Asian Journal of Chemistry; Vol. 31, No. 7 (2019), 1565-1568

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2019.22036

Monitoring of Organochlorine Pesticides Residues in Pumpkin (*Cucurbita maxima*), Brinjal (*Solanum melongena*), Cucumber (*Cucumis sativus*), Ridge Gourd (*Luffa aegyptiaca*) and Apple Gourd (*Praecitrullus fistulosus*) Vegetables by using QuEChERS Method

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Received: 27 February 2019; *Accepted*: 26 March 2019; *Published online*: 21 May 2019; AJC-19415

The continuous use of pesticides infected vegetables poses a leading threat to public health. Gas chromatography equipped with electron capture detector (ECD) was used to monitored 20 organochlorine pesticides including isomers of benzene hexachloride (α-BHC, β-BHC, γ-BHC, δ-BHC), heptachlor, aldrin, heptachlor epoxide, γ-chlordane, α-chlordane, endosulfan-I, 4,4′-DDE, dieldrin, endrin, 4,4′-DDD, endosulfan II, endrin aldehyde, 4,4′-DDT, endosulfan sulfate, methoxychlor and endrin ketone were monitored in five summer vegetables (pumpkin, brinjal, cucumber, ridge gourd and apple gourd) by using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method. All vegetables were found contaminated with pesticides. Only apple gourd was found contaminated with one pesticide, rest of the vegetables was found contaminated with four or more than four pesticides. . The concentrations of estimated pesticides were less than the maximum residue limit values but regular eating of pesticide contaminated vegetables may create serious health problems. The results of study indicated the need for strict guideline and regular monitoring of banned pesticide residues in vegetables to protect consumer′s health.

Keywords: Organochlorines, Pesticides, QuEChERS, Vegetables.

INTRODUCTION

Approximately 71 % of the Indian population is vegetarian, with a per capita availability of 135 g per day as against the suggested 300 g every day [1]. Vegetables in the daily diet have been strongly linked with good health, reduced risk of certain types of cancer [2], heart problems [3], diabetes [4], aplastic anemia [5], gastric ulcer [6], rheumatoid arthritis and other chronic diseases. Every year a number of insect pests attacked vegetables crops which may origin high damage to the productivity. Generally, vegetables are attacked by insects due to their liking nature and to complete their chain of life cycle. They harm each part of the plant due to this reason high economic loss has been observed by the farmers. Therefore, farming community are of great concern for these pests, as they could pressurize the agricultural and horticultural industries by raising the cost of production [7]. To protect the crop from pests certain pesticides are used. In India, the use of chemical pesticides, herbicides and fungicides were introduced during the mid-sixties to achieve economic benefits and to make enough supply of food, which are now being used on a large scale [8]. India is the highest consumer of pesticides in the South Asian countries and third in the world. According to Ministry of Agriculture & Farmers Welfare, Government of India [9] the consumption of chemical pesticides in various states was 57000 metric tons during 2016-2017. Among 29 Indian states, Punjab, Maharashtra and Uttar Pradesh are the top three states contributing 51 % of pesticide consumption in India, the rest 49 % pesticide is used by other states and union territories. Thus, pesticides are considered as an essential part of present farming and could play a main role to make better yield of their farm crops. Consequently, the broad use of pesticides to control pests has emerged as a main feature in high-input demanding agricultural production systems [10] as most pesticides are unsuccessful in natural degradation. The excess use of pesticides accompanied unacceptable technical study or idea and generated unfortunately numerous negative ecological impacts. In addition to environmental problems, humans are also exposed directly by pesticides, which are generally present in little amount in the foodstuffs. There are enormous evidences which confirm that exposure of pesticides caused an increased rate of chronic diseases like various types of cancers [11,12], neurodegenerative diseases such as Parkinson [13], amyotrophic lateral sclerosis [14] and Alzheimer, diabetes [15], liver damage, reproductive disorders [16,17] and birth defects [18]. The pesticide exposure also create some acute effects on human [19] like nose and throat irritation, headache, cyanosis, dermal irritation, rashes and blisters on the skin, sweating, stinging of the eyes and skin, faintness, abdominal cramps, diarrhea, unclear vision, blindness and infrequently death. These adverse effects of pesticides on health and environment have prompted to researchers [20-22] to monitor the different pesticides in various food commodities. Keeping these facts in mind it has been decided to monitor the pesticide residues in different vegetables. In continuation of our previous work [23,24] in this article, we have reported the monitoring of 20 organochlorine pesticides present in five vegetables namely pumpkin (*Cucurbita maxima*), brinjal (*Solanum melongena*), cucumber (*Cucumis sativus*), ridge gourd (*Luffa aegyptiaca*) and apple gourd (*Praecitrullus fistulosus*).

EXPERIMENTAL

All glasswares were carefully cleaned by double distilled water and after that rinsed with acetone and dried (150 ºC) for overnight in an oven before use. All the organic solvents such as acetonitrile, acetone, ethyl acetate, dichloromethane and *n*-hexane were distilled before use. Adsorbents neutral silica gel and charcoal were activated before use. The obtained purified extract of vegetables samples were investigated by gas liquid chromatography coupled with capillary columns with ^{63}Ni electron capture detector (GLC-ECD). During the extraction procedure, minor equipments such as mechanical shaker, rotary evaporator and waring blender were also used. A standard solution (2.0 μL) was injected to record the chromatogram of pesticides. Standard of organochlorine pesticides was purchased from RESTEK, USA.

Five vegetables pumpkin (*Cucurbita maxima*), brinjal (*Solanum melongena*), cucumber (*Cucumis sativus*), ridge gourd (*Luffa aegyptiaca*) and apple gourd (*Praecitrullus fistulosus*) were chosen for monitoring of pesticides.

Extraction of pesticides from vegetables has been carried out in the following steps:

Collection of samples: The sample consist of 250 g of each vegetable, *i.e.* pumpkin (*Cucurbita maxima*), brinjal (*Solanum melongena*), cucumber (*Cucumis sativus*), ridge gourd (*Luffa aegyptiaca*) and apple gourd (*Praecitrullus fistulosus*) were collected from local vegetable market.

Storage of samples: Each sample was stored in refrigerator at 5 ºC and analyzed within 72 h of collection. Each sample of vegetable was washed under tap water and dried by using filter paper.

Blending of sample: In order to calculate the accurate concentration of pesticide reaching within human body, only edible part was processed for the analysis of pesticide. After drying each vegetable was cut into little pieces. A representative sample 250 g of each vegetable was homogenously mixed with 25 g anhydrous sodium sulfate in waring blender to prepare a fine paste.

Extraction for ridge gourd (*Luffa aegyptiaca*): A fine paste of ridge gourd (50 g) and 100 mL acetonitrile was mixed with high speed blender and mixture was filtered. Filtrate was transferred into a separating funnel and shaken smoothly for 1 h with 100 mL mixture of *n*-hexane and dichloromethane (3:2 v/v). To obtain distinct layers the separating funnel was hold at vertical position for 30 min. Organic layer (*n*-hexane and dichloromethane) of solvent was separated out and collected in a beaker. This procedure was repeated in triplicate. The collected extract was dried up to dryness (5 mL) in a rotary evaporator and dissolved in 10 mL *n*-hexane.

Extraction for brinjal (*Solanum melongena*): A homogenize fine paste of brinjal (50 g) was treated with 100 mL acetone by using mechanical shaker for 2 h. The extract was filtered and passed through a layer of anhydrous sodium sulfate. This filtrate was transferred into a separating funnel and diluted with 50 mL aqueous solution of NaCl (10 %) and shaken gently for 30 min. To this solution, 50 mL ethyl acetate was added and again shaken for 4 h. Thereafter, separating funnel was allowed to be held at straight up position to get the two distinct layers. The organic layer was collected from the separating funnel. The extraction process was repeated three times using 50 mL ethyl acetate each time. Organic phases were assembled together and evaporated up to dryness (5 mL) by using rotary evaporator and dissolved in 10 mL *n*-hexane.

Extraction for pumpkin (*Cucurbita maxima*), cucumber (*Cucumis sativus*) and apple gourd (*Praecitrullus fistulosus*): A macerated sample of each vegetable (50 g) was taken and treated with 50 mL ethyl acetate by using mechanical shaker for 1 h. This procedure was repeated two times by using 50 mL ethyl acetate each time. The obtained mixture was filtered and evaporated upto dryness 20-30 mL and then exchanged into a separating funnel. To this, 50 mL dichloromethane was added and again shaken for about 3 h.This procedure was repeated thrice using 50 mL dichloromethane each time. The obtained extract was collected and concentrated up to dryness (5 mL) in a rotary evaporator and dissolved in 10 mL *n*-hexane.

Purification: The collected extracts were purified by using columns packed with silica gel:activated charcoal (5:1 w/w)/ silica gel. Each extract was first passed through column and then eluted by using 50 mL *n*-hexane. Eluted extract was further concentrated near to dryness and redissolved in 10 mL of *n*-hexane. Finally, the samples were subjected to GC-ECD analysis of pesticides.

RESULTS AND DISCUSSION

The pesticides present in the standard were run through the GC-ECD to find out retention time and peak area corresponding to 0.2 μg/μL concentration (Table-1). A gas chromatogram of standard (Fig. 1) exhibited the peaks of various isomers of benzene hexachloride (BHC) at R_t values 8.481, 9.775, 12.041 and 13.533 for α-BHC, γ-BHC, β-BHC and δ-BHC, respectively. The peaks at R_t value 15.042 and 18.379 was due to heptachlor and heptachlor epoxide, respectively. The aldrin exhibited its peak at R_t value 16.753. γ-Chlordane and α-chlordane peaks exhibited their peaks at R_t value 20.223 and 21.342.

TABLE-1
RETENTION TIME AND PEAK AREA OF ORGANOCHLORINE PESTICIDES OF STANDARD

Peak	Pesticides	Ret. time (min)	Area	Area (%)
1	α-BHC	8.481	747866	0.3123
2	γ-BHC	9.775	178423	0.0745
3	β-BHC	12.041	17788716	7.4293
4	δ-BHC	13.533	23197482	9.6882
5	Heptachlor	15.042	13099995	5.4711
6	Aldrin	16.753	15246076	6.3674
7	Heptachlor epoxide	18.379	15052379	6.2865
8	γ-Chlordane	20.223	13459524	5.6212
9	α-Chlordane	21.342	14431111	6.0270
10	Endosulfan I	21.925	25859986	10.8001
11	4,4'-DDE	23.068	6975083	2.9131
12	Dieldrin	23.154	18779776	7.8432
13	Endrin	24.095	13244260	5.5313
14	4,4'-DDD	24.667	12228021	5.1069
15	Endosulfan II	25.065	4210779	1.7586
16	Endrin aldehyde	25.353	8823023	3.6848
17	4,4'-DDT	26.463	10972013	4.5823
18	Endosulfan sulfate	26.718	8989607	3.7544
19	Methoxychlor	28.545	12990256	5.4252
20	Endrin ketone	29.325	2963671	1.2377

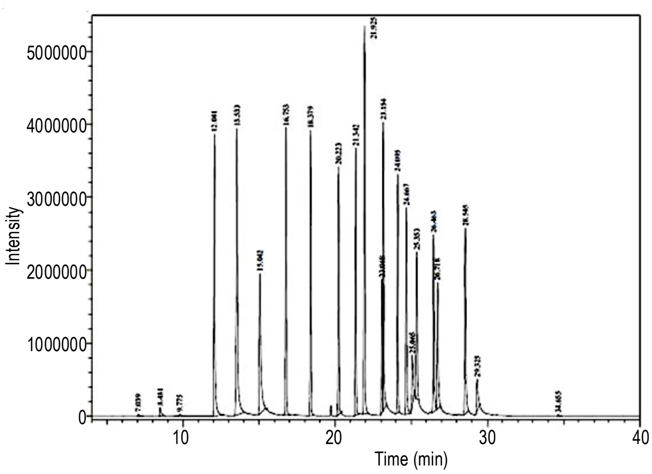

Fig. 1. GC-ECD chromatogram of standard

The peaks at R_t values 21.925, 25.065 and 26.718 were found for endosulfan I, endosulfan II and endosulfan sulfate, respectively. The peak of dieldrin was noticed at R_t value of 23.154. The detected peaks at R_t values 24.095, 25.353 and 28.545 were observed due to endrin, endrin aldehyde and methoxychlor, respectively. The peaks of 4,4′-DDE, 4,4′-DDD and 4,4′-DDT were found at R_t values 23.068, 24.667 and 26.463. The peak at R_t value 29.325 was found for endrin ketone.

The chromatogram of pumpkin (Fig. 2) exhibited a number of peaks from those five peaks at R_t value 9.769, 13.519, 23.078, 26.720 and 28.543 were close to the R_t value of γ-BHC, δ-BHC, 4,4′-DDE, endosulfan sulfate and methoxychlor, respectively which indicated the existence of above mentioned pesticides in pumpkin (Table-2). Similarly, in the chromatogram of brinjal (Fig. 3) number of peaks were obtained from those four peaks at the R_t values 23.051, 23.168, 26.712 and 28.542 were very near to the R_t values of 4,4′-DDE, dieldrin, endosulfan sulfate and methoxychlor, respectively which indicated the presence of aforesaid pesticides in brinjal (Table-2).

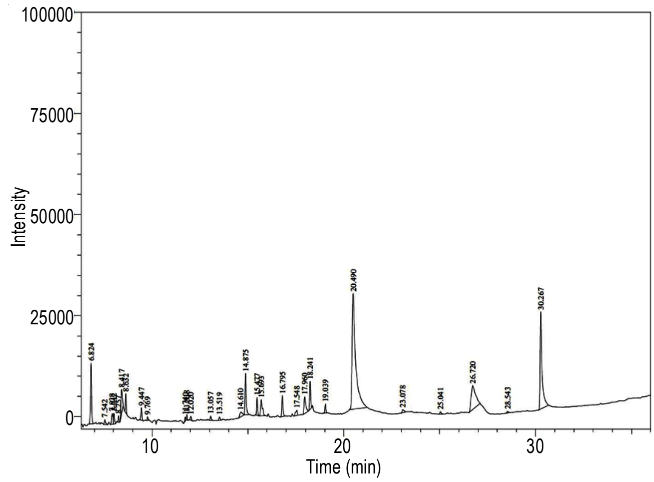

Fig. 2. GC-ECD chromatogram of pumpkin

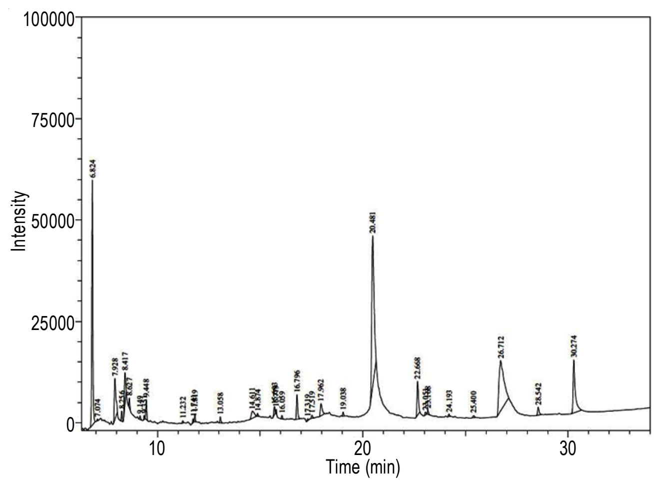

Fig. 3. GC-ECD chromatogram of brinjal

Gas chromatogram of cucumber exhibited (Fig. 4) four peaks at R_t values 13.516, 23.049, 28.531 and 29.307 very closed to the R_t values of δ-BHC, 4,4′-DDE, methoxychlor and endrin ketone, respectively which indicated the occurrence of these pesticides in sample of cucumber (Table-2). A number of peaks were obtained in the gas chromatogram of ridge gourd (Fig. 5) from those only four peaks at the R_t values 13.517, 18.364, 23.069 and 26.708 were similar to the R_t values of

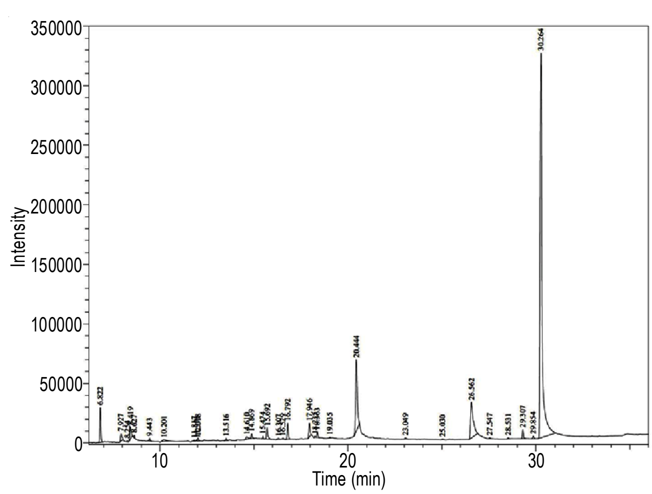

Fig. 4. GC-ECD chromatogram of cucumber

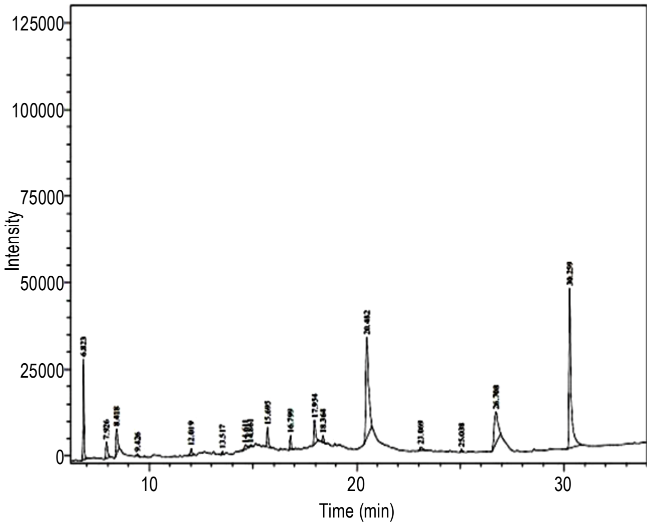

Fig. 5. GC-ECD chromatogram of ridge gourd

δ-BHC, heptachlor epoxide, 4,4′-DDE and endosulfan sulfate, respectively which showed the existence of aforesaid pesticides in ridge gourd. In case of the gas chromatogram of apple gourd (Fig. 6), only one peak at R_t value 28.536 resemble with the R_t value of methoxychlor, which suggested the occurrence of above pesticide in the sample of apple gourd (Table-2).

TABLE-2
DETECTED PESTICIDES AND CONCENTRATION OF PESTICIDE EVALUATED THROUGH GAS CHROMATOGRAPHY

Sample name	Name of detected pesticide	Retention value	Pesticides concentration (ppm)
Pumpkin	γ-BHC	9.769	0.006
	δ-BHC	13.519	0.014
	4,4'-DDE	23.078	0.010
	Endosulfan sulfate	26.720	0.002
	Methoxychlor	28.543	0.002
Brinjal	4,4'-DDE	23.051	0.017
	Dieldrin	23.168	0.014
	Endosulfan sulfate	26.712	0.006
	Methoxychlor	28.542	0.003
Cucumber	δ-BHC	13.516	0.017
	4,4'-DDE	23.049	0.001
	Methoxychlor	28.531	0.014
	Endrin ketone	29.307	0.018
Ridge gourd	δ-BHC	13.517	0.016
	Heptachlor epoxide	18.364	0.015
	4,4'-DDE	23.069	0.001
	Endosulfan sulfate	26.708	0.010
Apple gourd	Methoxychlor	28.536	0.009

ACKNOWLEDGEMENTS

The authors are thankful to Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi, India for gas chromatographic analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

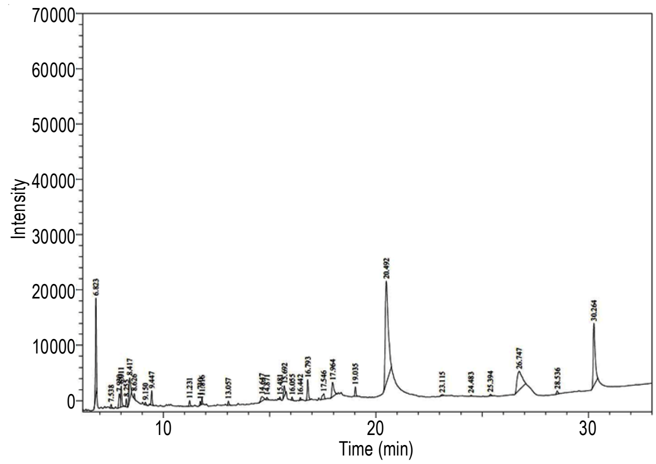

Fig. 6. GC-ECD chromatogram of apple gourd

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