CHROMATOGRAPHIC DETERMINATION OF RESIDUAL CONTENTS OF PESTICIDES IN RICE SAMPLES FROM DIFFERENT GEOGRAPHICAL REGIONS OF PUNJAB

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Abstract

During the past decade, the use of pesticides in agriculture has dramatically increased which is becoming a major threat to human health. This study was conducted to investigate the pesticide residues in Basmati and IRRI varieties of rice in different areas of Punjab. A total of 106 rice samples of different varieties were taken from rice mills of specified areas. The concentration of 4 pesticides (L-Cyhalothrin, Malathione, Monocrotophos and Cartap) was detected using high performance thin layer chromatography (HPTLC). The residual contents of L-Cyhalothrin, Monocrotophos and Cartap residues were detected in all while of Malathione in only 16 samples. The average concentration of pesticides residues, though ranged from 0.08 to 0.1 mg/L, were statistically insignificant in all growing areas of rice and all varieties of rice. Interestingly, the mean levels of all pesticides was below the standard lethal doses which point towards no risk of acute poisoning but may become problematic during long exposures.

Introduction

In Pakistan, most of the area in Central Punjab is used to cultivate rice, including Narowal, Sialkot, Gujranwala, Gujrat, Sheikhupura, Hafizabad and Mandi Bahauddin districts. (Golshan *et al.,* 2013). The private sector is playing a vital role in controlling trade in Pakistan including Rice Export Corporation (REC), Rice Exporters Association Pakistan (REAP) and Trading Corporation of Pakistan (TCP). All of these regard a vital role in maintaining a quality review committee to certify the worth of Pakistani Rice prior to exporting. (Alia *et al.,* 2011). Pest outbreaks are the major cause of heavy reduction in yield of rice production, can also have other detrimental impacts which are related to the health of human beings as well as the animals related to rice fields and the environment is also threatened. These pesticides may cause deterioration of the Nervous system of humans and animals, both in acute and chronic exposures (Toteja *et al.,* 2003). So preventive measures should be taken to avoid hazards and all the preventive measures are greatly dependent of international differences, but a mutual achievement is present to make environment and food free of contamination. They have also been meant for to prevent both sudden and late bad impacts for manufacturers, consumers and the population of the whole world. (Ahmed *et al.* 2008). The major pesticides used in rice crops in Punjab are Cyhalothrin (Karate), Malathione, Monocrotophos (Novacran) and Cartap (Padan) pesticides. The Stockholm Convention on Persistent Organic Pollutants addressed that the total nine out of the twelve most harmful and persistent organic chemicals are pesticides (Gilden *et al.,* 2010). Cartap is essentially a contact and stomach poison. It is generally considered to be a safe compound with oral LD_{50} in the monkey of 100-200 mg/kg body weight. We know a case of intentional ingestion of Cartap hydrochloride as a suicidal attempt in a farmer which subsequently resulted in severe respiratory failure (Boorugu and Chrispal, 2012). Special care should be done while spraying these pesticides because Malathione is a source for heavy indoor pollution and it can be fatal for in-living persons on inside the room exposure in any form. So it is greatly recommended that the doors and windows should be kept close during sparying of fields with Malathione (Bonner *et al.,* 2007). They have been using first time in rice paddy and remain in use till the ripening of the crops. The study was designed to investigate the absorbance level of these five different pesticides used in rice crops in different rice samples, collected from different geographical regions of Punjab, using High Performance thin layer chromatography (HPTLC).

Material and Methods

Sample Collection: A total of 106 samples collected from all specified areas. A 100 g of each rice sample was collected in plastic zipper bags, labeled with area of cultivation and variety of rice with date and time of collection. All the collected specimens were then transported to Quality Operation Laboratory at University of Veterinary and Animal Sciences for analysis. Well mixing of whole sample was done, then it was grinded and sieved and a fine particle sample was prepared by passing through 20-mesh size.

Extraction and Cleanup procedure: 20-50 g duplicate ground sample of rice was blended with 350 mL Acetonitrile (H_2OCH_3CN) mixture at high Speed in a stainless steel blender unless a well, homogenized blend was prepared. Then, it was filtered with suction through Buckner funnel in to 500 mL suction flask and filtrate of residues was transferred to Petroleum ether. The filtrate was transferred to 1 L separator, shacked vigorously for 1-2 min and 10 mL saturated sodium chloride and 600 Ml water were added. Then approx. 15 g anhydrous sodium sulfate (Na_2SO_4) was added and shacked vigorously. Solvent was concentrated to 15-10 mL in K-D concentrator by starting evaporation and the solution was directly transferred to Florisil column for cleanup prior to determination.

Determination of Pesticides: Thin layer plates were prepared by applying silica gel on glass plates and drying them in incubator. All the four pesticide standards were prepared 100 ppm solution in methanol. Acetyl cholinesterase enzyme was used to develop the HPTLC plates. The Substrate solution was prepared by taking 100 g of acetyl thiocholine iodide was mixed in 100 ml of distilled water to catalyze by the enzyme. Ethyl acetate used as mobile phase to develop the chromatograms. Then extorted samples were sampled on the plates along with their standards. After development in mobile phase, spot diameter was also calculated for each of the pesticides along with their standard. Quantitation of pesticides was done by comparing spot diameters of test samples with standards on TLC plate which was then converted to mg/L in each specimen from different regions. After the development of plates distance travelled by the ethyl acetate and the pesticide spots were measured (Iqbal *et al.,* 2009). A retention factor R_f was then calculated with the given formula.

Statistical Analysis: Data was entered and analyzed using SPSS 17.0. (SPSS Inc. Chicago, USA). The categorical values were expressed in the form of frequency and proportion. Bar Charts were used to display the data. A one way ANOVA was applied to compare the mean content level in the samples.

Results

In almost all rice samples from different geographical regions of Punjab, L-Cyhalothrin was detected in large quantity while Malathione was not detected in many samples. In Narowal and Kamoke region, the concentration of pesticides was more than 0.1mg/L for Cyhalothrin, less than 0.02 mg/L for Monocrotophos, ranged between 0.06 to 0.08 mg/L for Cartap while absent for Malathione in any of the samples (Fig.1 and 2). In Gujrat region, the concentration of pesticides was more than 0.08mg/L for Cyhalothrin, less than 0.02 mg/L for Monocrotophos, ranged between 0.06 to 0.08 mg/L for Cartap and less than 0.01 mg/L for Malathione (Fig.3). In Gujranwala and Dinga region, the concentration of pesticides ranged between 0.08mg/L to 0.1 mg/L for Cyhalothrin, between 0.06 to 0.08 mg/L for Cartap and less than 0.02 mg/L for Monocrotophos (Fig.4) however Malathione was not detected in samples from this Dinga (Fig.5). From Table 1-7 it is observed that the average concentration of pesticides residues were statistically insignificant in all growing areas of rice (i.e. P-value is greater than level of significant=0.05)

Discussion

The above mentioned results showed that there were no significant differences in type of pesticide with respect to area of rice cultivation (P>0.05). This means that almost same types of pesticides are being used in rice crops in all areas of the Punjab. Similarly, the use of pesticides for different varieties of rice is the same as revealed by our results (P>0.05). It was concluded that the pesticides residues were present in almost all samples of rice with the exception of Malathione which was detected only in 16 samples from Gujranwala and Gujrat regions. Parsons *et al.* 2010 conducted the study on Effects of Pesticide use in Rice Fields on birds and reported that rice has absorbed significant quantity of pesticide which has adverse effect for human and birds, which is also supported by our study. Ntow *et al*. (2006) reported that use of pesticide in all type vegetable crops has hazards for health of both the farmers and animals. The results showed that significant levels of pesticides in their specimens. The results were insignificant with respect to area and type of rice, that there was no difference in average concentration level of pesticides in different areas and different types of rice. The results were almost same in the rice cultivated in Narowal in comparison with Kamoke, Gujranwala, Gujrat and Dinga. There was

not any significant difference among different rice varieties like Basmati (Super kernel), IRRI 86 and other types of rice including Kainat, Superfan, and IRRI 6 etc. For different varieties of rice, same pesticides are used and it does not affect the concentration level of pesticide in rice. The study agrees with the research work of Ahmed *et al.* (2008), in which the researchers carried out their study in Kala Shah Kaku, Kasur, Sheikhpura and Batapur, and they found almost same results in specimens collected from different areas in different varieties i.e. Basmati Pk-385 and Irri-6 and also showed that pesticide residual level was present in all type Husked and Unhusked rice specimen. The current study showed that almost all type of rice including Basmati and IRRI varieties of rice have significant levels of various pesticides. Whereas, Alia *et al*. in 2011, reported different results in contrast to the result of this study. According to her study, estimated intake of pesticides through consumption of rice will be a major health risk for human beings. The mean level of all detected pesticides was below the standard lethal doses which indicate that there are no chances for acute poisoning of these pesticides by consuming the rice but may be a future threat on long exposures.

Acknowledgments

We highly acknowledge the persons from rice mills for collection of samples from all growing areas and the staff of Quality Operation Laboratory (WTO) of University of Veterinary and Animal Sciences, Lahore for helping out in laboratory work.

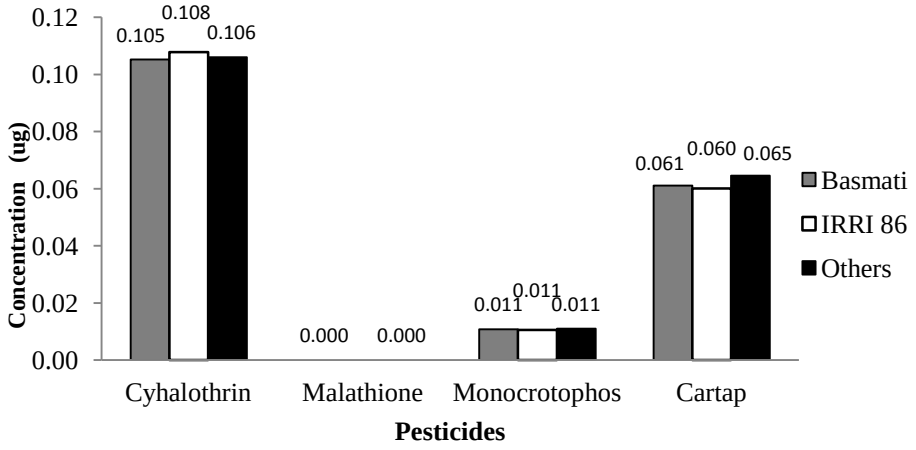

Fig.1. Concentration of Pesticides in rice specimens from Narowal Region

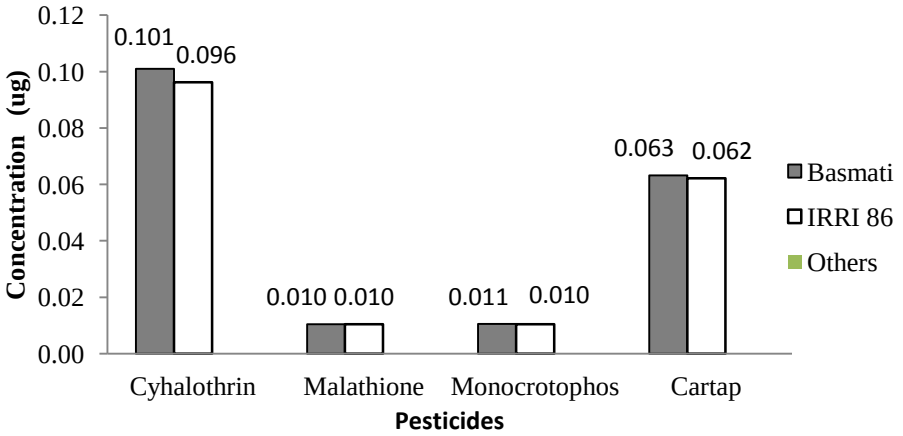

Fig.2. Concentration of Pesticides in rice specimens from Kamoke Region

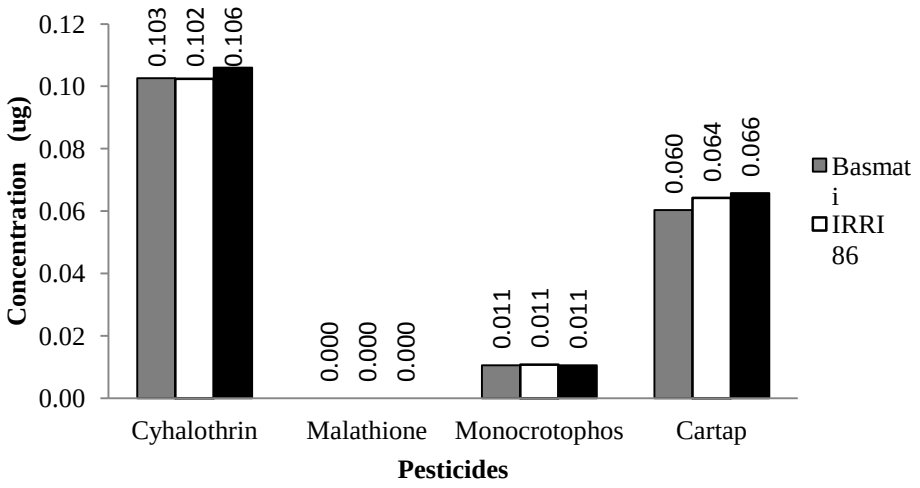

Fig.3. Concentration of Pesticides in rice specimens from Gujrat Region.

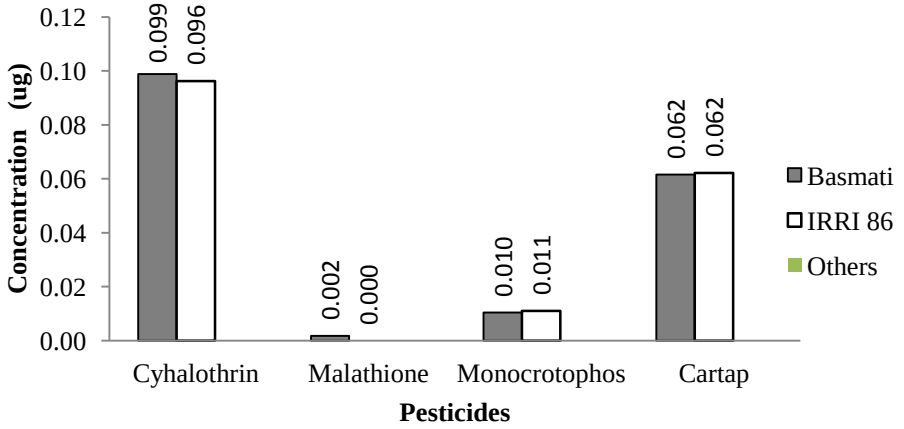

Fig.4. Concentration of Pesticides in rice specimens from Gujranwala Region.

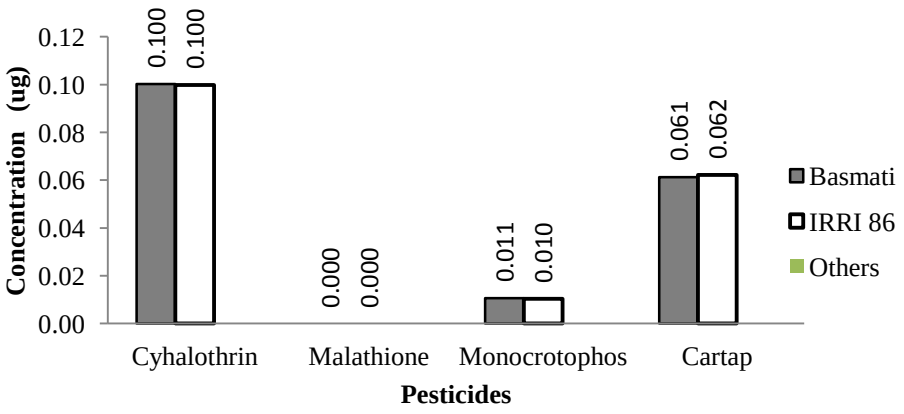

Fig.5. Concentration of Pesticides in rice specimens from Dinga Region.

Table 1. ANOVA Table for mean content level of L – Cyhalothrin with respect to area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.000	2	0.000	1.497	0.229
Within Groups	0.007	103	0.000		
Total	0.007	105			

Table 2. ANOVA Table for mean content level of Malathione with respect to area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.001	4	0.00	27.39	0.00
Within Groups	0.001	101	0.00		
Total	0.002	105			

Table 3. ANOVA Table for mean content level of Malathione with respect to area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.000	2	0.000	1.081	0.343
Within Groups	0.001	103	0.000		
Total	0.002	105			

Table 4: ANOVA Table for mean content level of Monocrotophos with respect to area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.000	4	0.00	0.149	0.963
Within Groups	0.000	101	0.00		
Total	0.000	105			

Table 5: ANOVA Table for mean content level of Monocrotophos with respect to area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.000	2	0.000	0.228	0.796
Within Groups	0.000	103	0.000		
Total	0.000	105			

Table 6: ANOVA Table for mean content level of Cartap with respect to area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.000	4	0.00	0.426	0.789
Within Groups	0.003	101	0.00		
Total	0.003	105			

Table 7: ANOVA Table for mean content level of Cartap with respect to area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.000	2	0.000	1.772	0.175
Within Groups	0.003	103	0.000		
Total	0.003	105			

References

Ahmed, S., Zia-Ul-Haq, M., Imran, M., Iqbal, S., Iqbal, J. and Ahmad, M. (2008). Determination of Residual Contents Of Pesticides In Rice (Oryza Sativa L.) Crop From Different Regions Of Pakistan. *Pakistan Journal of Botany*. 40(3), 1253-1257.

Alia, B.M., Hina, A.S., Fayyaz, A.A., Razia, B. and Tanzil, H.U. (2011). Multiresidue Analysis of Pesticides by Gas Chromatography with Electron-capture in Rice Samples from Different Geographical Regions of Pakistan. *Pakistan Journal of Nutrition.* 10 (9): 851-859.

Boorugu, H.K. and Chrispal, A. (2012). Cartap hydrochloride poisoning: A clinical experience. *Indian Journal of Critical Care Medicine.* 16 (1): 58-59.

Bonner, M.R., Coble, J. and Blair, A. (2007). Malathion Exposure and the Incidence of Cancer in the Agricultural Health Study. *American Journal of Epidemiology*. 166 (9): 1023–1034.

Gilden, R.C., Huffling, K. and Sattler, B. (2010). Pesticides and health risks. *Journal of Obstetrics Gynecological Neonatal Nursing*. 39 (1): 103–110

Golshan, H., Saber, M., Shilsar, F.M., Bagheri, M. and Mahdavi, V. (2013). Effects of Common Pesticides Used in Rice Fields on the Conidial Germination of Several Isolates of Entomopathogenic Fungus, Beauveria bassiana (Balsamo) Vuillemin. *Journal of Entomology*. 15(1): 17-22.

Iqbal, M.F., Maqbool, U., Perveez, I., Farooq, M. and Asi, M.R. (2009). Monitoring of pesticides in brinjal collected from market of NosheraVirkan, Pakistan. *Journal of Animals and Plant Sciences.*19 (2): 90-93

Ntow, W.J., Gijzen, H.J., Kelderman, P. and Drechsel, P. (2006). Farmer perceptions and pesticide use practices in vegetable production in Ghana. *Pest Management Sci*ences. 62(4): 356-65.

Parsons, K.C., Mineau, P. and Renfrew, R.B. (2010). Effects of Pesticide use in Rice Fields on Birds. *Waterbirds*. 33(1):193-218.

Toteja, G.S., Mukherjee, A., Diwakar, S., Singh, P. and Saxena, B.N. (2003). Residues of DDT and HCH pesticides in rice samples from different geographical regions of India: a multicentre study. *Food Additives and Contaminants Journal*. 20(10): 933-939.