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MONITORING OF PESTICIDE RESIDUES IN VEGETABLES COLLECTED FROM MARKETS OF SINDH, PAKISTAN

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Abstract- Pesticide residues were determined in marketed samples of different vegetables. Samples were collected from different markets of southern Sindh during 2012-13. Residues were extracted with QuEChERS method and were analyzed with Gas Chromatography (GC) coupled with Mass Spectrometry Detector. However, emamectin benzoate and imidacloprid were analyzed with High Performance Liquid Chromatography (HPLC) coupled with Ultraviolet (UV) detector. The insecticide residues in vegetable samples were quantified by using the standards.

Results showed that seven vegetables namely okra, bitter gourd, brinjal, tomato, onion, cauliflower, and chilies, were heavily contaminated with chlorpyrifos, profenofos, endosulfan, imidacloprid, emamectin benzoate, lufenuron, bifenthrin, diafenthiuron, and cypermethrin. Moreover, every vegetable was contaminated with more than one pesticide and majority of samples violated the Japanese MRLs.

Desi spinach, lettuce, bottle gourd, fenugreek, peas, and cluster bean are not sprayed with pesticides normally, but these were found contaminated with trace level residues within MRL(s). This could be due to contaminated soil (from previous crops) and/or may be due to vegetable vendors' mishandling as they use the same water for washing vegetables in series which is a common practice all over Sindh.

Present study recommends that vegetables may be washed thoroughly prior to use and water may be changed after each vegetable wash or washing of vegetables may be done under running tap water in order to minimize pesticide contamination .

Keywords- Vegetables, pesticides, residues, QuEChERS, GC-MS, HPLC-UV.

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Introduction

In modern agricultural practices, the use of pesticides provides unquestionable benefits by increasing the production of crops. However, it has the drawback of pesticide residues which remain on the vegetables, constituting potential health risks to consumers. This on one hand leads to violation of the established legal directives to control their levels within the maximum residue levels (MRLs) and simultaneously continue to search for pesticides, which are less persistent and less toxic for human beings on the other [1].

Wide range of pesticides are used for crop protection globally during the cultivation of vegetables due to heavy pest infestation throughout the crop season [2], Literature reveals that vegetables contaminated with pesticide residues above their respective maximum residue limit MRL [3] may pose health hazards to consumers [4-5].

Pesticide application is an essential component of modern crop production technology. Their use has been contentiously increasing over the past decades. In Pakistan, the pesticides application is at maximum on cotton crop followed by fruits and vegetables. Insecticides, herbicides and fungicides are commonly used for crop protection throughout the country [6]. But the overdose of pesticides makes the residue problem, which might pollute our food and be harmful for our health. It has been reported that some of the pesticides are being used in the country where no pre-harvest time frame after last application is maintained [7]. As a result of indiscriminate use of pesticides by the unskilled persons, only a small portion of applied pesticides reaches the targeted species; remainder enters in food chain and is indirectly passed on to human beings. Amongst food items, fresh fruits are the most vulnerable part of the diet, as they are mostly consumed directly after picking as compared to vegetables and grains that are cooked which in turn reduces and metabolizes the pesticide residues [8].

Indiscriminate use of pesticides is very alarming in Pakistan, and they are used in excessive quantities which makes a major food safety concern of consumers and government. Hence, monitoring and assessment of pesticide contamination in farm produce has become a necessity. Particularly, there is need to determine, quantify and confirm pesticide residues in vegetables for both research and regulatory purposes. The pesticides are generally analyzed by spectrophotometry [9-10], thin layer chromatography (TLC) [11-13], high performance liquid chromatography (HPLC) and high performance liquid chromatography-mass spectrophotometry (HPLC-MS) [14-16], gas chromatography (GC) [17-20] and GC-MS [21].

There are many vegetables grown in Sindh province some are sprayed with pesticides (non-organic vegetables) that are more prone to insects pests while some are not sprayed (organic vegetables). In this paper we have studied six organic vegetables viz. lettuce, fenugreek, bottle gourd, peas, cluster beans, and desi spinach and seven non-organic vegetables viz. okra, bitter gourd, brinjal, tomato, onion, cauliflower and chilies for determination of pesticide residues. Therefore, the purpose of the present study is to assess the multi-pesticide residues assessability through Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) methods [22-23] from collected vegetables of the local markets of Southern Sindh.

Materials and Methods

Chemicals and Solvents

QuEChERS kits were purchased from the Company Restek Corporation USA. Sodium sulphate was procured from Merck India. Acetonitrile (HPLC grade) used in this study, was obtained from Scharlau Company (Scharlau chemie S.A. La Jota, Barcelona Spain) and were glass distilled before use. Pesticide Standards were supplied by M/S Ali Akbar Group (Pvt.), Ltd. Hyderabad. Silica gel 60 was purchased from Sigma-Aldrich.

Sampling

Thirteen different vegetables were selected for analysis were okra, bitter gourd, brinjal, tomato, onion, cauliflower, chilies, lettuce, bottle gourd, fenugreek, peas, cluster bean and desi spinach. Twenty samples of each vegetable were collected from various vegetable shops of Jamshoro and Tando Mohammad Khan metropolis, packed in polyethylene bags; labeled and transported in ice preserved packs to the laboratory of Institute of Food Sciences and Technology Sindh Agriculture University Tandojam. The samples were kept in freezer at -20°C till extraction.

Extraction

The QuEChERS sample preparation method for pesticides (AOAC Official Method 2007.01) was applied to all the samples. The sample was homogenized by Homogenizer by the addition of 1g sodium sulfate and 20ml of acetonitrile and 1g salt mixture. The homogenized sample was transferred to 50ml tube shaken vigorously for 3 min and centrifuged at 4000 rpm for 5 min. The supernatant (5ml) were transferred to a 15 ml PTFE tube to which 750 mg $MgSO_4$ and 250 mg PSA were added. The extract was shaken using a vortex mixer for 1 min and centrifuged at 4000 rpm again for 5 min. Supernatant was then filtered through a 0.45 mm PTFE filter (13 mm diameter) and transferred to 10ml vials and sealed for quantification using gas chromatograph equipped with mass spectrometry (GC-MS).

Analytical Technique

For analysis, Agilent (6890N) gas chromatograph system equipped with a model 7673 auto-sampler Mass Spectroscopy (GC/MS) was used. Residues were separated through Agilent DB-1 capillary column (30 m X 0.2 5mm with 0.1 µm film) with nitrogen flow rate 30 ml / minute, air flow rate 60 ml/minute was used under the following conditions.

The inlet was set at 250°C and the MS source at 250°C. The oven was programmed: 40°C, 1.5 min., 15°C/min., 150°C; 7°C/min., 225°C; 25°C/min., 290°C, 15 min with a constant column flow rate of 1 mL/min.

High Performance Liquid chromatography coupled with ultra violet (UV) detector was used for imdacloprid and emamectin benzoate residues determination [Table-1]. Separation was carried out on a Supelco LC-18 column (250mm× 4.6mm ID, 5µm) (Supelco Park, Bellefonte, USA). The mobile phase was acetonitrile and de-ionized water.

Table 1- HPLC parameters for determination of HPLC amenable pesticides (emamectin benzoate and imidacloprid residues).

Imidacloprid	Emamectin Benzoate
Flow rate = 1.2ml/min	Flow rate = 1.2ml/min
Ratio: Acetonitrile : Water (de-ionized) 35:65	Ratio: Acetonitrile : Water (de-ionized) 98:02:00
Wavelength = 270nm	Wavelength =246nm
Injection volume = 20µl	Injection volume =30 µl

Results and Discussion

Pesticides are widely used to increase the productivity of agricultural commodities and hence are essential component in modern agriculture. These chemicals are actually produced and/ or developed for agriculture pest control. Pesticides spray on vegetable crops is very common practice which not only kills insect/ pests but also stuck/ get inside the vegetables through minute pores thereby becoming its component. These are called pesticide residues that remain on the surface or inside of the vegetables and may become a great health hazard after consumption. Contamination of vegetables result from pesticide spray, as well as from improper handling, contaminated environment (air, soil or water) and from cross contamination processes.

The present study was undertaken to evaluate the pesticide residues from market samples of Southern Sindh, the pesticide compounds in collected vegetable samples were identified by comparing their retention time with respect to their technical grade reference standards.

In the present research work an attempt has been made to estimate multi-residues via QuEChERS method, the results indicate the presence of pesticide residues in different vegetables. Vegetable samples were analyzed in triplicate for the presence of pesticides residues [Table-2] and [Table-3].

Twenty samples of each six vegetable, that is, lettuce, fenugreek, bottle gourd, peas, cluster beans, and desi spinach which are usually organically grown (without pesticide spray) were collected for pesticide residue analysis. It was observed that all the six vegetables were found contaminated with trace amount of pesticide residues. Lettuce was found contaminated with trace amounts of endosulfan (0.05ppm), imidacloprid (0.11ppm) and bifenthrin (0.2ppm). Fenugreek was contaminated with endosulfan (0.13ppm), imidacloprid (0.11ppm) and bifenthrin (0.013ppm). Bottle gourd contained the residues of chlorpyrifos (0.005ppm), endosulfan (0.07ppm), imidacloprid (0.21ppm) and bifenthrin (0.05ppm).

Peas had chlorpyrifos (0.01ppm), endosulfan (0.09ppm), imidacloprid (0.13ppm) and bifenthrin (0.01ppm). Cluster beans contained endosulfan (0.04ppm), imidacloprid (0.31ppm) and bifenthrin (0.01ppm) and desi spinach had residues of chlorpyrifos (0.003ppm), endosulfan (1.02ppm), imidacloprid (0.65ppm) and bifenthrin (0.07ppm).

Percent contamination and maximum residues were also calculated for each vegetable and results revealed that percent contamination in lettuce of endosulfan was 10% (0.1 max residues), imidacloprid

35% (0.51max residues) and bifenthrin was 30% (0.45 max residues). In fenugreek endosulfan was 25% (0.22 max residues), imidacloprid 35% (0.19 max residues) and bifenthrin was 30% (0.42 max residues). In bottle gourd chlorpyrifos 40% (0.008 max residues), endosulfan 35% (0.13 max residues), imidacloprid 30% (0.49 max residues) and bifenthrin was 35% (0.15 max residues). In peas chlorpyrifos 30% (0.045 max residues), endosulfan 25% (0.17 max residues), imidacloprid 25% (0.34 max residues) and bifenthrin was 20% (0.04 max residues). In cluster beans endosulfan 15% (0.05 max residues), imidacloprid 20% (0.8 max residues) and bifenthrin was 10% (0.03 max residues) and in desi Spinach chlorpyrifos 25% (0.007 max residues), endosulfan 55% (1.28 max residues), imidacloprid 35% (1.2 max residues) and bifenthrin was 35% (0.13 max residues).

Although these vegetables are organically grown but our findings [Table-2] showed that these vegetables had trace amount of residues which may be due to the reason that the retailers contaminate these vegetables by washing with same water which they use for washing heavily sprayed vegetables. The other possible reason for contamination of organic vegetables may be growing these vegetables on the soil contaminated from previous crop. This is also in conformity with Hill [25] who proposed that the fruits are usually mixed in the lots in trade, the residue data from these composite samples were therefore, potentially misleading.

Table 2- Pesticide residues (ppm) in organic vegetables

Pesticides	Parameter	Lettuce	Fenugreek	Bottlegourd	Peas	Cluster Beans	Desi Spinach
chlorpyrifos	Residue found	ND	ND	0.005	0.01	ND	0.003
	mrl	ND	ND	0.01	0.05	ND	0.01
	% + ve (%violate)	ND	ND	40- ND	30- ND	ND	25- ND
	Max - min	ND	ND	0.008- ND	0.045- ND	ND	0.007- ND
Endosulfan	Residue found	0.05	0.13	0.07	0.09	0.04	1.02
	mrl	1	0.5	0.5	0.5	0.5	2
	% + ve (%violate)	10- ND	25- ND	35- ND	25- ND	15- ND	55- ND
	Max - min	0.1- ND	0.22- ND	0.13- ND	0.17- ND	0.05- ND	1.28- ND
Imidacloprid	Residue found	0.11	0.06	0.21	0.13	0.31	0.65
	mrl	3	5	1	3	3	15
	% + ve (%violate)	35-ND	35--ND	30- ND	25- ND	20- ND	35- ND
	Max - min	0.51- ND	0.19- ND	0.49- ND	0.34- ND	0.8- ND	1.2- ND
Bifenthrin	Residue found	0.2	0.013	0.05	0.01	0.01	0.07
	mrl	3	2	0.4	0.05	0.2	0.2
	% + ve (%violate)	30-ND	30-ND	35-ND	20-ND	10-ND	35-ND
	Max- min	0.45-ND	0.42-ND	0.15-ND	0.04-ND	0.03-ND	0.13-ND

() = MRLs ppm; ND = Non Detected   Note: 20 samples were analyzed for each vegetable

For each pesticide 1st row shows residue detected in ppm, 2nd row shows Japanese MRL[1], 3rd row shows percent samples found positive, 4th row shows percent samples violating MRL.

Similarly, twenty samples of each seven non-organic vegetables namely okra, bitter gourd, brinjal, tomato, onion, cauliflower and chilies were also purchased from markets for pesticide residues determination. [Table-3] shows that nine pesticides, that is, chlorpyrifos, profenofos, endosulfan, imidacloprid, emamectin benzoate, lufenuron, bifenthrin, diafenthiuron and cypermethrin, which are most commonly used on different vegetables, were detected and majority of them were found with residues above their respective MRLs. Results [Table-3] revealed that okra contained the residues of chlorpyrifos (0.09ppm), endosulfan (0.93ppm), imidacloprid (0.29ppm) and bifenthrin (0.031ppm). Bitter gourd was found with residues of endosulfan (0.28ppm), imidacloprid (0.38ppm), emamectin benzoate (0.06ppm), diafenthiuron (0.015ppm) and cypermethrin (0.022ppm). Brinjal was contaminated with endosulfan (0.33ppm), imidacloprid (0.74ppm), emamectin benzoate (0.04ppm) and diafenthiuron (0.017ppm). Tomato had residues of endosulfan (0.35ppm), imidacloprid (0.82ppm), bifenthrin (0.26ppm) and diafenthiuron (0.008ppm). Onion was contaminated with profenofos (0.046ppm), endosulfan (0.17ppm) and imidacloprid (0.15ppm). Cauliflower was found contaminated with chlorpyrifos (0.04ppm), endosulfan (0.25ppm), imidacloprid (0.25ppm), emamectin benzoate (0.11ppm) and cypermethrin (0.58ppm). Chilies were contaminated with endosulfan (0.22ppm), emamectin benzoate (0.07ppm), lufenuron (0.37ppm) and cypermethrin (0.29ppm).

Percent contamination ratio and max residues with percent violated samples were also determined of each vegetable. It was noted that contamination of okra was 60% with chlorpyrifos and 25% samples violated the MRLs (0.13 max residues), 55% with endosulfan and 25% samples violated the MRLs (1.5 max residues), 35% with imidacloprid and 25% samples violated the MRLs (0.74 max residues) and 35% with bifenthrin and 25% samples violated the MRLs (0.098 max residues). In the case of bitter gourd 35% contained endosulfan and 15% samples violated the MRLs (0.57 max residues), 45% with imidacloprid and 20% samples violated the MRLs (1.32 max residues), 35% with emamectin benzoate and 10% samples violated the MRLs (0.14 max residues), 56% with diafenthiuron and 30% samples violated the MRLs (0.05 max residues) and 30% in cypermethrin with no samples violated the MRLs (0.08 max residues). Similarly, for brinjal 40% with endosulfan and 15% samples violated the MRLs (0.59 max residues), 30% with imidacloprid and 15% samples violated the MRLs (2.65 max residues), 15% with emamectin benzoate with no samples violated the MRLs (0.07 max residues), and 45% with diafenthiuron and 35% samples violated the MRLs (0.03 max residues). 25% tomato samples had endosulfan and 10% violated the MRLs (0.68 max residues), 40% had imidacloprid and 10% violated the MRLs (2.12 max residues), 30% had bifenthrin and 15% violated the MRLs (0.66 max residues) and 10% had diafenthiuron and 10% violated the MRLs (0.03 max residues).

Onion grows underground and is prone to accumulate more pesticide residues and 20% samples were polluted with profenofos and 10% violated the MRLs (0.07 max residues), 35% with endosulfan and 10% violated the MRLs (0.42 max residues), 35% with imidacloprid and 10% violated the MRLs (0.5 max residues). Cauliflower whose leaves have waxy layer to roll of spray droplets and its 25% samples had chlorpyrifos and 10% violated the MRLs (0.06 max residues), 35% had endosulfan and 15% violated the MRLs (0.53 max residues), 45% had imidacloprid and 15% violated the MRLs (0.55 max residues), 30% had emamectin benzoate and 10% violated the MRLs (0.92 max residues) and 25% had cypermethrin and 10% violated the MRLs (1.67 max residues; whereas 45% chilies samples were contaminated with endosulfan and 15% violated the MRLs (0.57 max residues), 25% with emamectin benzoate and 5% violated the MRLs (0.28 max residues), 35% with lufenuron and 10% violated the MRLs (0.67 max residues) and 35% with cypermethrin and 20% violated MRLs (0.94 max residues).

Out of 13 different vegetable samples collected from Jamshoro and Tando Muhammad Khan markets, it was noted that organochlorine, organophosphate, nicotinoid and pyrethroid pesticides are most commonly used in Sindh and found frequently in vegetable samples collected from markets with the exception of lufenuron (insect growth regulator) which was found only in brinjal, tomato, chilies and lettuce.

Majority of the samples violated MRLs and these results are in conformity with the findings of earlier study [24] in which fruit samples of Karachi market were taken and most of the samples were found contaminated with multiple pesticide residues.

The persistent nature of different pesticides, mishandling, environmental pollution and presence of pesticide residues in vegetables has now become a global concern. Organophosphorous, organochlorine and nicotinoid pesticides, along with mixture of different pesticides in fruits and vegetables were also reported all over the world by many researchers [26-34].

Table 3- Pesticide residues (ppm) in non-organic vegetables

Pesticides	Parameters	Okra	Bittergourd	Brinjal	Tomato	Onion	Cauliflower	Chilies
Chlorpyrifos	Residues Found	0.09	ND	ND	ND	ND	0.04	ND
	mrl	0.1	ND	ND	ND	ND	0.05	ND
	% + ve (%violat)	60 (25)	ND	ND	ND	ND	25(10)	ND
	Max - min	0.13- ND	ND	ND	ND	ND	0.06-ND	ND
Profenofos	Residues Found	ND	ND	ND	ND	0.046	ND	ND
	mrl	ND	ND	ND	ND	0.05	ND	ND
	% + ve (%violat)	ND	ND	ND	ND	20(10)	ND	ND
	Max-min	ND	ND	ND	ND	0.07-ND	ND	ND
Enosulfan	Residues Found	0.93	0.28	0.33	0.35	0.17	0.25	0.22
	mrl	1	0.5	0.5	0.5	0.2	0.5	0.5
	% + ve (%violat)	55(25)	35(15)	40(15)	25(10)	35(10)	35(15)	45(15)
	Max-min	1.5-ND	0.57-ND	0.59-ND	0.68-ND	0.42-ND	0.53-ND	0.57-ND
Imidacloprid	Residues Found	0.29	0.38	0.74	0.82	0.15	0.25	ND
	mrl	0.5	1	2	2	0.2	0.4	ND
	% + ve (%violat)	35(25)	45(20)	30(15)	40(10)	35(10)	45(15)	ND
	Max-min	0.74-ND	1.32-ND	2.65-ND	2.12-ND	0.5-ND	0.55-ND	ND
Emamectin Benzoate	Residues Found	ND	0.06	0.04	ND	ND	0.11	0.07
	mrl	ND	0.1	0.1	ND	ND	0.5	0.2
	% + ve (%violat)	ND	35(10)	15-ND	ND	ND	30(10)	25(5)
	Max-min	ND	0.14-ND	0.07-ND	ND	ND	0.92-ND	0.28-ND
Lufenuron	Residues Found	ND	ND	ND	ND	ND	ND	0.37
	mrl	ND	ND	ND	ND	ND	ND	0.5
	% + ve (%violat)	ND	ND	ND	ND	ND	ND	35(10)
	Max-min	ND	ND	ND	ND	ND	ND	0.67-ND
Bifenthrin	Residues Found	0.031	ND	ND	0.26	ND	ND	ND
	mrl	0.04	ND	ND	0.5	ND	ND	ND
	% + ve (%violat)	35(25)	ND	ND	30(15)	ND	ND	ND
	Max-min	0.098-ND	ND	ND	0.66-ND	ND	ND	ND
Diafenthiuron	Residues Found	ND	0.015	0.017	0.008	ND	ND	ND
	mrl	ND	0.02	0.02	0.01	ND	ND	ND
	% + ve (%violat)	ND	56(30)	45(35)	10(10)	ND	ND	ND
	Max-min	ND	0.05-ND	0.03-ND	0.03-ND	ND	ND	ND
Cypermethrin	Residues Found	ND	0.022	ND	ND	ND	0.58	0.29
	mrl	ND	0.05	ND	ND	ND	1	0.5
	% + ve (%violat)	ND	30-ND	ND	ND	ND	25(10)	35(20)
	Max-min	ND	0.08-ND	ND	ND	ND	1.67-ND	0.94-ND

() = MRLs ppm; ND = Non Detected; Note: 10 samples were analyzed for each vegetable

For each pesticide 1st row shows residue detected in ppm, 2nd row shows Japanese MRL[1], 3rd row shows percent samples found positive, 4th row shows percent samples violating MRL.

Organochlorine (Endosulfan) pesticide is banned due to its highly toxic and persistent nature but unfortunately it is still used by local farmers of Sindh. Results [Tables-2] and [Table-3] showed that all the 13 vegetable samples were contaminated with toxicant endosulfan. Similarly, organophosphate poisoning cases are also global health problem with annually 0.2 million deaths, because of their direct effect on Central Nervous System, cardiovascular system and reproductive system [35]. Chiron, et al [36] also reported the toxic effects of carbamates and declared them as potential environmental pollutants.

The observations of different compounds [Table-2] and [Table-3] were compared with recommended Maximum residue limits (MRLs). The comparison of results with their respective MRLs have led to an insight which suggests that majority of vegetables had residual levels far above the MRLs, hence were unfit for human consumption. It was further observed that same water was being used for washing of different vegetables which increased the contamination ratio.

Conclusion

It was concluded that pesticide spray is most common practice in Lower Sindh and single vegetable was found with more than one pesticide with residual level above mentioned MRLs. Present study recommends that vegetable may be thoroughly washed prior to use and water may be changed after each vegetable wash or washing of vegetables may be done under running tap water in order to minimize pesticide contamination ratio. The study further recommends that.

Conflict of Interest : None Declared

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