Bull Environ Contam Toxicol (2011) 87:303–306
DOI 10.1007/s00128-011-0325-9

Determination of Multi-residue Insecticides of Organochlorine, Organophosphorus, and Pyrethroids in Wheat

Riazuddin · Muhammad Farhanullah Khan · Sajid Iqbal · Muhammad Abbas

Received: 1 February 2011 / Accepted: 25 May 2011 / Published online: 8 June 2011

Abstract The undesirable effects of green revolution include residues of extensively used pesticides in various food commodities. Several studies showed that pesticides could cause health problems. Keeping in view the problem of pesticide residues in various food commodities, the present study was conducted on domestic stored wheat as well as on imported wheat for the qualitative and quantitative analysis of organochlorine, organophosphorus and pyrethroids. Among the imported wheat, 22.5% samples were found contaminated by organophosphorus (chlorpyrifos 0.073–0.230 μg/g, malathion 0.0419–0.1003 μg/g) and pyrethroids (cypermethrin 0.1404–0.2005 μg/g, permethrin 0.0140–0.0480 μg/g) while in domestic wheat 6.7% samples were found contaminated by pyrethroids (deltamethrin 0.0650–1.2903 μg/g) only. Method used for extraction and analysis of insecticides was validated both by recovery studies and inter laboratory comparison proficiency test. The method recovery results show that the average recovery of the fortified wheat samples was in the range of 73.77%–100.17% with the RSD in the range of 2.21–9.27 whereas, the Z-scores of the inter laboratory comparison proficiency test's result was less than 2.

Keywords Insecticides · Domestic wheat · GC-ECD

Riazuddin (✉) · S. Iqbal · M. Abbas
Grain Quality Testing Laboratory, Southern zone Agricultural Research Centre, Pakistan Agricultural Research Council, Karachi University Campus, Karachi 75270, Pakistan
e-mail: riaz_1969@yahoo.com

M. F. Khan
Department of Zoology, University of Karachi, Karachi 75270, Pakistan

Wheat is one of the most important cereal crop produced and consumed around the world. It is the staple diet of most of the countries including Pakistan. Pakistan is the 8th largest wheat producer, contributing about 3.17% of the world wheat production from 3.72% of the wheat growing area. It contributes 12.7% to the value added in agriculture and 2.6% to the Gross Domestic Products (GDP) of Pakistan. Wheat grains in the form of flour and its processed products have become vital and essential part of human diet. Its consumption is increasing everyday throughout the world (Khan et al. 2007). Pesticides from a broad range of classes are being world widely used in various combinations at different stages of cultivation and during post-harvest storage to protect food commodities against a wide range of pests and to provide quality protection. It has been estimated that if pesticides were not used, the crop loss in the world would have been around 40%. Stored-product insects can cause post harvest losses, estimated from up to 9% in developed countries to 20% or more in developing countries (Phillips and Throne 2010). The presence of insecticides residues in cereals represents a great risk for the consumer, because they are used at a large scale to protect stored grains from the attack of pests. Insecticide residues in food generally arise from their indiscriminate use in various agricultural practices, grain storage and public health (Newsome et al. 1998, Schade and Heinzow 1998).

With the constant increase of insecticides uses, people from all over the world are paying more attention to the potential hazard of insecticides to food safety and human health which is also a problem to address in international trade (Pang et al. 2009). Thus importing countries are rejecting contaminated food resulting in heavy losses to the exporters. In this scenario, the presence of insecticides residues is a major hurdle in the acceptance of the food

commodities by the importing countries. With the increasing human health concerns, most developed countries of the world have established Maximum residue Levels (MRL's) of insecticides to protect consumers from exposure to unacceptable levels of insecticide residues. In addition, the Codex Alimentarius Commission of the FAO recommends maximum levels for commodities which move in international trade, in the hope that these MRL's will be accepted by the National governments. Therefore, less developed countries including Pakistan which do not currently possess facilities to develop their own maximum residue levels often use those set by the Codex Commission as a basis for their own regulations.

The purpose of present study was to investigate the occurrence and magnitude of insecticides residues both in domestic stored wheat grains as well as in imported wheat.

Materials and Methods

Acetone, n-hexane, methanol, dichloromethane, anhydrous sodium sulphate and activated graphitized charcoal were of analytical grade, purchased from Merck (Germany). Acidic aluminum oxide pH 4.5 ± 0.5, Brockmann activity was purchased from Fluka (Switzerland). Insecticide standards were purchased from AccuStandard, Inc (USA). All standards were at least 99.9% pure. Aluminum oxide was activated at 500°C for 3 h while charcoal was activated at 300°C for 3 h and anhydrous sodium sulphate was dried at 500°C. Cotton wool washed with a mixture of acetone and hexane (1 + 1). Stock standard solution of the pesticides was prepared in n-hexane. Working standard solutions were prepared as per requirement from insecticide stock solution in hexane. Filter papers of Whatman # 542, chromatography glass column of 58 cm length and 2.4 cm id, centrifuge machine Jouan, Inc (USA) Model # CR412 and a rotary evaporator Model BUCHI V-512, with chiller were used.

Besides, from forty imported wheat grains samples received from importers/trading corporation, sixty domestic samples were collected in June–July, 2009 from two majors storage godowns located in Landhi and Pepri areas of Karachi. Sampling of domestic grain was performed by using a steel probe. The probe was introduced in different fabric grain bags to get one kilogram each a representative and homogenous sample. One kilogram each sample was placed in a plastic bag and labeled. Thereafter, samples were brought to the laboratory where they were cleaned and immediately stored at −20°C until further analysis.

Samples were analyzed according to our own laboratory developed method (Uddin et al. 2011). According to this method, 4 g ground grain sample was taken in a centrifuge tube along with extraction solvents mixture of 40 mL of acetone and methanol (1:1). After through hand shaking, the samples were centrifuged at 2,500 rpm for 3 min. The supernatant solvents were decanted through a filter funnel into a 500 mL separating funnel. A further 35 mL of extraction mixture was added to the sample, shaking thoroughly for 1 min and again centrifuged. Again the supernatant solvents were decanted into the same 500 mL separating funnel. Two hundred mL of sodium sulphate solution (2.5 g/100 mL) and 25 mL of dichloromethane were added to the separating funnel, followed by vigorously hand shaking. Phases were allowed to separate and the lower dichloromethane phase was passed through 25 g of anhydrous sodium sulphate glass column. The partitioning of aqueous extract was repeated with two further 25 mL of dichloromethane. The moisture free extract was concentrated to approximately 1 to 2 mL on a rotary evaporator. The concentrated dichloromethane extract was then transferred to a glass column packed with 16 g of a mixture of acidic aluminum oxide and activated charcoal (15 + 1) followed by elution with 160 mL of dichloromethane. Elute was concentrated to dryness on a rotary evaporator and dissolved the residues in 2 mL hexane.

Samples were injected into an Agilent Technologies Model GC 6890N, USA, gas chromatograph equipped with Ni^{63} electron capture detector and an auto injector (Model 7683 series). The fused silica capillary column (30 m × 0.25 mm i.d) coated with HP-5MS (5% phenyl, 95% dimethylpolysiloxane) having a film thickness of 0.25 μm (J & W Scientific) was used for the determination of insecticides. The column temperature was maintained at 80°C for 1 min, ramped at a rate of 30°C/min to 180°C then at 3°C/min to 205°C hold for 4 min and finally at a rate of 20°C/min to 290°C, which was hold for 5 min. Injector and detector temperatures were kept at 280 and 320°C respectively. Nitrogen was used both as a carrier and as makeup gas. Insecticides residues were quantified from individually resolved peak areas with corresponding peak areas of the standards. For every set of four samples, a procedural blank consisting of all the reagents and glassware used during analysis was run to check for interferences and cross contamination.

Before the analysis of samples, the efficiency of the method was evaluated by fortification of the control ground grain samples (free from any pesticide) with known quantities of each studied pesticides separately as well as in a mixture and were allowed to stand for at least 24 h before extraction in order for the insecticides to be absorbed on to the sample. Recoveries of the studied insecticides by this method were ranged from 73.77% to 100.17%.

Results and Discussion

In order to ascertain the accuracy of the method used for analysis of insecticide residues, recovery studies were also

Table 1 Percent recovery of studied insecticides in wheat at 0.1 and 0.5 μg/g fortification levels

Insecticides	Limit of quantification (μg/g)	Average recovery and %RSD	
		0.1 μg/g	0.5 μg/g
Alpha-BHC	0.002	91.07[a] ± 2.21[b]	98.13[a] ± 2.39[b]
Beta-BHC	0.003	88.93 ± 4.17	95.70 ± 2.86
Lindane	0.005	88.83 ± 2.98	91.40 ± 2.29
Delta-BHC	0.004	91.57 ± 3.06	95.13 ± 4.60
Alpha-endosulfan	0.050	83.20 ± 4.64	89.40 ± 2.79
Chlorpyrifos	0.050	97.23 ± 4.32	100.17 ± 3.95
Malathion	0.050	89.30 ± 3.43	91.20 ± 2.47
Bifentherin	0.050	87.17 ± 9.27	93.63 ± 3.58
Cypermethrin	0.050	92.90 ± 2.55	94.70 ± 6.41
Fenvalerate	0.050	77.03 ± 4.07	82.47 ± 3.84
Deltamethrin	0.050	84.20 ± 5.46	89.07 ± 4.04
Lemda-Cyhalothrin	0.050	73.77 ± 3.72	89.33 ± 6.18
Permethrin	0.050	79.80 ± 2.38	80.23 ± 3.34

[a] Average value of three replicates

[b] RSD

Table 2 Results and Z-score for carbendazim, lamda-cyhalothrin and deltamethrin in wheat flour test material of FAPAS proficiency test 0967, 2010

Lab. number	Carbendazim assigned value 179 μg/kg			Lamda-cyhalothrin assigned value 102 μg/kg			Deltamethrin assigned value 188 μg/kg		
	Result (μg/kg)	Recovery (%)	Z-score	Result (μg/kg)	Recovery (%)	Z-score	Result (μg/kg)	Recovery (%)	Z-score
026	#			139.00	87.43	1.6	212.09	79.41	0.6

Not analyzed

Table 3 Insecticide residues concentrations found in wheat samples

Insecticide	N	Insecticide residues (μg/g)	Origin	MRLs (μg/g)
Chlorpyrifos	3	0.1286[a] (0.0735–0.2300)[b]	Imported	1.0
Malathion	2	0.0711 (0.0419–0.1003)	Imported	8.0
Permethrin	2	0.0310 (0.0140–0.0480)	Imported	5.0
Cypermethrin	2	0.1704 (0.1404–0.2005)	Imported	1.0
Deltamethrin	4	0.6392 (0.0650–1.2903)	Domestic	2.0

n Number of samples found contaminated

[a] Average value of three replicates

[b] Minimum and maximum values, within the parenthesis

conducted. Recoveries of insecticides were determined at two fortification levels of 0.1 and 0.5 μg/g. Recoveries were found to be acceptable (73.77%–100.17%) with relative standard deviation (RSD) below 10%. Recoveries of insecticides were measured by comparing peak areas of the samples with external standards in hexane and matrix matched control prepared from unfortified samples. The insecticides recovery results are presented in Table 1.

Laboratories analyzing food samples for the determination of pesticide residues need to ensure that the reported results are within defined uncertainty limits. One important measure is applying a quality system such as ISO 17025 (1999), showing that the analytical procedures are carried out following specific rules. In addition, laboratories should participate in proficiency tests (PTs) to demonstrate sufficient quality of the analytical results. PTs are designed to inform laboratories about the difference between the result of their analysis and the assigned value of concentration when analyzing test material of unknown analyte concentration. This difference is expressed in terms of Z-score (ISO/IEC 43-1, 1997). ISO Guide 43-1 introduces the Z-score as the most important performance score for laboratories. The quality of analysis increases with the decrease in the Z-score value. $|Z| \leq 2$ good, $2 < |Z| \leq 3$

satisfactory and $|Z| > 3$ unsatisfactory (Sanyal and Rani 2009). Therefore, for assessing the method accuracy, the method performance has been tested in the year 2010 by participating in the FAPAS proficiency test sample program (wheat flour test material) and obtained the Z-score values below 2. The results are shown in Table 2 with author's laboratory No: 026.

Insecticides residues found in this study were chlorpyrifos, malathion, cypermethrin, permethrin and Deltamethrin. Insecticides found in imported wheat grains were chlorpyrifos, malathion, cypermethrin and permethrin while in domestic wheat grain stored in government godowns of Landhi and Pepri areas of Karachi, Pakistan were found contaminated by deltamethrin only. The results are shown in Table 3.

All insecticide residues were found below the maximum limits permitted for stored grains (FAO/WHO 2008). The results of this study indicated that only deltamethrin insecticide was used for the maintenance of stored wheat grains in Pakistan and the residue levels found were within the permissible limits.

In the domestic samples a low level concentration of only deltamethrin is probably due to the fact that in our country insect pests seldom reach to the economic threshold level on wheat crop. Therefore, on growing crop the insecticide usage is almost none, however at post harvest level grain protection measures are required. At this stage because of easy availability and low cost application, phosphine fumigation measures are employed (Aslam et al. 1999). Insecticides are used in godowns and its surrounding for the control of pest habitat and mice destruction. Since being a safe, deltamethrin was employed. For this purpose, from the present results it seems that in the storage area wheat bags were either stored just after applying deltamethrin or godowns disinfestation was made during the storage period. That results in presence of the said insecticide in the wheat grains.

Acknowledgments We thank the chairman Pakistan Agricultural Research Council and Project-Director National Animal and Plant Health Inspection Project (NAPHIS) for providing the financial support to carry out the present study. The corresponding author is also grateful to the Chairman – Department of Agriculture and Agribusiness Management, University of Karachi, Pakistan for his kind cooperation.

References

Aslam MS, Shaukat SS, Ahmed M, Iqbal S, Ahmed A (1999) A survey of resistance to phosphine in some coleopterous pests of stored wheat and rice grain in Pakistan. Pakistan J Biol Sci 2:623–629

FAO/WHO (2008) Pesticide residues in food. Maximum residue limits; Extraneous maximum residue limits. Codex Alimentarius Commission, FAO/WHO Food standards. http://www.codexalimentarius.net/mrls/

FAPAS Proficiency Test 0967 (2010) The Food and Environment Research Agency Sand Hutton, York. www.fapas.com

International organization for standardization, ISO/IEC 17025 (E) (1999) General requirements for the competence of testing and calibration laboratories ISO, Geneva

International organization for standardization, ISO/IEC 43-1:(E) (1997) Proficiency testing by inter laboratory comparisons, Part 1: Development and operation of proficiency testing schemes. ISO Geneva

Khan IAT, Riazuddin, Parveen Z, Ahmed M (2007) Multi-residue determination of synthetic pyrethroids and organophosphorus pesticides in whole wheat flour using gas chromatography. Bull Environ Contam Toxicol 79:454–458

Newsome WH, Devis DJ, Sun WF (1998) Residues of polychlorinated biphenyls (PCB) in fatty foods of the Canadian diet. Food Addit Contam 15:19–29

Pang GF, Cao YZ, Fan CL, Liu YM (2009) Analysis method study on 839 pesticide and chemical contaminant multiresidues in animal muscles by gel permeation chromatography cleanup, GC/MS and LC/MS/MS. JAOAC Int 92:933–940

Phillips TW, Throne JE (2010) Biorational approaches to managing stored–product insects. Annu Rev Entomol 55:375–397

Sanyal D, Rani A (2009) Proficiency test for chemical laboratories for the analysis of a pesticide in a formulated product: inter laboratory study. JAOAC Int 92:271–278

Schade G, Heinzow B (1998) Organochlorine pesticides and polychlorinated biphenyls in human milk of mothers living in northern Germany: current extent of contamination, time trend from 1986 to 1997 and factors that influence the levels of contamination. Sci Total Environ 215:31–39

Uddin R, Iqbal S, Khan MF, Parveen Z, Ahmed M, Abbas M (2011) Determination of pesticide residues in rice grain by solvent extraction, column cleanup, and gas chromatography-electron capture detection. Bull Environ Contam Toxicol 86:83–89