



Monitoring and risk assessment of pesticide residues in agricultural / horticultural commodities

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ABSTRACT: A study on monitoring and risk assessment of pesticide residues in agricultural/horticultural commodities revealed that out of 33 samples detected with pesticides, 22 samples showed presence of multiple pesticides and most of these were not having label claim/approval for use in India by CIB&RC in that specific commodity. Chlorpyrifos was the most frequently detected insecticide followed by profenophos. None of the detected pesticides in commodities monitored during the study period resulted in an intake of >50 per cent of ARfD value which indicated that their consumption does not cause acute health risk. Among the different agricultural/horticultural commodities like cardamom, cumin seed and curry leaf, the highest detected level of pesticides viz., lambda cyhalothrin and ethion in cardamom, profenophos in cumin seed and chlorpyrifos, profenophos and ethion in curry leaf exceeded 4 per cent of ADI value, which was considered as a margin indicating chronic health risk. Among the different pesticides studied, profenophos was present in levels of the ADI which represented a high level of chronic health risk to consumers. The results call for an investigation into the levels of pesticide residues in cardamom, cumin seed, curry leaf and for tighter regulation and regular monitoring by government and industry.

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Key words: Agricultural/horticultural commodities, pesticide, residues, risk assessment

INTRODUCTION

Pesticides are used globally for the protection of food, fibre, feed and human health. If the

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credits of pesticides include enhanced economic potential in terms of increased production of food and fibre and amelioration of vector borne diseases, their debits have resulted in serious health implications to man and his environment. In India, where meeting food demand is a big challenge, use of chemicals like pesticides, antibiotics and fertilizers are unavoidable inputs to ensure a sustained production of food grain to meet the increasing demand.

Food and health authorities around the world are continuously monitoring pesticide residues in different agricultural commodities by setting Maximum Residue Levels (MRLs) of pesticide in foods. The results of monitoring studies focus on the proper use and exact concentration of pesticides. MRLs encourage food safety by restricting the concentration of a pesticide residue permitted on a commodity (Claeys *et al.*, 2011).

Among the different sources of exposure to pesticides, food appears to be the most significant as pesticide residues were constantly detected in some of the raw agricultural commodities (Mathew *et al.*, 2012). Therefore, assessing the risk of pesticide residues in agricultural commodities intended for human consumption is necessary. The potential health risks from acute and chronic dietary exposure to pesticides can be assessed by comparing the daily intake with the toxicological reference dose *ie.* Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD) (WHO, 1997).

The aim of the present study is to monitor the presence of residues of pesticides (organochlorines, organophosphates and synthetic pyrethroid group) commonly used on agricultural commodities. The results of the monitoring data in combination with food consumption data were taken into consideration to evaluate whether the ADI and ARfD of pesticides through the consumption of agricultural commodities is a cause of toxicological concern according to the recommended dose by the Food and Agriculture Organization (FAO) and World Health Organization (WHO).

MATERIALS AND METHODS

Sampling

One sample each of agricultural commodities like parboiled rice, raw rice and basmathi rice, branded rice flour, wheat, atta and maida (one kilogram each), cardamom and cumin seed (500 g each), capsicum, okra and curry leaf (2 kilogram each) were collected from Thiruvananthapuram district at monthly intervals for a period of six months (January 2012 - June 2012). Samples were analyzed within 24 hr and stored at 4°C until the moment of extraction.

Chemicals

Certified Reference Materials (CRM) of different pesticides used in the present study having purity ranging from 95.10 to 99.99 per cent were purchased from M/s Sigma Aldrich and stored in a freezer at low temperature, with light and moisture excluded. Standard stock solutions

were made by dissolving each analytical standard in distilled acetone and diluted with n-hexane: toluene (1:1) to obtain a stock solution of 1000 mg L⁻¹. The stock, intermediate standards and working standard solutions were prepared and stored at “20°C until analysis.

Analytical procedure

The whole quantity of each commodity is blended and a representative sample of 25 g (parboiled rice, raw rice, basmathi rice, wheat, leaflets removed from curry leaf, capsicum and okra), 5 g (rice flour, atta and maida) and 8 g (cardamom and cumin) were analyzed for the presence of pesticide residues following QuEChERS method.

a. Rice and wheat grains

Twenty five gram of coarsely ground samples of rice and wheat grain were taken in 200 ml centrifuge. To this, 25 ml distilled water and 50 ml acetonitrile was added and the mixture was placed on a mechanical shaker for 30 min at 1200 strokes min⁻¹. A total of 12-15 g of activated sodium chloride was added. After centrifuging at 2500 rpm for 4 min, 16 ml supernatant was transferred into a 50 ml centrifuge tube containing 2.0 g sodium sulphate and 2.0 g magnesium sulphate and vortexed at full speed for 30 s and then centrifuged for 5 min at 2500 rpm. Twelve ml of upper organic phase was transferred to another 15 ml centrifuge tube containing 0.75 g and 0.10 g each of anhydrous magnesium sulphate and Primary Secondary Amine (PSA) respectively. After centrifuging at 2500 rpm for 5 min, an aliquot of 4.0 ml supernatant was concentrated using Turbovap (50°C) and final volume was made up to one ml using n-hexane and analyzed by Gas Chromatograph.

b. Rice flour, atta and maida

Five gram of rice flour, atta and maida samples were taken in 50 ml centrifuge tubes in four replicates each and soaked in 10 ml of water for 10 min. Then, the samples were extracted using 15 ml acetonitrile in a 50 ml centrifuge tube with 150 µL of acetic acid. Subsequently, 6.0 g anhydrous magnesium sulphate and 1.5 g sodium acetate were added, immediately shaken for one min and then the extract was centrifuged at 1500 rpm for 5 min. Ten ml of the upper layer was transferred to a 15 ml centrifuge tube containing 500 mg of Primary Secondary Amine (PSA) and 1.5 g of anhydrous magnesium sulphate. The centrifuge tube was shaken for 30 seconds followed by centrifugation for one min at 1500 rpm. Six ml from the upper layer was taken and concentrated to dryness using Turbovap (50°C) and final volume was made up to one ml using n-hexane and analyzed by GC.

c. Cardamom and Cumin seed

Eight gram of coarsely ground cardamom and cumin seed samples taken in 50 ml centrifuge tubes To this, 4.0 g activated magnesium sulphate and 1.0 g sodium chloride were added. Then 10 ml of chilled distilled water (4°C) and 15 ml of acetonitrile were added and the samples

were shaken for one min in a vortex and centrifuged at 3500 rpm for 2 min. A dispersive solid phase extraction cleanup process was carried out by transferring the supernatant (6.0 ml) to a centrifuge tube (15 ml) containing 1.0 g magnesium sulphate (hydrated) and 0.30 g PSA (Primary Secondary Amine) and 0.50 g florisil. These tubes containing the supernatant and the reagents were shaken for a few seconds followed by centrifugation at 3500 rpm for 2 min. The cleaned supernatant extract was evaporated to dryness using Turbovap (50°C). The dry residue was reconstituted to one ml with a mixture of n-hexane: acetone (7:3, v/v basis) and analyzed in a Gas Chromatograph

d. Capsicum, curry leaf and okra

Twenty five gram each of blended curry leaf, capsicum and okra were taken in 200 ml centrifuge tubes. A volume of 50 ml acetonitrile was added to the mixture and then homogenized at 14000 rpm for one min. Ten gram of sodium chloride was added to the mixture and centrifuged at 2000-2500 rpm for 4 min. From this, 16 ml supernatant was transferred to a 50 ml centrifuge tube containing 6.0 g sodium sulphate and vortexed. A total of 12 ml supernatant was then transferred to a 15 ml centrifuge tube containing 1.2 g magnesium sulphate and 0.2 g Primary Secondary Amine (PSA) and vortexed again at full speed for 30 s and centrifuged at 2500 rpm for 3 min. After that, 4.0 ml of upper layer was evaporated to dryness using Turbovap at 50°C. The dry residue was reconstituted to one ml using n-hexane and analyzed in a Gas Chromatograph.

Instrument analysis

Gas Chromatograph – (Shimadzu GC 2010 A) equipped with ⁶³Ni Electron Capture Detector (ECD), fitted with DB-5 capillary column (dimethyl polysiloxane, 30m x 0.25mm i.d. x 0.5µm film thickness) was used for the analysis. Ultra high Purity (99.999 %) nitrogen was used as carrier gas with a column flow rate of 0.79 ml min⁻¹ and linear velocity 26.00 cm S⁻¹. A column temperature programme was developed to get proper separation of all pesticides used in the analysis. The operating parameters of the instrument were: oven temperature 170°C (5 min) '1.5°C min⁻¹ '1220°C (10 min) '14°C min⁻¹ '1280°C (7 min), injection port at 250°C and detector at 300°C and the total run time as 70 min. and split ratio of 1: 10.

Residue Quantification

$$\text{Pesticide residue in substrate (mg kg}^{-1}\text{)} = \frac{\text{Peak area of sample} \times \text{Concentration of standard injected} \times \text{Final volume of sample injected} \times \text{Dilution Factor}}{\text{Peak area of standard} \times \text{Volume of sample injected}}$$

RESULTS AND DISCUSSION

Pesticide residues in agricultural/horticultural commodities

In this study, Multi Residue Methods (MRM) for pesticide residue analysis in agricultural/horticultural commodities was validated by conducting recovery studies. The results demonstrated that the method followed had a satisfactory analytical performance in terms of selectivity and linearity. Good linearity was found within the range of 0.01-0.5 mg kg⁻¹ for the pesticides belonging to OC, OP and SP insecticide groups. Satisfactory recoveries and RSDs were achieved for all the pesticides evaluated even at the lowest level of fortification. The mean recovery of all the pesticides under study were in the range 70 - 110 per cent and the repeatability of the recovery results, as indicated by the RSD < 20 % confirmed that the method is sufficiently reliable for pesticide residue analysis in different agricultural commodities.

Monitoring study revealed the presence of 14 different pesticides (Table 1) *viz.*, malathion, chlorpyrifos, fenvalerate, methyl parathion, cypermethrin, quinalphos, profenophos, bifenthrin, lambda cyhalothrin, ethion, alpha endosulphan, triazophos, fenpropathrin and beta cyfluthrin belonging to organophosphate (7), synthetic pyrethroid (6) and organochlorine (1) group in the samples analysed.

Among the different pesticides, chlorpyrifos (O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) was the most frequently detected insecticide and it may be due to its preference by farmers because of its broad spectrum activity as insecticide, acaricide and nematocide. Profenophos (O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate) has been the second frequently detected organophosphate (19 samples) pesticide, registered for use only in cotton and tea in India. Because of its translaminar, ovicidal and growth stimulating nature, it is widely used by the farmers. Being a pesticide banned for sale and use in Kerala state, its presence in curry leaf sample to the tune of 0.033 to 25.63 mg kg⁻¹ has to be viewed seriously (AICRP(PR), 2012).

Considering pesticide groups, it may be concluded that insecticides belonging to organophosphate group predominated over synthetic pyrethroid and organochlorine compounds. This trend is supported by the consumption pattern of pesticides which also indicated greater use of organophosphates when compared with synthetic pyrethroids and organochlorines (Adityachaudhury *et al.*, 1997).

The study revealed the presence of pesticide residues like chlorpyrifos, malathion, methyl parathion, quinalphos, cypermethrin and fenvalerate in cereals like basmathi rice and wheat (Table 1). Among the different insecticides, chlorpyrifos and malathion were the major contaminants. Malathion, chlorpyrifos, dichlorovos (DDVP), fenitrothion and synthetic pyrethroids like cypermethrin are reported to be misused widely as grain protectants during storage (Zhang *et al.*, 2010). Basmathi rice being the most expensive brand of rice, grain

Table 1. Pesticide residues in various agricultural/horticultural commodities collected from market during January - June 2012

Commodity	Insecticide detected	Concentration (mg kg ⁻¹)	Commodity	Insecticide detected	Concentration (mg kg ⁻¹)
Parboiled rice	ND	-	Atta	ND	-
Raw rice	ND	-	Maida	ND	-
Basmathi rice	Malathion Chlorpyriphos Fenvalerate Methyl parathion Cypermethrin	0.06 -0.08 0.025 0.052 0.046 0.011	Rice flour	ND	-
Wheat	Malathion Chlorpyriphos Quinalphos Methyl parathion	0.024 -0.19 0.047 -0.31 0.039 – 0.046 0.065	Okra	Malathion Profenophos	0.038 0.121
Cumin seed	Chlorpyriphos Profenophos Quinalphos Alpha endosulphan	0.04-0.270 0.488-1.45 0.139 0.115-0.135	Capsicum	Chlorpyriphos Profenophos	0.024 -0.047 0.033
Cardamom	Chlorpyriphos Profenophos Quinalphos Cypermethrin Lambda cyhalothrin Ethion Bifenthrin	0.057-0.353 0.139-0.954 0.137-2.044 0.061-0.461 0.058-0.364 0.344 0.106	Curry leaf	Chlorpyriphos Profenophos Quinalphos Alpha endosulphan Triazophos Cypermethrin Fenpropathrin Beta cyfluthrin Methyl parathion Malathion Ethion Bifenthrin	0.014-1.34 1.62-25.63 0.209-0.259 0.015 0.36-1.58 0.12-1.44 0.14-0.143 0.08 0.113 0.078-0.439 1.15 0.104

ND – Not Detected

protectants are more likely to be applied which might have resulted in pesticide residue. In contrary to this, none of the samples of raw rice, parboiled rice, rice flour, atta and maida showed the residues of any pesticide. Fractionation of residues in different wheat and rice portions (bran, germ, semolina, grout and flour) during milling and polishing could be the reason for the absence of pesticide residues in atta, maida and rice flour. Our findings are in line with those of Uygun *et al.* (2005) reported reduction of malathion residues about 95 per cent in wheat through milling (to flour).

The data generated through monitoring studies in cardamom have established the overdependence and abuse of pesticides as evident from the range of chemically different pesticides like chlorpyrifos, quinalphos, profenophos, cypermethrin, lambda cyhalothrin, ethion and bifenthrin that were identified and quantified. Presence of multiple residues has undoubtedly established rotational spraying of these pesticides directly on capsules. All the cardamom samples analyzed during the study period contained quinalphos residues above MRL fixed by FSSAI and all the insecticides detected except quinalphos were not at all registered for use in cardamom. Usha (2007) reported that there has been an increase in the pesticide consumption in cardamom during the last ten years and the results of a survey showed an unscientific and non judicious use of pesticides by farmers in Kattapana block of Idukki district. Cumin seed samples tested in the present study were found to be frequently contaminated with residues of profenophos, chlorpyrifos, quinalphos and alpha endosulphan for which no FSSAI MRL exists which means none of the pesticides detected were registered for use in this commodity.

Monitoring of pesticide residues in curry leaf revealed the presence of 12 different pesticide molecules *viz.*, chlorpyrifos, quinalphos, profenophos, triazophos, methyl parathion, cypermethrin, alpha endosulphan, malathion, fenprothrin, cyfluthrin, bifenthrin and ethion at varying levels. A level as high as 25.63 mg kg⁻¹ of profenophos was detected in one sample of curry leaf. Eventhough it is not registered for use in curry leaf, it is widely used against psyllids, citrus butterfly and citrus leaf roller in curry leaf because of its high bioefficacy, translaminar and growth promoting action.

Most of the samples tested in the present study had multiple residues with some samples containing three to six pesticides. However, most of these detected pesticides were not registered for use in India by CIB (RC) on that specific commodity. All the cardamom, cumin seed and curry leaf samples showed multiple pesticide residues at varying levels. None of the pesticides detected in curry leaf and cumin seed were registered for use in these commodities. Another important factor to consider is the presence of pesticides like methyl parathion, profenophos and endosulphan in basmathi rice, cardamom, cumin seed, curry leaf, capsicum and okra samples tested which were banned for sale and use in Kerala state (Report of Government of Kerala, 2011).

Table 2. Monitoring and risk assessment of pesticides detected in different agricultural/horticultural commodities

Commodity	*Amount consumed per day (g/day/person)	Pesticides detected (mg kg ⁻¹)	Highest residue level (mg kg ⁻¹)	Average daily intake (mg kg ⁻¹ bodyweight)	**ADI (mg kg ⁻¹ body weight)	% of ADI based on highest residue level	**ARfD (mg kg ⁻¹ bw)
Basmathi rice	275.00	Malathion	0.08	3.6 x 10 ⁻⁴	0.03	0.26	0.3
		Methyl parathion	0.046	2.1 x10 ⁻⁴	0.003	1.53	0.3
		Chlorpyriphos	0.025	1.14 x10 ⁻⁴	0.01	0.25	0.1
		Cypermethrin	0.011	5.04 x10 ⁻⁵	0.05	0.02	0.2
		Fenvalerate	0.052	2.38 x10 ⁻⁴	0.02	0.26	NA
Wheat	172.80	Malathion	0.19	5.4 x10 ⁻⁴	0.03	0.63	0.3
		Methyl parathion	0.065	1.8x10 ⁻⁴	0.003	2.16	0.3
		Chlorpyriphos	0.31	8.9 x10 ⁻⁴	0.01	3.1	0.1
		Quinalphos	0.039	1.12 x10 ⁻⁵	NA	-	NA
Cardamom	0.8	Chlorpyriphos	0.353	4.7 x10 ⁻⁹	0.01	3.53	0.1
		Quinalphos	2.044	2.72 x10 ⁻⁵	NA	-	NA
		Profenophos	0.954	1.27 x10 ⁻⁶	0.03	3.18	1.0
		Lambda cyhalothrin	0.364	4.85 x ⁴ 10 ⁻⁶	0.005	7.28	0.0074
		Cypermethrin	0.461	6.14 x10 ⁻⁶	0.05	0.92	0.2
		Ethion	0.344	4.58 x10 ⁻⁶	0.002	17.20	0.015
		Bifenthrin	0.106	1.41 x10 ⁻⁶	0.015	0.70	0.03
Cumin seed	0.8	Chlorpyriphos	0.27	3.6 x10 ⁻⁶	0.01	2.7	0.1
		Quinalphos	0.139	1.85 x10 ⁻⁶	NA	-	NA
		Profenophos	1.45	1.33 x10 ⁻⁵	0.03	4.83	1.0
		Alpha endosulphan	0.135	1.80 x10 ⁻⁵	0.006	2.25	0.02
Capsicum	8.7	Profenophos	0.033	4.7 x10 ⁻⁶	0.03	0.11	1.0
		Chlorpyriphos	0.047	6.81 x10 ⁻⁶	0.01	0.47	0.1

Commodity	*Amount consumed per day (g/day/person)	Pesticides detected (mg kg ⁻¹)	Highest residue level (mg kg ⁻¹)	Average daily intake (mg kg ⁻¹ bodyweight)	**ADI (mg kg ⁻¹ body weight)	% of ADI based on highest residue level	**ARfD (mg kg ⁻¹ bw)
Okra	4.1	Profenophos	0.121	8.26 x10 ⁻⁶	0.03	0.40	1.0
		Malathion	0.038	2.59 x10 ⁻⁶	0.03	0.12	0.3
Curry leaf	2	Chlorpyriphos	1.34	4.46x10 ⁻⁵	0.01	13.40	0.1
		Malathion	0.439	1.46x10 ⁻⁵	0.03	1.46	0.3
		Quinalphos	0.259	8.63x10 ⁻⁶	NA	-	NA
		Methyl parathion	0.113	3.76x10 ⁻⁶	0.003	3.76	0.3
		Profenophos	25.63	8.54x10 ⁻⁴	0.03	85.43	1.0
		Cypermethrin	1.44	4.8x10 ⁻⁵	0.05	2.88	0.2
		Ethion	1.15	3.8x10 ⁻⁵	0.002	57.50	0.015
		Bifenthrin	0.104	3.46x10 ⁻⁶	0.015	0.69	0.03
		Fenpropathrin	0.143	4.76x10 ⁻⁶	0.03	0.47	NA
		Alpha endosulphan	0.015	5x10 ⁻⁷	0.006	0.25	0.02

*Assuming a 60 kg person, total intake of each commodity is estimated from cluster diets compiled by the Global Environment Monitoring System–Food Contamination Monitoring and Assessment Programme (WHO/GEMS/FOODS) on <http://www.who.int/foodsafety/chem/gems/en/index1.html>.

** PPDB: Pesticide <http://sitem.herts.ac.uk/aeru/footprint/en/index.html>.

Risk assessment

Risk assessment was conducted based on monitoring results of the pesticide residue to determine the degree of risk by the detected pesticide residues in samples. WHO has recommended to compare the maximum detected level of pesticide with percentage of ARfD and percentage of ADI for assessing acute and chronic health risk. If pesticides detected resulted in an intake of >50 per cent of percentage of ARfD and >4 per cent of percentage of ADI value, it can be considered to cause acute and chronic health risk (Dalvie and London, 2008)

The highest detected level of the pesticides like lambda cyhalothrin and ethion in cardamom, chlorpyrifos, profenophos and alpha endosulphan in cumin seed and chlorpyrifos, profenophos, malathion, cypermethrin, ethion and bifenthrin in curry leaf exceeded the ARfD values (Table 2). However, none of the detected pesticides resulted in an intake of >50 % of ARfD value which gave an impression of no acute health risk, as per the guidelines of WHO.

Among the different agricultural/horticultural commodities like cardamom, cumin seed and curry leaf, the highest detected level of pesticides like (lambda cyhalothrin and ethion in cardamom, profenophos in cumin seed and chlorpyrifos, profenophos and ethion in curry leaf exceeded >4 % of ADI value fixed for the representative insecticides (Table 2). Among the different pesticides studied, profenophos was present in levels of >85.00 % of the ADI which represented a very high level of a chronic health risk to consumers as indicated by more than 20 times that of the safe margin. So it may be concluded that consumption of cardamom, curry leaf and cumin seed for a longer period of time can cause chronic health risk to consumers. High extent of pesticide residues in agricultural/horticultural commodities calls for improved management of residues at production, post harvest and marketing of food commodities. So, great significance has to be given to simple cost effective strategies like dipping in different decontaminating solutions like 2% tamarind, 2% acetic acid, 2% common salt, washing, cooking and decortications of cardamom capsules to eliminate harmful pesticides which could be practiced by home makers.

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