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MONITORING OF MULTIPLE PESTICIDE RESIDUES IN SOME FRUITS IN KARACHI, PAKISTAN

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Abstract

One hundred and twenty samples of different fruits including apple, apricot, persimmon, chiku, citrus, grapes, guava, mango, papaya, peach, plum and pomegranate procured from different selling points of Karachi, Pakistan during 2008-2009, were analyzed for monitoring of multiple pesticide residues using GC/FID and HPLC/UV. The results showed exceeding level of contamination. On an average 62.5% samples contained residues of pesticides while 22% samples exceeded the maximum residue limits (MRLs) as given by FAO/WHO (Anon., 2000).

Introduction

Analysis of pesticide residues in food products is gaining momentum due to consumer demands for safe food and to serve the trade related obligations. There are increasing concerns about the potential of pesticides as harmful agents to human health and non-target populations (Chitanat *et al*., 2008). Pesticide residues may enter the food chain causing serious hazards to human and animal lives (Khan *et al*., 2007). Despite the fact that pesticides are used with the aim of controlling insects, diseases, fungi and other pests along with their other beneficial effect, leave trace amounts of residues on fruits and vegetables (Basa Cesnik *et al*., 2007; Anwar *et al*., 2011).

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 maximum on cotton crop followed by fruits and vegetables. Insecticides, herbicides and fungicides are commonly used for crop protection throughout the country. However, among all categories, insecticides are used more frequently. After ban, over the use of organochlorine insecticides in 80's (Parveen & Masud 2003), organophosphates, synthetic pyrethroids and carbamates are presently in use with less persistence. Selectively, with minimum mammalian toxicity to minimize the pesticide residue levels in the agricultural produce. The Integrated Pest Management (IPM) strategies, biological control techniques and pest resistant varieties are utilized on limited scale and chemical control is still the most preferred technique to mitigate the pest problem throughout the country. 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 (Newsom *et al*., 2000).

Primary factors - affecting the accumulation of pesticide residues in food include the frequency of pesticide application, quantity of crop that receives pesticides, and pesticide application intervals from treatment to harvest (Suhre, 2000). Residue levels may be reduced to some extent by manipulating the above factor(s) but storage and processing also reduces the levels of such poisons.

Several countries have initiated residue reserved projects to evaluate the extent of danger (if any) and health of their food. Grain Quality Testing Laboratory of Pakistan Agricultural Research Council (PARC) has a mandate to monitor the pesticide residues in foods. In this regard a study was carried out to evaluate the multiple pesticide residues in fruits procured from retail markets of Karachi during 2008-2009.

Materials and Methods

For the analysis of samples, analytical reagent grade chemicals were used and all the solvents were redistilled in a glass system before use. One hundred-twenty samples of different fruits were procured from different selling points of Karachi during 2008-2009. One kilogram sample of each commodity was purchased as per standard procedure of FAO/WHO (Anon., 1993). All the samples were sliced or peeled off and homogenized. Sub-samples were taken for extraction that was undertaken on the same day of sub-sampling. Extraction was carried out on the same day as the sampling was done. Samples were analyzed in accordance with described procedures (Parveen & Masud, 2002). Each sample (30g) was extracted with 75ml of extraction mixture (Toulene + n-Hexane + Ethylacetate) in the ratio of 3:1:1. The decanted extract was concentrated to approximately 2-3ml and poured on a mini column of Florisil plus activated charcoal and eluate was collected for each sample. The cleaned up extract was evaporated to dryness in a rotary vacuum evaporator and taken up in 2ml acetone for Gas Chromatographic determination. For High Performance Liquid Chromatographic determination, evaporated dry residues were taken up in 2ml methanol (HPLC grade).

For the instrumental analysis, a Shimadzu Model LC-10AT High Performance Liquid Chromatograph equipped with Deuterium Lamp with chargeable wavelength and Agilent Gas Chromatography (GC) Model 6890N Network GC System equipped with detector – FID, were used. Light Source: Deuterium Lamp with changeable wavelength, wavelength: 223nm and 254nm, Pressure: 200psi, Column: Stainless steel column packed with C-18 5µm 25cm x 0.46cm, Injection volume: 20µl, Mobile Phase: Methanol + Water (3:1) with a flow rate: 0.5ml min^{-1}, were used as instrumental parameters for HPLC.

Column: Capillary Column HP5-MS (fused silica capillary) i.d.: 0.25mm, length: 30m, Column temperature: 230ºC, Injector temperature: 250ºC, Detector temperature: 300ºC, Attenuation: 12, Nitrogen flow rate: 13ml min-1, Hydrogen flow rate: 4.5ml min-1 and Air flow rate: 175ml min-1 were used as instrumental parameters for GC.

Results and Discussion

Fourteen types of fruits analyzed in this study consist of the main part of dietary intakes of Pakistani people. The pesticides targeted, were related on basis of their frequency of application. One hundred and twenty samples of fruits were screened for 25 different pesticides during 2008-2009 (Tables 1 & 3). The results indicate the presence of pesticide residues. About 62% samples contained residues of pesticides while 22% samples exceeded the MRLs. These results are in conformity with the finding of our earlier study (Parveen *et al*., 2005) in which vegetables samples of Karachi market were taken and most of the samples were found contaminated with multiple pesticide residues. These excess of contamination at such a level needs advocating good agricultural practices. Presence of pesticide residues up to 54% in fruits in Denmark (Andersen & Poulsen 2001), 36.6% in Slovenia (Basa Cesnik *et al*., 2007), 24.5% in Brazil (Araujo *et al*., 2001) and 49% for domestic and 78% in imported food products in Japan (Akiyama *et al*., 2002) have been reported but the violation of MRLs are very low 4%, 6.4%, 3% and less than 0.5% in Denmark, Slovenia, Brazil and Japan respectively. In India organochlorine pesticides i.e., HCH, DDT and endosulfan were detected in almost all the fruits samples of ber, grapes and guava (Madan & Kathpal, 2006).

Table 1. Multiple pesticide residues detected in different fruits.

S. No.	Pesticides	Fruit														
		Apple	Apricot	Persimmon	Banana	Chiku	Citrus	Grapes	Guava	Mango	Melon	Papaya	Peach	Plum	Pomegranate	Total
	Organochlorine															
1.	Endosulfan	1	ND	1	1	ND	2	ND	2	2	ND	ND	1	ND	ND	10
	Organophosphorus															
2.	Chlorpyrifos	3	ND	ND	ND	ND	ND	ND	ND	1	4	1	1	ND	ND	10
3.	Dimethoate	1	ND	ND	ND	1	1	1	1	1	ND	ND	ND	ND	1	7
4.	Ethion	11	ND	ND	3	ND	1	ND	ND	ND	ND	ND	ND	ND	1	16
5.	Fenitrothion	ND	ND	ND	4	ND	1	ND	ND	ND	ND	ND	ND	1	ND	6
6.	Malathion	ND	ND	ND	1	1	ND	ND	ND	ND	ND	ND	ND	ND	1	3
7.	Methamidophos	2	1	1	2	ND	2	2	4	2	2	ND	2	1	1	22
8.	Methylparathion	1	ND	ND	ND	ND	2	1	ND	ND	ND	ND	ND	ND	ND	4
9.	Monocrotophos	1	ND	ND	ND	ND	ND	1	ND	ND	ND	ND	ND	ND	ND	2
10.	Profenofos	1	ND	1	ND	ND	ND	ND	1	2	3	3	ND	ND	ND	11
11.	Quinalphos	1	ND	ND	ND	ND	ND	ND	ND	ND	1	ND	ND	ND	ND	2
	Synthetic Pyrethroids															
12.	Bifenthrin	5	ND	2	ND	1	3	ND	ND	1	4	ND	ND	1	ND	17
13.	Cyhalothrin	4	1	ND	2	1	3	ND	1	1	2	ND	2	2	1	20
14.	Cypermethrin	7	2	ND	2	1	ND	1	ND	1	2	4	3	2	ND	25
15.	Cyfluthrin	4	ND	ND	ND	ND	2	ND	ND	ND	ND	ND	ND	ND	ND	6
16.	Deltamethrin	1	1	ND	1	ND	ND	1	1	3	ND	ND	ND	ND	ND	8
17.	Fenpropathrin	2	ND	1	ND	ND	ND	ND	3	ND	ND	ND	ND	ND	ND	6
18.	Fenvalerate	9	1	ND	4	ND	2	ND	1	ND	ND	ND	1	ND	ND	18
19.	Permethrin	ND	ND	ND	ND	2	ND	1	ND	ND	ND	ND	ND	ND	ND	3
	Carbamates															
20.	Carbofuran	3	ND	ND	ND	ND	5	ND	1	ND	6	1	ND	ND	ND	16
21.	Carbosulfan	ND	ND	1	4	ND	1	ND	ND	ND	ND	ND	2	ND	ND	8
	Fungicides															
22.	Benomyl	1	ND	1	ND	3	2	ND	2	ND	ND	ND	1	1	ND	11
23.	Metalaxyl	3	1	ND	5	ND	1	ND	ND	ND	3	2	3	1	1	20
24.	Thiabendazole	2	ND	ND	ND	1	ND	ND	ND	ND	2	ND	ND	1	1	7

ND: Not Detected

Table 2. Statistical data of multiple pesticide residues in fruits.

S. #	Pesticide	n*	Range (ppm)		Mean	Median	SD**	CV***
			Min	Max				
	Organochlorine							
1.	Endosulfan	10	Traces	13.3	2.18	0.46	8.75	209.30
	Organophosphorus							
2.	Chlorpyrifos	10	0.01	3.04	0.66	0.40	0.81	123.35
3.	Dimethoate	7	0.09	91.30	15.39	1.95	38.02	247.07
4.	Ethion	16	Traces	24.90	2.75	0.13	6.02	218.79
5.	Fenitrothion	6	0.16	4.90	2.39	2.21	1.59	66.83
6.	Malathion	3	9.68	60.80	26.93	10.31	23.95	88.93
7.	Methamidophos	22	Traces	127.00	14.45	2.91	8.93	131.00
8.	Methylparathion	4	0.17	1.42	0.67	0.55	0.53	79.50
9.	Monocrotophos	2	0.18	5.50	2.84	0.53	2.65	93.60
10.	Profenofos	12	0.04	15.60	3.30	0.65	5.74	174.20
11	Quinalphos	2	0.32	0.90	0.61	ND	0.29	47.50
	Synthetic Pyrethroids							
12.	Bifenthrin	17	Traces	41.60	5.82	1.90	11.33	194.60
13.	Delta-Cyhalothrin	21	0.04	5.90	1.35	0.20	3.53	261.11
14.	Cypermethrin	26	0.08	20.67	1.44	0.80	4.24	189.40
15.	Cyfluthrin	7	0.32	19.00	5.29	1.89	6.37	120.40
16.	Deltamethrin	7	0.01	0.27	0.08	0.04	0.09	115.74
17.	Fenpropathrin	7	0.07	8.60	1.94	0.78	2.99	153.90
18.	Fenvalerate	18	0.01	57.50	16.09	2.08	37.63	233.89
19.	Permethrin	3	0.01	0.16	0.09	0.10	0.08	87.45
	Carbamates							
20.	Carbofuran	16	Traces	32.00	8.50	7.07	10.29	121.06
21.	Carbosulfan	8	0.01	51.20	8.28	0.26	16.75	202.40
	Fungicides							
22.	Benomyl	11	0.04	11.21	2.45	1.01	3.30	134.57
23.	Metalaxyl	20	0.04	13.10	2.54	0.80	3.93	155.03
24.	Thiabendazole	7	0.01	1.07	0.32	0.05	0.41	128.80

* = Total number of samples, ** = Standard Deviation, *** = Coefficient of Variation

Table 3. Fruit samples contaminated with pesticide residues and exceeding MRLs.

S. No.	Fruits	n*	No. of samples contaminated	% of contaminated samples	No. of samples exceeding MRLs	% of samples exceeding MRLs
1.	Apple	21	17	86	6	28
2.	Apricot	5	2	40	1	20
3.	Persimmon	5	3	60	2	40
4.	Banana	16	8	50	3	18
5.	Chiku	5	3	60	1	20
6.	Citrus	10	7	70	2	20
7.	Grapes	5	2	40	1	20
8.	Guava	11	5	45	2	18
9.	Mango	5	4	80	-	0
10.	Melon	20	11	55	3	15
11.	Papaya	4	4	100	1	25
12.	Peach	5	5	100	2	40
13.	Plum	5	2	40	2	40
14.	Pomegranate	3	2	66	1	33
	Total	**120**	**75**	**62.5**	**27**	**22**

* Total number of samples

Statistical analysis of data (Table 2) indicates much variation among several samples while the standard deviation also indicates the most of observations remain very much diverse from mean value. Analysis of composite samples gives overestimates as a lot of variation exists within individual fruits. Residues in individual roots of carrot vary up to 25 times (Harris, 2000), when the fruits are mixed in the lots in trade, the residue data from these composite samples are potentially misleading (Hill, 2000).

Being highly persistent organochlorine insecticides are banned in Pakistan. But Endosulfan is still on registration list. Ten samples were found to contain this toxicant in the range of 0.018-13.3 ppm with an average value of 2.18 ppm.

Organophosphate insecticide poisoning is a global health problem with approximately 3 million poisoning and 0.2 million deaths annually; these are irreversible inhibitors of acetylcholinesterase affecting the CNS (Central Nervous System), cardiovascular system and reproductive system producing wide range of effects (Aardema *et al*., 2008).

Fruit samples were screened for 11 different organophosphates commonly used for pest control in Pakistan. Methamidophos was the most dominant brand followed by Ethion and Profenofos and other brands in relatively less frequency. General tendency was Methamidophos> Ethion> Profenofos> Chlorpyrifos> Dimethoate> Fenitrothion> Methyl parathion. Persistence studies confirm that the Methyl parathion; Methamidophos and Monocrotophos have high persistence.

Pyrethroids are relatively less persistent, their residues in plant products are not as high as those of organophosphates because they hydrolyze into alcohol and acid moieties which link to glucose and other long chain polysaccharides resulting in decline of parent compounds but their conjugates and metabolites persist longer (Mortimer & Shields, 1995).

Fruits samples were screened for 8 different synthetic pyrethroids. As compared to organophosphates these insecticides were more frequently detected. General trend was Cypermethrin> λ-Cyhalothrin> Fenvalerate > Bifenthrin> Deltamethrin> Cyfluthrin and Fenpropathrin> Permethrin.

Like organophosphates, carbamates are also inhibitors of acetylecholinesterase enzyme system but due to their high mammalian toxicity and even greater toxicity of their transformation products, carbamates are potential environmental concern (Chiron *et al*., 1995). Eighty % and 37% samples were contaminated with carbofuran and carbosulfan respectively that exceeded MRLs.

Although fungicide application is not so common in plant protection strategies yet few fungicides are applied in the field for controlling diseases or in post-harvest protection against fruit rots. Metalaxyl, Benomyl and Thiabendazole were detected in descending order, Metalaxyl was found to exceed MRLs while Thiabendazole was found within the safe limit.

Conclusion

Pesticide usage in Pakistan is rapidly increasing and cases of misuse or over-use of pesticides are simultaneously on the increase. In the backdrop of such a situation, it is essential to impart proper education to the farming community about hazards involved in the misuse of toxic/persistent pesticides and formulation of laws and their enforcement. In this way, risks to human beings, livestock, poultry etc., can be minimized considerably. Furthermore, periodical monitoring of food commodities for pesticides is essential to assess the level of their contamination.

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