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Quantification of Pesticide Residues in Vegetables by Different Chromatographic Techniques

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Abstract

Cypermethrin, chlorpyrifos and imidacloprid residues were determined in different vegetables. During 2013, samples were collected from different six major vegetable markets from Lahore. Ethyl acetate was used for extraction, whattman fluted filter and charcoal was used for cleaning procedure. High performance thin layer chromatography was used for detection, as well as quantification of pesticides. Confirmation of results was done by using gas chromatography/mass spectrophotometer. From results, it was concluded that different vegetables contain different concentration of pesticides means adsorption rate is different for each pesticide and vegetable. 79% samples were contaminated with cypermethrin, 70% from imidacloprid and 65% samples were contaminated from chlorpyrifos. Pumpkin, okra, egg plant, cucumber spinach and cabbage were contaminated with pesticides. Peeling of vegetables resulted in less concentration of pesticide.

Keywords: Imidacloprid; Chlorpyrifpos; Cypermethrin; HPTLC; Maximum residue limit; Ethyl acetate

Introduction

Pests are the main affecting agents of our crops. Different types of chemical sprays are used to repel and control the spread of these pesticides (Table 1). Pesticides may be of any kind, synthetic or natural chemicals. Due to the attack of pests, different crops destroy. There is a big damage due the pre-harvest and post-harvest of crops. It is studied that in advanced countries, the damage is less as compare to the developing countries, but still a little bit damage is present. There is no doubt that due to the use of pesticides, production is increased day by day. Due to this reason, the pesticides are come in market on large scale. And their adverse effects are also increasing as their use is increased. Vegetables and fruits are the main source of human diet. Different kinds of elements are present in vegetables that are healthful for human being [1].

Pesticides contain different kinds of active reagents, on which basis the pesticides are classified. These active reagents possess different types of action on pests that their action is not specific for pests. If it enters in unintended individual, then it can also attack on them same as pests. Some pesticides are more persistent and reside in our environment for a long duration. Mainly the pesticides act on the neurotransmitter of pests. If the pesticides contaminated food is used by the human being, then these pesticides can also attack on the neurotransmitter ofhuman being. There is no differentiating characteristic of pesticides, to differentiate between pests and human beings [2]. When pesticides applied on crops, pesticides can absorb in soil, eatable elements and water. Through different routes, the pesticides can act on human being oral, dermal and through inhalation. Due to the side effects of pesticides, it becomes essential to control the use of pesticides within the potential limit. Pakistan economy is dependent on the production of fruits and vegetables. And no doubt, Pakistan is one from those countries who are using pesticides in large amount to increase their production. A large part of fruits and vegetables production in Pakistan being export to other countries. Pakistan is one from the developing countries. So, there is lack of information about the use of pesticides. But, now a days, different labs are developed to develop study on different pesticides residues and their effect on human beings. Organo chlorine class of pesticides is now banned. Due to the excessive use of this class, leaves large concentration in vegetables [3].

Pesticides name	Molecular formula	Classification
Cypermethrin	$C_{22}H_{19}Cl_2NO_3$	Pyrethroid
Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	Organophosphate
Imidacloprid	$C_9H_{10}ClN_5O_2$	Neonicotinoid

Table 1: Selected pesticides.

Maximum residue levels represent the maximum concentrations of pesticide residues, which are legally permitted in food crop, and they are interpreted as the maximum concentrations expected to be found, if pesticides are applied to the crop according to the guidelines. Pesticides can cause both acute and chronic diseases. Skin rashes and blurred vision are the main acute effects of pesticides. Heat stress, heat cramps and neurological diseases are the main effect of repeated exposure to the pesticides. Maximum residue is set by each country, before a pesticide came in market. Measurement of the level of toxin that can cause disease is called toxicity. Maximum residue limit of each country should be same because the health of each country human being is precious. Integrated pests management control is the best way, but it is not known by the illiterate peoples [4].

Lahore is the second largest city relative to the population size in Pakistan. Peoples belong to different classes are resident of Lahore. Vegetables are common in use. A market based survey was conducted to determine the contamination of vegetables in different major markets of Lahore.

Materials and Methods

Experimental design

Residues of different pesticides were checked in vegetables samples

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by using the High Performance Thin Layer Chromatography (HP-TLC) [5].

Pesticides selection

It was studied that which pesticides are now common in use were selected and use in high concentration. Standards were collected from agriculture department of Pakistan. Standards were in powder form. 1% solution of each pesticide was prepared.

Samples collection

To determine the residues of different pesticides in vegetables, different samples were collected from different markets. Six different sites were selected for the collection of vegetables. Site A, site B, site C, site D, site E and site F were selected on the basis of major markets of vegetables. Selected vegetables samples are given in Table 2.

Samples were packed in polythene bags and stored at 4°C to avoid any damage. And transfer to lab for further processing.

Extraction methodology

Samples were stored in refrigerator. After passing half an hour, take off the samples for further processing. Thawed the samples and then chopped these samples within the blender. Weigh 100 g of the chopped vegetable. 500 ml Erlenmeyer flask was selected, add weighed vegetable. Add 2.5 g of NaCl to concentrate the extract. Add 20 g of anhydrous sodium sulphate to remove extract from vegetables. 70 ml of ethyl acetate was added to dissolve pesticides in it. For one an hour flask, keep in moving condition [6]. Dissolve salts in mixture, so pesticides dissolve in ethyl acetate.

Clean up methodology

Then pass this material through a proper clean up procedure to take the extract. Whattmann fluted filter paper no 42 was used to exclude the solid part from extract. At 105°C charcoal was activated, the extract was passed through it [5]. The extract was in diluted form, rotatory evaporator was used to concentrate the extracted sample [4].

Standards preparation

Different pesticides that were selected contain different percentages, and were in powder form. 1% solution of each pesticide was prepared (Table 3), and it was analyzed that either 1% was sufficient for comparison or not. The pesticides solutions were prepared in ethanol. Weighed standards, prepared and stored at low temperature. And used within one week.

High Performance Thin Layer Chromatography (HPTLC)

High performance thin layer chromatography is a cheap and effective method for the detection of different pesticides. It can be used for detection, as well as quantification of pesticides. Quantification was done by comparison with standards.

Common name	Scientific name
Pumpkin	*Curcurbita maxima*
Cucumber	*Cucumissativus*
Egg plant	*Solanummelongena*
Cabbage	*Brassica oleracea*
Okra	*Hibiscus esculenta*
Bitter gourd	*Momordicachorantia*
Spinach	*Spineraciaoleracea*
Cauliflower	*Brassica oleracea*
Turnip	*Brassica rapa*
Bell pepper	*Capsicum annuum*

Table 2: Selected vegetables for analysis.

Standard % available	Amount taken of standard (g)	Total volume (solvent)
Imidacloprid (95.03%)	0.105	10 ml
Chlorpyrifos (97%)	0.103	10 ml
Cypermethrin (92%)	0.108	10 ml

Table 3: Pesticides standards preparation.

Pesticide name	Distance travelled by spot (cm)	Distance travelled by the solvent (cm)	Retention factor
Cypermethrin	8.8	13	0.67
Chlorpyrifos	8.7	13	0.669
Imidacloprid	3	13	0.23

Table 4: Retention factor of pesticides.

Imidacloprid standard volume (µl)	Concentration (µg)	Average spot diameter (mm)
0	0	0
2 µl (1 spot)	21	2.2
4 µl (2 spots)	42	2.4
6 µl (3 spots)	63	2.6
8 µl (4 spots)	84	2.8
10 µl (5 spots)	105	3

Table 5: Spot diameter versus concentration for Imidacloprid.

Evaluation of standards: First of all, HPTLC plate was activated at 105˚C. Different spotting (1 spot, 2 spots……,5 spots) of pesticides was done. Different concentrations spots were applied, then develop plate in methanol and ammonia and checked the diameter difference. The end spot of higher concentration produce larger diameter and brighter color and fluorescence. A standard curve was drawn between concentration and spot diameter for comparison with the sample. And also calculate the retention factor (Table 4) for these standards [6].

Sample spotting: Samples extract was spotted along with the standards and compare with the standards. Methanol and ammonia was used as a mobile phase (Table 7).

Development of plates: Firstly, plate was placed under UV light for half an hour to check the fluorescence. UV light source was chromadex (USA) dual epi-UV, ranging from 254 and 365 nm used. And spots were developed by applying coloring technique. Potassium iodide and O-tolidine was applied, yellowish brown color was developed. Diameter was measured and then compared with the diameter of spots and analyzed the concentration [6]. UV at 254 nm for half an hour was used.

Results

Results that are obtained from samples analysis through HPTLC is given below in appendices.

Retention factor

Calculated retention factors are drawn from this following formula.

Distance travelled by the spot/distance travelled by the solvent

Retention factor of each pesticide remain same, it will never be changed. Different number of spotting was done to calculate the spot diameter and calculated the concentration.

Quantification of sample

Spot diameter versus concentration was measured to compare with the sample spot diameter to analyze for concentration of pesticides in vegetables samples (Tables 5-7). Concentration was calculated from the initial preparation of standards and spot diameter was measured after the development of plate. Results calculated from HPTLC for different vegetable samples collected from different markets are given in Table 8.

Confirmation of results

The extracted samples were analyzed by GC/MS (chromadex USA). From final results, it was concluded that minimum detectable quantity is variable for these chromatographic techniques. HPTLC can also used for quantitation and can provide reliable results.

Statistical analysis

By applying F test and least significant difference test, it was concluded that different vegetables possess different absorption rate pesticides. By applying least significant difference test, it was concluded that pumpkin, okra, eggplant, cucumber, spinach and cabbage.

Discussion

Pesticides are dangerous for human health if it exceed from the calculated maximum residue limit [4]. Due to spray of pesticides on vegetables, it leaves their residues [7]. It is studied that the pesticides become visible under UV and develop yellowish to brown color; this development procedure is also used by Asi et al. [6]. Detection of these pesticides in vegetable samples by using HPTLC is a convenient and cheap method. This method is also used for detection of pesticides is previously used by Khan et al. [4]. Retention factor of pesticides will never be changed, either it is previously determined or determine in future [8]. Imidacloprid and cypermethrin residues were also studied by Manzoor et al. [8], and it is concluded that cypermethrin was present in high rate. 79% vegetables were contaminated from cypermethrin, 70% contamination was of imidacloprid and 65% contamination of chlorpyrifos was determined. Results for the determination of uimidacloprid was somewhat have resemblance with the results of Obana et al. [7]. Results are indicating that cypermethrin possess a high absorption rate, as compared to other pesticides [9]. Detection of pesticides through HPTLC is a common process. Quantitation of pesticides in different vegetables in comparison with the standard is

Chlorpyrifos standard volume (µl)	Concentration (µg)	Average spot diameter (mm)
0	0	0
2 (1 spot)	20.6	2
4 (2 spots)	41.2	2.2
6 (3 spots)	61.8	2.4
8 (4 spots)	82.4	2.6
10 (5 spots)	103	2.8

Table 6: Spot diameter versus concentration for Chlorpyrifos.

Cypermethrin standard volume (µl)	Concentration (µg)	Average spot diameter (mm)
0	0	0
2 (1 spot)	21.6	2.3
4 (2 spots)	43.2	2.6
6 (3 spots)	64.8	2.8
8 (4 spots)	86.4	3
10 (5 spots)	108	3.2

Table 7: Spot diameter versus concentration for Cypermethrin.

Vegetables	Markets (lahore)	Pesticides mg/kg		
		Imidacloprid	Cypermethrin	Chlorpyrifos
Pumpkin	Site A	N.D	N.D	N.D
	Site B	0.125	N.D	0.076
	Site C	N.D	0.04	0.250
	Site D	N.D	0.132	0.030
	Site E	0.130	0.130	N.D
	Site F	0.120	N.D	0.090
Okra	Site A	0.070	0.126	N.D
	Site B	0.080	N.D	0.171
	Site C	0.160	0.075	N.D
	Site D	0.165	0.162	0.147
	Site E	0.124	N.D	0.047
	Site F	0.185	0.010	0.164
Egg plant	Site A	0.245	0.246	N.D
	Site B	0.153	0.075	N.D
	Site C	0.060	N.D	0.171
	Site D	N.D	0.165	0.167
	Site E	0.134	0.010	0.082
	Site F	0.060	0.078	0.264
Bitter gourd	Site A	0.161	0.137	0.075
	Site B	0.010	0.152	0.031
	Site C	N.D	N.D	0.170
	Site D	N.D	0.130	N.D
	Site E	N.D	0.015	N.D
	Site F	0.010	0.021	0.041
Bell pepper	Site A	0.050	0.160	N.D
	Site B	0.120	0.080	0.246
	Site C	0.053	0.170	0.167
	Site D	0.130	0.010	N.D
	Site E	N.D	0.052	0.036
	Site F	0.153	0.030	N.D
Turnip	Site A	0.035	0.052	0.045
	Site B	N.D	0.085	0.050
	Site C	0.136	N.D	0.071
	Site D	0.053	0.063	N.D
	Site E	0.030	N.D	0.058
	Site F	0.010	0.075	N.D
Spinach	Site A	0.240	0.153	N.D
	Site B	0.165	0.030	0.150
	Site C	N.D	0.140	0.010
	Site D	0.025	0.031	N.D
	Site E	0.052	0.010	0.173
	Site F	N.D	0.156	0.184
Cucumber	Site A	N.D	0.160	0.241
	Site B	0.151	0.080	0.020
	Site C	0.071	0.170	N.D
	Site D	0.160	0.010	0.250
	Site E	N.D	0.052	0.082
	Site F	0.050	0.030	N.D
Cabbage	Site A	0.020	0.052	0.060
	Site B	0.164	0.040	0.031
	Site C	N.D	0.140	0.052
	Site D	0.127	N.D	N.D
	Site E	N.D	0.071	0.075
	Site F	N.D	N.D	N.D
Cauliflower	Site A	0.145	0.064	N.D
	Site B	0.137	0.030	0.046
	Site C	N.D	0.164	N.D
	Site D	0.145	N.D	0.230
	Site E	0.050	N.D	0.063
	Site F	N.D	0.050	N.D

N.D indicates not detectable.

Table 8: Results of HPTLC.

easy to handle and cheap way. Standard curve drawn by concentration calculated from the standard solution, and the spot diameter after development. There are present many ways to quantify pesticides on HPTLC plate by observing the color intensity, but it is not for the new user of HPTLC. By digging that portion of HPTLC plat, then dissolve in methanol or acetone, and then observe under UV-visible spectrophotometer. One way is to compare the sample spot diameter with the diameter of standard and determine the concentration. It is an easy way to calculate the concentration in samples. This method of quantification of pesticides is used in this research project, same as used by the Asi et al. [6]. It was analyzed that pesticides residues are present in high concentration in leafy portion, as compared to the edible portion. If vegetables use after peeling, then less residues will be remained on vegetable, because upper portion contain and absorb maximum pesticides, and it protects the edible portion from contamination with pesticides [4]. Vegetables also possess different rate of inside leeching of pesticides. From this research project, it was concluded that pumpkin, okra, eggplant, cucumber, spinach and cabbage were contaminated from pesticides. Cucumber and okra contamination in Pakistan is also discussed by Khan et al. [4]. Residues somewhat vary, it may be due to the difference in collection area (personal observation). Peeling of cucumber resulted in less pesticides. Cucumber and eggplant results are indicating that pesticides are present; imidacloprid and cypermethrin are present in high concentration [7]. Okra contamination with different pesticides is common. High rate of absorption of pesticides in okra was determined. Okra is used without peeling, that is why risk of action of residues on human is high. According to this research study, okra contamination with different pesticides was recorded [10]. Eggplant contamination was also determined in this research. It was concluded from this research that imidacloprid and chlorpyrifos was present in eggplant, same as the pesticides residues determined by the Iqbal et al. [5].

Different methods can be used for determination and quantification of pesticides, but by using HPTLC, it is convenient and easy.

Conclusion

It is concluded from this research work that pesticides can be adsorb in pulpy portion of vegetables. But the rate of adsorption is different in different vegetables. Pesticides also leech in vegetables with different rate. If vegetables used after peeling the pesticide residues become in less concentration. Checking for pesticides residues in different vegetable samples should be done after regular intervals.

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