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Unfolding organochlorine pesticides toxicity in seasonal fruits and vegetables of Agra region using QuEChERS method coupled with gas chromatography detection

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Abstract

Worldwide, Organochlorine pesticides are banned for agricultural use due to their toxicity and highly persistence that were introduced into the environment, biomagnifying and bioaccumulation in food chains and long range transport led to extensive contamination of various environmental matrices such as air, water, soil and biota. But at the large quantity, the compound residues and metabolites are persist in crops due to illegal or historical applications. This study investigated residual concentrations and human health implications of legacy Organochlorine pesticides in fruits and vegetables of wholesale market of Agra region. Samples of 8 vegetables (Okra, cucumber, sponge gourd, pointed gourd, favabeans, green peas, cabbage and spinach) and 8 fruits (Plum, mosambi, avocado, custard apple, grapes, Cape gooseberry, strawberry and sapota) were collected from Agra market. The pesticides were extracted using modified QuEChERS method and analyzed by GC-ECD. Estimate organochlorine pesticides α -BHC, β -BHC, γ -BHC, δ -BHC, endosulfan-I, endosulfan sulfate, endrin, dieldrin, heptachlor, heptachlor epoxide, aldrin, α -chlordane, γ -chlordane, 4,4'-DDE, 4,4'-DDD, endrin aldehyde, 4,4'-DDT, endrin ketone, endosulfan-II and methoxychlor. The fruits and vegetables samples contained α -BHC, γ -BHC, β -BHC, Heptachlor, Aldrin, Endosulfan-I, γ -chlordane and dieldrin were detected at varying frequencies. However, all residues were detected below or above the quantification limit of 0.05 mg/kg and none exceeded FAO/WHO maximum residue limits. This study will provide data regarding emphasizing the importance of user education, enhanced regulatory frameworks and integrated pest management; sponsored for a strategic shift towards practices that minimize environmental harm and safeguard human health. Executing these procedures can significantly contribute to the advancement of sustainable agriculture by reducing pesticide dependence and endorse ecological stability.

Keywords: Organochlorine pesticide, gas chromatography, QuEChERS method, fruits and vegetables

Introduction

The aim of this study is to depict the presence of organochlorine pesticide residues in fruits and vegetables, mainly how they are introduced, dispersed, ruined, distressed by food dispensation system and their risk assessment. Vegetables and fruits are vital for human being diet while they afford vital nutrients that are required for most of the reactions occurring in the body. High intake of these commodities has been expectant not only to prevent vitamin deficiency but also to reduce the occurrence of major diseases. In the year 2026, as per estimation the worldwide production of vegetables and fruits was 2.1 billion metric tons; india is the second largest producer of vegetables and fruits. In 2025, the global market of fruits was 19.35 billion US dollars and expected to grow 27.31 billion in 2030 at a compound annual growth rate of 7.1%. When the pests are attacked during the crop production that reduced the quality and yield of the crops. To reduce the loss and maintain the quality of vegetables and fruits, the use of pesticides is one of the tools. Pesticides are used with other pest management techniques during cropping to prevent diseases and destroy pests. About 1000 compounds (inorganic and organic) are used as active ingredients of pesticides.

Organochlorine pesticides which were banned 40 years ago are still found in fruits and vegetables. The uses of organochlorine pesticides have increased because they have effectiveness on crop production and low-cost in market. Intensive fruits and vegetable production using pesticides has biggest threat to growers and the consumers. In recent years, the widespread use of pesticides has led to serious health concern for humans and animals. As they are intrinsically toxic and deliberately spread in the environment, manufacture, distribution and use of pesticides necessitate strict instruction and control. Before they can be authorized for use, pesticides should first be tested for possible health effects and the results should be analyzed by experts to assess the risk to humans. In collaboration with FAO, WHO is responsible for assessing the risk to humans of pesticides. They have been through direct exposure and through residues in fruits and vegetables and for recommending adequate protections. Risk assessments for pesticide residues in food are conducted by an independent, international expert scientific group, the joint FAO/WHO meeting on pesticide residues (JMPR). In such order the accumulation of pesticide residues is increased due to relatively short pre-harvest interval. Use of pesticides without knowing the label claim information increases the cost of production, number of spray and labour cost, ultimately leading to decrease in farmers profitability. Hence, the adoption of pesticide as per label claim is very much essential. The metabolites of pesticides exhibit different toxicological profiles compared to their parent compounds and in some cases they can be more harmful. The exposure to DDT or DDE may cause adverse health effects for example, diabetes, impaired neuro-development in children, breast cancer and spontaneous abortion. Heptachlor epoxide accumulates in the liver and is associated with long-term effects such as the development of liver tumor. This study involves the determination of concentration of organochlorine pesticide residues in seasonal (summer and winter) fruits and vegetables procured from wholesale market of agra region. The pesticides were extracted using modified QuEChERS method and analyzed by GC-ECD and GC-MS techniques. The application of organochlorine pesticides on crops because of protect crop and increase their yield because of their effectiveness on crop production, low-cost, ease of application and ease of use. The pesticides are accumulated in vegetables and fruits from water and soil. These contaminated commodity consumed by human being cause adverse health effects. The scope of the study included the evaluation of seasonal trends in pesticide contamination in vegetables and fruits to provide insight into patterns of pesticide application and potential consumer exposure risks in the study area. That was hypothesized that the concentrations of residues in vegetables and fruits varied significantly between the season (summer and winter) reflecting variations in agricultural practices, environmental degradation and pest pressure processes.

Materials and Methodology

The fruits and vegetables were collected randomly from Agra region from the different fruits and vegetables salesperson (Okra, cucumber, sponge gourd, pointed gourd, favabeans, green peas, cabbage and spinach) and fruits (Plum, mosambi, avocado, custard apple, grapes, cape gooseberry, strawberry and sapota). The total number of

fruits and vegetables samples collected from the April 2025 to February 2026 was total of 16 different types of fruits and vegetables and was a total 16 samples were collected. Each sample was collected and placed in a polybag and transfer to the laboratory for extraction and experimental work.

Chemicals and Reagents

All chemical and reagents were obtained from Merck grade (India) i.e, n-hexane, acetonitrile, acetone, ethyl acetate, cyclohexane, dichloromethane, anhydrous sodium sulphate silica gel, activated charcoal whereas the technical grade pesticide standards were of Restek 110 Benner Circle, Bellefonte, PA 16823 USA. For preparing stock solution, standard was dissolved in hexane. The standard was stored in a freezer at -4°C.

Sampling

Fruits and vegetables samples were washed for few minutes under running water to remove dirt. Then, the samples were dried with filter paper. After drying, each sample was chopped into small pieces and to make a fine paste in warring blender.

Extraction and Clean Up

Extraction Method for Vegetables

Extraction of pesticides from all vegetables was prepared and using a modified method. Take 50 g of blended standardized fine paste of each vegetables sample in separating funnel and subjected to shaken with 100 ml acetonitrile for 2-3 hr. The extract was filtered through filter paper, then filtration the remaining filtrate were transferred into separating funnel and adding 50 ml acetonitrile again and shaken for 2 hr. Again filter the solvent and adding 50 ml n-hexane and acetone with 3:2 v/v then shaken for 3-4 hr. After some times, the separating funnel was permitted at vertical position for about 2 hr to obtain the two different layers. The upper layer; there were high density and more non-polar solvent and high chances to accumulated pesticide residues in that layer. So, that layer was separated out from the separating funnel. This extraction process was repeated three times using 50 ml n-hexane and acetone with 3:2 v/v each step. Then, the collected extract was concentrated at 40°C temperature by using rotatory evaporator; the solvent concentrated upto 2 ml and in that solvent dissolved the n-hexane 10 ml and then take it for analysis of pesticide residues in laboratory.

Extraction Method for Fruits

Extraction of pesticides from all fruits was prepared and using a modified method. Take 50 g of blended standardized fine paste of each fruits sample in separating funnel and subjected to shaken with 100 ml acetonitrile for 2-3 hr. The extract was filtered through filter paper, then filtration the remaining filtrate were transferred into separating funnel and adding 50 ml acetonitrile again and shaken for 2 hr. Yet again, filter the solvent and adding 50 ml n-hexane and ethyl acetate with 3:2 v/v then shaken for 3-4 hr. After some times, the separating funnel was permitted at vertical position for about 2 hr to obtain the two different layers. The upper layer; there were high density and more non-polar solvent and high chances to accumulated pesticide residues in that layer. So, that layer was separated out from the separating funnel. This extraction process was repeated three times using 50 ml n-hexane and acetone with 3:2 v/v

each step. Then, the collected extract was concentrated at 40°C temperature by using rotatory evaporator; the solvent concentrated upto 2 ml and in that solvent dissolved the n-hexane 10 ml and then take it for analysis of pesticide residues in laboratory.

Purification

The extracts were collected and subjected for clean up by column packed with silica gel and activated charcoal with (5:1 w/w) silica gel : activated charcoal : silica gel and eluted by solvent. Then, eluted extract was further concentrated upto dryness and re-dissolved in 10 ml n-hexane and then subjected to GC-ECD for separation, detection and confirmation of pesticides in samples.

Results and Discussion

The chromatographic analyses for the organochlorine pesticide residues; the recovery percentage, limit of detection for that are presented in Table 1. The detection limits ranged from 0.001 to 0.005 mg/kg and the recovery percentage ranged from 80 to 100%. All calibration curves for all organochlorine pesticide residues of interest showed good linearity with regression coefficients indicating reliable analytical performance.

Analytical results

In 8 out of 16 analysed fruits and vegetables samples, no detectable pesticide residues were found, although 8 samples were contaminated with residues. 3 samples were contained residues above MRLs, while 5 samples contained the residues at or below MRLs as set by the European pesticides database. The fruits and vegetables samples such

as, Okra, cucumber, pointed gourd, green peas, cabbage and spinach have been contaminated with highest percentage of residues. No pesticide residues were detected in any of the sponge gourd and favabeans, Samples. Out of 100% samples, 50% samples showed the highest number of samples that despoiled MRLs.

Table 1: GC-ECD report of peaks of Organochlorine pesticides and Concentration of residues in Organochlorine pesticide standard.

Peaks	Pesticides	Retention Time	Area	Concentration (µg/ml)
1	α-BHC	7.632	2061994	200.4
2	γ-BHC	10.213	4276211	200.0
3	β-BHC	11.087	2591367	199.9
4	δ-BHC	12.414	2014959	200.8
5	Heptachlor	12.550	2185845	200.8
6	Aldrin	15.758	5510055	200.4
7	Heptachlor epoxide	17.374	5931942	200.0
8	γ-Chlordane	19.226	5083279	200.4
9	α-Chlordane	20.339	5165043	200.0
10	Endosulfan I	20.924	8865666	200.0
11	4,4'-DDE	22.090	6964698	200.0
12	Dieldrin	22.155	5479407	200.3
13	Endrin	23.096	4956250	200.0
14	4,4'-DDD	23.662	3182235	200.0
15	Endosulfan II	24.055	1956382	200.0
16	Endrin aldehyde	24.355	3421332	200.0
17	4,4'-DDT	25.147	1018304	200.0
18	Endosulfan sulfate	25.754	1693570	200.3
19	Methoxychlor	27.549	2386315	200.4
20	Endrin ketone	28.386	1462610	200.4

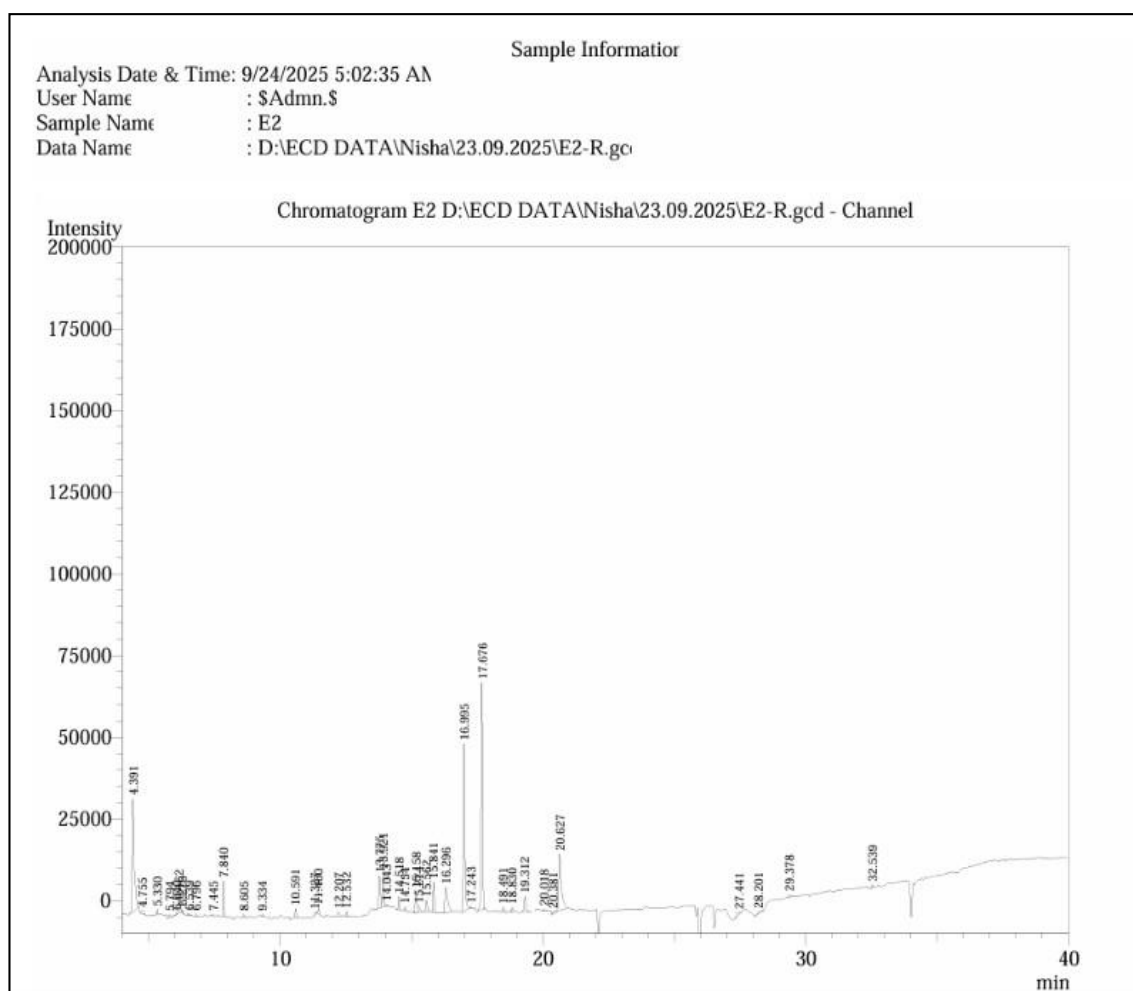
Table 2: Detected concentration of Organochlorine pesticide residues in seasonal vegetables.

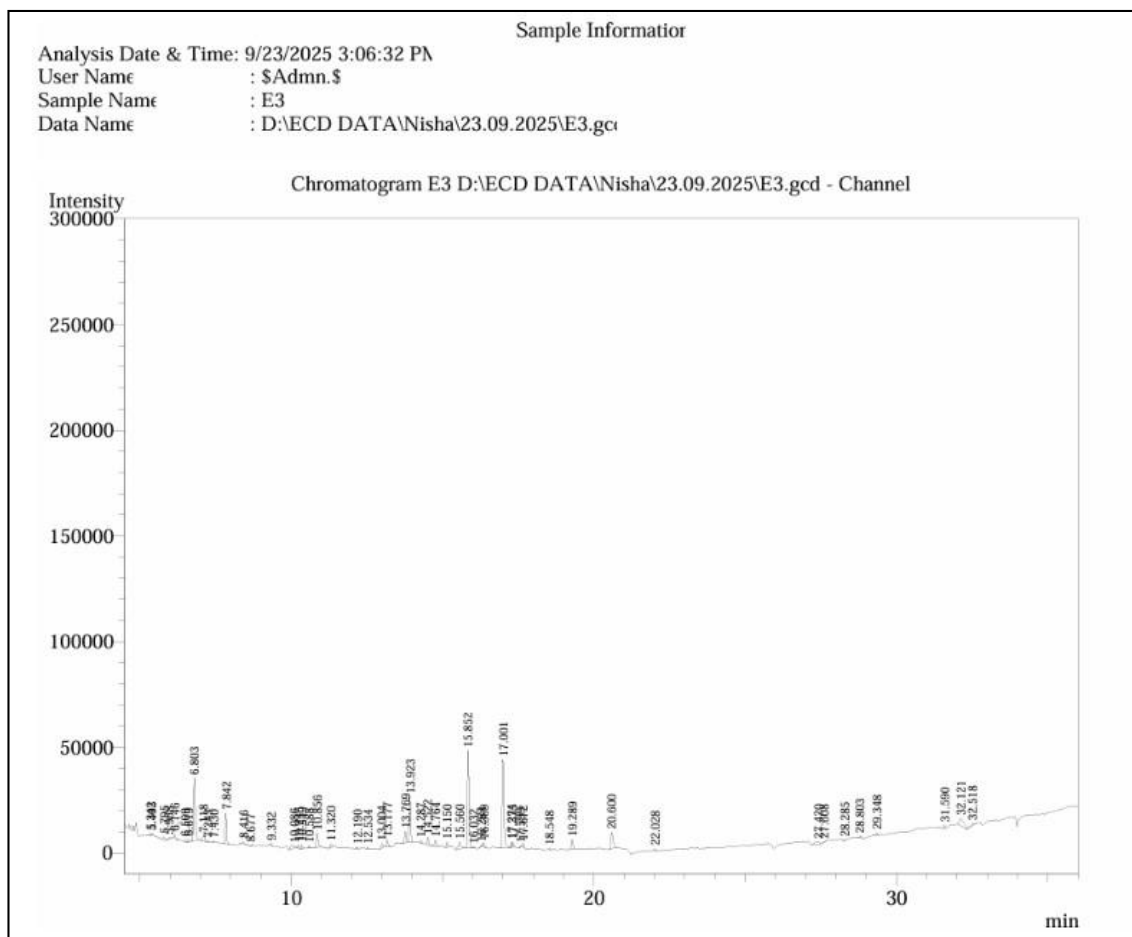
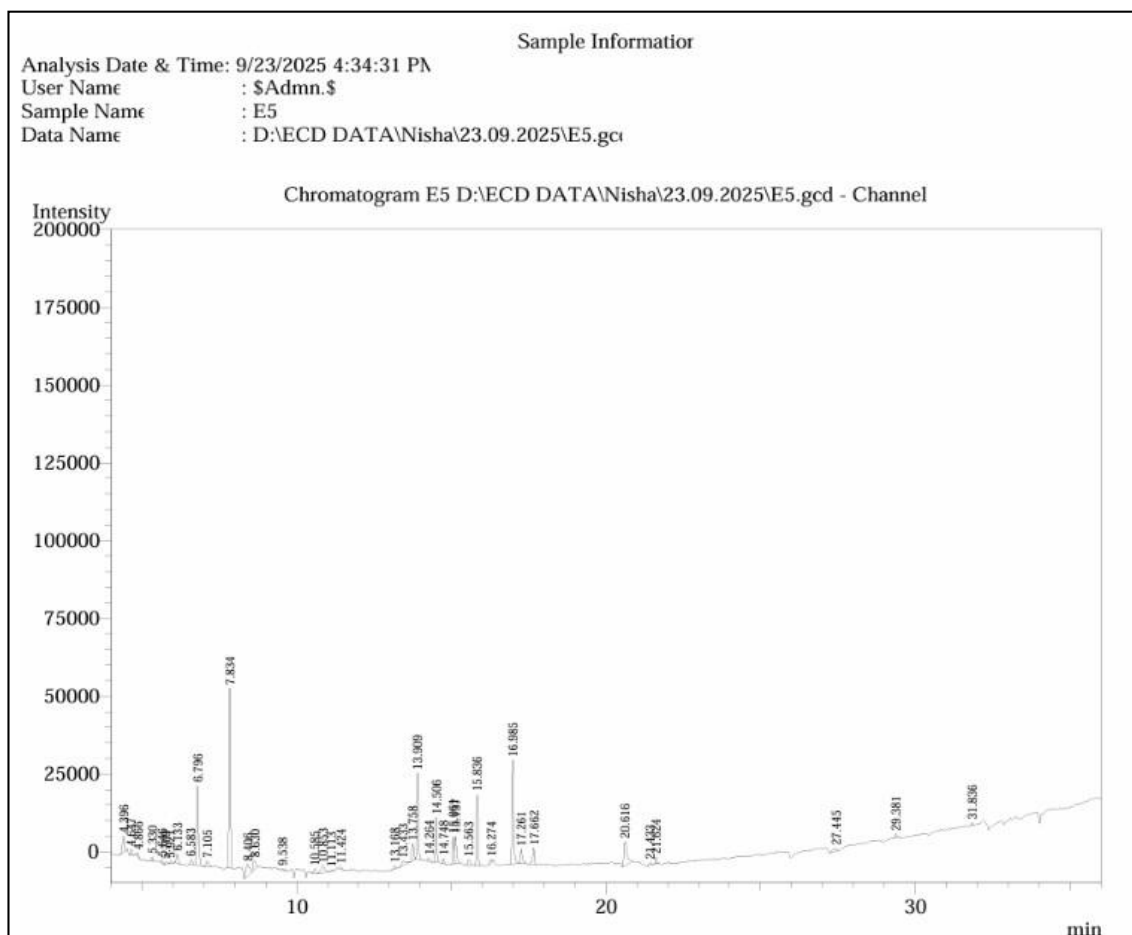
Pesticides	Okra	Cucumber	Sponge gourd	Pointed gourd	Favabeans	Green Peas	Cabbage	Spinach	MRLs
α-BHC	"0"	"0"	"0"	"0"	"0"	0.788	"0"	"0"	1.00
γ-BHC	"0"	1.486	"0"	"0"	"0"	"0"	1.098	0.201	1.00
β-BHC	"0"	"0"	"0"	"0"	"0"	"0"	0.150	"0"	1.00
δ-BHC	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	1.00
Heptachlor	"0"	"0"	"0"	"0"	"0"	"0"	0.452	"0"	0.02
Aldrin	1.138	0.684	"0"	"0"	"0"	"0"	"0"	"0"	0.02
Heptachlor epoxide	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.02
γ-Chlordane	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	-
α-Chlordane	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	-
Endosulfan I	2.836	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.05
4,4'-DDE	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.50
Dieldrin	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.50
Endrin	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.01
4,4'-DDD	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.50
Endosulfan II	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.10
Endrin aldehyde	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.50
4,4'-DDT	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.50
Endosulfan sulfate	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.10
Methoxychlor	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.10
Endrin ketone	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	-

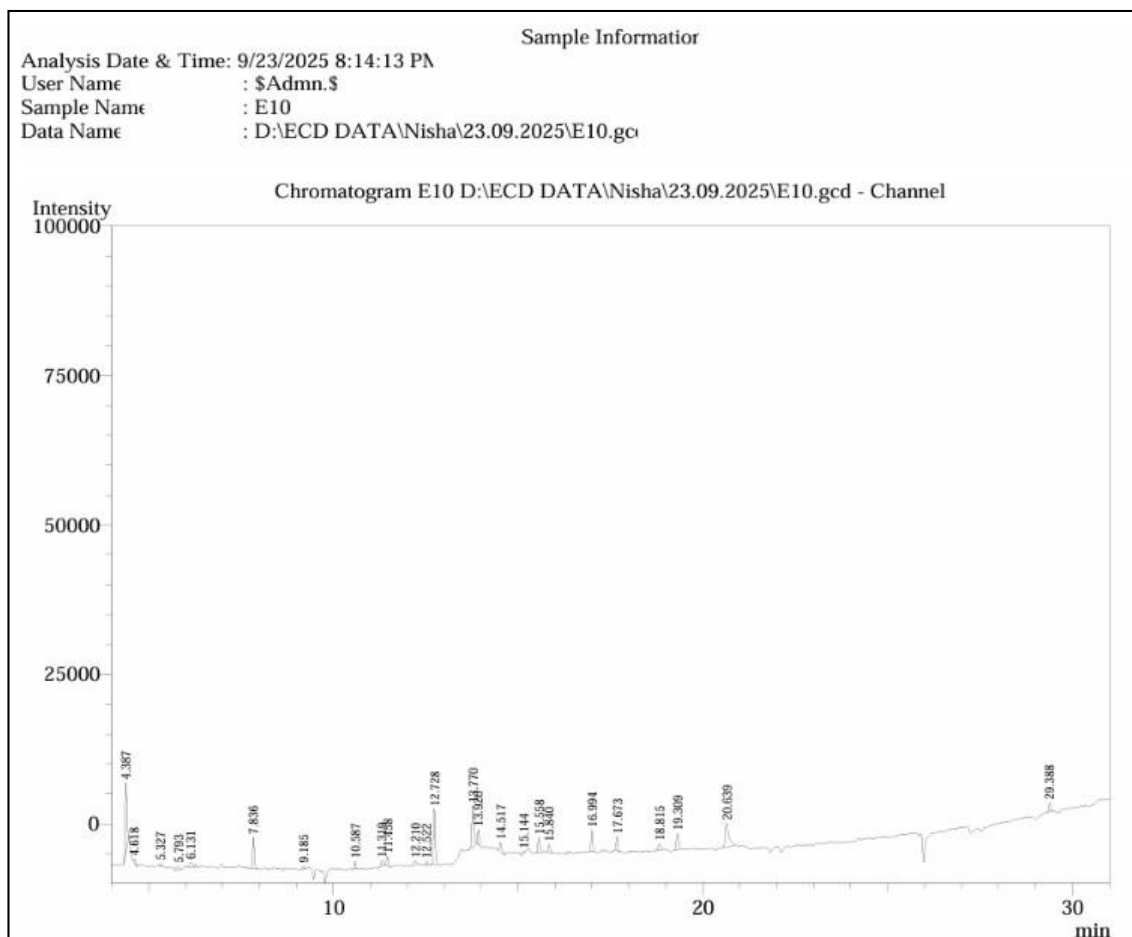
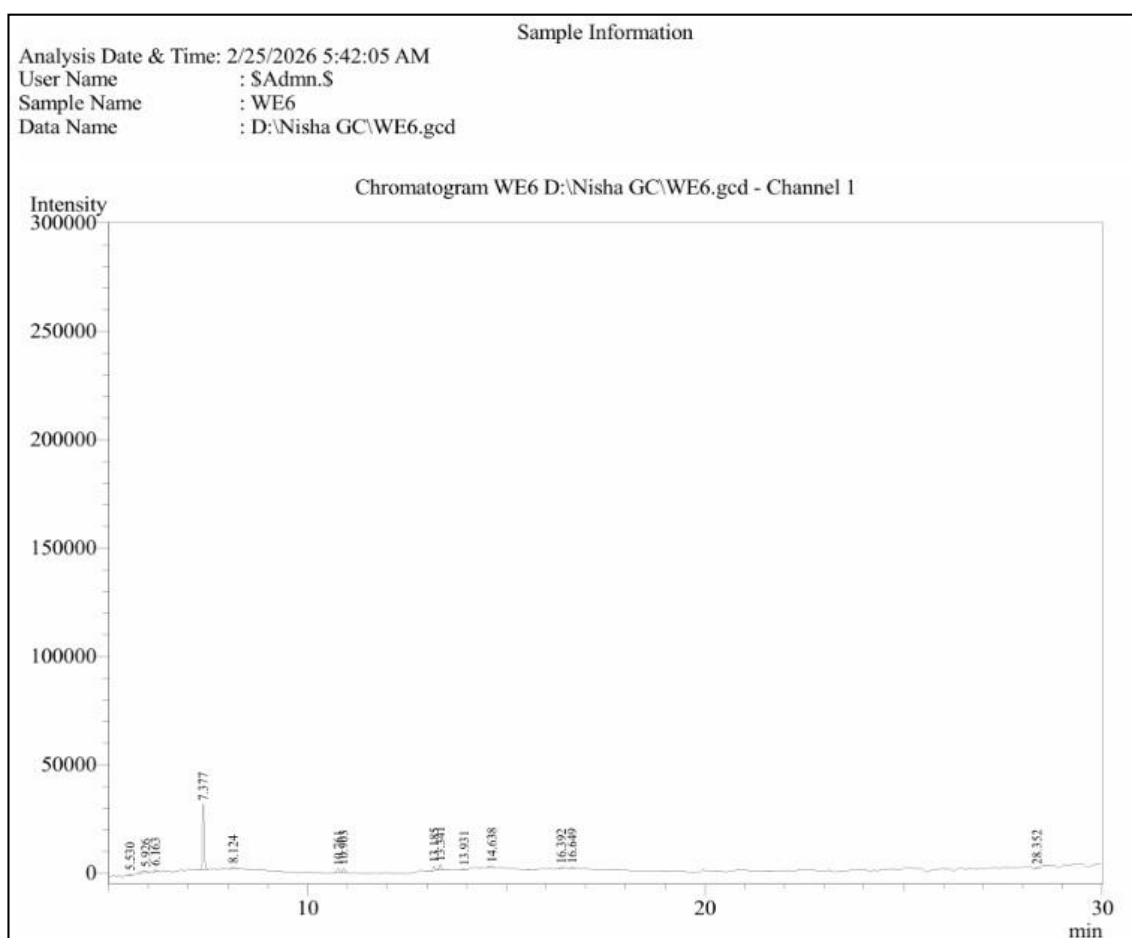
Table 3: Detected concentration of Organochlorine pesticide residues in seasonal fruits.

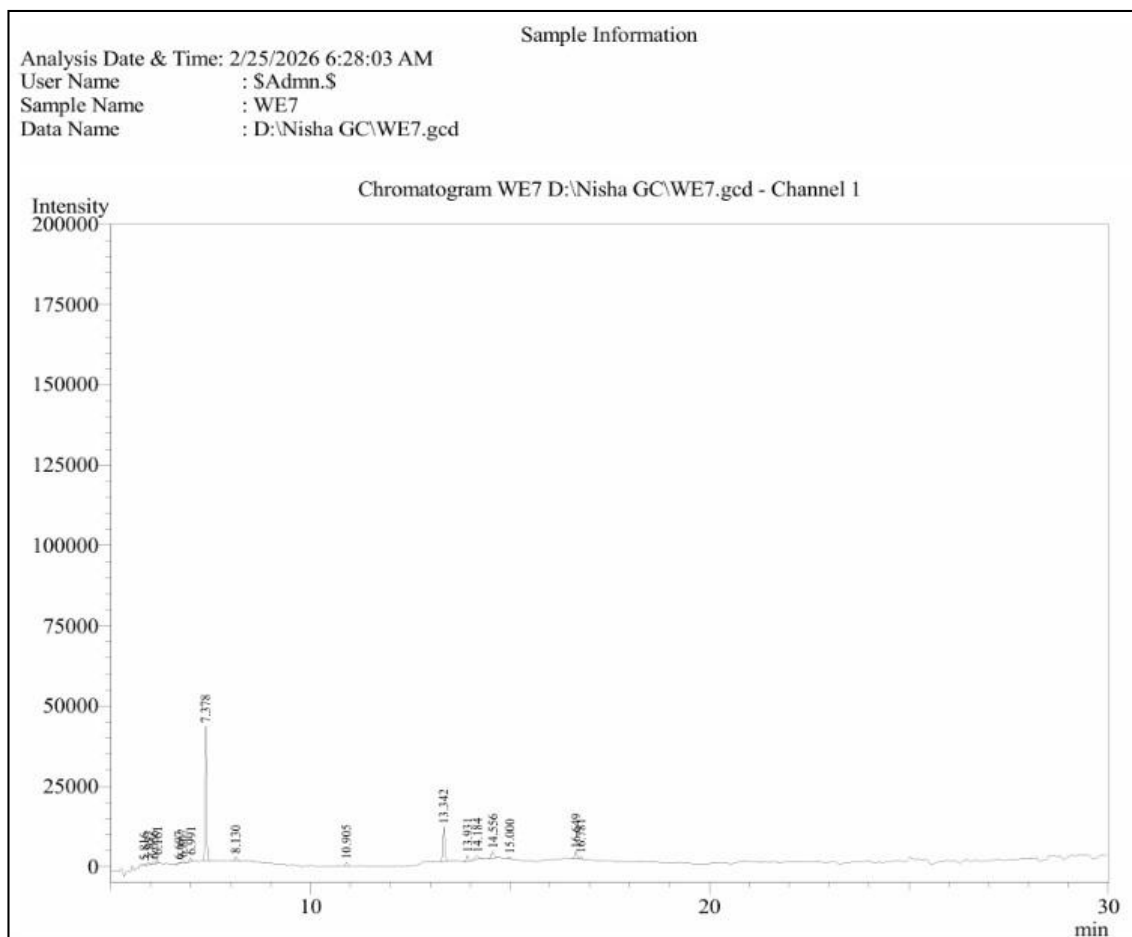
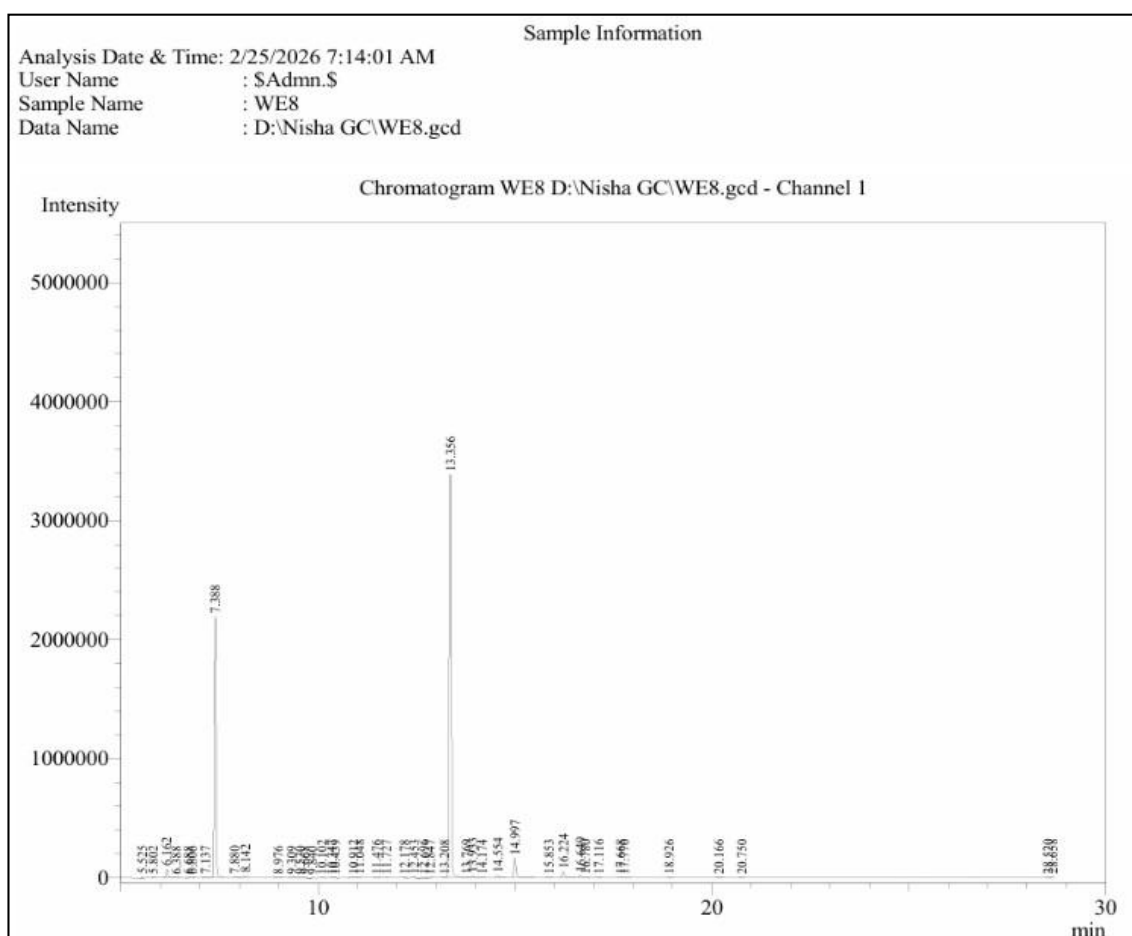
Pesticides	Plum	Mosambi	Avocado	Custard apple	Grapes	Cape gooseberry	Strawberry	Sapota	MRLs
α -BHC	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.01
γ -BHC	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.01
β -BHC	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.01
δ -BHC	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.01
Heptachlor	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.05
Aldrin	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.10
Heptachlor epoxide	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.05
γ -Chlordane	0.3060	0.4773	"0"	"0"	"0"	"0"	0.052	"0"	0.05
α -Chlordane	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.1
Endosulfan I	"0"	"0"	"0"	"0"	"0"	0.102	"0"	"0"	0.1
4,4'-DDE	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	-
Dieldrin	"0"	"0"	"0"	"0"	"0"	0.094	"0"	"0"	0.01
Endrin	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.05
4,4'-DDD	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.05
Endosulfan II	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.05
Endrin aldehyde	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.01
4,4'-DDT	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.1
Endosulfan sulfate	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.2
Methoxychlor	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	-
Endrin ketone	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	0.05

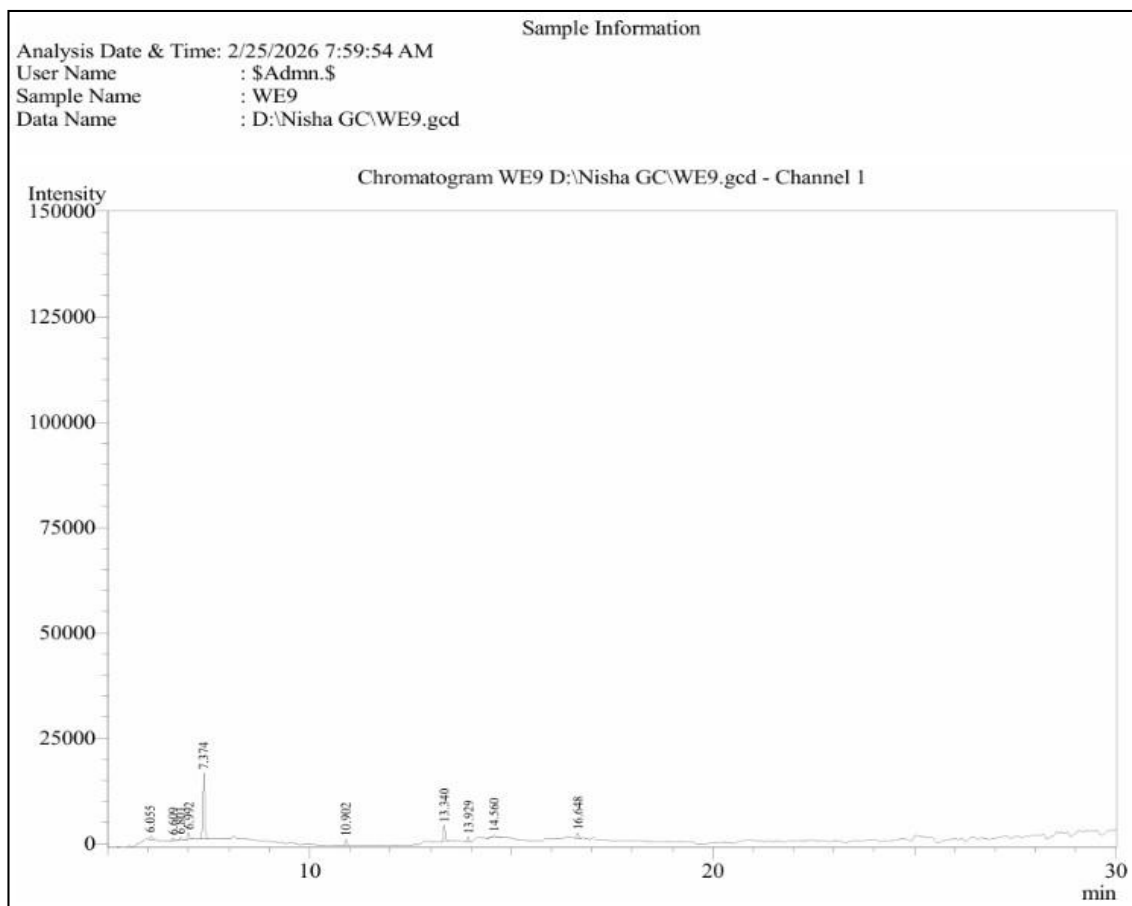
Gas chromatograms of vegetables samples

**Fig 1:** Gas Chromatogram of Okra

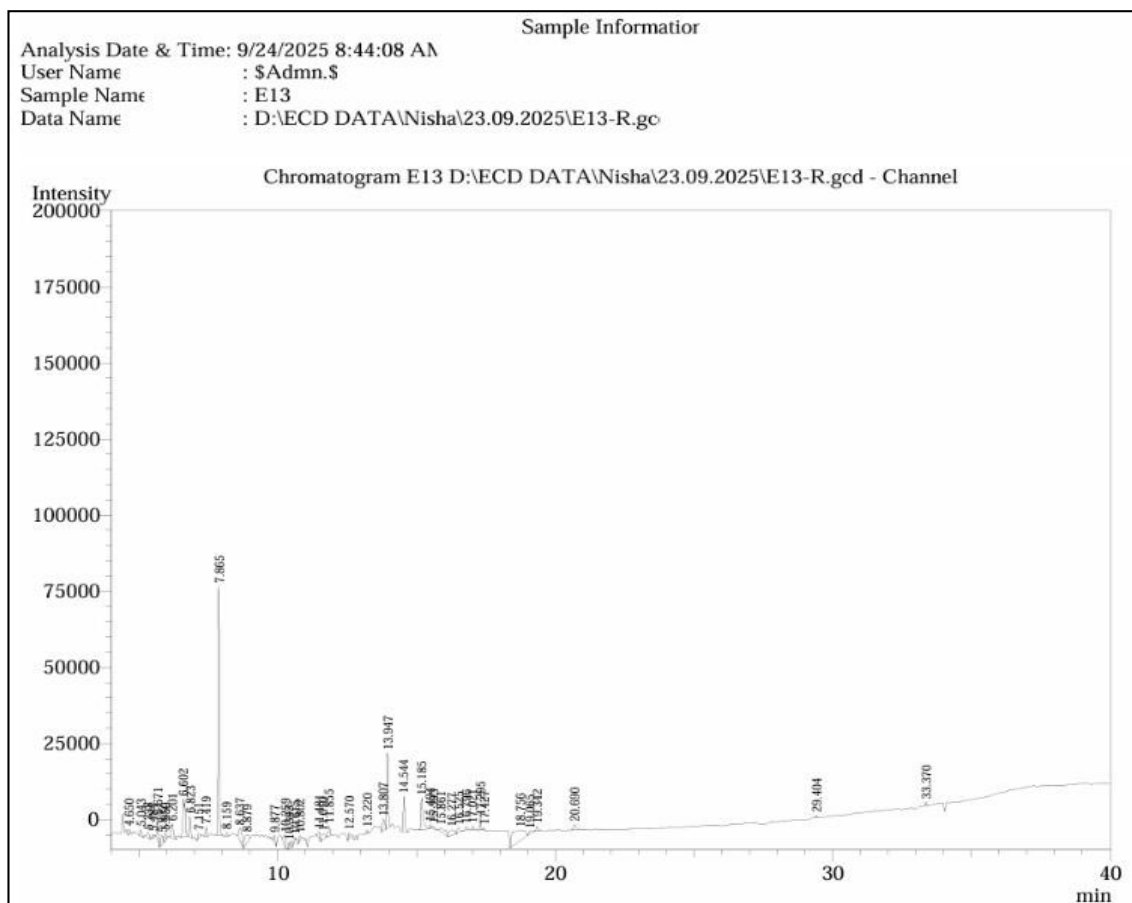
**Fig 2:** Gas Chromatogram of Cucumber**Fig 3:** Gas Chromatogram of Sponge Gourd

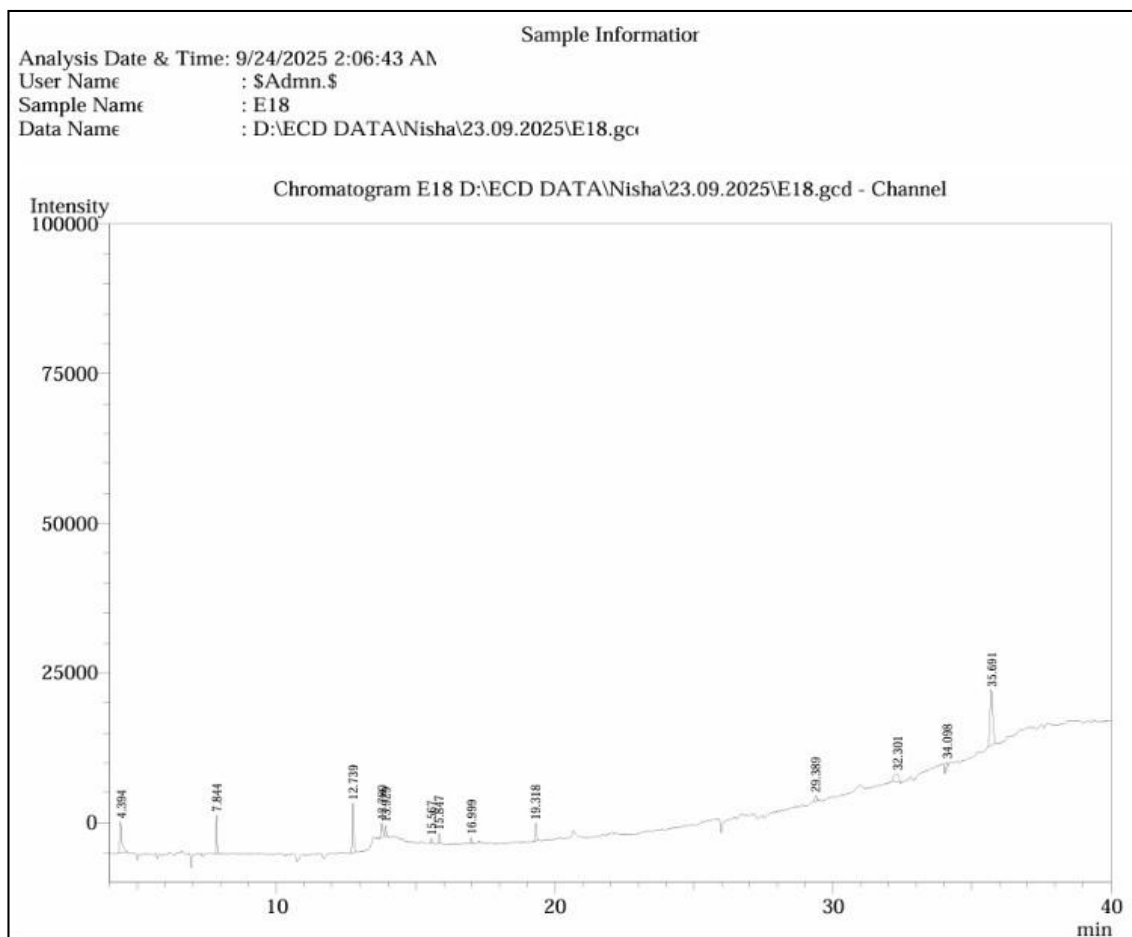
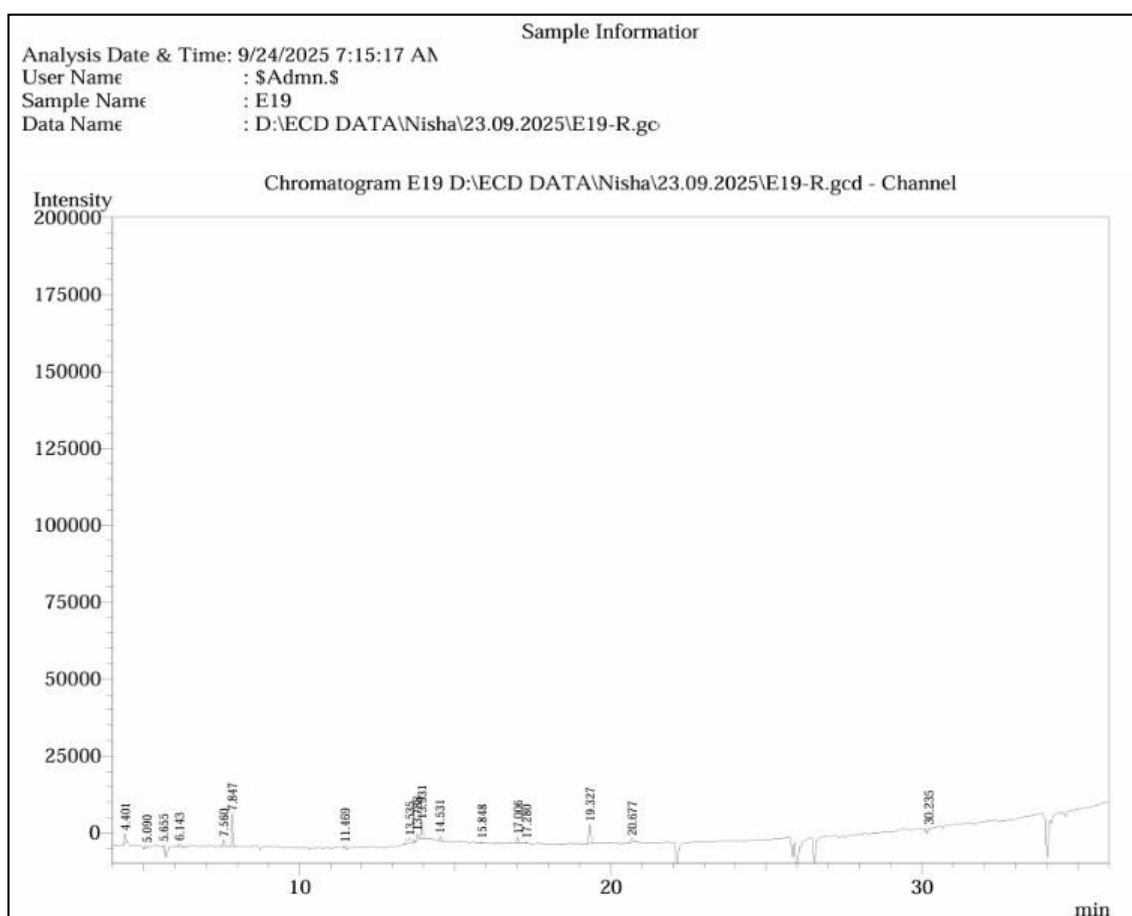
**Fig 4:** Gas Chromatogram of Pointed Gourd**Fig 5:** Gas Chromatogram of Favabeans

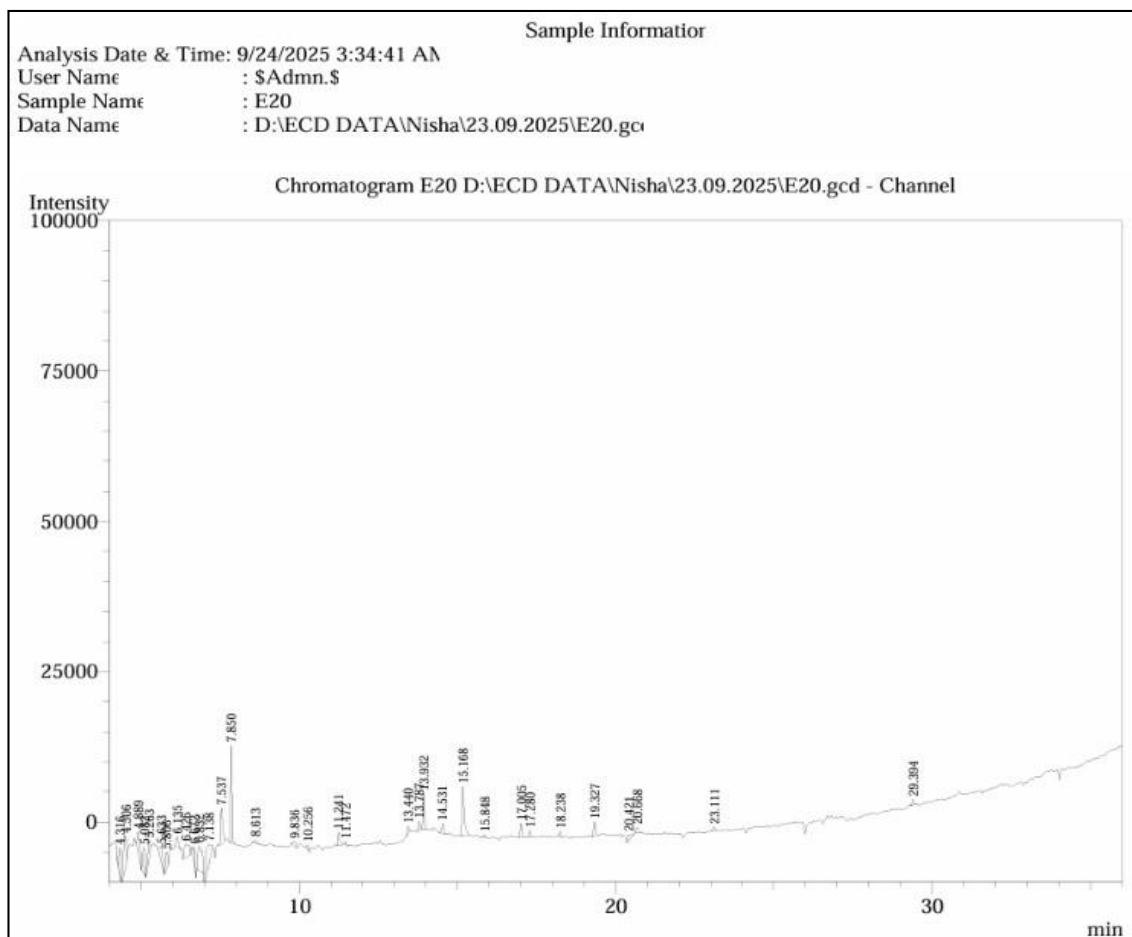
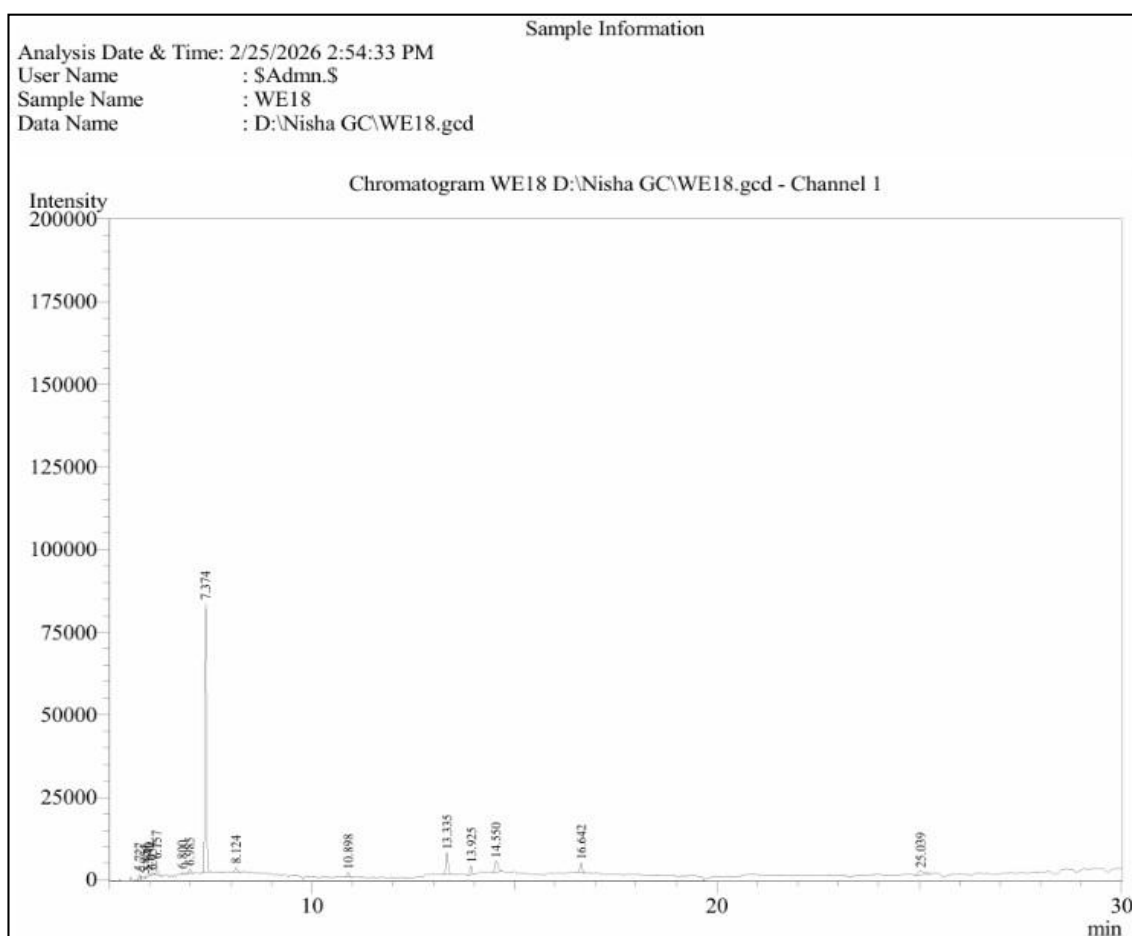
**Fig 6:** Gas Chromatogram of Green Peas**Fig 7:** Gas Chromatogram of cabbage

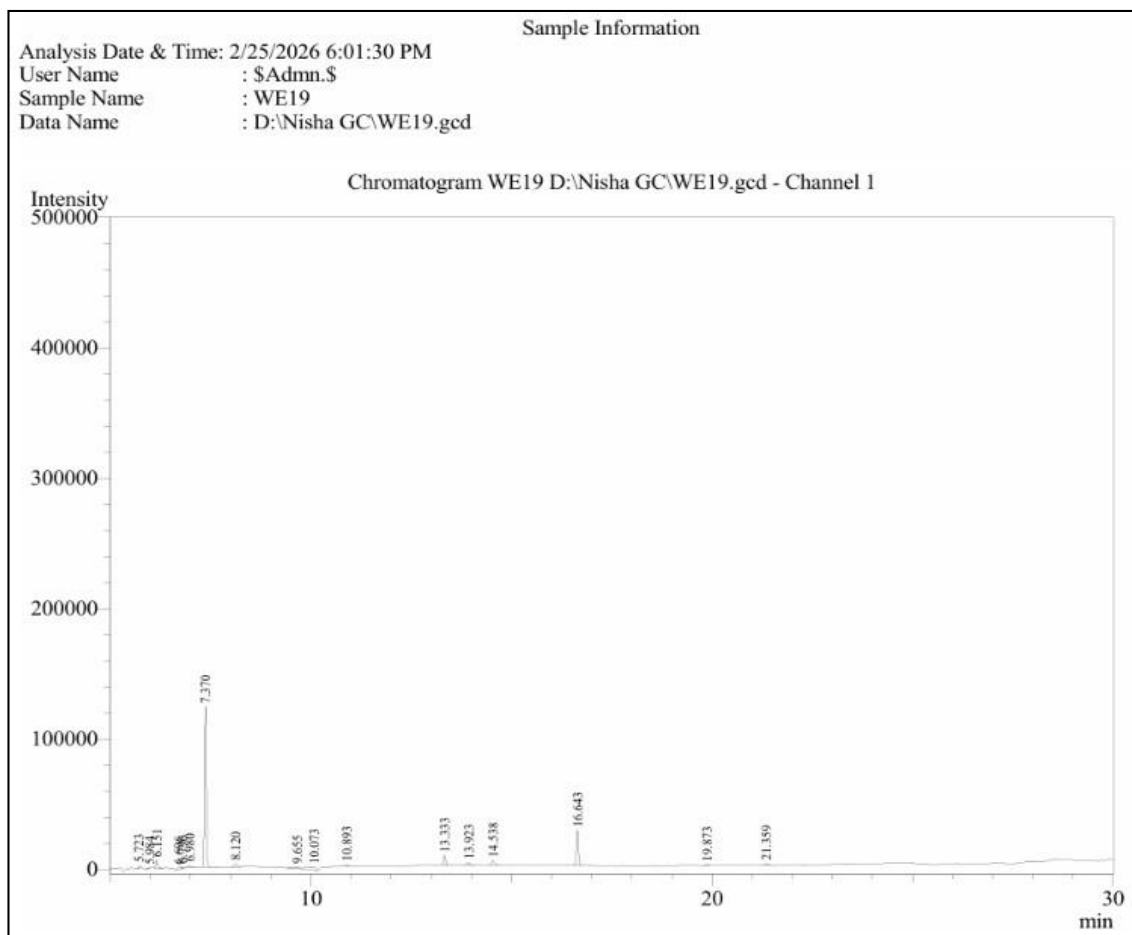
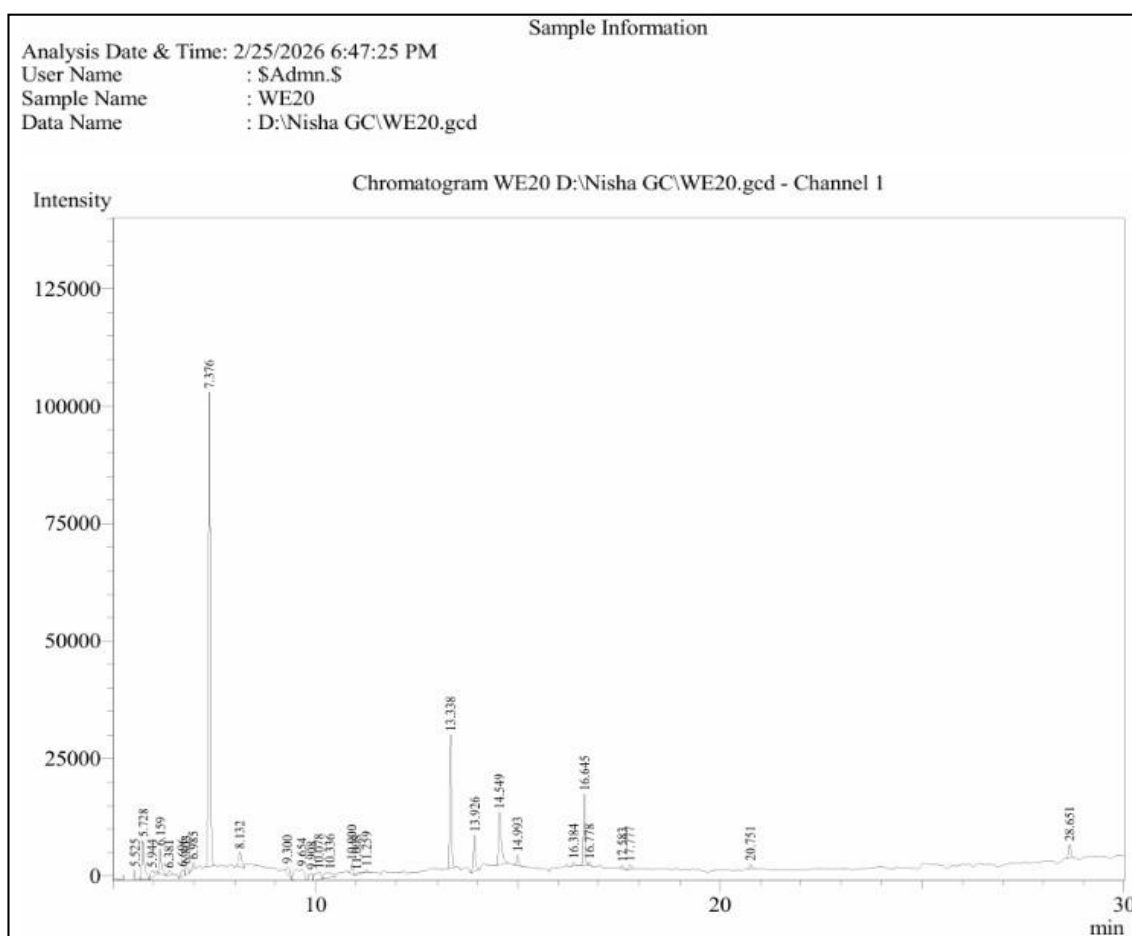
**Fig 8:** Gas Chromatogram of Spinach

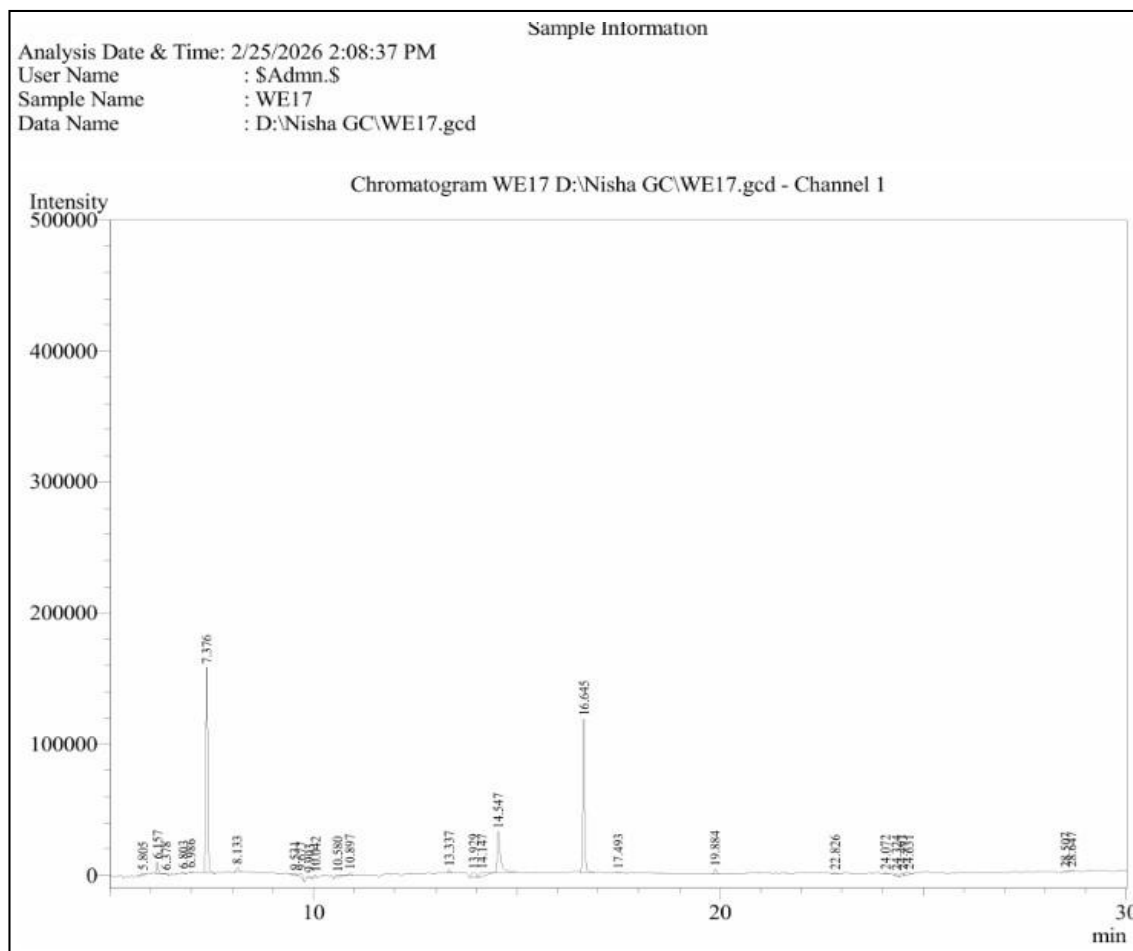
Gas chromatograms of fruits samples

**Fig 9:** Gas chromatogram of Plum

**Fig 10:** Gas Chromatogram of mausambi**Fig 11:** Gas Chromatogram of Avocado

**Fig 12:** Gas Chromatogram of Custard Apple**Fig 13:** Gas Chromatogram of grapes

**Fig 14:** Gas Chromatogram of Cape Gooseberry**Fig 15:** Gas Chromatogram of Strawberry



monitoring of pesticide residues in fruits and vegetables and evaluating their potential health impacts is essential to ensure effective consumer protection against the harmful effects of pesticide contamination.

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