Evaluation of Pesticide Residues in Vegetables from the Eight Districts of Haryana State (Northern India)

Mishra Sudeep[1], Singh Mukesh K.[1], Saini Mahesh K.[1], Pande Mangesh A.[1], Doetale Vinod D.[1], Alam Samsul[1*], Ahluwalia Vivek [1], Sherawat B.S.[2] and Thakur Lalitesh K.[1]

1. Institute of Pesticide Formulation Technology, Udyog Vihar, Gurgaon – 122016, Haryana, INDIA
2. Directorate of Horticulture, Panchkula, Haryana, INDIA

*alam_samsul@rediffmail.com

Abstract

Haryana is one of important States of Northern India for the vegetable production and supply to National Capital in the region. The study was conducted to evaluate the presence of pesticide residue levels of some most frequently used pesticides on vegetables in the State of Haryana. For this study, 217 samples were collected from market and farm from Ambala, Rohtak, Jind, Fatehabad, Kaithal, Faridabad, Hisar and Sirsa districts of Haryana. Study showed presence of residue of at least one pesticide in 65 samples. The pesticides like chlorpyrifos, butachlor, pretilachlor, ethion, atrazine, profenofos, lambda cyhalothrin, cypermethrin and fenvalarate were detected in at least one commodity.

Among all the pesticides detected, chlorpyrifos was detected as major contaminant of vegetables investigated (farm samples 64.5 %; market samples 79.4 %) whereas butachlor was detected in 12.9 % of farm samples and 14.7 % of market samples. Chilli, brinjal, cauliflower and ladyfinger were found more contaminated as compared to other vegetable crops. Among different districts, samples collected from Hisar (4.9 %) and Rohtak (13.3 %) were least contaminated whereas Faridabad (78.9 %) and Jind (48.8 %) were more contaminated.

Keywords: Maximum residue limits, Monitoring multiresidues, Pesticide residue, Residual concentration, Vegetables.

Introduction

India is fastest growing economy of the world and now on the edge of being called a developed nation. India is the second largest producer of horticultural crops and fruits in the world. Various types of pesticides including organochlorines, organophosphorus, carbamate, phthalimide and pyrethroid are used to protect the crop from different types of pests and diseases in India. Use of pesticides has significantly enhanced the crop production, thus enhancing the income of farmers in India, but some studies have shown that unscientific use has negatively affected the human health and environment.[8]

Recent studies have shown that chemical contamination is likely on higher side for vegetables grown in peri-urban areas than in rural areas. Due to this, the human health risk in the population dependent on these vegetables has also risen.

Exposure of pesticides to human has led to increased health risk.[10] Dietary exposure estimation of pesticides on human health requires data on the daily vegetable consumption and incidence of presence of contaminant. This data then must be compared with relevant health-based supervision value for the chemical of concern. Children are most affected by exposure to different types of pesticides due to increased use of pesticides in agriculture and household. Among different methods of exposure to children, dietary intake is possibly one of important pathways for exposure to young children.[21]

Literature shows that higher level of pesticides residues was found in fruits and vegetables among different region of world, making them unsuitable for human consumption. Therefore, different countries have put in place different monitoring programs for pesticide use and residues to improve food safety.[4] European Union and Codex has suggested the maximum residue limits (MRL) to avoid health threat due to consumption of pesticide residues. In India, Food Safety and Standards Authority of India (FSSAI) fixes MRLs for different pesticides.[19]

Several validated methods for routinely quantify pesticide residue through assessment of sensitivity, linearity, limit of determination (LOD), limit of quantitation (LOQ) and accuracy and precision of recoveries in vegetables and fruits by gas chromatography (GC), gas chromatography mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods are available.[20]

There are several reports showing measurement of pesticides of different classes in vegetable samples in India.[5,10,14,16] Another study concluded the presence of total HCH and DDT in bitter gourd. Study found that DDT residues concentration was on higher side than HCH residues. p,p′-DDT was found most noticeable among four metabolites of DDT whereas p,p′-DDE was found in slight concentration.[9]

Recently, Sivaperumal et al[15] evaluated pesticide residue in vegetable and fruit samples including brinjal, cabbage, cauliflower, guava, okra, onion, potato apple, banana, grape, mango orange and pomegranate selected at random from the local markets at in the State of Gujarat in the western part of India.

Study showed that no significant amount of pesticides were present in the studied samples.[15] Based on the above findings, we aim to evaluate the pesticide residue concentration of the individual pesticides, commonly used on vegetables of selected district of the State of Haryana in Northern India.

Materials and Methods

Study area: The study area falls under the Northern Western Himalayan region of India and shares the boundary with four States *viz.* Uttar Pradesh, Himanchal Pradesh, Punjab and Rajasthan and National Capital Region, Delhi. Major vegetables produced in the region include onion, tomato, chilli, pea, capsicum, cauliflower, brinjal etc. This region is main supplier of vegetables to Delhi.

Sample collection: Vegetables samples were collected from the farms and markets of eight districts *viz.* Ambala, Rohtak, Jind, Fatehabad, Kaithal, Faridabad, Hisar and Sirsa of Haryana for the present study for analysis of pesticide residues. Samples were collected in sterilized polybags and brought to the laboratory within 24 h and stored in a refrigerator at 4°C until the analysis. Sampling and preservation protocol was as per standard protocol.

Chemicals and Reagents: ACS grade anhydrous Na_2SO_4 (99%), anhydrous $MgSO_4$ (98%) and anhydrous NaCl (99%) were purchased from Merck (Merck Life Science Private Limited, Mumbai, Maharashtra, India).

Na_2SO_4 was heated at 650 °C for 4 h and kept in a desiccator until use. Certified reference materials (CRMs) of targeted pesticides were procured from Sigma-Aldrich (Germany). Discovery® DSC18 and primary secondary-amine (PSA) used for extraction and cleanup were purchased from Supelco, USA. ACS grade n-hexane (99%) from Merck (Merck kGaA, Darmstadt, Hesse, Germany) and HPLC grade acetonitrile (99.9%) from Merck (Merck kGaA, Darmstadt, Hesse, Germany) were procured. PTFE syringe filters (0.22 mm) were procured from Cole Parmer, India.

The standard operating procedures were followed for obtaining, labelling, storing and handling of pesticide standards. Reference standards were initially stored in deep freezer (- 20 °C) under dry storage conditions and were brought to room temperature prior to preparing stock solutions, while the liquid standards were sonicated for proper mixing. The working standard solutions were prepared from the intermediate solutions. All chemicals and reagent used were of analytical grade.

Sample extraction and analysis: Modified QuEChERS (quick, easy, cheap, effective, rugged and safe) method as recommended for matrices having 75% of water content was used for sample preparation and pesticide extraction[1,7]. Sample (10 g) was homogenized in high speed homogenizer and taken in 50 ml centrifugation tube. 10 mL of acetonitrile (HPLC grade) and anhydrous Na_2SO_4 (10 g) was added to it, vortex shaken for 1 min and centrifuged for 5 min at 5000 rpm. 1.5 mL supernatant was transferred to 15 mL of teflon centrifuge tube which contained the mixture of 0.125 g anhydrous $MgSO_4$, 0.037 g primary secondary amine (PSA) and 0.037g C18 sorbent.

The mixture was first shaken manually and then homogenized in vortex shaker for 1 min and centrifuged at 5000 rpm for 5 min to remove all interfering components with pesticides. A fraction of 2 mL of the extracted sample in glass tubes was evaporated in Turbo Vap-evaporator at 35°C using N_2 gas of 10 psi pressure. The residues were reconstituted using n-hexane for the analysis in GC-MS.

Instrumentation: Samples were analysed using gas chromatography system (Agilent Technology 7890A) equipped with mass spectrometer (5975C inert XL EI/CI MSD). The chromatographic separation was performed with DB-5MS fused silica capillary column (Agilent J and W, GC Column, 30 m × 0.25 mm × 0.25 µm). 1µl of sample was injected with linear flow at 1 ml/min in splitless mode with helium as carrier gas. Gases were passed through gas purification filters that contain oxy trap and moisture trap before supply to the column.

Operating condition for column oven was programmed from 50°C (kept for 2 min) and then increased to 280°C (at the rate of 8 °C per min) (kept for 15 min). The injector was operated in splitless mode at 280^0C temperature. The interface temperature was kept at 280^0C, ion source at 250^0C and quadruple temperature at 150^0C.

The MS was operated in Electron Impact Mode (EI) with electron energy 70 ev using selective ion monitoring (SIM) mode with solvent delay time 3min. The presence of pesticides residues in collected vegetables was quantified by comparing the retention time, peak area and peak height of the sample with those of the standards.

Results and Discussion

Evaluation by commodity: Table 1 gives an overview of the details of the sample collection from different districts of Haryana. 217 samples in total were collected from eight districts. Highest numbers of sample were collected from Rohtak district followed by Jind, Hisar. Lowest numbers of sample were collected from Kaithal district.

Table 2 gives an outline of data obtained after analysis of 217 samples. With regards to 217 samples analyzed, 116 samples were collected from the farmers at their farm lands and 101 samples were collected from different markets in identified districts.

Only 65 samples were detected with presence of pesticides accounting for 30.0 % of total samples analyzed. Presence of pesticide residues was detected more on market samples (34.7 %) as compared to farm samples (25.9 %).

Table 1
Details of the sample collections from different districts of Haryana, India

Commodity	Collection District								Total
	Ambala	Rohtak	Jind	Fatehabad	Kaithal	Faridabad	Hisar	Sirsa	
Onion	2	1	1	0	0	0	0	1	5
Tomato	2	7	8	1	2	3	3	1	27
Chilli	1	6	5	4	1	2	3	3	25
Pea	2	1	0	0	0	0	0	0	3
Capsicum	1	0	1	0	0	0	0	1	3
Brinjal	2	6	4	2	2	3	8	3	30
Cauliflower	2	5	4	3	0	2	11	3	30
Cabbage	0	2	3	1	0	1	5	0	12
Bitter gourd	0	0	2	1	0	0	0	0	3
Bottle gourd	0	9	1	1	2	2	0	0	12
Round gourd	0	1	1	1	0	0	0	0	3
Raddish	0	1	0	0	2	0	0	0	3
Carrot	2	0	0	0	2	0	0	0	4
Cucumber	0	2	2	1	0	1	1	2	9
Potato	6	2	0	0	1	1	0	0	10
Ridge gourd	0	2	2	1	2	2	2	1	12
Ladyfinger	0	3	7	2	2	2	8	2	26
Total	20	45	41	18	16	19	41	17	217

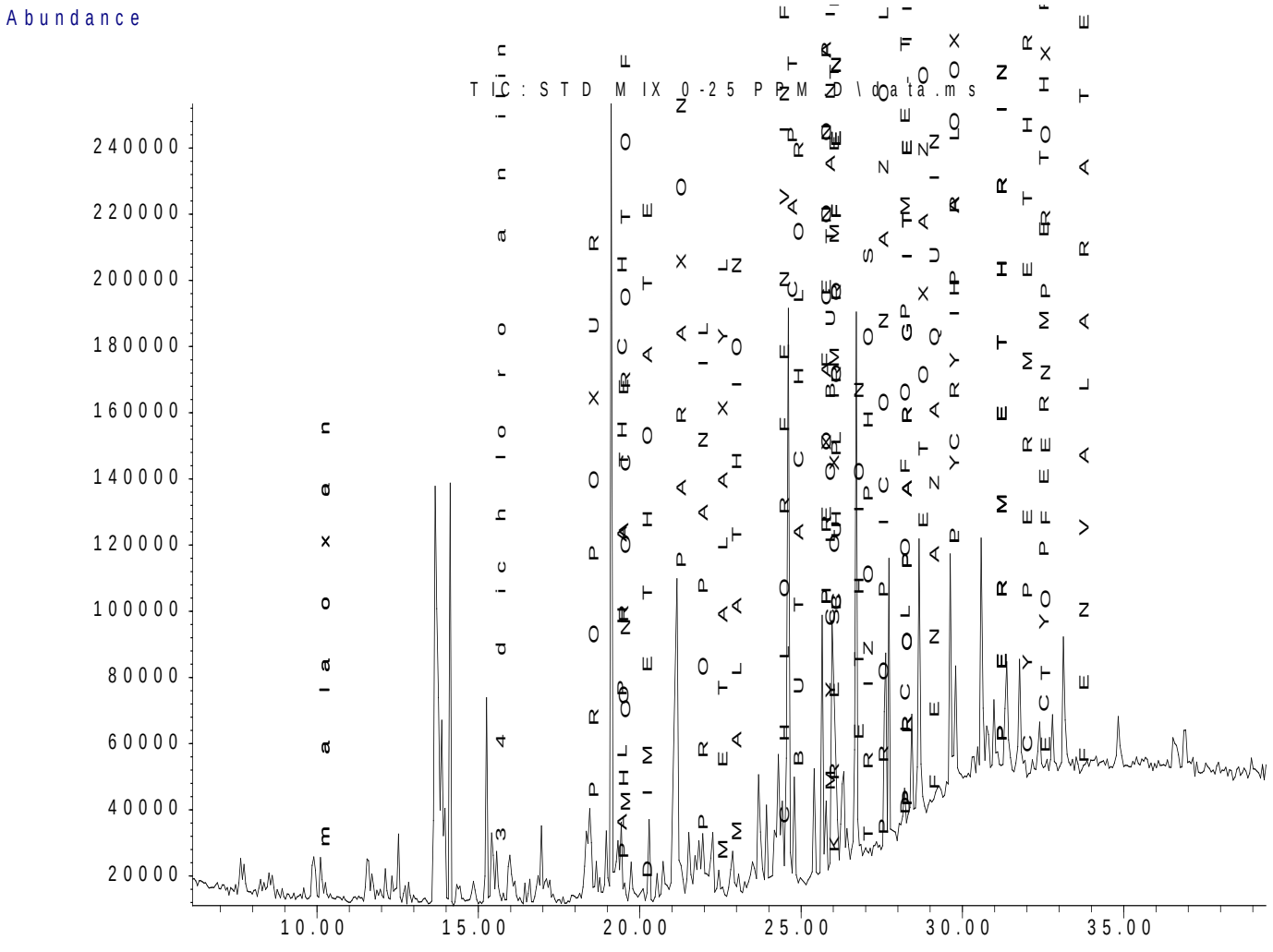

Figure 1: Total ion chromatograms (TIC) of mixture of pesticides

Table 2
Number and percentage of contaminated samples, name of pesticide detected in different vegetables collected from Haryana, India

Commodity	Total Number of samples analyzed		Number of samples with detectable residue		Name of pesticide detected	Number of samples with detectable residue		Tolerance limit/MRL (mg/kg) as per FASSI*	No. of Samples exceeding MRL
	Farm	Market	Farm	Market		Farm	Market		
Onion	2	3	2 (100.0 %)	1 (33.3 %)	Chlorpyrifos	0	1	0.01	1
					Butachlor	1	0	-	-
					Pretilachlor	1	0	-	-
Tomato	10	17	0 (0 %)	4 (23.6 %)	Chlorpyrifos	0	4	0.2	4
Chilli	13	12	4 (30.8 %)	7 (58.4 %)	Ethion	0	1	1.0	1
					Butachlor	0	1	-	-
					Chlorpyrifos	3	5	0.2	7
					Fenvalarate	1	0	-	-
Pea	1	2	1 (100.0 %)	1 (50.0 %)	Butachlor	1	0	-	-
					Chlorpyrifos	0	1	0.2	1
Capsicum	1	2	0 (0 %)	1 (50.0 %)	Chlorpyrifos	`0	1	0.2	1
Brinjal	19	11	6 (31.6 %)	2 (18.2 %)	Butachlor	1	1	-	-
					Cypermethrin	1	0	0.2	1
					Chlorpyrifos	3	1	0.2	4
					L-cyhalothrin	1	0	-	-
Cauliflower	17	13	5 (29.4 %)	4 (30.8 %)	Atrazine	1	0	-	-
					Chlorpyrifos	3	3	0.01	6
					Cypermethrin	0	1	-	-
					Profenofos	1	0	-	-
Cabbage	7	5	2 (28.6 %)	1 (20.0 %)	Chlorpyrifos	1	1	0.01	2
					Profenofos	1	0	-	-
Bitter gourd	2	1	1 (50.0 %)	1 (100.0 %)	Chlorpyrifos	1	1	0.2	2
Bottle gourd	10	2	3 (30.0 %)	1 (50.0 %)	Butachlor	1	1	-	-
					Chlorpyrifos	2	0	0.2	2
Round gourd	2	1	0 (0 %)	1 (100.0 %)	Butachlor	0	1	-	-
Raddish	3	0	2 (66.7 %)	0 (0 %)	Chlorpyrifos	2	0	0.2	2
Carrot	1	3	0 (0 %)	2 (66.7 %)	Chlorpyrifos	0	2	0.2	2
Cucumber	6	3	1 (16.7 %)	0 (0 %)	Chlorpyrifos	1	0	0.2	1
Potato	5	5	1 (20.0 %)	2 (40.0 %)	Chlorpyrifos	1	2	0.01	3
Ridge gourd	8	4	1 (12.5 %)	1 (25.0 %)	Chlorpyrifos	1	1	0.2	2
Ladyfinger	9	17	2 (22.2 %)	6 (35.3 %)	Chlorpyrifos	2	5	0.2	7
					Butachlor	0	1	-	-
Total	116	101	30 (25.9 %)	35 (34.65 %)					

Evaluation by pesticides: Chlorpyrifos was most frequent pesticide detected in farm and market samples. 80 % of total market samples detected with pesticides showed presence of chlorpyrifos, similarly percentage of farm samples (66.7 %) with chlorpyrifos was high (Table 3). In farm samples, pretilachlor, atrazine, L-cyhalothrin, cypermethrin and fenvalarate were detected in at least one commodity while ethion was not detected in any farm commodity. Market samples lacked presence of pretilachlor, atrazine, profenofos, L-cyhalothrin and fenvalarate.

Evaluation by district wise: Study showed that percentages of samples contaminated with pesticide residue were highest in Faridabad (78.9 %) and least in Hisar (4.9 %) districts (Table 4).

Table 3
Pesticide residues range in vegetables from farm and market samples.

Name of Pesticide detected	Number of Samples with pesticide Residue		Ranges (mg/Kg)	
	Farm	Market	Farm	Market
Butachlor	4	5	1.28-1.49	1.34-1.45
Chlorpyrifos	20	27	0.45-5.47	0.18-5.17
Pretilachlor	1	0	BDL-1.45	-
Ethion	0	1	-	BDL-2.72
Atrazine	1	0	BDL-0.085	-
Profenofos	2	0	0.69-3.98	-
L-cyhalothrin	1	0	BDL-4.20	-
Cypermethrin	1	1	BDL-6.18	BDL-2.08
Fenvalarate	1	0	BDL-1.39	-
Total	**31**	**34**		

Table 4
Number of contaminated samples in different districts

Collection District	Total number of contaminated samples	% of samples contaminated with pesticide residue
Ambala	7	35.0
Rohtak	6	13.3
Jind	20	48.8
Fatehabad	6	33.3
Kaithal	6	37.5
Faridabad	15	78.9
Hisar	2	4.9
Sirsa	3	17.6
Total	**65**	**30.0**

The study was conducted to analyze presence of pesticide residues in seventeen common vegetables (onion, tomato, potato, chilli, pea, brinjal, cauliflower, ladyfinger, cucumber, ridge gourd, cabbage, bottle gourd, bitter gourd, round gourd, carrot etc.). Total 217 samples were collected from eight districts (Ambala, Rohtak, Jind, Fatehabad, Kaithal, Faridabad, Hisar and Sirsa) of Haryana (Table 1). The sampling was done randomized and according to availability of the vegetables in market.

Five samples of onion were collected from different districts of Haryana. Three pesticides i.e. pretilachlor (1.45 mg/Kg), chlorpyrifos (1.22 mg/Kg) and butachlor (1.46 mg/Kg) were detected. Twenty five samples of tomato were collected from all eight districts which include ten samples collected from farm and seventeen samples were collected from market. Samples collected from the farm were free from pesticide residue, while four samples from market showed presence of pesticide residue. Only 14.8 % of samples were contaminated with pesticide residues. Samples contaminated with pesticides were collected from Jind (2 Samples) and Faridabad (2 Samples) markets. All four samples showed presence of chlorpyrifos in range from 0.743 – 1.52 mg/Kg.

Similarly, twenty five samples of chilli were collected from farms and markets of sampling districts. Percentage of samples detected with pesticide residues was more in markets (58.3 %) as compared to farms (30.8%). Chilli samples were found to be contaminated with four pesticides i.e. ethion, chlorpyrifos, butachlor and fenvalarate. The concentration of ethion, chlorpyrifos, butachlor and fenvalarate ranged from BDL to 2.72 mg/Kg, BDL to 4.24 mg/Kg, BDL to 1.45 mg/Kg and BDL to 1.39 mg/Kg respectively. Out of eleven samples detected with pesticide residue, eight samples were found contaminated with chlorpyrifos.

Three samples were collected for pea from two districts Ambala and Rohtak. Out of three pea samples, two samples were detected with pesticides. From Ambala district, one pea sample each from market and farm was detected with pesticides. Sample collected from market was contaminated with chlorpyrifos (1.22 mg/Kg), while farm sample showed the presence of butachlor (1.28 mg/Kg). One sample of capsicum collected from Sirsa district was found contaminated with chlorpyrifos (1.74 mg/Kg).

Thirty samples of brinjal were collected from different designated sampling sites. Four samples were found contaminated with chlorpyrifos. The observed concentrations of chlorpyrifos in brinjal samples ranged from 0.34 to 2.09 mg/Kg. One sample of brinjal collected from farm of Ambala district was contaminated with butachlor (1.49 mg/Kg). L-cyhalothrin (4.20 mg/Kg) and cypermethrin (6.18 mg/Kg) residues were detected in brinjal sample collected from farm of Jind district. Only two

samples collected from districts of Rohtak and Faridabad markets were detected with pesticides.

Thirty two samples of cauliflower collected from farm and market of eight districts of Haryana were investigated for the presence of pesticide residue. Analysis showed presence of pesticide residue traces in seven samples. Two market samples each from Fatehabad and Faridabad district were contaminated with chlorpyrifos and one market sample from Rohtak was contaminated with cypermethrin. Five samples from farm were contaminated with chlorpyrifos (1.82-4.13 mg/Kg), profenofos (3.98 mg/Kg) and atrazine (0.86 mg/Kg).

The three samples of cabbage out of twelve total collected samples were contaminated with chlorpyrifos (2 No.) and profenofos (1 No.). Profenofos (0.69 mg/Kg) was detected in farm sample from Hisar district. Vegetables like bitter gourd, raddish, carrot, cucumber, potato and ridge gourd showed presence of only chlorpyrifos residues in the samples. Bottle gourd and ladyfinger were contaminated with butachlor and chlorpyrifos. Results showed the presence of butachlor in one farm sample of round gourd. Chlorpyrifos (0.73; 0.57 mg/Kg) was detected in two market samples collected from market (Table 2).

It was found that 20 samples from Jind district were contaminated with pesticide residue which accounted for 48.8 % of total samples analyzed. Similarly, in case of Faridabad district, 15 samples were found to be contaminated for pesticides. Faridabad district was found to have highest percentage of samples with pesticide residue. Hisar district was found with least number of samples with pesticide residue. Forty one samples were processed for detection of pesticide residues, only two samples were detected positive which accounted for 4.9 % (Table 4). So, from this study we can infer that vegetables from Hisar district are least contaminated and safe for the human use.

The residue levels and the detection rate of pesticides indicate that number of some vegetables from market had higher residue level than farm. There is a possibility that the vegetables samples in the farm are left in open at the mercy of the sun which might have undergone degradation of some of the pesticides on the vegetables. Moreover, Haryana is extremely hot in summer at around 45°C. The low temperature conditions or shady conditions in the markets favored less degradation of the pesticides and high contamination levels of residues as compared with that of farm samples. Similar results were found in another study where vegetables in the supermarket were found to be more contaminated with pesticides as compared to open markets and roadside grocery shops.[19]

There were some vegetables which showed contradictory results with more number of farm samples showing residue than market. Likely reason for low residue in commodity could be pre-processing. There are studies which also suggested that market samples are having less residue than farm samples.[3,14]

Frequencies and pesticide residue concentration range of tested vegetable samples are tabulated in table 4. Traces of chlorpyrifos were detected in maximum samples (73.5 %). The chlorpyrifos was detected in 66.7 % of farm samples and 80 % of market samples investigated. Similar results were also reported in a study conducted to investigate pesticide residues in vegetables from farms, markets and supermarket of Northern Thailand. Study concluded that chlorpyrifos was the most common pesticide detected at the highest level. Also, chlorpyrifos is one of chemicals used in maximum countries globally for use in agricultural to control insects because of its applicability in a widespread range of pests.[20,21]

The butachlor was second highest pesticide detected after chlorpyrifos. The butachlor was detected in 12.1 % of farm samples and 14.2 % of market samples. The pesticide residues in farm samples were detected more as compared to market samples. Among farm samples analyzed for the pesticide residue, butachlor was detected in 6 farm samples with varying range of 1.28-1.46 mg/kg while chlorpyrifos was detected in 20 farm samples with concentration range 0.45-5.45 mg/kg. Pretilachlor, atrazine, L-cyhalothrin, cypermethrin and fenvalarate were also detected in farm samples. The farm samples detected with presence of pesticide residues of L-cyhalothrin, cypermethrin and fenvalarate were found above maximum residue limit (MRL). Among market samples, butachlor was detected in three samples while chlorpyrifos was detected in twenty six market samples with range of 0.18-5.17 mg/kg. Ethion and cypermethrin were detected in only one market sample and were found above maximum residue limit (MRL).

Conclusion

Study showed presence of pesticide residue of different pesticide in variable concentrations in both farm and market samples. Chlorpyrifos found in samples of farm and market from study area can affect human health. Study showed more samples with pesticides collected from market than farm. It is recommended to have continuous pesticide monitoring in samples from the region or transported from region to build a proper database for effective health monitoring of population. Determination of pesticide residues in vegetables is necessary for ensuring that human exposure to contaminants, especially by dietary intake, does not exceed acceptable levels for health.

Acknowledgement

The authors would like to thank Director, Institute of Pesticide Formulation Technology for providing necessary research facilities. The authors also thank B. S. Sehrawat, Department of Horticulture, Govt. of Haryana for providing samples. The authors also thank Department of Horticulture, Govt. of Haryana for financial assistance.

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(Received 06th April 2021, accepted 19th June 2021)