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Determination of malathion, chlorpyrifos, λ-cyhalothrin and arsenic in rice

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Abstract

Presented study focused on the incidence of pesticides: malathion, chlorpyrifos and λ-cyhalothrin and heavy metal, arsenic in the rice samples collected from the three main rice growing areas. 30 samples of brown Super Kernel Basmati rice and Super Basmati rice varieties, irrigated through tube well irrigation were selected. Highly sensitive and selective method of High Performance Liquid Chromatography was used for determination of pesticides. Results of analysis showed the presence of malathion in one sample in concentration of 18.26 mg/kg, chlorpyrifos in two samples in concentration of 3.3 mg/kg and 1.45 mg/kg respectively and λ-cyhalothrin in one sample in concentration of 1.848 mg/kg. Amount of pesticides detected in these samples were exceeding their maximum residual limits thus showed their strong potential to pose significant health risk. The presence of arsenic in rice was determined by Atomic Absorption Spectrophotometer and it was detected in 12 samples out of 30 in range of 0.04-0.28ppm. The detected arsenic concentration of arsenic in all samples was within the permissible limits set by WHO/FAO.

Keywords: malathion; chlorpyrifos; λ-cyhalothrin; arsenic; rice; high performance liquid chromatography.

Practical Application: Due to a substantial escalation of population growth over the past few decades, there has been an extensive application of pesticides and other agrochemicals in order to produce a larger crop yield, resulting in the accumulation of pollutants in the environment. Presented study focused on the incidence of pesticides: malathion, chlorpyrifos and λ-cyhalothrin and heavy metal, arsenic in the rice samples. Focus of this study was also to formulate and adopt easy and convenient methodology for extraction of these pesticides from rice samples and their successful qualitative and quantitative analysis on HPLC which can be practically implemented.

1 Introduction

Recent decades have seen an increase in the demand of one of the most consumed foods worldwide, i.e. rice. However, due to a substantial escalation of population growth over the past few decades, there has been an extensive application of pesticides in order to produce a larger crop yield, resulting in the accumulation of pollutants in the environment. Agrochemicals including pesticides and fertilizers, which are used to prevent pest attacks on grains and increase production per unit area, have the ability to migrate into grain due to the chemical properties they possess (Dors et al., 2011). Pesticides, a group of environmental pollutants, are widely used to control pests and disease causing vectors in agriculture, in order to increase food productivity. Every year, 4.6 million tons of pesticides are used worldwide (Zhang et al., 2011). Pesticides have the potential to cause cancer and their prolonged usage can leave behind toxic residues, which can contaminate air, water, soil and food, affect non target organisms, human health and can cause economic losses (Hashmi et al., 2011; Ahmed et al., 2011; Anwar et al., 2012). Organophosphate pesticides (OPs), a class of insecticides, affect the nervous system of the insect, by inhibiting the functioning of the acetylcholinesterase enzyme. Human exposure to the OPs through ingestion, inhalation and dermal contact can result in weakness, nausea, seizures, dizziness, sweating, headache, diarrhea and lack of coordination (Eleršek & Filipič, 2011; Stokes et al., 1995); chronic exposure can result in an increased risk of cancer (Eleršek & Filipič, 2011; Ingelse et al., 2001). Malathion, an OP, is widely used in agriculture, residential, and public recreational settings for controlling animal parasites, mosquitoes, insects, flies and lice. Malathion is classified as slightly toxic; but it is metabolized to highly toxic malaoxon when absorbed or ingested in the human body (Arava et al., 2013). Chlorpyrifos, is moderately toxic, targets the peripheral and central nervous system and inhibits the working of the acetylcholinesterase enzyme (Smegal, 2000; Phung et al., 2012). Pyrethroids are synthetically produced organic compounds. Exposure to these can result in seizures, vertigo, nausea, headache, ulcers in the mouth, vomiting, chest tightness, sore throat, increased secretions, palpitations, blurred vision, pain in the abdomen and coma (Bradberry et al., 2005). Rice is an important food for 50% of the world's population, due to being relatively cheaper and nutritious (Shabbir et al., 2013). Basmati rice is one of the most popular varieties of rice worldwide, because of its long grains, aroma and fine quality (Bhattacharjee et al., 2002). Literature reports presence of pesticide residues in rice in different areas. Carbamate (carbofuran and carbaryl) and organophosphate (malathion, diazinon and chlorpyrifos) have been detected in twenty seven water samples collected from vegetable and paddy fields in Bangladesh (Chowdhury et al., 2012). Likewise, organophosphorus

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pesticide residues were determined in samples of milled rice, purchased from China's local markets. The results showed that organophosphate pesticides (parathion-methyl, dichlorvos, chlorpyrifos, parathion, methamidophos, triazophos and omethoate) were found in 9.3% of the samples. The study proposed that national safety standards for milled rice should be implemented and regular monitoring of organophosphorus pesticides should be made essential (Chen et al., 2009). In Pakistan, rice is predominantly grown in the Punjab and Sindh provinces. This Kharif crop requires lots of water and a frequent spray of pesticides, thus making the crop vulnerable to pesticide and arsenic poisoning. Investigation of pesticide residues in 400 different rice samples from Punjab have shown presence of Karate, Padan, Malathion and Novacran in varying concentrations (19 ppm to 148 ppm); though mean concentrations mostly below or just above threshold levels (Ahmad et al., 2008). In addition to pesticide contamination, arsenic pollution in rice is also documented by many researchers. Recent studies have exposed that arsenic contaminated groundwater irrigation is the leading cause of arsenic in crops, mostly in rice and vegetables (Qian et al., 2010). Arsenic contamination in water is a serious problem in Bangladesh. Studies document that Bangladeshi people were affected by arsenic through seed grains and fodder (Chakma et al., 2012). Arsenic, lead, mercury and cadmium determination in 100 rice samples, purchased from the stores of Iran's Khorasan province showed, arsenic to be the most abundant of the trace elements in rice with a mean concentration of 51.85 ng/g (Fakoor et al., 2011). Four varieties of arsenic: arsenate, dimethylarsenic acid, arsenite and monomethylarsonic acid were determined in processed rice; grown in Korea's mining areas by HPLC-ICP-MS. Methanol-water (1:1) solution was used to extract the arsenic compounds. Detection limits on dry weight basis for the four varieties were: 5.3, 3.7, 19.2, and 3.7 mg/kg, respectively (Paik et al., 2010). Use of arsenic contaminated irrigation water results in Reduction in rice yield, plant height and the development of the root growth (Abedin et al., 2002a). A noteworthy increase has been detected in the concentrations of arsenic present in the rice husk, straw and root, due to an increase in the strength of arsenate in the irrigation water. Whereas, the number of filled grains, grain weight, plant height, grain yield and root biomass were notably decreased (Abedin et al., 2002b). Moreover the distribution and accumulation of arsenic in various parts of rice varies as depicted in study in Bangladesh which show the order of arsenic concentration as; rice hull > bran-polish > brown rice > raw rice > polish rice, for parts of parboiled and non-parboiled rice grain. The results showed that traces of arsenic were less in parboiled rice, than in non-parboiled rice grain, while arsenic's concentration in the study, was below the World Health Organization's allowable limit for arsenic in rice (1.0 mg/kg) (Rahman et al., 2007). Therefore, the purpose of the study was to determine the presence of pesticide and arsenic residues in rice samples collected from the study areas.

2 Materials and methods

The present study was designed in order to determine malathion, chlorpyrifos, λ-cyhalothrin and arsenic residues in the selected rice samples collected from three districts of Kasur, Sheikhupura and Nankana Sahib districts.

2.1 Primary data collection

Primary data was collected from the agricultural farmlands of Kasur, Sheikhupura and Nankana Sahib districts, through detailed visits and interview based surveys and information regarding varieties of rice grown, the type and reason for the application of various pesticides on the rice crop and the frequency of the pesticide application and irrigation of crops through groundwater was gathered.

2.2 Sample collection

A total of 30 rice samples (100g per sample) were collected for this study, out of which 10 samples were from the farmlands of Kasur, which were coded from K1…. K10. 10 samples were from Sheikhupura S1…. S10 and the remaining 10 were from Nankana Sahib N1….N10, districts of Punjab. The gathered samples were brown Super Kernel Basmati rice and Super Basmati rice varieties and they were collected from only those rice fields where the rice crop was grown through tube well irrigation.

2.3 Selection of the pesticides and arsenic

Interview based surveys were conducted with the farmers of the areas in order to get an idea about the types of organophosphates (malathion and chlorpyrifos) and pyrethroid (λ-cyhalothrin) pesticides which were commonly sprayed on the rice crops in the study areas. On the basis of the survey and previous studies malathion, chlorpyrifos and λ-cyhalothrin were selected.

Similarly, as per the documentation by previous researches, the presence of arsenic in the groundwater of the study areas has been reported. Therefore, arsenic was also selected as one of the parameters to be analyzed in the collected samples.

3 Materials and methods

HPLC was carried out using Dionex Ultimate 3000 Autosampler, Model 2013. Methanol, acetonitrile, standards and all other chemicals were purchased from Merck. For the determination of arsenic residues in rice, BUCK Scientific Model 210 VGP Atomic Absorption Spectrophotometer was used at a wavelength of 193.7 nm.

3.1 Extraction of pesticide residues in rice

Brown rice was extracted from the collected husked rice, by using a mortar and pestle. 10 grams of the extracted brown rice from each sample was weighed and put in a conical flask. The weighed sample was immersed for 5 days in 40 ml methanol: water solution (1:1 v:v) (Rahman et al., 2007). After that, 5 g of Sodium Chloride (NaCl) was added in the flask and the sample was left for 24 more hours. Then the extracted solvent from the sample was filtered twice with a 10 mm filter paper, and was stored in a vial for further analysis.

3.2 Detection of pesticide residues in rice

Three different volumes (0.25 mL, 0.5 mL and 1 mL) of each standard i.e. malathion, chlorpyrifos and λ-cyhalothrin were diluted with methanol, to make a standard solution of 100 mL each. A gradient rapid and sensitive HPLC-UV method was developed and validated to determine malathion, chlorpyrifos and λ-cyhalothrin byproducts in the samples. For achieving method development, optimization studies were done on each HPLC parameter such as solvent ratio, pH, temperature of column, sample and injection volume, flow rate, wavelength and retention time etc. Each sample was analyzed in triplicate to record the variation (if any), so that precision in method could be achieved. Calibration experiments were conducted in order to achieve linearity. Samples and standards were analyzed on HPLC Dionex Ultimate 3000 Autosampler Gradient System using a UV Detector, C_{18} column, at a wavelength of 254 nm, pressure of 102 bar, a temperature of 28 °C and a retention time of 20 minutes. Mobile phase was 50% acetonitrile: water in (1:1) v/v ratio. Previous study also support the use of similar type of solvent system (Niaz et al., 2016). The set flow rate was 1.0 ml/min and the injection volume was 10 μL.

3.3 Qualitative and quantitative analysis

Qualitative analysis was carried out, in order to check the presence of the selected organophosphates (malathion and chlorpyrifos) and pyrethroid (λ-cyhalothrin) in the rice samples. To accomplish this, the retention times of the attained standard peaks were noted and compared with the retention times of the peaks detected in the samples, in order to mark the existence of each individual standard in the samples.

Quantitative analysis was carried using the formula formulated by chromo academy.

$$\text{Response Factor} = \frac{\text{Peak Area of the Standard}}{\text{Standard Amount}} \quad (1)$$

$$\text{Quantity of the Sample} = \frac{\text{Peak Area of the Sample}}{\text{Response Factor}} \quad (2)$$

3.4 Extraction of arsenic residues in rice

5 gram of each rice sample was taken and made into powder form with a grinder. Samples were transferred in a 100 ml flask containing 0.1 N HCL for 24 hours (Fakoor et al., 2011). Then solution was stirred for 30 minutes by a magnetic stirrer and was filtered using 10 mm filter paper. Further analysis was conducted on BUCK Scientific Model 210 VGP for detecting arsenic at a wavelength of 193.7 nm, in the filtrate of the rice samples. The presence of arsenic was determined on the mechanism that the rate of the absorbed UV light passed through the sample is in direct proportion to the concentration of the element, within it (García & Báez, 2011). Microsoft Office Excel 2007 was used to represent the arsenic concentrations, determined using the Atomic Absorption Spectrophotometer, in tabular and graphical form.

4 Results and discussion

The agricultural sector is mainly dependent on the usage of pesticides and tube well irrigation, in order to enhance crop yield. In the rice farmlands of Punjab, extensive use of organophosphates (malathion and chlorpyrifos) and pyrethroids (λ-cyhalothrin) is a common practice in spite of the fact that their use on rice crop is not very much recommended due to their potential effects towards environment and health. Thus the present study was designed to determine their concentration in the rice samples collected from various districts of Punjab and also to determine the arsenic levels in rice. Focus of this study was also to formulate and adopt easy and convenient methodology for extraction of these pesticides from rice samples and their successful qualitative and quantitative analysis on HPLC.

HPLC method was beautifully employed in order to determine the concentration of selected pesticides in rice samples as shown in Table 1.

Qualitative analysis of rice showed that out of the 30 collected rice samples, malathion (is a slightly toxic compound in EPA toxicity class III) was detected in only one sample which was coded as K7 in a concentration of 18.26 mg/kg, which is far above the MRL value of malathion in rice i.e., 8 mg/kg thus having the potential to pose significant health risk towards the consumers of the rice. Malathion is toxic via skin contact, ingestion, and inhalation exposure (Tomlin, 2006).

Usage of chlorpyrifos as a pesticide on various crops is very common throughout the world (Cardoso et al., 2010; Li et al., 2015). It was detected in two samples which were coded as S16 and S17 in concentration of 3.3 mg/kg and 1.45 mg/kg respectively exceeding the MRL value of chlorpyrifos in rice (0.5mg/mL) showing its potential to pose significant health risk to the consumers. Some previous literature also reported the presences of chlorpyrifos in different crops of Punjab (Baig et al., 2009; Islam et al., 2010). Chlorpyrifos itself is not significantly much toxic, but when it enters in the body via food ingestion, the body breaks it down, and creates a toxic form called chlorpyrifos oxon which has the ability to bind permanently to enzymes which control the messages that travel between nerve

Table 1. Pesticide residues in samples.

Standard	Samples in which Pesticides are Detected	Identification codes of the Detected Samples	Quantity in Rice (mg/kg)	MRL Value (mg/kg)
Malathion	Kasur District	K7	18.26	8
Chlorpyrifos	Sheikhupura District	S16	3.3	0.5
	Sheikhupura District	S17	1.45	0.5
Λ-Cyhalothrin	Nankana Sahib	K4	1.848	1

MRL: Maximum Residue Limit.

Table 2. Concentration of Arsenic in Rice Samples.

Sample (Kasur District)	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
Arsenic Concentration (mg/kg)	0.28	BDL	0.08	0.08	BDL	BDL	0.06	BDL	BDL	0.2
Sample (Sheikhupura District)	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
Arsenic Concentration (mg/kg)	0.2	BDL	BDL	0.04	0.18	0.2	BDL	BDL	BDL	BDL
Sample (Nankana Sahib Dist)	N21	N22	N23	N24	N25	N26	N27	N28	N29	N30
Arsenic Concentration (mg/kg)	BDL	BDL	0.04	BDL	0.04	BDL	0.06	BDL	BDL	BDL

BDL: Below detection limit. WHO/FAO Standard <1 mg/kg (1 ppm) (World Health Organization, 2010).

cells and results in effecting muscles as muscles do not function correctly. The body then must make more enzymes so that normal nerve function can resume. The body can break down and excrete most of the unbound chlorpyrifos in feces and urine within a few days (Christensen et al., 2009).

λ-Cyhalothrin was determined only in one sample which was coded as K4 in concentration of 1.848 mg/kg which exceeded the prescribed MRL value i.e., 1mg/kg. The EPA identifies lambda cyhalothrin as a group D carcinogen, an undetermined human carcinogen but its ingestion with food above MRL may cause abdominal pain and coughing (Pesticide Action Network North America, 2010). Malathion and chlorpyrifos are organophosphate pesticides, which do not persist in the environment nor bioaccumulate and biomagnify (Hashmi et al., 2011). λ-cyhalothrin, which is a pyrethroid, is easily biodegradable and it also does not bioaccumulate therefore they were hardly detected in the collected rice samples. The only possible reason that could be drawn for the concentrations of detected pesticides exceeding the MRL values, is their frequent and indiscriminate use by the farmers, in order to produce surplus amounts of the crop during its growing season, so that it could be provided abundantly to other countries, for the purpose of boosting economic progress. This however, has resulted in the contamination of rice.

Rice is the food stuff which is widely used in Pakistan and all over the world in one or the other form. The occurrence of pesticides above MRLs in the rice samples is an indication of some form of direct misuse/abuse of the targeted compounds. Noncompliance with MRLs will definitely pose negative impacts on international trade in agricultural as each commodity must meet international standards or standards of the producing and receiving country. Thus monitoring of pesticide in rice farmlands must be conducted to ensure the appropriate use of recommended products and avoid the potential health effects associated with them.

Second part of this study was the detection of Arsenic in collected rice samples as previous studies reported the presence of arsenic in water of study area (Ahmad et al., 2013). The results demonstrated in Table 2 shows that out of ten samples collected from Kasur district, five samples were found contaminated with arsenic. Which were coded as K1, K3, K4, K7 and K10 in concentration of 0.28 mg/kg, 0.08 mg/kg, 0.08 mg/kg, 0.06 mg/kg and 0.2 mg/kg respectively. Arsenic residues were detected in four samples of Sheikhupura district; S1: 0.2 mg/kg, S4: 0.04 mg/kg, S5: 0.18 mg/kg and S6: 0.2 mg/kg, while in only three samples of Nankana Sahib district which were coded as N3, N5 and N7 in concentration of 0.04 mg/kg, 0.04 mg/kg and 0.06 mg/kg. The detected values were all within the WHO/FAO permissible limits i.e. 1.0 mg/kg. However, the rest of the 18 samples were below Detection Limit (BDL). In the present study the concentrations of arsenic were within the permissible limits. It is because the rates of absorption and distribution of arsenic in the rice plant fluctuate. The absorption of arsenic in the rice plant is in the order: straw >husk> brown rice grain > polished rice grain. Its accumulation is 28 times higher in root than in the shoot and 75 times higher than the rice grain. Since, only the rice grain was opted for the research study, therefore the results demonstrated fewer concentrations of arsenic in the collected rice samples. Another reason could be the fluctuations in the soil and water arsenic concentrations, which vary considerably with the locations.

Previous study reported District Sheikhupura's drinking water being contaminated with arsenic and its health impacts on the female population of that area (Abbas & Cheema, 2015). According to WHO (World Health Organization, 2010), the main sources of human exposure to arsenic are water and food. Rice is a crop plant which can absorb more arsenic than other cereals thus it is very important to monitor the contamination of rice with arsenic and to estimate the risk to human health as its ingestion may cause various health problems and increase the risk of chronic diseases like various types of cancer, hypertension and heart diseases etc.

5 Conclusion

In this research, the first target was to determine the concentration of malathion, chlorpyrifos and λ-cyhalothrin by HPLC and secondly to determine the presence of arsenic in rice samples collected from various parts of district Punjab. Results revealed the presence of malathion in only one sample, chlorpyrifos in two samples while λ-cyhalothrin in only one sample. All detected concentrations were found to be above the maximum residue limits with legal implications and the ranges of concentrations were wide. There is a need for the relevant agency to strictly control the importation, sale, use and disposal of these toxic chemicals. Arsenic residues were detected in only 12 samples in concentration within the range of WHO/FAO permissible limit for rice i.e. 1.0 mg/kg.

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