vegetables samples were contaminated with different pesticides but few of them were found to be contaminated with higher % of inhibition (i.e. >45%) and most of the vegetable contaminated in safer limit (i.e. <35% of inhibition). This observation showed that due to presence of the pesticide residue analysis laboratory on the Kalimati fruit and vegetable market farmers became quite aware about the time interval of pesticide use and harvesting before supply in market. The application of pesticide close to harvesting time could be the main reason for the contamination of vegetables with pesticides.

The finding of the present study of 16 vegetable samples showed that out of them 3 vegetables i.e. one bean sample of Banasthali local market, one Tomato sample of Kirtipur local market and one cauliflower sample of Suryabinayak local market were contaminated with pesticide (Cypermethrin). In which bean and cauliflower sample were found below the MRLs value i.e. 118.334 and 47.091 ppb respectively but one sample of tomato found above the MRLS value i.e. 306.103 ppb. Sample with residue is above MRL may pose health hazards to the consumers. It may be due to lack of awareness of farmer about the application dose, method of application and withholding period.

A new modified QuEChERS method for the analysis of vegetables sample is easy method which could be affordable and feasible in context to Nepal for further studies.

Data availability Statement

The authors declare that all the relevant data generated and analyzed during this study, such as experimental, Gas chromatographic and computational data, are included and are available within the paper. Any raw data files if needed in another format, they can be made available from the first and corresponding authors upon reasonable request.

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