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Residue Analysis of an Organophosphate Pesticide in Wild Plants in Lahore Area

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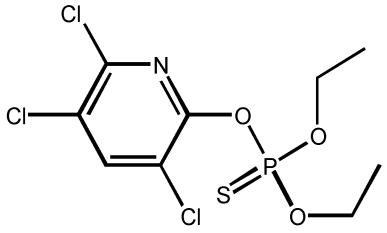
Abstract: Residue analysis of chlorpyrifos (an organophosphate pesticide) was investigated in different wild plants. Chlorpyrifos was analyzed in different samples of plants collected from different sites of Lahore. Analysis was carried out using High Performance Liquid Chromatography (HPLC). Ethyl acetate, acetonitrile (HPLC), methanol, dichloromethane are the solvents were used as mobile phase to achieve the best separation. Investigation of all sample showed that no residue was found in 31.8% samples while 54.54% samples contained chlorpyrifos residue at or below MRL and 13.6% samples contained chlorpyrifos residue above MRL. Chlorpyrifos was detected in 0.02-0.71 mg/kg concentration range and the results showed that concentration of chlorpyrifos varies from 0.02 mg/kg to 0.71 mg/kg. Maximum limit of chlorpyrifos residue in these plants established by either World Health Organization (WHO) or European Union (EU) is 0.05, 0.5, 0.5 mg/kg. The highest concentration of chlorpyrifos residue was 0.71 mg/kg in Melilotus Indica. Study of residue analysis of chlorpyrifos in plants has the significant role to solve the environmental problems related to pesticides.

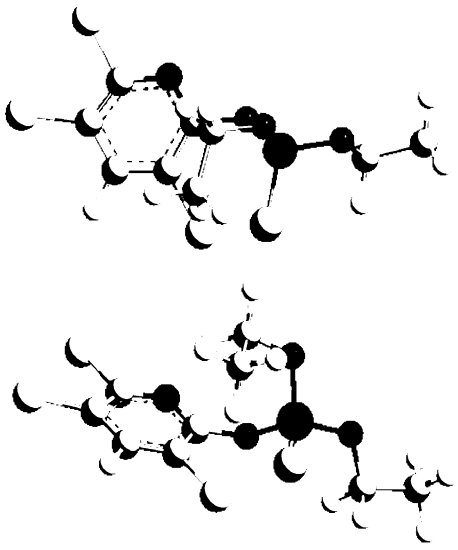
Key words: Residue Analysis · Pesticide · Chlorpyrifos · Wild Plants · HPLC

INTRODUCTION

Agrochemicals have importance in agriculture but may also harmful for the human health and safety of mammals due to their persistence and toxic nature. Chlorpyrifos is a toxic crystalline organophosphate insecticide. Structural formula and 3D representation of the chlorpyrifos are shown as:



Chlorpyrifos [*o, o* diethyl-*o*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate]



Side 3D views of Chlorpyrifos

It is a white, granular, crystalline solid. Its melting point is 42-43°C and vapour pressure is 1.87×10^{-5} mm Hg at 25°C and 8.87×10^{-5} mm Hg at 35°C. Chlorpyrifos is soluble in the solvents like benzene (7900g/kg), acetone (6500g/kg), chloroform (6300g/kg), methanol (450g/kg), ethyl acetate (2000g/kg). It is slightly soluble in water (0.4ppm). Chlorpyrifos is stable in air (non volatile) and is not sensitive to UV radiation. It is stable to neutral and weakly acidic solutions, but is hydrolyzed by strong bases. It underwent hydrolysis in presence of water to

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liberate 3, 5, 6-trichloro-2-pyridinol which underwent further decomposition to diols and triols and ultimately cleavage of ring to fragmentary products [1]. Hydrolysis in water occurs least readily at about pH-6 and very readily above pH-8. Chlorpyrifos is thermally sensitive to temperature over 50°C and undergoes violent exothermic decomposition at 130°C. The half life of aqueous methanolic solution at pH-6 is 1930 days. The rate of hydrolysis increases with both pH and temperature.

Chlorpyrifos inhibits acetyl cholinesterase and used to control pests. It kills animals by attacking their nervous system. Chlorpyrifos inhibits the action of cholinesterase, a natural enzyme produced by both insects and mammals that turns off signals sent by nervous system. When cholinesterase is blocked by chlorpyrifos, the body cannot turn off these signals, which essentially locks the nervous system in an "ON" position. Chlorpyrifos is a neurotoxin and suspected endocrine disrupter [2].

Its poisoning usually affects many organs of the body. Among the most commonly affected are the central and peripheral nervous system, eyes, respiratory system and the digestive tract. Symptoms of acute and chronic chlorpyrifos exposure in humans include headache, dizziness, vomiting, weakness, twitching, sweating, stomach cramps and hypertension and at high doses include unconsciousness and coma and even death. The estimated accepted daily intake (ADI) for humans was set at 0-0.1 mg/kg bw^{-1} d^{-1} by the WHO/PCS and by the FAO/WHO.

It is used in agriculture. The crops with the most intense chlorpyrifos use are cotton, corn, almonds, vegetables and fruit trees including oranges and apples [3]. Depending upon the type of application and pesticide formation, chlorpyrifos residue may be detectable in water, soil and on surfaces for months to years [4, 5].

Concerning the analysis of chlorpyrifos residue in wild plants, there are few works in the literature dealing this topic [6-8]. Our findings are new, particularly in the area of Lahore and will be helpful to update the scientific knowledge in the field of pesticides.

MATERIALS AND METHODS

Materials: Organic solvents (residue analysis grade) for dissolving and extracting were methanol, ethyl acetate, dichloromethane, acetonitrile (HPLC grade), acetic acid purchased from Merck (Germany). Reference material was obtained from Dr. Ehrenstorfer GmbH Germany. Stock standard solutions were prepared by accurately weighing 0.0104gm of standard chlorpyrifos and dissolved it in methanol and made the volume 100ml.

Working standard solutions of 0.1 ppm, 0.5ppm, 1ppm and 2ppm were prepared by dilution with methanol. For each standard solution took 50µl, 250µl, 500µl and 1ml of stock standard solution and made the volume 50ml.

Stock standard solution and working standard solutions were stored under refrigeration (4°C). Purity of standard was higher than 95%. Anhydrous sodium sulphate and sodium chloride (extra pure) were purchased from Merck (Germany).

Apparatus: A high performance liquid chromatograph (HPLC), Agilent 1100, equipped with diode array detector (DAD) was used for the analysis of residues of chlorpyrifos. HPLC column, Zorbax RX C_{18} (4.6mm ID; 250mm length; 5µm particle size, stainless steel) was used for chlorpyrifos residue analysis. Mobile phase was a combination of acetonitrile (82.5%), water (17%) and acetic acid (0.5%). Injection volume was 20µ liters.

Sampling: Total 22 samples of different wild plants (Ageratum Conyzoide, Melilotus Indica and Parthenium Hysterophorus) were collected from the fields of Raiwind Area near Lahore. These wild plants (Ageratum Conyzoide, Melilotus Indica) are used in the preparation of different medicines (herbal). These samples were analyzed after 24 hours being stored at 4°C until analysis. These samples were collected from November 2008 to June 2009.

Extraction: The entire sample (50gm of plant) was grinded in a grinder and made a paste of it. Then 10gm of grinded sample was weighed. 20gm anhydrous sodium sulphate and 2.5gm of extra pure sodium chloride was added. Dissolved the above sample in 100ml of solvent (methanol, ethyl acetate, dichloromethane). Blended it on high speed varying blender for 3 minutes. Filtered it through 0.4µm filter paper. Added 25ml solvent mentioned above in the residue and blend it for 10 minute. Filtered it again. Concentrated the filtrate on rotary evaporator. Dried it on hot plate. Dissolved in 100% solvent and analyzed by HPLC (Fig. 1).

RESULTS AND DISCUSSION

Pesticide was identified from its retention time and confirmed by comparison with authentic standard. A total of 22 samples of different wild plants (ageratum conyzoide, melilotus indica, parthenium hysterophorus) were analyzed. Frequency and concentration of pesticide in the analyzed samples are outlined in table 2. It was found from the results that in 7 (31.8%) samples no

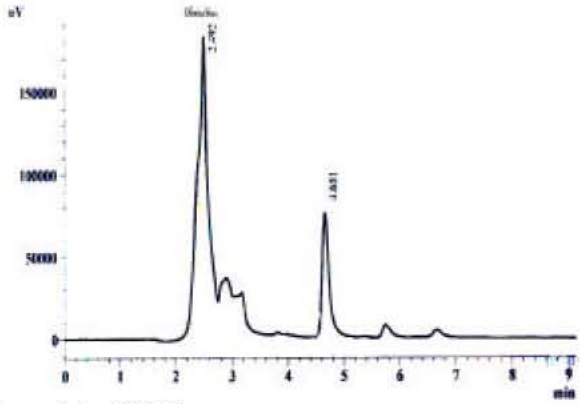

Fig. 1: Chromatogram of a plant sample by HPLC

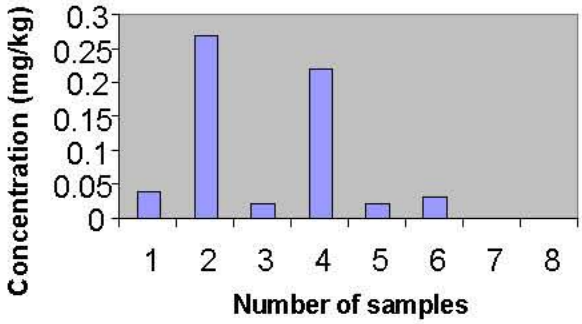

Fig. 2: Plot of concentration of chlorpyrifos residue in *Ageratum Conyzoide*

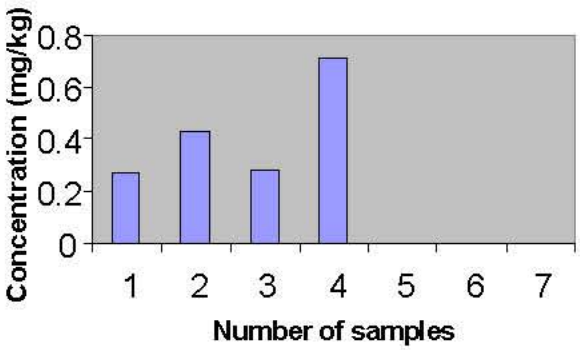

Fig. 3: Plot of concentration of chlorpyrifos residue in *Melilotus Indica*

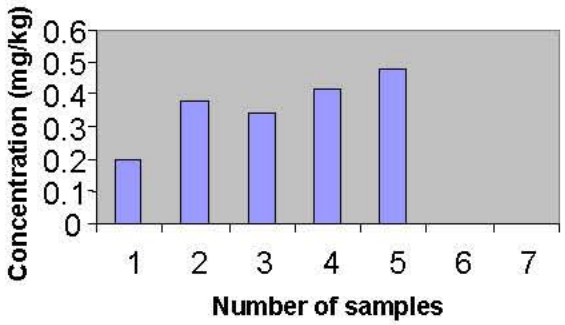

Fig. 4: Plot of concentration of chlorpyrifos residue in *Parthenium Hysterophorus*

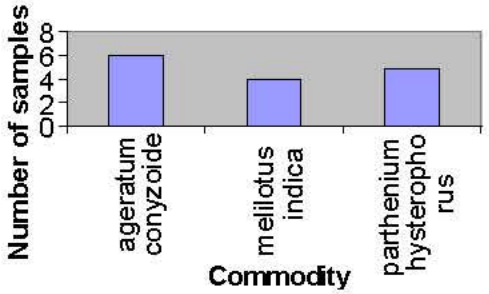

Fig. 5: Total number of samples containing chlorpyrifos residue

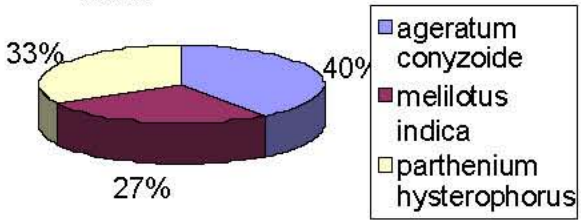

Fig. 6: Percentage of samples containing chlorpyrifos residues

residue was found, 12 (54.54%) samples contained chlorpyrifos residue at or below MRL (maximum residue limit) established by either World Health Organization (WHO) or European Union (EU). 3 (13.8%) samples contained chlorpyrifos residue above MRL. The most exceeded MRL value is 0.71mg/kg of melilotus indica.

Chlorpyrifos residue was detected in 15 samples out of 22 samples. These samples are, 6 from ageratum conyzoide plant (Table 1, Fig. 2), 4 samples of melilotus indica (Table 1, Fig.3) and 5 samples of parthenium hysterophorus (Table 1, Fig. 4). The total number of commodities containing residue is presented in Fig. 5. The maximum concentration was detected is 0.71mg/kg in melilotus indica. Concentration of chlorpyrifos was greater in melilotus indica than ageratum conyzoide and

Table 1: Concentration of chlorpyrifos in three different wild plants, HPLC data and their MRL values

No of plant	Plant Name	Sample Location	Solvent	Retention time (minutes)	Area (m^2)	Plate Height	Theoretical Plate	Tailing Factor	Conc. mg/kg	MRL values mg/kg
1	Ageratum	Raiwind 1	Methanol	2.44	396309	26634	721.028	1.057	0.04	0.05
	Conyzoide	Raiwind 1	Ethyl Acetate	2.5	2565355	184228	1148.92	0	0.27	0.05
		Raiwind 2	Methanol	2.45	170076	10036	520.252	0.847	0.02	0.05
		Raiwind 2	Ethyl Acetate	2.53	2027786	149440	791.403	0	0.22	0.05
		Raiwind 3	Methanol	2.43	204278	12377	403.638	0.869	0.02	0.05
		Raiwind 3	Ethyl Acetate	2.53	285633	30862	2447.325	1.119	0.03	0.05
2	Melilotus	Raiwind 4	Methanol	2.45	2509816	27884	1830514	0	0.27	0.5
	Indica	Raiwind 4	Dichloro methane	2.52	4088370	320782	177.815	0	0.43	0.5
		Raiwind 5	Dichloro methane	2.52	2637518	167991	535.141	0	0.28	0.5
		Raiwind 6	Dichloro methane	2.51	6703549	327453	100.061	0	0.71	0.5
3	Parthenium	Raiwind 7	Methanol	2.44	1859625	432483	60.306	0	0.2	0.5
	Hysterophorus	Raiwind 7	Dichloro methane	2.45	3554438	395303	406.788	0	0.38	0.5
		Raiwind 8	Dichloro methane	2.45	3232764	358097	98.305	0	0.34	0.5
		Raiwind 9	Dichloro methane	2.51	3993050	359591	46.882	0	0.42	0.5
		Raiwind 10	Dichloro methane	2.5	4552743	413248	27.957	0	0.48	0.5

Table 2: Concentration limits of chlorpyrifos residues in wild plants (Total samples 22)

Sr. No.	Plant Name	No. of samples (Detected)	No. of samples (Not Detected)	Concentration range mg/kg
1	Ageratum Conyzoide	6	2	0.02-0.27
2	Melilotus Indica	4	3	0.27-0.71
3	Parthenium Hysterophorus	5	2	0.20- 0.48

parthenium hysterophorus. Different solvents were used for the preparation of extracts of these plants. Methanol used as a common solvent for each plant sample while ethyl acetate and dichloromethane were also used to study the effect of solvent on the separation of residues. Ethyl acetate and dichloromethane gave the best separation as compared to methanol. Ethyl acetate and dichloromethane absorbed more pesticide from the plants because this pesticide (chlorpyrifos) is more soluble in these solvents than methanol. Results obtained by using ethyl acetate and dichloromethane are presented in Table 1 and shown in Fig. 6.

CONCLUSION

A total of 22 samples were analyzed and chlorpyrifos was detected in most of the samples (68%). The present study shows the high incidence rate of chlorpyrifos in the samples. The results reveal that the solubility of pesticide (chlorpyrifos) plays a very important role in the extraction and detection of that pesticide in any sample of plant, water or soil. The results also emphasize the need for regular monitoring of pesticide residues in agriculture. This work represents the occurrence of chlorpyrifos in wild plants from Lahore Area.

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