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Research Article

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Multi-class pesticide residues in apple *(Malus domestica)*, pyrus *(Pyrus calleryana)*, grapes *(Vitis vinifera)*, sweet orange *(Citrus sinensis)* and guava *(Pasidum guajava)* fruits by gas chromatography

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ABSTRACT

Multi-class pesticides viz. isomers of benzene hexachloride (α-BHC, β-BHC, γ-BHC and δ-BHC), heptachlor, heptachlor epoxide, aldrin, γ-chlordane, endosulfan-I+α-chlordane, dieldrin+p,p'-DDE, endrin, endrin aldehyde, endrin ketone, endosulfan II, endosulfan sulphate, p,p'-DDT, p,p'-DDD, methoxychlor, decachlorobiphenyl (DCBP) and synthetic pyrethroids were monitored by an improved extraction method from apple (Malus domestica), pyrus (Pyrus calleryana), grapes (Vitis vinifera), sweet orange (Citrus sinensis) and guava (Pasidum guajava) using Gas Chromatography coupled with Electron Capture Detector (ECD). In the sample of apple three pesticides β-BHC, aldrin & malathion ; in pyrus four pesticides β-BHC, aldrin, heptachlor epoxide & malathion; in sweet orange four pesticides β-BHC, aldrin, heptachlor epoxide & malathion ; in guava four pesticides β-BHC, aldrin, heptachlor epoxide & malathion and in grapes two pesticides δ-BHC & aldrin were found. All the results were compared with Codex MRLs. It was found that all fruits were safe for human consumption. Only the residual concentration of malathion was higher than MRL value. It can cause serious health hazards to human health and environment as well so its use on fruits should be limited.

Key words: Multi-class pesticides, Gas-chromatography (GC-ECD), Fruits

INTRODUCTION

Importance of fruits in human diet is well recognized. They have important role in the diet for maintenance of health and prevention [1] of disease. They are essential sources of vitamins, minerals and antioxidants. India is the second largest fruits producer in the world. To obtain the better yield and quality of fruits a wide range of pesticides (13–14%) are applied during the entire period of growth and sometimes even at the fruiting stage [2]. Many researchers have reported the pesticide residues in various fruits including banana, mango, apple, peach, watermelon, melon, grapes, orange, lemon, pear, pineapple, strawberry, raspberry, kiwi, beet, papaya and litchi etc. [3-12] and in some samples concentrations of pesticide residues have been found more than maximum residue level (MRL) values recommended by European union (EU), world health organization (WHO) and food and agricultural organization (FAO). Long term consumption of such fruits can cause several diseases in human, like immune suppression [13], neurological dysfunction [14], endocrine disrupting [15], reproductive abnormalities [16] and carcinoma [17]. Thus, in continuation of our previous work [18-21] in the present study it has been planned to monitor the residual concentration level of selected multi-class pesticide residues in five fruits namely apple, pyrus, grapes, sweet orange and guava.

EXPERIMENTAL SECTION

Materials

All glassware were thoroughly washed with deionized water and then rinsed with acetone and dried in oven (150 ^{o}C) for overnight before use. Solvents like acetone, dichloromethane, ethyl acetate and n-hexane were distilled before use. Adsorbent neutral alumina, florisil and charcoal were activated before use. Anhydrous sodium sulfate was purified with acetone and heated for 4 h at 600 ^{o}C in a muffle furnace to eliminate possible phthalate impurities. Purified extracts of fruits were analyzed by GLC equipped with capillary column using ^{63}Ni electron capture detector (ECD). A stock solution of standard pesticides used for GC study was procured from Dr. Ehrenstorfer Gmbh Chemicals, Germany.

Sample collection and preparation

Samples consist of 1kg of each fruit, i.e. Apple, Grapes, Pyrus, Guava and Sweet orange, was collected from local market. Each sample was refrigerated at 5^{o}C and analyzed within two days of collection. In order to determine the right concentration of pesticides reaching within the human body, the household processing such as washing and peeling off covering etc. were carried out. Each fruit was washed for few minutes under tap water and dried by using filter paper. After drying each fruit was peeled and cut into small pieces. After that these fruits were blended with the high speed warring blender to make a fine paste.

(A) Extraction for apple, pyrus and grapes

50 g fine paste was taken out from the fruit sample of apple and subjected to extraction with 300 ml acetone (3×100 ml) . The extract was filtered with the help of Buchner funnel. The filtrate was concentrated in vacuum up to 5 ml and then transferred to a 500 ml separating funnel. 50 ml saline water (2%, w/v) was added and shaken for 50 min. The extract was exchanged into dichloromethane layer by liquid-liquid partitioning (3×50 ml). The organic layer was separated out from the separating funnel and passed through a layer of sodium sulphate (5 g). The extract was evaporated to dryness (2-5 ml) in rotary evaporator. The concentrated extract was redissolve in 10 ml n-hexane. Similar procedure was adopted for pyrus and grapes.

(B) Extraction for sweet orange

50 g fine paste, 250 ml ethyl acetate and 80 g anhydrous sodium sulphate was taken in a warring blender and mixture was blended for 5 min. The obtained suspension was filtered with the help of Buchner funnel and then filtrate was passed through the layer of sodium sulphate (20 g). The obtained extract was concentrated to dryness (2-5 ml) by using rotary evaporator. The volume of extract was then adjusted by n-hexane to 10 ml.

(C) Extraction for guava

25 g blended sample of guava was macerated with 10 g of sodium sulphate in warring blender. The macerated sample was extracted with 100 ml acetone (2×50 ml) by using separating funnel. Extract was filtered, concentrated up to 50 ml and subjected to liquid-liquid partitioning with 50 ml ethyl acetate for three times after diluting with 10 % aqueous NaCl solution. The extract was concentrated up to 2-5 ml by using rotary evaporator. Finally the concentrated extract was re-dissolved in 10 ml n-hexane.

Purification:

Purification of extracts was carried out by using column chromatography. Columns were packed with silica gel and activated charcoal (5:1 w/w). Extracts were eluted with 25 ml mixture of acetone: hexane (1:9 v/v). After concentrating, final volumes of the elutes were made to 10-15 ml for analysis by gas liquid chromatography.

RESULTS AND DISCUSSION

In Gas-Liquid Chromatographic analysis, chromatogram is drawn between response (mv) and retention time (Rt). First by running solution of standards, we have determined retention time and their peak areas corresponding to 5 ppm concentration.

Gas chromatogram (Fig.1) of standard pesticides exhibited the peaks of different isomers of benzene hexachloride (BHC) at Rt values 5.210, 5.834, 6.003 and 6.676 which correspond to α-BHC, β-BHC, γ-BHC and δ-BHC respectively. The peaks of heptachlor and heptachlor epoxide were found at Rt value 8.285 and 11.637 respectively. The peak at Rt value 9.731 was found for aldrin. The peak of γ-chlordane was found at Rt value 12.934. The peak at Rt value 13.825 was found for endosulfan I + α-chlordane. Dieldrin + p,p'-DDE exhibited peak at 15.241. The peaks at Rt values 16.704, 18.787 and 24.278 correspond to endrin, endrin aldehyde and endrin ketone respectively. The peaks of endosulfan II and endosulfan sulphate were found at Rt values 17.385 and 20.584. The peaks of p,p'-DDD and p,p'-DDT were found at Rt values 18.173 and 21.026. The peak at Rt value 26.181 was found for

methoxychlor. The Rt value of the peak of decachlorobiphenyl (DCBP) was found at 42.525. The peak for malathion [22] has been reported at RT value 10.800.

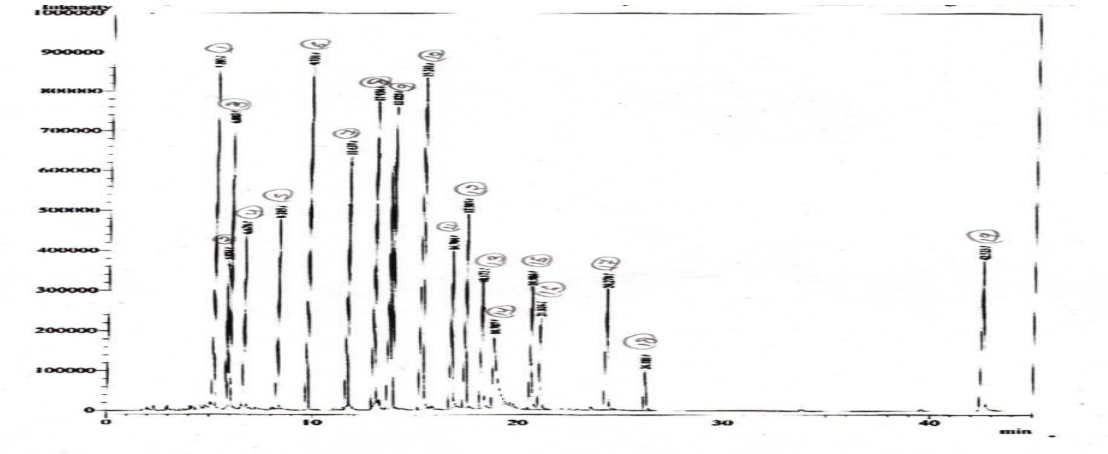

Fig. 1: Gas Chromatogram of standard of organochlorine pesticides

Table No 1: Rt values and peak areas of standard organochlorine pesticides corresponding to 5 ppm concentration

Peak	Pesticides	Ret. Time	Area	Area %
1	α-BHC	5.210	3703640	5.8074
2	β-BHC	5.834	1528709	2.9921
3	γ-BHC	6.003	3264915	5.761
4	δ-BHC	6.676	2085353	3.8096
5	Heptachlor	8.285	2292675	3.9475
6	Aldrin	9.731	4346046	6.9554
7	Heptachlor Epoxide	11.637	3269756	5.5584
8	γ-Chlordane	12.934	4300941	6.8968
9	Endosulfan-I +α Chlordane	13.825	7686728	10.85
10	Dieldrin,+ p,p' DDE	15.241	756334	10.6925
11	Endrin	16.704	2655909	4.7616
12	Endosulfan-II	17.385	3188425	5.3839
13	p, p'-DDD	18.173	2250920	3.9055
14	Endrin Aldehyde	18.787	2598185	4.6178
15	Endosulfan Sulphate	20.584	2186191	3.7103
16	p,p'-DDT	21.026	1608239	3.042
17	Endrin ketone	24.278	2388342	3.9727
18	Methoxychlor	26.181	751951	1.976
19	DCBP	42.525	3456786	5.3595
			55572167	100

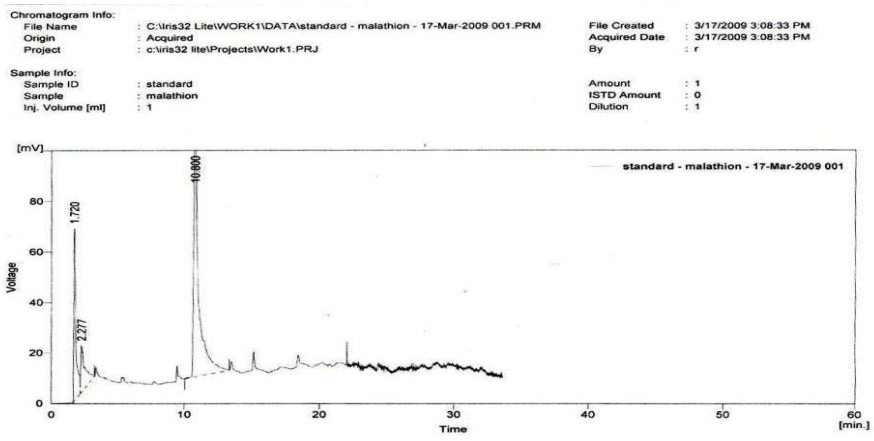

Fig. 2: Gas chromatogram of standard malathion

Table No 2: Rt values and peak areas of melathion standard [22] corresponding to 5 ppm concentration

Peak No.	Reten. Time [min]	Area [m V.s]	Height [mv]	Area [%]	Height [%]
1	1.720	752.370	68.253	18.9	29.6
2	2.277	409.213	18.351	10.3	7.9
3	10.800	2824. 911	144.254	70.9	62.5
	Total	3986.494	230.858	100.0	100.0

On comparing the chromatogram (Fig.3) of apple with standards it has been noticed that three peaks at Rt value 5.84, 9.71 and 10.86 were very close to β-BHC, aldrin and malathion respectively which indicated that these pesticides are present in the apple.

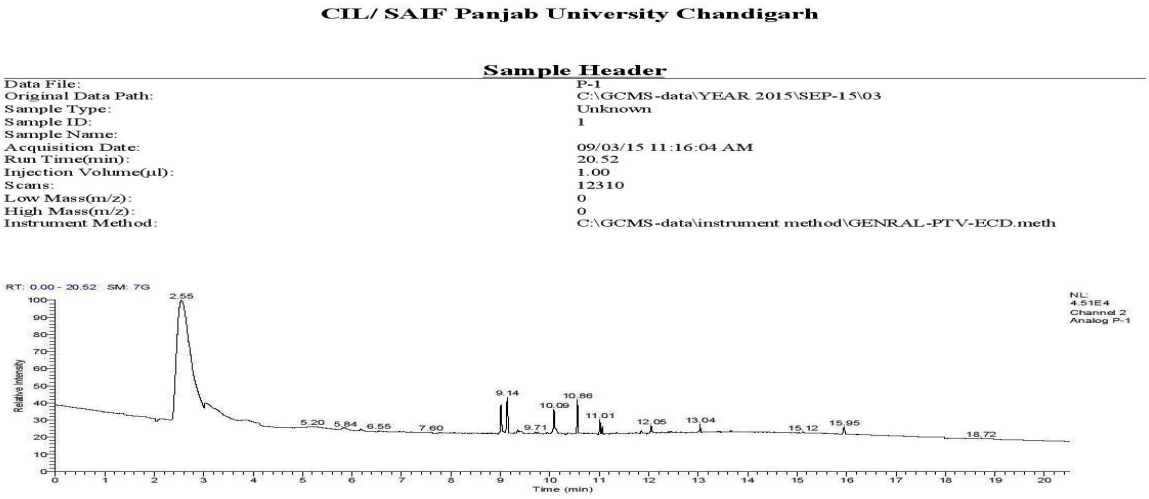

Fig. 3: Gas chromatogram of apple

In the chromatogram (Fig.4) of pyrus four peaks at Rt value 5.84, 9.76, 10.85 and 11.60 were very near to the Rt values of β-BHC, aldrin, malathion and heptachlor epoxides respectively which suggested that the above pesticides were present in the pyrus.

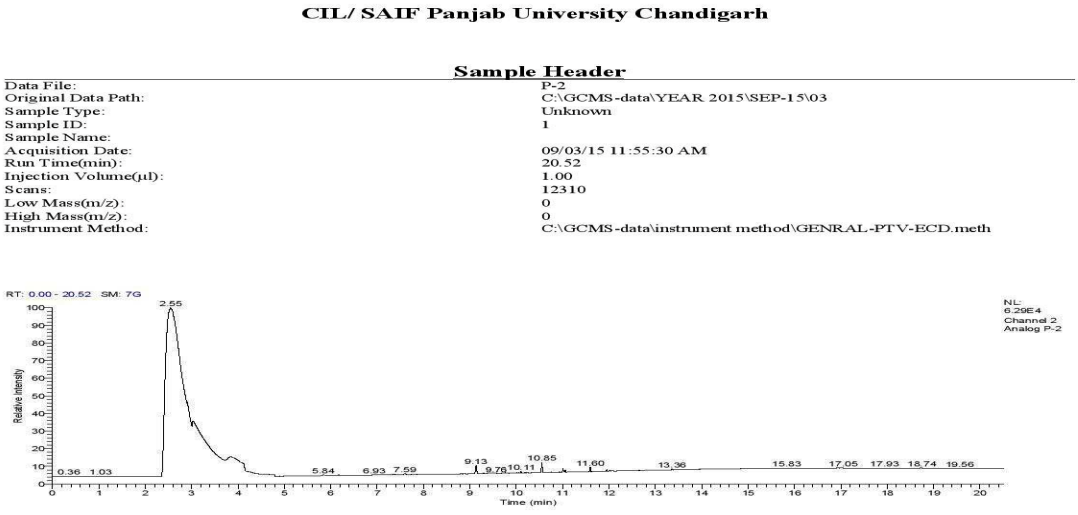

Fig. 4: Gas chromatogram of pyrus

In the chromatogram (Fig.5) of sweet orange four peaks at Rt value 5.85, 9.76, 11.65 and 10.85 were very close to the Rt value of β-BHC, aldrin, heptachlor epoxide and malathion respectively which indicated the presence of β-BHC, aldrin, heptachlor epoxide and malathion in the sample of sweet orange.

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Sample Header

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Injection Volume(µl):	1.00
Scans:	12310
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High Mass(m/z):	0
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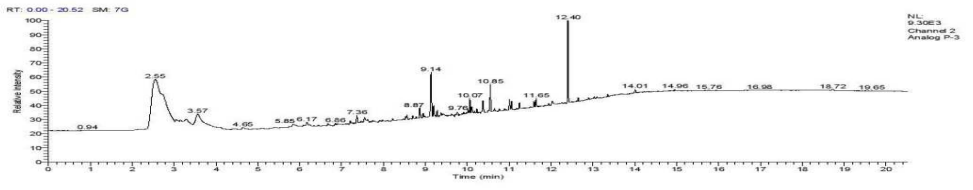

Fig.5: Gas chromatogram of sweet orange

In the chromatogram (Fig.6) of guava four peaks at Rt value 5.84, 9.76, 11.64 and 10.84 were very near to the Rt values of β-BHC, aldrin, heptachlor epoxide and malathion respectively which indicated that these pesticides were present in the guava.

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Sample Header

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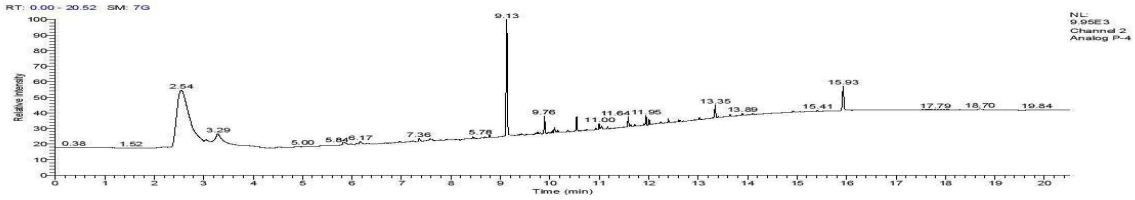

Fig. 6: Gas chromatogram of guava

In the chromatogram (Fig.7) of grapes two peaks at Rt value 6.64 and 9.74 were very close to the Rt values of δ-BHC and Aldrin respectively which indicating the presence of δ-BHC & aldrin in the sample of grapes.

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Sample Header

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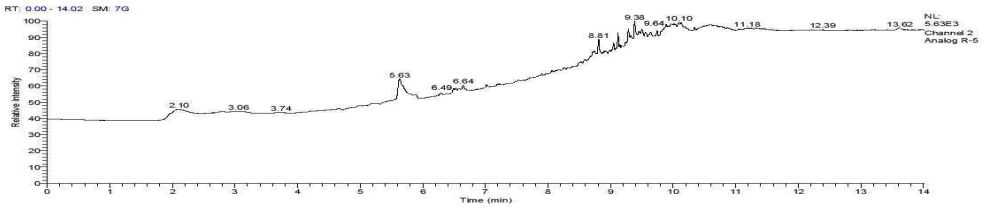

Fig. 7: Gas chromatogram of grapes

Table 3: Rt values, peak areas and concentrations of detected pesticides

Name of Sample	RT values	Peak Areas	Conc. of pesticides (ppm)	Name of Pesticides
APPLE	5.84	1671.29	0.005 4	β – BHC
	9.71	386.47	0.00044	Aldrin
	10.86	10927.09	19.340	Malathion
PYRUS	5.84	285.83	0.00093	β – BHC
	9.76	229.02	0.00026	Aldrin
	11.60	1876.44	0.00028	Heptachlor Epoxide
	10.85	4359.52	7.716	Malathion
SWEET ORANGE	5.85	246.84	0.00080	β-BHC
	9.76	381.50	0.00043	Aldrin
	11.65	618.69	0.00094	Heptachlor Epoxide
	10.85	2143.33	3.793	Malathion
GUAVA	5.84	206.91	0.00067	β – BHC
	9.76	111.02	0.00012	Aldrin
	11.64	115.49	0.00017	Heptachlor Epoxide
	10.84	1049.95	1.858	Malathion
GRAPES	6.64	218.53	0.00052	δ– BHC
	9.74	203.82	0.00023	Aldrin

CONCLUSION

Pesticide contamination poses significant risks to the human, environment and non-target organisms ranging from useful soil microorganisms to insects, plants, fish, and birds. In the present monitoring study all the fruit samples were contaminated either two or more pesticides. However, the concentrations of detected pesticides were far below the maximum residue limit (MRL) values prescribed by Codex Alimentarius 2016 except malathion. The environmental deterioration due to pesticides is endangering the situation of future. Thus, the use of these pesticides should be limited.

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