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GC-MS ANALYSIS OF MULTICLASS PESTICIDE RESIDUES FOUND IN FARMLAND WATER OF HARYANA DISTRICTS

Sudeep Mishra[1], V. D. Deotale[1], M. A. Pande[1], M. K. Saini[1], M. K. Singh[1], S. Alam*[1], B. S. Sehrawat[2], L. K. Thakur[1] and S. K. Raza[1]

[1]Institute of Pesticide Formulation Technology, Sector-20, Udyog Vihar, Opp. Ambience Mall, NH-8, Gurgaon, Haryana-122016, India.

[2]Haryana Horticulture, Panchkula, Haryana.

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***Corresponding Author**
Dr. S. Alam
Institute of Pesticide Formulation Technology, Sector-20, Udyog Vihar, Opp. Ambience Mall, NH-8, Gurgaon, Haryana-122016, India.

ABSTRACT

Presence of pesticide residue in irrigation water is major problem in India. Only 10-15% of the pesticides reach the target and rest goes to the environment and shows cumulative effects with time. Pesticide may affect the pest population, nature of growth & production of crops. The present study was aimed to monitor irrigation water samples from various districts of Haryana over a period of two years. Samples were extracted for pesticides using Liquid-Liquid extraction. Extract was evaporated to dryness and reconstituted with n-hexane. Pesticides residues were determined qualitative and quantitatively using gas chromatography mass spectrometry (GCMS). Of total tested 112 numbers of water samples. 70 samples were found contaminated with pesticide residue among which 25 samples were found contaminated with multi-residue. Positive samples were found contaminated with Malathion, Chlorpyrifos, O P DDE, Pretilachlor, Butachlor, Atrazine and Trizophos with concentration range 0.044 -0.079 mg/kg, 0.01-0.10 mg/kg, 0.07 mg/kg, 0.01-17.09 mg/kg, 0.012-0.91 mg/kg, 0.012-0.91 mg/kg and 0.05-0.17 mg/kg respectively.

KEYWORDS: Pesticide residue, Water, Liquid- Liquid extraction, GCMS.

INTRODUCTION

Pesticides are commonly used in agricultural production to check or control pests, diseases weeds and other plant pathogens in an effort to reduce or eliminate yield losses and preserve high product quality.[1] Most pesticides show a high degree of toxicity because they are

intended to kill certain organisms and thus create some risk of harm.[2-3] Nowadays pesticide use are of great concern for human health, wildlife and ecosystems.[4] Due to lack of proper training, badly maintained and unsuitable spraying equipment and inadequate storage causes risks to exposure. Pesticide residues in plants are unavoidable in agriculture.[5] Intensive agricultural practices included the use of pesticides to enhance crop yields, which is one of the cause of occurrence and persistence of pesticide residues in water.[6]

Contamination of surface water and groundwater is major environmental problem. Various steps were taken to control pollution of surface waters and groundwaters. Pesticide contamination of surface waters and groundwaters from agricultural use of organochlorine insecticides. Pesticides are primarily moved from agricultural fields to surface waters in surface run-off.[7] The amount lost from fields and transported to surface waters depends on several factors, including soil characteristics, topography, weather, agricultural practices and chemical and environmental properties of individual pesticides Pesticides which are sufficiently resistant to degradation and are adequately soluble to be transported in water may reach the sea in significant amounts. Water run-off and river transport are the main processes involved in the land–sea transfer of pesticides.[8] Ground water can be contaminated by domestic, industrial and agricultural wastes, including fertilizers and pesticides. Several studies have demonstrated a positive correlation between concentrations of pesticides and nitrates, suggesting that these contaminants are linked through land use practice. Monitoring studies have been the most widely used method for assessing groundwater contamination with pesticides. Government and private laboratories around the world have monitor the levels of pesticide residues in imported and locally grown agricultural produce and varying levels of pesticide residues have been reported in the produce.[9]

Study in this paper is based on distributions of pesticides in surface water and ground waters for a period of 2 year (from 2012-2013) from 18 districts of Haryana. Monitoring and assessment of pesticide contamination in farmland water has become a necessity. Particularly, there is need to determine, quantify and confirm pesticide residues in water for both research and regulatory purposes. The pesticides are analyzed by gas chromatography Mass spectrometry.[10-12]

MATERIALS AND METHODS

Individual pesticide stock standard solutions were prepared by exact weighing of high-purity pesticide analytical standards (Sigma-Aldrich) in 10 mL volumetric flasks and filled up with

an appropriate solvent (Merck Chromatography grade) like acetone and n-hexane. Standard stock solution and working standards were stored in deep freezer at −20°C. A working standard prepared for analysis having mixture of all 13 detected pesticides each at 2 ppm concentration are shown in Figure-1. Study of the variations, distributions of pesticides in surface and ground water from 18 districts of Haryana for a period of 2 year (from March 2012 to Dec 2013). Total numbers of 112 farmland water samples were collected from Haryana i.e (Sirsa, Fatehabad, Hisar, Biwani, Rewari, Jhajjar, Rohtak, Jind, Kaithal, Kurukshetra, Ambala, Yamunanagar, Mewat, Faridabad, Jhajjar, Sonipat, Panipat, Karnal by Haryana horticulture, Panchkula. Samples of water were collected in 1 litre amber colored glass bottles, transported to the laboratory in cool-box with ice packs, stored at 4°C and extracting within 48 h.

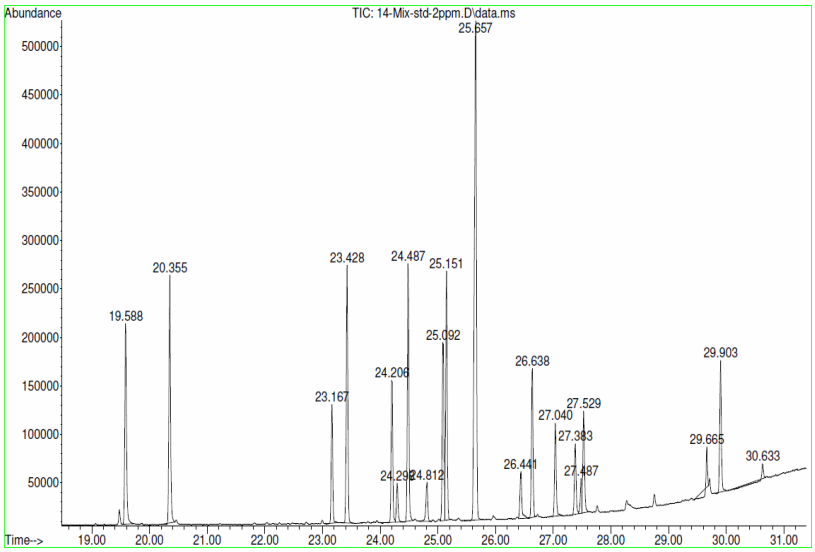

Fig. 1. GC-MS chromatogram of standard mixture of 13 pesticides and its metabolites at 2 ppm level.

Collected water samples were filtered with Whatman no. 1 filter paper to remove debris. 500 mL of water sample was transferred into a 1 liter glass-separating funnel. Then, 100 g of NaCl was added to produce a salt out effect. It was thoroughly mixed by inverting the flask three to four times. The sample was extracted thrice with (100:50:50) mL dichloromethane: Hexane (80:20) mix; shaken for 4–5 min each time with periodic venting. The combined organic phase was dried by passing it through anhydrous Na_2SO_4. The organic phase was dried in a vacuum rotary evaporator (Buchi make) and further sample was reconstituted in 5 mL of n-hexane which was analyzed by GC–MS (Gas Chromatography-Mass Spectroscopy). Matrix of water samples in GCMS is given in Figure 2.

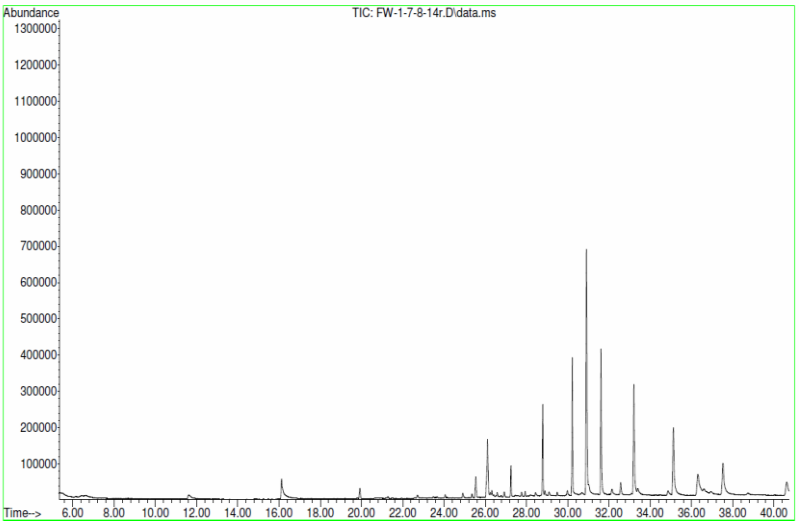

Fig. 2. GC-MS chromatogram of water matrix (Control).

GC Model 7890A (Agilent Technologies) with mass **(**5975C inert XL EI/CI MSD) triple axis detector was used for pesticide residue analysis. Injector port temperature was set at 280^{o}C. DB-5MS fused silica capillary column (Agilent J&W GC column, 5% Phenylated methyl siloxane, 30 m length × 0.25 mm i.d. × 0.25 µm film thickness) was used with linear flow at 1ml/min. 1 micro litre of concentrated extract was injected in splitless mode with carrier gas helium (grade-1). The pesticides were separated with 40.75 min oven programming of initial temperature 50^{o}C for 2 min followed by a ramp rate of 8^{o}C /min up to a temperature of 280°C with a hold time of 10 min. The injector was operated in splitless mode at 280^{o}C temperature. The interface, ion source and quadruple temperatures were set at 280^{o}C and 230^{o}C and 150^{o}C respectively. The mass spectrometer was operated in electron impact (70ev), selected ion monitoring (SIM) mode with solvent delay time 3 min. The scanning mode offer enhances selectivity over either full scan or selected ion monitoring (SIM). The retention time, peak area and peak height of the sample was compared with those of the standards for quantization.

RESULTS AND DISCUSSION

Main occupation of peoples in Haryana are agriculture. Haryana is one of the most rice and wheat producing area of India. Maximum use of the pesticide in these crops by farmers from soaing to harvest and storage to raise heavy production. Direct effect by use of pesticides occurs in water, that's why monitoring is necessary. Monitoring of pesticides in water from 18 districts of Haryana shows that water samples are found contaminated by pesticides of daily use in agriculture. Pesticides of different groups detected in water i.e organochlorine,

organophosphorous, herbicides and synthetic pyrithroides with their respective retention time of each pesticide. are given in Table 1.

Table 1: The usage, chemical group, molecular weight, retention times and fragmentation of detected pesticides from agriculture water samples of Haryana districts.

S.No.	Pesticide	Chemical group	Molecular Weight	R.T.	Qualifier Ions (m/z)		
					Q1	Q2	Q3
1.	Malathion	Organophosphate	330	23.34	173	125	93
2.	Chlorpyriphos	Organophosphate	350	23.5	97	197	199
3.	Butachlor	Herbicide	312	25.27	160	176	57
4.	Atrazine	Insecticide	215	20.73	215	200	58
5.	Pretilachlor	Organochlorine	312	25.76	162	238	176
6.	Trizophos	Organophosphate	313	27.68	172	161	97

For analysing samples, analytical method was set by optimizing G C parameters like; oven temperature programming, carrier gas flow rate and split control to get the better chromatographic separation in run time of 40.75 minutes. GC-MS instrument gave better peaks separation, peak shape at lower concentration. Limit of Detection (LOD) and Limit of Quantification (LOQ) was calculated as the lowest concentration of a pesticide in a water matrix. LOD and LOQ values were found below the MRL value of individual pesticides set by European Union for water sample. Recovery study at 0.5 mg/kg concentration was done for the pesticide detected in the samples and it was found that Malathion, Chlorpyrifos, Butachlor, Atrazine, Pretilachlor, Trizophos were recovered at 80.3, 90.5, 91.5, 75.5, 90.5 and 85.5 percent respectively. The pesticides residue recorded below the detection limit were considered as non detectable (ND). Recovery percentage was found with in Codex acceptable range (67-128%).

Table-2. Recovery percent, LOD and LOQ values of detected pesticides in water samples.

S.No.	Pesticide	Fortification level (mg kg−1)	Recovery (%)	Limit of detection (µg kg−1)	Limit of Quantification (µg kg−1)
1.	Malathion	0.5	80.3	0.25	0.5
2.	Chlorpyriphos	0.5	90.5	0.1	0.3
3.	Butachlor	0.5	91.5	2	6
4.	Atrazine	0.5	75.5	5	15
5.	Pretilachlor	0.5	90.5	0.1	0.3
6.	Trizophos	0.5	85.5	10	30

Total 112 water samples were collected from major crop producing 18 distrists of Haryana. From the total samples collected, 7 samples were collected from villages of Faridabad from

which 2 samples were found contaminated with pesticides (Butachlor). 12 samples were collected from villages of Karnal from which 5 samples were found contaminated with pesticides (Chlorpyrifos, Pretilachlor) and 2 samples of them were above MRL. 19 samples collected from villages of Panipat from which 8 samples were found contaminated with pesticides (Chlorpyrifos, Pretilachlor and Trizophos) and 5 samples of them were above MRL. 4 samples collected from villages of Bhiwani from which 2 samples were found contaminated with pesticides (Pendimethlin, Chlorpyrifos). 5 samples collected from villages of Sonipath were found contaminated with pesticides (Pretilachlor, Butachlor) and 2 samples were above MRL. 5 samples collected from villages of Hisar from which 3 samples were found contaminated with pesticides (Pretilachlor) and 1 sample of them were above MRL. 5 samples collected from villages of Sirsa from which 3 samples were found contaminated with pesticides (Atrazine, Pretilachlor, Butachlor). 5 samples collected from villages of Palwal from which 2 samples were found contaminated with pesticides (Atrazine). 5 samples collected from villages of Meewat from which 3 samples were found contaminated with pesticides (Butachlor, Pretilachlor). 5 samples collected from villages of Jind from which 2 samples were found contaminated with pesticides (Pretilachlor, Butachlor). 5 samples collected from villages of Narnaul from which 1 samples were found contaminated with pesticides (Butachlor). 5 samples collected from villages of Fatehabad from which 3 samples were found contaminated with pesticides (Pretilachlor). 5 samples collected from villages of Kaithal from which 5 samples were found contaminated with pesticides (Chlorpyrifos, Pretilachlor) and 1 sample of them were above MRL. 4 samples collected from villages of Kurukshetra from which 4 samples were found contaminated with pesticides (Chlorpyrifos, Pretilachlor) and 1 sample of them were above MRL. 5 samples collected from villages of Jhajjar from which 4 samples were found contaminated with pesticides (Malathion, Butachlor, Pretilachlor, Chlorpyrifos) and 3 samples of were above MRL. 5 samples collected from villages of Rewari from which 5 samples were found contaminated with pesticides (O P-DDE, Malathion, Butachlor, Pretilachlor) and 2 samples of them were above MRL. 5 samples collected from villages of Ambala were found contaminated with pesticides (Chlorpyrifos, Pretilachlor) and 1 sample of them were above MRL. 5 samples collected from villages of Yamunanagar from which 5 samples were found contaminated with pesticides (Pretilachlor, Butachlor, Malathion) and 1 sample of them were above MRL. 5 samples collected from villages of Rohtak were found contaminated with pesticides (Pretilachlor, Malathion, Butachlor) and 1 sample of them were above MRL. Water samples collected from Sonipat, Kurukshetra, Rewari, Ambala, Yammuna Nagar and Rohtak were found totally

contaminated with pesticides whereas from Faridabad, Karnal, Panipat, Hisar, Sirsa, Palwal, Meewat, Jind, Narnaul, Fatehabad were found least contaminated.

From tested water samples frequencies and pesticide concentration ranges are shown in Table-3. Tested water samples were found contaminated with pesticides, out of 112 water samples 70 samples of them were found positive. From positive samples, 8 of them were found contaminated with Malathion with concentration range 0.044 -0.079 mg/kg and above MRL (0.0005mg/kg) where as 16 samples were found contaminated with Chlorpyrifos with conc. range 0.01-0.10 mg/kg and all above MRL(0.0003 mg/kg). single sample was found contaminated with O P DDE with conc 0.07 mg/kg above MRL value (0.00004 mg/kg). 17 samples were contaminated with Butachlor with concentration range (0.012-0.91 mg/kg); 8 samples were contaminated with Atrazine with conc. range 0.002-0.021 mg/kg; 45 samples were found contaminated with Pretilachlor with conc range 0.01-17.09 mg/kg; 3 samples were found contaminated with Trizophos with conc. range 0.05-0.17 mg/kg.

Table 3: Frequencies and pesticide concentration ranges found in the tested water samples.

S.No.	Pesticide	Number of Positive samples	Detection %(Total=70 Detected)	Range(mg/kg)	Maximum Residue Limit(MRL) of Pesticides µg/l	Number of samples exceed MRL
1.	Malathion	8	11.42	0.044-0.079	0.5	8
2.	Chlorpyriphos	16	22.89	0.01-0.01	0.3	16
3.	Butachlor	17	24	0.012-0.91	-	-
4.	Atrazine	8	11.43	0.002-0.021	-	-
5.	Pretilachlor	45	64.3	17.09- 0.01	-	-
6.	Trizophos	3	4.2	0.05-0.17	-	-

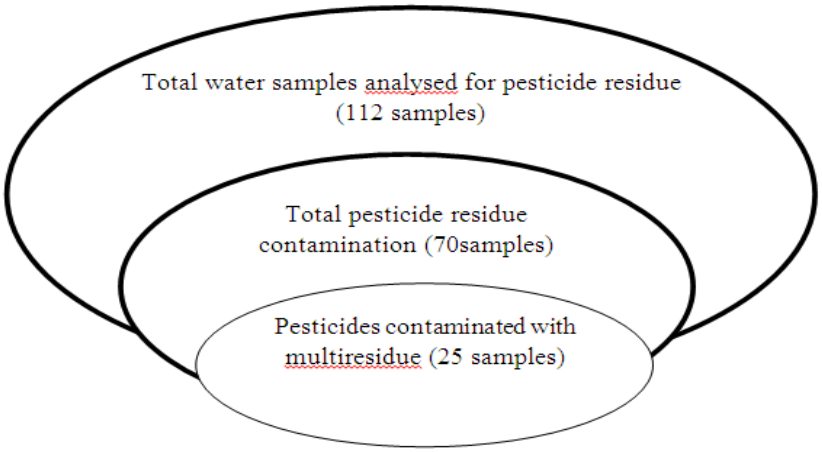

Fig. 3. Representing the pesticide contamination in water samples

CONCLUSION

Pesticides were detected in about 70% of the samples collected from different locations. Results of monitoring of pesticide in water of Haryana 18 districts for the year 2012-2013 are given in Figure.3. From total 112 water samples 70 samples were found contaminated with pesticide among which 25 samples were found contaminated with multiresidue. Malathion, Chlorpyrifos, Butachlor Atrazine, Pretilachlor, Trizophos, O P DDE detected in 8, 16, 17, 8, 45, 3, 1 water samples with detection percentage 11.42, 22.89, 24, 11.43, 64.3, 4.2, 1.7 percent respectively, out of 70 positive water samples, Pretilachlor , Chlorpyrifos , Butachlor were detected in maximum numbers of tested water samples. Pretilachlor, Chlorpyrifos, Malathion, Butachlor were detected in many samples. Atrazine, Trizophos and isomers of DDT were also detected in some samples. Many samples were found contaminated with multiresidue. Trizophos, Butachlor and Pretilachlor were detected in high concentration in some samples.

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