ISSN: 2347-4688, Vol. 11, No.(1) 2023, pg. 204-211

Current Agriculture Research Journal

www.agriculturejournal.org

Tracing of contamination level of organochlorine pesticides in Bottle gourd (*Lagenaria siceraria*), Sponge gourd (*Luffa aegyptiaca*), Brinjal (*Solanum melongena*), Plum (*Prunus domestica*), Kiwi (*Actinidia deliciosa*) and Pineapple (*Ananas comosus*)

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Abstract

Fruits and vegetables have nutritional value, but they can also be source of toxic contaminants such as pesticide residues. The aim of this study was to estimate the contamination level of pesticide residues in summer season fruits and vegetables. The constant use of pesticides contaminated fruits and vegetables pose a major risk to community health. An electron capture detector was used in Gas chromatography analysis to monitor 20 organochlorine pesticides including α-chlordane, γ-chlordane, isomers of benzene hexachloride (α-BHC, β-BHC, γ-BHC, δ-BHC), 4,4'-DDT, 4,4'-DDE, 4,4'-DDD, aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone, heptachlor, heptachlor epoxide, endosulfan-I, endosulfan II, endosulfan sulfate and methoxychlor in six types of fruits and vegetables (bottle gourd, sponge gourd, brinjal, plum, kiwi and pineapple) of summer season. It was found that plum and pineapple were found contaminated with 4,4'-DDD and other fruits and vegetables were found contaminated with more than one pesticides. During the tracing it was noticed that the estimated pesticides concentrations were lower than the (MRL) values but constant eating of infected pesticide fruits and vegetables may produce severe health complications. The findings of the present research showed that the existence of strict rules and observance of pesticide residues in fruits and vegetables is a basic need.

Article History
Received: 11 October 2022
Accepted: 04 April 2023

Keywords
Fruits; GC-ECD; Organochlorine Pesticides; Vegetables.

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Doi: http://dx.doi.org/10.12944/CARJ.11.1.17

Introduction

Fruits and vegetables are an important component of our food because of their nutritional significance and have a main role in the diet from the point of view of health and prevention of disease.[1] The majority of Indians are vegetarian and their average diet constitutes about 150–250 g of fruits and 400g of vegetables in the total meal per day.[2] As several pesticides are toxic substances to human health and persist in the surrounding for a long time. Therefore, for improved crop production[3] and quality, pesticides are constantly used throughout the whole period of growth time and also at the ripening stage.[4] In a country like India, the use of pesticides has become predictable to sustain and improve current level of crop production by protecting the crop from pests.[5] However approximately, per year two million tons pesticides was consumed around the world, out of which, 45% of pesticides is used by Europe, and 25% in USA, the rest of the pesticide is consumed in the remaining world. India's share is just 3.75%. Now a days, India has turned into the second largest producer of pesticides in Asia after China and it ranks 12th worldwide.[6] As reported by the Agriculture Ministry & Farmers Welfare, Government of India, the utilization of pesticides in different states and union territories was 58720 metric tons pesticides for the duration of 2021-2022. In all the Indian states, Maharashtra, Haryana, Uttar Pradesh and Telangana are the topmost four states contributing to 57.93 % of pesticide intake in India. The 35.10 % of pesticide used by other states and 6.97 % used by union territories.[7] These pesticides are absorbed by the fruits and vegetables and finally by human beings due to which they may be hazardous.[8]

Organochlorine pesticides (OCPs) belong to the class of persistent organic pollutants (POPs) with long term residual effect in the environment. These pesticides have many problems including broad spectrum toxicity for both target and non-target species by alter the exact function of insects nervous system leading to disorders such as convulsions and paralysis followed by eventual death.[9] However, most of these pesticides have been banned in the developed countries but they are still used in developing countries of Asia via illegal routes. High global demands of Organochlorine pesticides in agricultural practice, in regard to environmental regulations, are due to their easy availability, cost-effectiveness and excellent efficiency in pest control and to increased yield of the crop in agriculture sector.[10] OCPs, including DDTs, hexachlorocyclohexanes (HCHs), aldrin, dieldrin, endrin, chlordane and heptachlor are still in use via illegal route.[10]

Organochlorine pesticides are associated with various human health's problems, ranging from short-term impacts including headaches and nausea, to chronic impacts,[11] including different cancers,[12,13] birth defects,[14] infertility,[15] metabolic disorders,[16] parkinson,[17] amyotrophic lateral sclerosis,[18] Alzheimer,[19] neurodegenerative disorders,[20] chronic lymphocytic leukemia (CLL), multiple myeloma and endocrine disruption.[21] These negative impacts of pesticides on human health have advised to researchers to monitor a variety of pesticides in many food materials. Keeping these points in mind and in continuation of our preceding work[22] in this article,20 organochlorine pesticides monitoring have been reported in selected fruits and vegetables of summer season viz., bottle gourd, sponge gourd, brinjal, plum, kiwi and pineapple.

Materials and Methods

All glassware was carefully cleaned using distilled water and dipped in acetone and then dried in oven at 150^{o}C temperature before use. Distillation of acetonitrile, acetone, n-hexane, ethyl acetate and dichloromethane (DCM) solvents has been carried out before use for the extraction of pesticide residues from fruits and vegetables. Then, Adsorbents such as silica gel and charcoal were activated before use for cleanup. Purified extracts were examined by GLC equipped with ^{63}Ni-Electron Capture Detector from AIRF JNU, New Delhi. The small equipments like mechanical shaker, warring blender and rotary evaporator etc. were also used during extraction. Stock solution of standard was prepared in n-hexane.

Extraction

Liquid-liquid extraction of pesticides from fruits and vegetables has been achieved in the subsequent stages.

Sampling

250g of all fruit and vegetable samples, i.e. Bottle gourd (*Lagenaria siceraria*), Sponge gourd (*Luffa aegyptiaca*), Brinjal (*Solanum melongena*), Plum (*Prunus domestica*), Kiwi (*Actinidia deliciosa*) and Pineapple (*Ananas comosus*) were collected directly from native market.

Storage of Samples

All samples were brought in lab and stored in a refrigerator at 5ºC. These samples were studied within 3 days of collection. Collected samples of fruits and vegetables have been washed for few minutes with water and dried with tissue paper.

Blending of Samples

To establish the exact concentration of pesticides in human beings, only the eatable parts of fruits and vegetables was keep for tracing of pesticide residue. Each fruit and vegetable has been cut into small pieces after drying process. A representative sample 250g of fruit and vegetable was uniformly blended with 25g of dehydrated sodium sulfate in a warring blender to make a thin ultra-fine paste.

Liquid-Liquid Extraction of Bottle Gourd and Sponge Gourd

50g homogenize thin paste of each vegetable sample was subjected to shaken with 100 ml acetonitrile with a mechanical shaker for 2h. It was filtered and 50 ml n-hexane and dichloromethane (3:2 v/v) was added to the filtrate and shaken for 3h. Then, separating funnel was permitted at perpendicular position for about 3h to get the two different layers. n- Hexane layer was removed in a conical flask. The extraction process was again repeated in three more times using 50 ml n-hexane and dichloromethane (3:2 v/v) each time. The collected extract was reduced up to dryness at 45^{o}C and then again 10 ml n- hexane was mixed to the dried solution.

Liquid-Liquid Extraction of Kiwi Fruit and Brinjal

50g homogenize paste of Kiwi/ brinjal was mixed with 100 ml acetonitrile and shaken for 2 h. The obtained extract was filtered then filtrate was exchanged to liquid –liquid extraction. To this, 50 ml n-hexane was mixed and agitated for 3h. Thereafter, to obtain the two dissimilar layers, separating funnel was permitted at vertical location for about 3h. The upper layer (n-hexane) was separated out. The extraction method was redone thrice using 50 ml n-hexane every time. The collected extract solution was reduced to 5ml at 40^{o}C temperature and 10 ml n- hexane was again mixed.

Liquid-Liquid Extraction of Plum

50g homogenize paste of plum was shaken for 2h on a mechanical shaker using 100 ml acetonitrile. This extract was filtered, and mixed with 50 ml cyclohexane. After that this solution was agitated for 3h. Thereafter, two distinct layers were obtained on hold up separating funnel at straight position for about 3h. The upper layer of cyclohexane was separated out and the whole method was again repeated three times more using 50 ml cyclohexane at every stage. The collected extract was concentrated at 45ºC temperature up to dryness (5 ml) and again mixed in 10 ml n-hexane.

Liquid-liquid Extraction of Pineapple

50g homogenize fine paste of pineapple was subjected to shaken with 100 ml acetonitrile for 2 h. The extract was filtered and filtrate was combined with 50 ml acetone and n-hexane (1:4). This solution was shaken for 3h. Thereafter, to obtain the two different layers, separating funnel was allowed to put at straight up position for about 3h. The upper layer (n-hexane) was separated out from the separating funnel. The extraction process was constantly repeated in the ratio 1:4 using acetone and n-hexane (50 ml) three more times. The collected extract was reduced up to dryness (5 ml) at 30ºC and dissolve in 10 ml n-hexane.

Purification

These extracts were subjected for purification by packed-column chromatography over silica gel and activated charcoal in the ratio 5:1 (w/w) or using silica gel. Every extract was first run via column using 50 ml n-hexane for elution. The obtained extracts were then concentrated to reduce up to dryness and again mixed with 10 ml n-hexane and subsequently, exposed to GC-ECD for pesticides analysis.

Results and Discussion

GC-ECD analysis of pesticide standard was carried out to find out (RT) retention time and peak area equivalent to 0.2 µg/µL concentration (Table-1).

Table.1: Peak area and RT value of standard of organochlorine pesticides

Peak No.	Name of pesticides	RT value	Area	Area %
1	α- Benzene hexachloride	7.264	4056398	0.3107
2	γ- Benzene hexachloride	10.666	69248549	5.3044
3	β- BHC	12.108	110194348	8.4409
4	δ-BHC	13.553	67783682	5.1922
5	Heptachlor	15.23	63367599	4.854
6	Aldrin	16.828	87205582	6.6799
7	Heptachlor epoxide	18.093	4789654	0.3669
8	γ- Chlordane	18.655	65779983	5.0387
9	α- Chlordane	19.761	82997615	6.3576
10	Endosulfan I	20.356	115947358	8.8816
11	4,4'-DDE	21.56	122612908	9.3921
12	Dieldrin	22.46	60365744	4.624
13	Endrin	23.041	74179902	5.6822
14	4,4'-DDD	23.459	60226898	4.6134
15	Endosulfan II	23.731	59156527	4.5314
16	Endrin aldehyde	24.837	82799420	6.3424
17	4,4'-DDT	25.122	70574949	5.406
18	Endosulfan sulfate	26.861	69382904	5.3147
19	Methoxychlor	27.713	34287889	2.6265
20	Endrin ketone	32.942	525912	0.0403
Total			1305483821	100

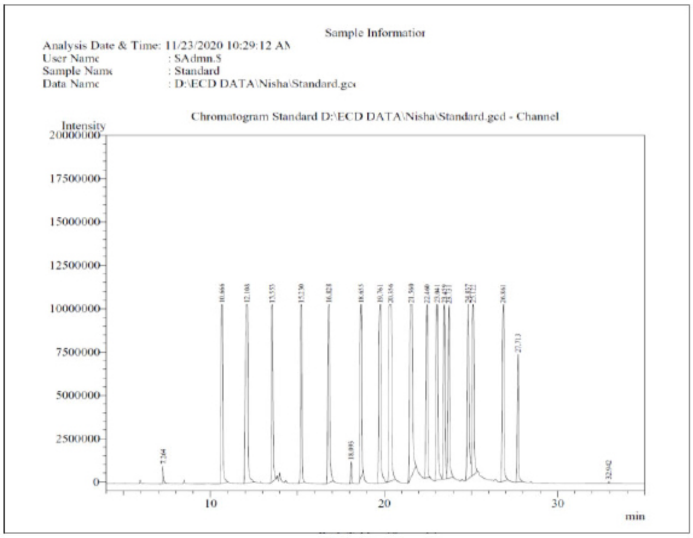

Figure 1: Gas chromatogram of Standard

Chromatogram of Bottle gourd (*Lagenaria siceraria*) (Fig.2) revealed three peaks at Rt values 13.540 16.858 and 27.685 resemble with δ- benzene hexachloride (δ-BHC), aldrin and methoxychlor correspondingly which specified that aforesaid pesticides were exist in the sample of bottle gourd. The chromatogram of Sponge gourd (*Luffa aegyptiaca*) exhibited numeral peaks (Fig.3), among them three peaks at Rt value 13.554, 23.448 and 26.831were adjacent to the peak of δ-BHC, 4,4'-DDD and endosulfan sulphate which indicated that existence of these pesticides in the Sponge gourd sample.

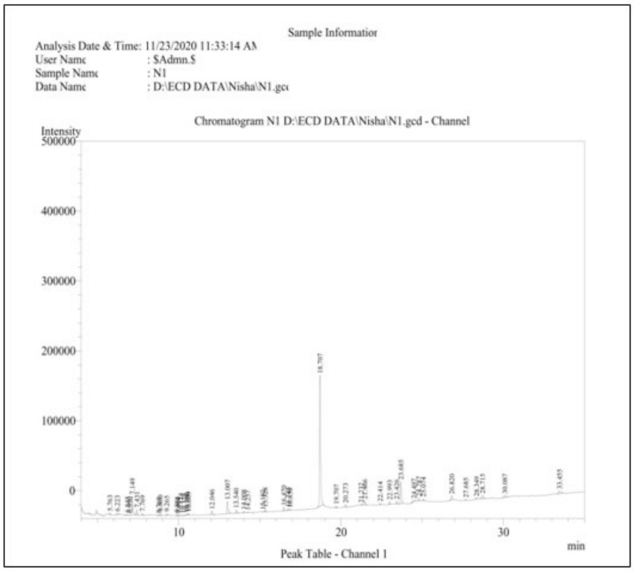

Figure 2: Gas chromatogram of Bottle gourd

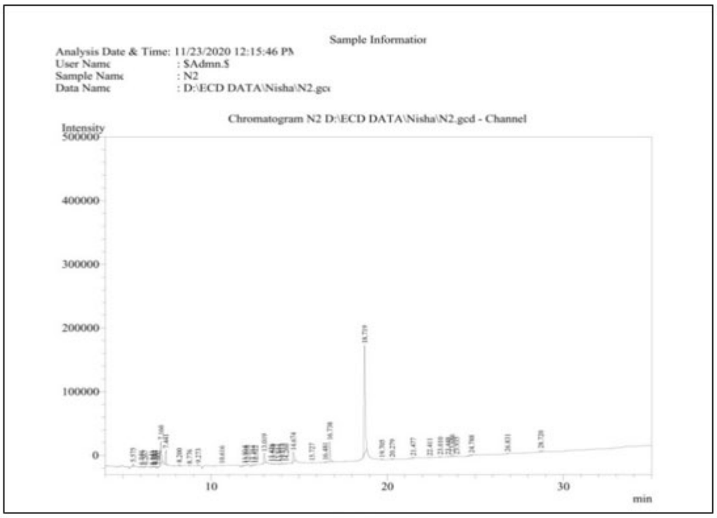

Figure 3: Gas chromatogram of Sponge gourd

Gas chromatogram of Brinjal (*Solanum melongena*) (Fig.4) displayed two peaks at Rt values 23.014 and 23.450 corresponding to the Rt value of endrin and 4,4'-DDD which point out the occurrence of these pesticides in the sample of Brinjal. The chromatogram of Plum (*Prunus domestica*) (Fig.5) consisted one peaks at the Rt value 23.464 which was resemble with Rt value of 4,4'-DDD, indicated the existence of 4,4'-DDD pesticide in the sample of Plum. In case of the gas chromatogram of Kiwi fruit (*Actinidia deliciosa*) (Fig.6) three peaks were exact nearby with Rt values 13.530 of δ-BHC, 16.853 of aldrin and 23.013 of endrin which showed the presence of these pesticides in the sample of Kiwi fruit. However, Gas chromatogram of Pineapple (Ananas comosus) (Fig.7) showed one peak at Rt value 23.458 which was adjacent to the Rt value of 4,4'-DDD indicated presence of 4,4'-DDD in the sample of Pineapple. Detected pesticides and their concentration have been given in Table-2.

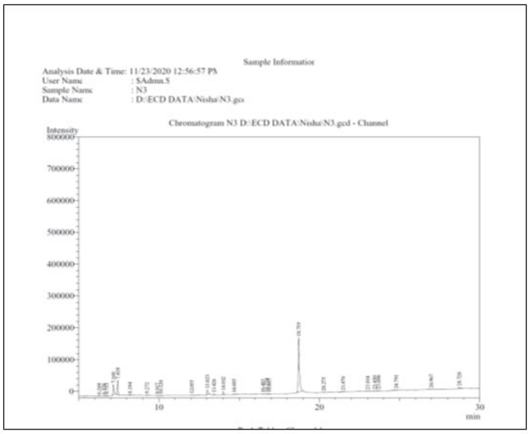

Figure 4: Gas chromatogram of Brinjal

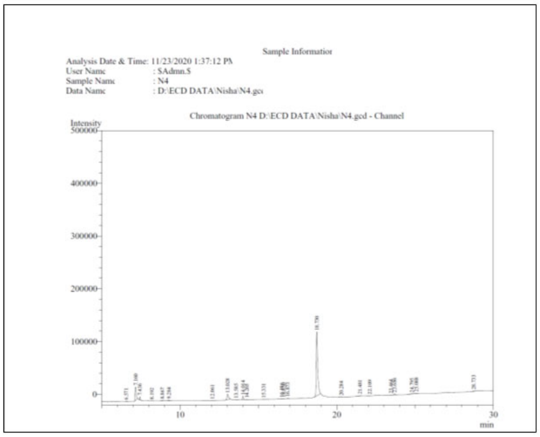

Figure 5: Gas chromatogram of Plum

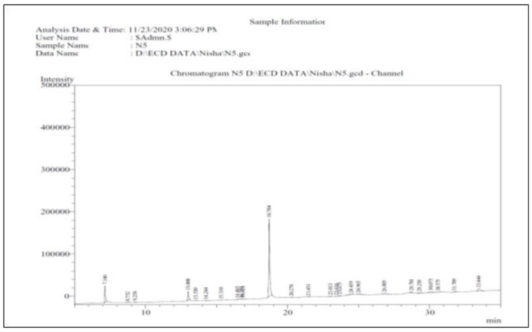

Figure 6: Gas chromatogram of Kiwi fruit

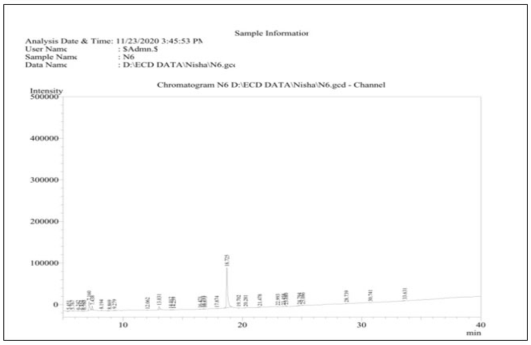

Figure 7: Gas chromatogram of Pineapple

Table 2: Discovered pesticides and their concentration evaluated through GC-ECD

S. No.	Sample Name	Pesticide found	Rt. value	Pesticides concentration (mg/kg)
1	Bottle gourd	δ-BHC	13.540	0.0652
		Aldrin	16.858	0.0051
		Methoxychlor	27.685	0.022
2	Sponge gourd	δ-BHC	13.554	0.01419
		4,4'-DDD	23.448	0.0065
		Endosulfan sulphate	26.831	0.0054
3	Brinjal	Endrin	23.014	0.01763
		4,4'-DDD	23.450	0.01403
4	Plum	4,4'-DDD	23.464	0.00649
5	Kiwi fruit	δ-BHC	13.530	0.0044
		Aldrin	16.853	0.0110
		Endrin	23.013	0.0087
6	Pineapple	4,4'-DDD	23.458	0.00373

Aldrin is an alicyclic chlorinated hydrocarbon. It was found in the concentration range 0.0110-0.00513 mg/kg in Kiwi fruit and Bottle gourd, δ-BHC was found in the range 0.0652-0.0044 mg/kg in bottle gourd, sponge gourd and kiwi fruit. Endrin was estimated in the range 0.01763-0.0087 mg/kg in brinjal and kiwi fruit. 4,4'-DDD were recorded in the range (0.01403-0.00373 mg/kg in brinjal, plum and pineapple. Methoxychlor was found 0.022 mg/kg and endosulfan sulphate 0.0054 mg/kg in sponge gourd. Among the different organochlorine pesticide residues found in various vegetables and fruits, δ-BHC was found with the highest concentration 0.0652 mg/kg in bottle gourd followed by methoxychlor 0.022 mg/kg in bottle gourd, endrin 0.01763 mg/kg in brinjal, 4,4'-DDD 0.01403 mg/kg in brinjal, aldrin 0.0110 mg/ kg in kiwi fruit, endosulfan sulphate 0.0054 mg/kg in sponge gourd.

Many researcher[23,24] reported the presence of organochlorine pesticides in food commodities in different range. Although the use of these pesticides has been banned or restricted in last few decades. The presence of lower amounts of pesticides were detected in present studies may be attributed due to previous use of organochlorine pesticides.

Conclusion

In order to minimize health hazard, pesticide residues tracing in food commodities is important and essential. The results of the study have indicated that all fruits and vegetables selected for tracing work were contaminated with pesticides. However, contamination level was below MRL values set by FAO/WHO Codex Alimentarius Commission but regular consumption of pesticide infected fruits and vegetables may create serious health issues.

Acknowledgements

We thanks Head, Chemistry Department, I.B.S., Dr. B.R. Ambedkar University, Khandari Campus, Agra for providing indispensable facilities during research work and also thankful to AIRF, JNU, New Delhi, India for providing GC analysis for samples.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

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