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Research Paper

Monitoring of Organochlorine Pesticides in Guava (*Psidium guajava*), Apple (*Malus domestica*), Pear (*Pyrus*), Pomegranate (*Punicagranatum*) and Plum (*Prunus domestica*) by Using QuEChERS Method

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A B S T R A C T

Eighteen organochlorine pesticides (OCP) viz. isomers of benzene (α-BHC, β-BHC, γ-BHC and δ-BHC), heptachlor, aldrin, heptachlor epoxide, γ-chlordane, endosulfan-I+α-chlorodane, dieldrin,+p,p'DDE, endrin, endosulfan-II, p,p'-DDD, endrin aldehyde, endosulfan sulphate, p,p'-DDT, endrin ketone and methoxychlor have been monitored in fruit samples: guava (*Psidium guajava*), apple (*Malus domestica*), pear (*Pyrus*), pomegranate (*Punica granatum*) and plum (*Prunus domestica*) by gas liquid chromatography coupled with Electron Capture Detector (GC-ECD). During the study it has been found that most of the samples were contaminated with either two or more OCP, but residual concentrations were below the MRL values prescribe by (Codex Alimantarius, 2016) but their continuous consumption can produce severe health problems for human.

***Key words*:** Organochlorine, Pesticides, Gas chromatography (GC-ECD), Fruits

Fruits are important part of the human diet as they provide essential nutrients that are required for most of the reactions occurring in the body. High intake of fruits not only prevents consequences due to vitamin deficiency but also reduces the incidence of major diseases such as cancer, cardiovascular and obesity. To increase the yield and to improve the quality of fruits certain pesticides are used. However, pesticides are beneficial for modern farming to feed the world's growing population, but quality is of equally important as quantity. The main role of pesticides is to prevent pests that cause damage to fruits. A wide range of pesticides is used to protect agriculture crop due to heavy infestation throughout the cropping season [1]. But the excess use of pesticides is a major issue of concern for human health and environment as pesticide residues, which remain after an agricultural treatment, may penetrate plant tissues and found in the pulp and the juice of the fruits [2]. Pesticides have been linked to a wide spectrum of human health hazards, ranging from short-term impacts such as headaches and nausea to chronic impacts such as genotoxicity [3], inhibition of acetyl cholinesterase activity [4], hepatic [5], renal toxicity [6], dermatological conditions [7], cancer [8], neurological deficiencies [9], [10], limb defects [11], reproductive harm [12] and endocrine disruption [13]. Chronic health effects may occur years after even minimal exposure of pesticides in the environment or result from their residues ingested through food and water. Owing to their potential adverse effects on human health and the environment, the contamination of pesticide residues in food commodities has always been a matter of serious concern. Keeping these facts in mind it has been decided to monitor organochlorine pesticides in different fruits. In continuation of our previous work [14], [15], [16] an effort has been made to monitor the residual concentration level of 18 organochlorine pesticides in five fruits viz. guava, apple, pear, pomegranate and plum by using quick, easy cheap effective, rugged and safe (QuEChERS) method.

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MATERIALS AND METHODS

All glassware was washed with deionized water, rinsed with acetone and dried in oven (150°C) for overnight before use. Solvent like n- hexane, acetone and ethyl acetate were distilled in vacuum before use. Column adsorbents like neutral alumina, florisil and charcoal were activated before use. Anhydrous Na_2 SO_4 was purified with acetone and heated for few hours at 600°C in muffle furnace to remove possible phthalate impurities. Purified extracts were analyzed by GLC equipped with capillary columns using 63Ni electron capture detector (ECD). Minor equipment such as mechanical shaker, rotatory evaporator and Warring blender etc. were also used during extraction. 2.5 µl solution of standard with concentration 0.025µg in hexane was injected to record the chromatogram of pesticides.

Extraction of pesticides

Sample collection and preparation: 200 g sample of each fruit was collected from local market, refrigerated at 5°C and analyzed within few days of collection. In order to estimate the right concentration of pesticides reaching within human body, the household processing like washing and peeling off covering etc. were carried out for each sample. A representative sample of each fruit (200 g) was macerated with 25g anhydrous sodium sulphate in warring blender to make fine paste.

Extraction and clean up: 25g fine paste of each fruit was treated with 100 ml acetone by using mechanical shaker for 2 h. The obtained mixture filtered, transferred into a separating funnel and diluted with 50 ml aqueous solution (10%) of NaCl and shaken gently for 30 min. To this, 25 ml ethyl acetate was added and again shaken for 2 h and separating funnel was allowed to stand at vertical position for about 2h to obtain two distinct layers. The ethyl acetate layer was separated out from the separating funnel. This procedure was repeated three times using 25 ml ethyl acetate each time. The collected extract was concentrated up to dryness (2-5 ml) in a rotary evaporator under vacuum.

Purification

The concentrated extracts were dissolved in 25 ml n-hexane separately and subjected for clean up by column packed with silica gel: activated charcoal (5:1 w/w) /silica gel. Each extract was eluted with 50 ml ethyl acetate. Eluted extract was further concentrated up to dryness and re-dissolved in 10 ml n-hexane and then subjected to GC-ECD for analysis of pesticides.

RESULTS AND DISCUSSION

First by running standard of pesticides, chosen for monitoring work, chromatogram has been recorded (Fig 1) for different organochlorine pesticides corresponding to the concentration 0.025µg. The retention time (Rt value), peak area and area % have been presented in (Table 1).

Table 1 Retention time and peak area of organochlorine pesticides standard

Peak	Pesticides	Ret. Time	Area	Area %
1	α- BHC	4.579	679542	0.4303
2	β- BHC	5.101	11954128	7.5691
3	γ- BHC	5.971	15917295	10.0785
4	δ- BHC	7.002	9364725	5.9296
5	Heptachlor	8.270	9881833	6.2570
6	Aldrin	9.805	11567303	7.3242
7	Heptachlor Epoxide	12.068	113517553	7.1914
8	γ- Chlordane	13.999	10287602	6.5139
9	Endosulfan-I+ α- Chlordane	15.631	10532047	6.6687
10	Dieldrin, +p,p'DDE	15.744	12105992	7.6653
11	Endrin	17.200	8961685	5.6744
12	Endosulfan-II	18.235	9522071	6.0292
13	p,p'-DDD	18.983	8387370	5.3107
14	Endrin Aldehyde	19.430	8167879	5.1717
15	Endosulfan sulphate	21.399	6655253	4.2140
16	p,p'-DDT	21.906	5688523	3.6019
17	Endrin ketone	25.223	1476567	0.9349
18	Methoxychlor	27.175	3332483	2.1101

Table 2 Detected pesticides and their concentration

Name of sample	Ret. time	Area of peak	Concentration of sample (µg)	Name of Pesticides
Guava	5.260	3793	0.00001	β- BHC
	7.053	10180	0.00003	δ- BHC
	9.605	15660	0.00003	Aldrin
Apple	4.527	1418	0.00005	α- BHC
	5.266	4067	0.00001	β- BHC
	7.060	7580	0.00002	δ- BHC
	8.082	17208	0.00004	Heptachlor
Pear	7.052	10349	0.00003	δ- BHC
	9.606	44541	0.000096	Aldrin
Pomegranate	4.375	4813	0.00018	α- BHC
	7.063	20835	0.00006	δ- BHC
	8.082	61781	0.00016	Heptachlor
	9.612	28215	0.00006	Aldrin
	13.869	2454	0.00001	γ- Chlordane
	15.440	1794	0.00001	Endosulfan-I+ α -Chlordane
Plum	7.053	7475	0.00002	δ- BHC
	9.606	31354	0.00007	Aldrin

During the monitoring work in the chromatogram of guava (Fig 2) number of peaks are obtained from those three peaks at Rt values 5.260, 7.053 and 9.605 resemble with the Rt values of β-BHC, δ-BHC and Aldrin respectively which indicated the presence of β-BHC, δ-BHC and Aldrin in this sample. In the chromatogram of apple (Fig 3) four peaks at Rt values 4.527, 5.266, 7.060 and 8.082 were very near to the Rt values of α-BHC, β-BHC, δ-BHC and heptachlor respectively which indicated the presence of α-BHC, β-BHC, δ-BHC and heptachlor pesticides in the sample of apple [17]. The chromatogram of pear (Fig 4) exhibited a number of small peaks from those only two peaks at Rt values 7.052 and 9.606 were very close to the Rt values of δ-BHC and Aldrin respectively which indicated the presence of above pesticides in the sample of pear. In the chromatogram of pomegranate (Fig 5) six peaks at the Rt values 4.375, 7.063, 8.082, 9.612, 13.869 and 15.440 resemble with the Rt values of α-BHC, δ-BHC, Heptachlor, Aldrin, γ-chlordane, Endosulfan–I + α-chlordane respectively which showed the presence of above pesticides in the sample of pomegranate. In the chromatogram of plum (Fig 6) two peaks at the Rt values 7.053 and 9.606 were very near to the Rt values of δ-BHC and Aldrin respectively which indicated the presence of δ-BHC and Aldrin pesticides in the sample of plum [18], [19], [20].

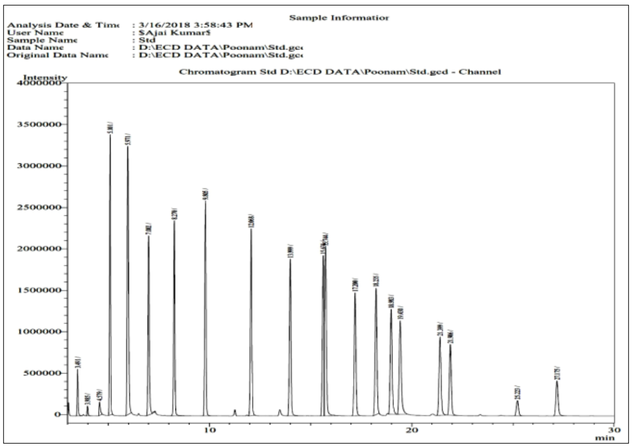

Fig 1 Gas chromatogram of standards

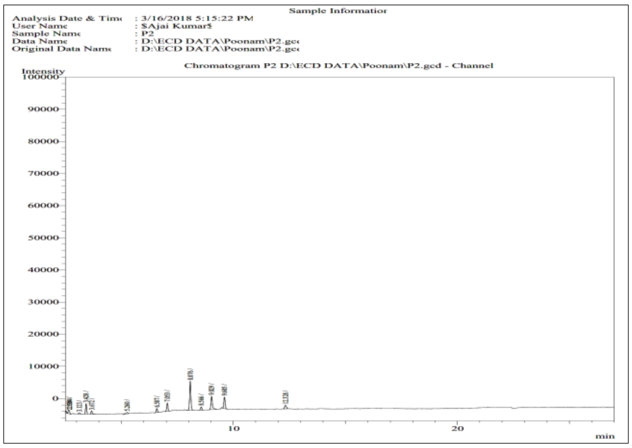

Fig 2 Gas chromatogram of guava

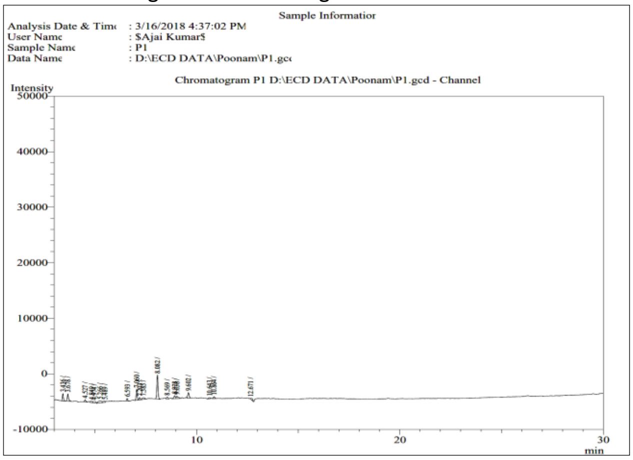

Fig 3 Gas chromatogram of apple

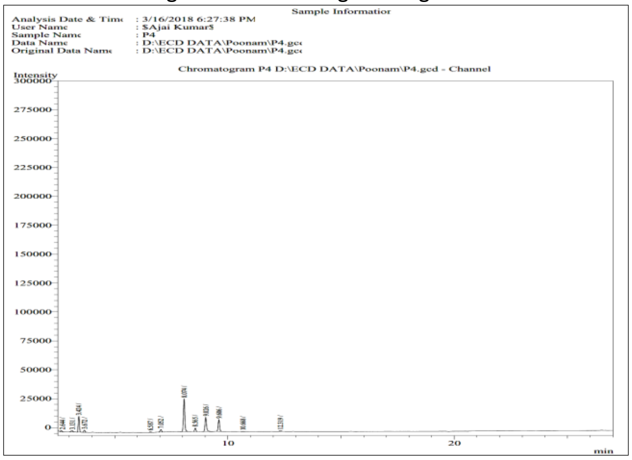

Fig 4 Gas chromatogram of pear

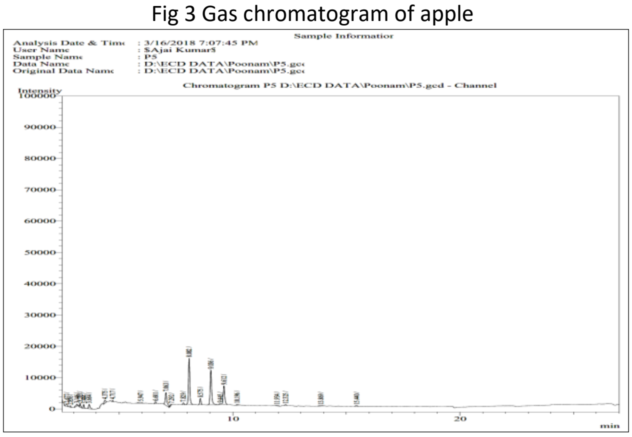

Fig 5 Gas chromatogram of pomegranate

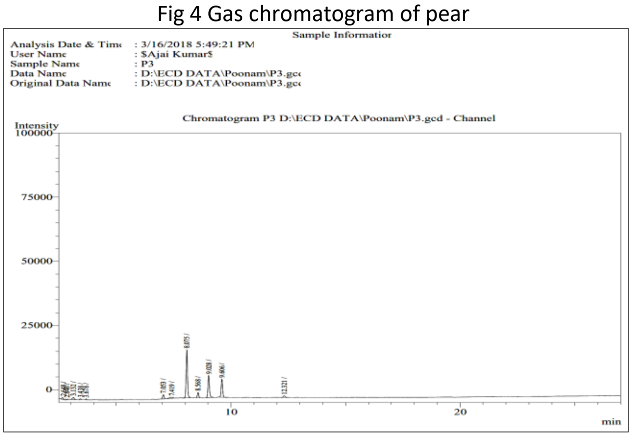

Fig 6 Gas chromatogram of plum

CONCLUSIONS

A simple, cheap and quick method using GC-ECD has been developed for monitoring the different organochlorine pesticides. It was found that all fruits were contaminated with pesticides. Among the all fruits only pear and plum was contaminated with only two pesticides, other fruits were found contaminated more than two pesticides. However, their concentration is very low i.e. below the MRL values, but continuous consumption of pesticide contaminated fruits can cause serious health problems. Our study showed that several banned pesticides were still being used. The results of this study can aware consumers for protection point of view therefore the use of these pesticides should be limited.

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