

Detection of Pesticide Residues in Fruits and Vegetables from Multan, Punjab Pakistan

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ABSTRACT

Pesticide residues are most frequently found in fruits and vegetables, which are hazardous to human health and may cause serious diseases. However, little information is available on the occurrence of pesticides in fruits and vegetables in Multan, Pakistan. A study was conducted to detect the possible pesticide residues in fruits and vegetables available at the local markets of Multan. A total of 40 fruits and vegetables were selected, and samples were collected from the local market of Multan from 2021 to 2022. Moreover, the samples were analyzed by the multi-residue pesticide scan method using gas chromatography-mass spectrometry (GC-MS) in the Central Laboratory MNS University of Agriculture, Multan. The finding of the study showed traces of Carbaryl, Linuron, and phorate which were detected in the samples of pear, lemon, and cabbage. However, this is a matter of concern that pesticide residues are detected in the samples of the market, indicating that extensive efforts are required to reduce the possible health risk of pesticides to consumers due to the residue in fruits and vegetables. It is concluded that pesticide residues should be monitored regularly on national level, and the farming community must educate about improved pesticide safety procedures, particularly the need to adhere to the pre-harvest intervals.

Keywords: Vegetables, Fruits, Multan, Pesticide residue, Gas Chromatography.

INTRODUCTION

Pesticides are essential to today's agriculture since they help to maintain high agricultural output. As a result, the extensive usage of pesticides to control pests has evolved as a dominating element of high input demanding agricultural production methods. Plant pests and diseases reduce agriculture productivity by 20 to 40% worldwide [1]. Farmers use pesticides to combat these circumstances as pesticides play an important role in controlling insect pests and diseases, moreover, protecting and promoting agricultural productivity. The use of pesticides and their detrimental effects are rising daily [2]. On the

other hand, pesticides have several negative consequences on human health and climate [3]. Furthermore, pesticide usage is difficult to maintain due to unforeseen long-term negative impacts on the ecosystem, particularly human health. Pesticide residues are found in all agroecosystems, but exposure to residues in primary and derived agricultural foodstuffs pose the highest risk to human health [4]. Pesticides are interconnected to numerous human health issues, from short-term effects like nuisance and sickness to long-term impacts, including cancer, birth deformities, infertility, and endocrine disruption. In addition, both short-term and long-term, pesticide exposure poses a greater risk to children and old-aged people, who are at the most significant threat from pesticide residues [5]. Vitamins, minerals, phytochemicals, and dietetic fiber are all found in fruits and vegetables, making them essential to a well-balanced diet. Fruits and vegetables in the daily diet have been linked to improved gastrointestinal health, clear eyesight, a lower threat of heart disease, chronic diseases including diabetes, and several types of cancer [6]. They serve a vital part of the human diet, particularly as sources of Phyto-nutraceuticals such as vitamins (vitamins A, B1, B6, B9, C, and E), minerals,

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dietary fiber, and phytochemicals [7]. Vegetables come in various shapes and sizes; however, they might be roots, stems, leaves, or edible seeds. Each category makes a unique contribution to the diet [8]. Like other nations attempting to enhance food self-sufficiency, the application of pesticides to control pests has quickly expanded in agricultural production in Pakistan, particularly in fruits and vegetable crops. Most of the fruits and vegetables grown in Pakistan are produced in a controlled environment of a greenhouse, accounting for more than 90% of total greenhouse crop output [9]. Pesticides are used extensively in these controlled environments, the crops cultivated in such protected settings may have higher pesticide residue levels than crops grown in the open field [10]. In 2007, Pakistan’s pesticide use was at 4.5 kg ha⁻¹ per year, but by 2015, it had climbed to 12.8 kg ha⁻¹ per year. There are 76 active pesticide chemicals in use, including pyrethroids, organophosphates, and carbamates, and 9 percent of them are classified as very dangerous by the World Health Organization (WHO). Due to a lack of information about pesticides' unthrifty and inappropriate application, farmers often make pesticide applications too close to the stage of crop maturity or when they are almost near harvest, possibly contaminating the product before shipping it to the market [11]. As a result, pesticide residues have been found in various fruits, vegetables, and other by-

product foods. Although several residual levels remain below the maximum allowable levels, there is a sure opportunity for the pesticide residue to remain in the food above the limits set [12]. Therefore, keeping in view the human health, food security, and climate change impact [13] the issue of pesticide residue needs to be seriously addressed. This research aimed to determine the presence of pesticide residues in widely consumed fruits and vegetables in Multan so that future monitoring could be based on them. The current study determined pesticide residues in fruits and vegetables using Gas Chromatography. The fruit and vegetable selection were based on their regular use among the public. The data obtained through this research will also help to create policies to lower or eliminate human health hazards from pesticide residues in primary and secondary agricultural products.

MATERIAL AND METHODS

Study Area

The samples of fruits and vegetables were collected from the local market of Multan, Punjab Pakistan (Fig 1). The selection of fruits and vegetables was based on the criteria of the regularly consumed fruits and vegetables in the locality of Multan (Table.1). After collection, samples were brought to the Central Laboratory of MNS University of Agriculture, Multan, Pakistan, for further processing.

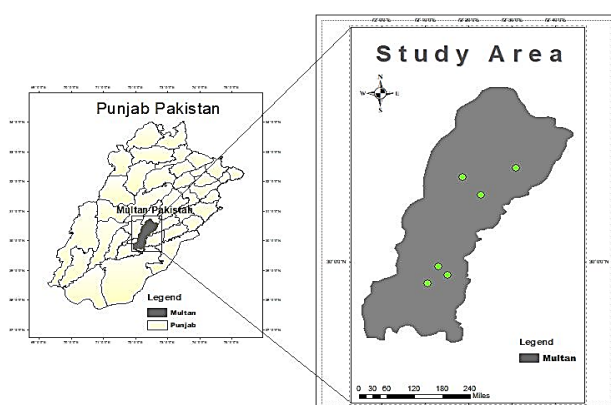


Figure 1. Location of the study area.

Table 1. Fruits and Vegetables selected for this study.

Sr. No	Fruits samples	Vegetables samples
1	Apple	Onion
2	Pear	Pumpkin
3	Guava	Potato
4	Lemon	Cabbage
5	Orange	Cauliflower
6	Banana	Tomato
7	Strawberry	Chilies
8	Chikoo	Turnip
9	Peach	Carrot
10	Grapes	Ginger

Instrument, Chemicals, and Reagents

Gas chromatography (GC) system (Agilent 8890) equipped with a mass spectrophotometer (MS) detector (Agilent 7000 MS system), silica capillary column (DB-5 MS), Mass hunter software, and autosampler attached with a loading capacity of 120 samples at a time was used for the detection of pesticide residue in fruits and vegetables. The data

analysis of samples was carried out by the NIST library equipped with GC-MS system. The extraction solvent and reagents used in this study were HPLC-grade methanol, acetone, and acetonitrile were acquired from (Merck Darmstadt, Germany). The glassware used in the study was Class A. The analysis conditions of the instrument are given in Table 2 [14].

Table. 2 Instrument Condition for Multi residue Pesticide Scanning.

Parameters	Conditions
Columns	Agilent DB-5 MS
Injection mode	Spitless Inlet
Carrier gas	Helium 99.99% pure
Gas Flow rate	0.75 mL/min
Injection volume	1.0 µL
Inlet port temperature	250°C
Oven Program	The oven temperature was programmed to start from 90°C withhold for 2 minutes and increase from 90 to 160°C (15°C/min), hold for 10 minutes, then to 250°C (20°C/min), for 15, and a final ramp to 270°C (20°C/min) hold for 20 minutes.

Extraction and Clean Up

For sample extraction and cleaning, the chemical extraction method was utilized. The procedure includes a single methanol extraction which only needs 10 to 15 g of sample. A representative 10-gram sample of completely homogenized material was weighed in a 50-ml centrifuge tube. After that, 10 mL of methanol was added to the tube. A mechanical shaker was used to vibrate the tube for 1-2 minutes after it was properly closed. The extract was then centrifuged at 6000 rpm for 5 minutes. The clean supernatant was subsequently transferred to a fresh tube after centrifugation. The centrifugation procedure was carried out once more under identical conditions. After centrifugation, a 1 mL supernatant was filtered using a 0.45 µm syringe filter and injected into clean GC vials [15].

RESULTS

Pesticide Residues in Analyzed Samples

It can be observed from the spectra of the studies that only a few fruits and vegetables have shown the

residues of pesticides in them. Environmental variables have little effect on pesticide absorption in fruits and vegetables that are not treated with pesticides but are near to pesticide application. Non-point source contamination also has the probability as few samples of fruits and vegetables show the residues of those pesticides which are not commonly used for that commodity. As a result, it is critical to determine if pesticides remain on fruit and vegetables. The studies identified and validated pesticide residues in fruit and vegetable samples using molecular weights. For additional validation of pesticides found, the molecular weights of pesticides were matched to those listed in the GC/MS data library. Pesticides, including fungicides, insecticides, and herbicides, were identified in a few samples. Most fruits and vegetables samples were determined with almost no pesticide residue in them (Table 3). The most prevalent pesticide discovered in all fruit and vegetable samples were organophosphorus and carbamates (Table 4) shows the pesticides identified in the cabbage, pear, and samples, with their retention, time, and molecular weights.

Table 3. Pesticides Screening Analysis of Fruits and Vegetables.

Sr. No	Sample ID	Sample Name	Total Peaks	Pesticide Name
1	GCMS-5977B_044G.D	Onion	2	No Pesticide
2	GCMS-5977B_043B.D	Tomato	1	No Pesticide
3	GCMS-5977B_042D.D	Cabbage	4	CARBARYL
4	GCMS-5977B_041C.D	Potato	1	No Pesticide
5	GCMS-5977B_040Q.D	Banana	18	No Pesticide
6	GCMS-5977B_039X.D	Peach	4	No Pesticide

7	GCMS-5977B_038K.D	Pear	53	LINURON
8	GCMS-5977B_037J'. D	Orange	37	No Pesticide
9	GCMS-5977B_036I.D	Chikoo	37	No Pesticide
10	GCMS-5977B_035I.D	Lemon	45	PHORATE
11	GCMS-5977B_035.D	Carrot	6	No Pesticide
12	GCMS-5977B_034I.D	Ginger	22	No Pesticide
13	GCMS-5977B_033I.D	Grapes	37	No Pesticide
14	GCMS-5977B_032.D	Cauliflower	11	No Pesticide
15	GCMS-5977B_031.D	Turnip	9	No Pesticide
16	GCMS-5977B_030.D	Strawberry	40	No Pesticide
17	GCMS-5977B_029.D	Guava	39	No Pesticide
18	GCMS-5977B_028.D	Apple	41	No Pesticide
19	GCMS-5977B_027.D	Chilies	5	No Pesticide
20	GCMS-5977B_026.D	Pumpkin	29	CONIINE

Table 4. Pesticides identified in Cabbage by GC/MS.

Sample	Pesticides	Retention Time	Molecular Formula	Molecular Weight	Category
Cabbage	Carbaryl	5.901	C ₁₂ H ₁₁ NO ₂	201.22	Insecticide
Pear	Linuron	19.617	C ₉ H ₁₀ C ₁₂ N ₂ O ₂	249.1	Herbicide
Lemon	Phorate	7.291	C ₇ H ₁₇ O ₂ PS ₃	260.4	Insecticide

Carbaryl was identified in the cabbage sample at a retention time of 5.901. Carbaryl is not applied to cabbage, but the residue of Carbaryl found in the

sample show that it could be absorbed in the vegetable from a non-point source.

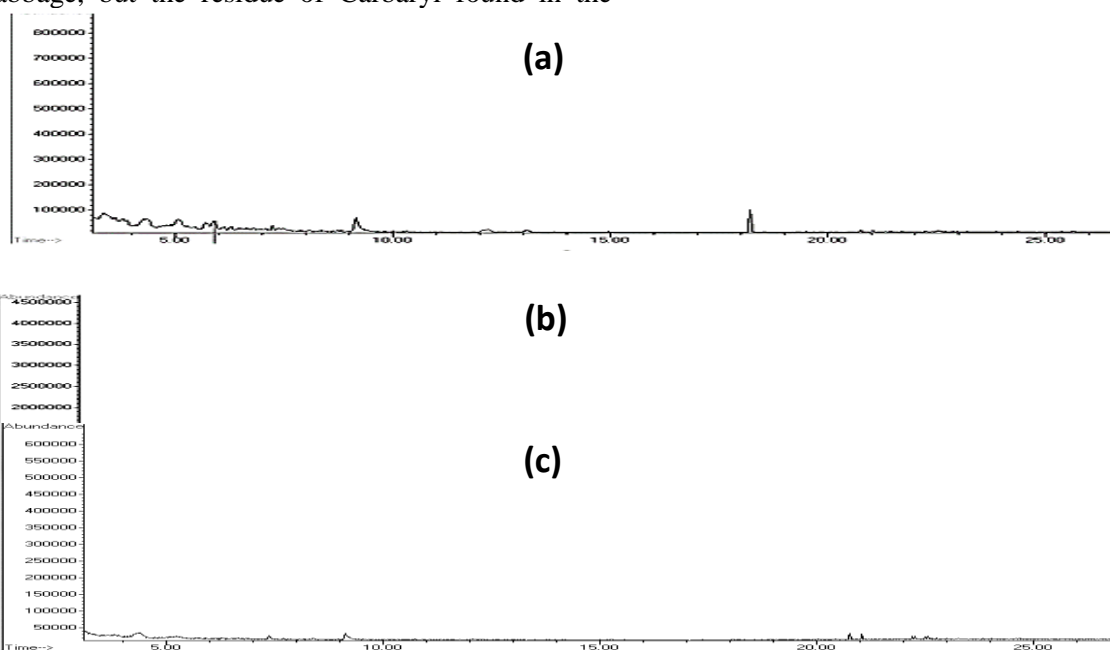


Figure 2. Gas chromatography and mass spectrometry analysis of pesticide residues in fruit and vegetable samples; (a) GC/MS Spectra of Identified pesticide Carbaryl in Cabbage Sample; (b) GC/MS Spectra identified Pesticide Linuron in Pear Sample; (c) GC/MS Spectra identified Pesticide Phorate in Lemon Sample.

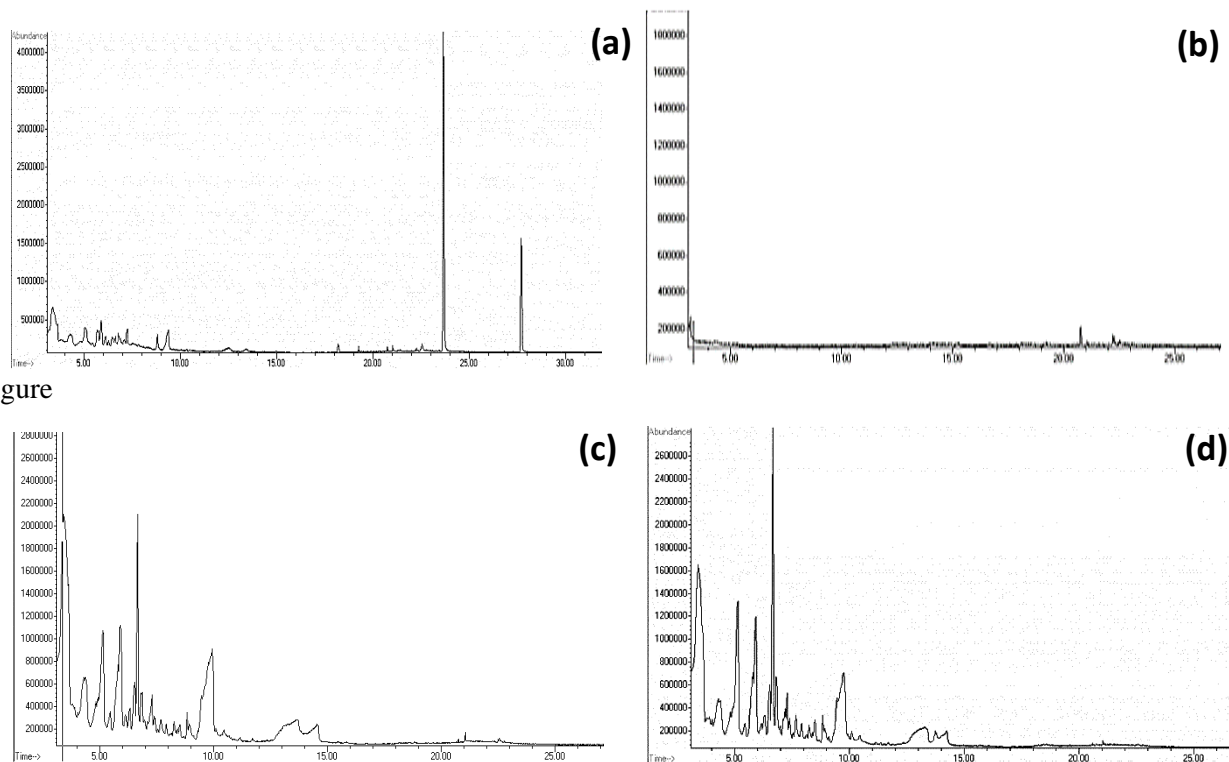
Samples Without Pesticide Residues

The analysis revealed that only three commodities had pesticide residues, while all seventeen fruits and vegetables were found to be free of pesticide residues. A total of eight fruits and nine vegetables were found pesticide residue-free. The GC/MS spectra for all fruits and vegetable samples are given below, showing no

pesticide traces. The pumpkin sample had many compounds, including d-Mannose, Glucose, Guanosine, Melezitose, Desulphosinigrin, Dodecanoic acid 3-hydroxy, Norephedrine, Levetiracetam, Ropivacaine, Phloroglucinol, etc. None of the peaks shows any pesticide compound in the samples of pumpkin (Fig 3a). The chillies sample was found to

have many compounds including, -carboxylic acid, Benzeneethanamine, Actinobolin, 1-Gala-1-ido-octose, pyrimidine, Chloroamphetamine, Atomoxetine, Tocainide, Hydrox-3-methoxyamphetamine, tetradecane, pentadecane, etc. None of the peaks shows any pesticide compound in the sample of chillies (Fig 3b). The Apple sample had many compounds,

including Sucrose, Glucopyranose, Fructose, d-Mannose, Lactose, Phendimetrazine, Phloroglucinol, Carbromal, etc. None of the peaks show any pesticide compound in the sample of Apple (Fig 3c). The Guava sample was found to have many compounds like Sucrose, Fructose, Maltose, etc. None of the peaks shows any pesticide compound in guava (Fig 3d).



Figure

3(a). GC/MS Spectra of Pumpkin Sample (b) GC/MS Spectra of Chillies Sample (c) GC/MS Spectra of Apple Sample (d) GC/MS Spectra of Guava Sample.

The strawberry sample was found to have many compounds, including Hydroxymethylfurfural, furaldehyde, 3-n-Propyl-2-pyrazolin-5-one, Fructose, Fatty Acid, Phendimetrazine, Cathinone, etc. No pesticide residues were detected in strawberries (Fig 4a). The Turnip sample had many compounds, including 1-Guanidinosuccinimide, 6-Acetyl- β -d-mannose, Trifluoroacetoxytridecane, Arginine Fructose, Phendimetrazine, Epinephrine, Amphetamine, etc. None of the peaks show any pesticide compound in the sample of turnip (Fig 4b). The Cauliflower sample had many compounds, including Azodicarbonamide, Formic acid, ethenyl ester, Acetic acid, 3-Piperidinol ethanamine, Atomoxetine Bupropion, Epinephrine, Phenylephrine, etc. None of the peaks show any pesticide compound in the sample of cauliflower (Fig 4c). The grapes sample were found to have many compounds, Ethylamine, Pterin-6-carboxylic acid, Formic acid, ethenyl ester, Phenylephrine, Azodicarbonamide, Phendimetrazine, Phloroglucinol Epinephrine, Amphetamine Phenylephrine Duloxetine, etc. None of

the peaks shows any pesticide compound in the sample of Grapes (Fig 4d).

The Ginger sample was found to have many compounds, including, Galacto-cellulose, d-Glycero-d-tallo-heptose, Sucrose, d-Mannose, 2-Deoxy-D-galactose, β -D-Glucopyranose, 4-O- β -D-galactopyranosyl, L-Glucose, Coniine, Amphetamine Benzylamine, etc. None of the peaks shows any pesticide compound in the sample of ginger (Fig 5a). The chikoo sample was found to have many compounds, including β -D-Glucopyranose, Melezitose, Desulphosinigrin, Lactose, Maltose Sucrose, Melibiose, Fatty Acid, etc. Pesticide residue was not observed in chikoo (Fig 5b). The orange sample was found to have many compounds, including, β -D-Glucopyranose, 4-O- β -D-galactopyranosyl, d-Mannose, L-Glucose Melezitose, Desulphosinigrin, Galacto-cellulose Lactose, Melibiose, Phendimetrazine, Phloroglucinol, Duloxetine, etc.

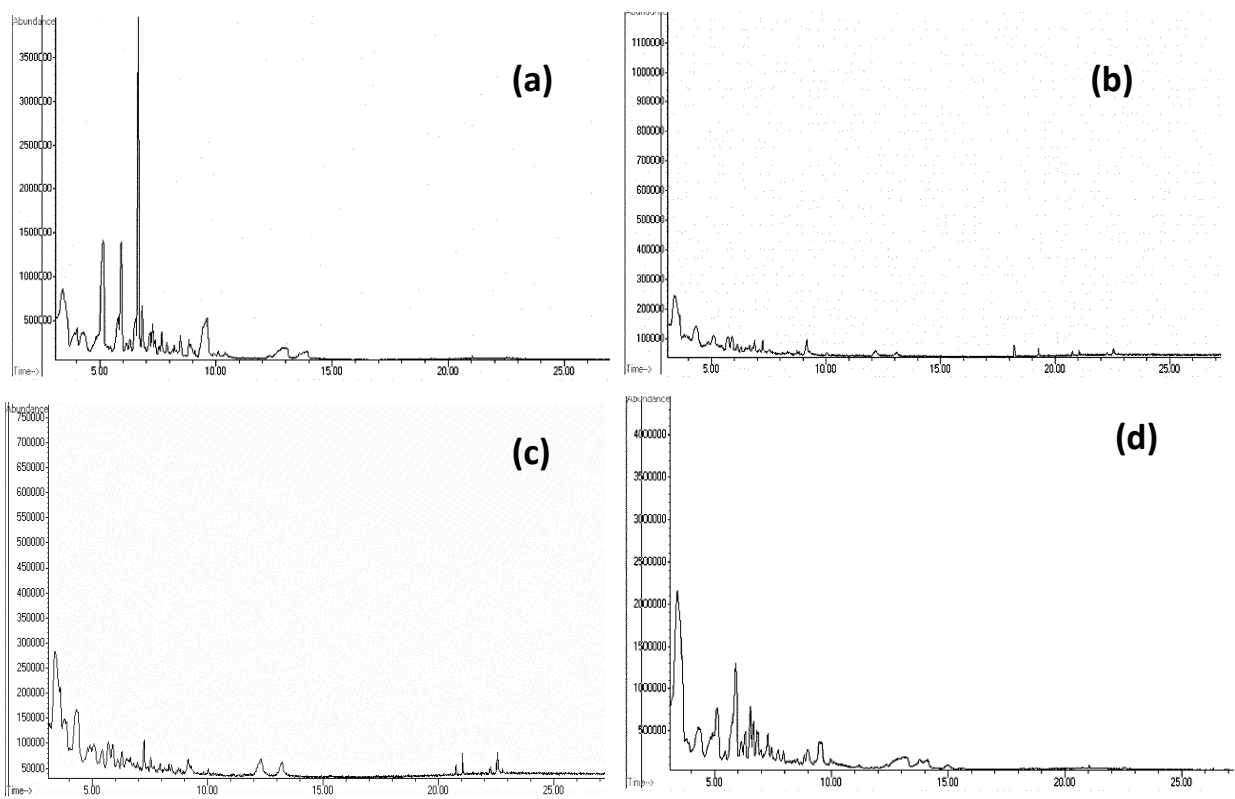


Figure 4(a). GC/MS Spectra of Strawberry Sample (b) GC/MS Spectra of Turnip Sample (c) GC/MS Spectra of Cauliflower Sample (d) GC/MS Spectra of Grapes Sample.

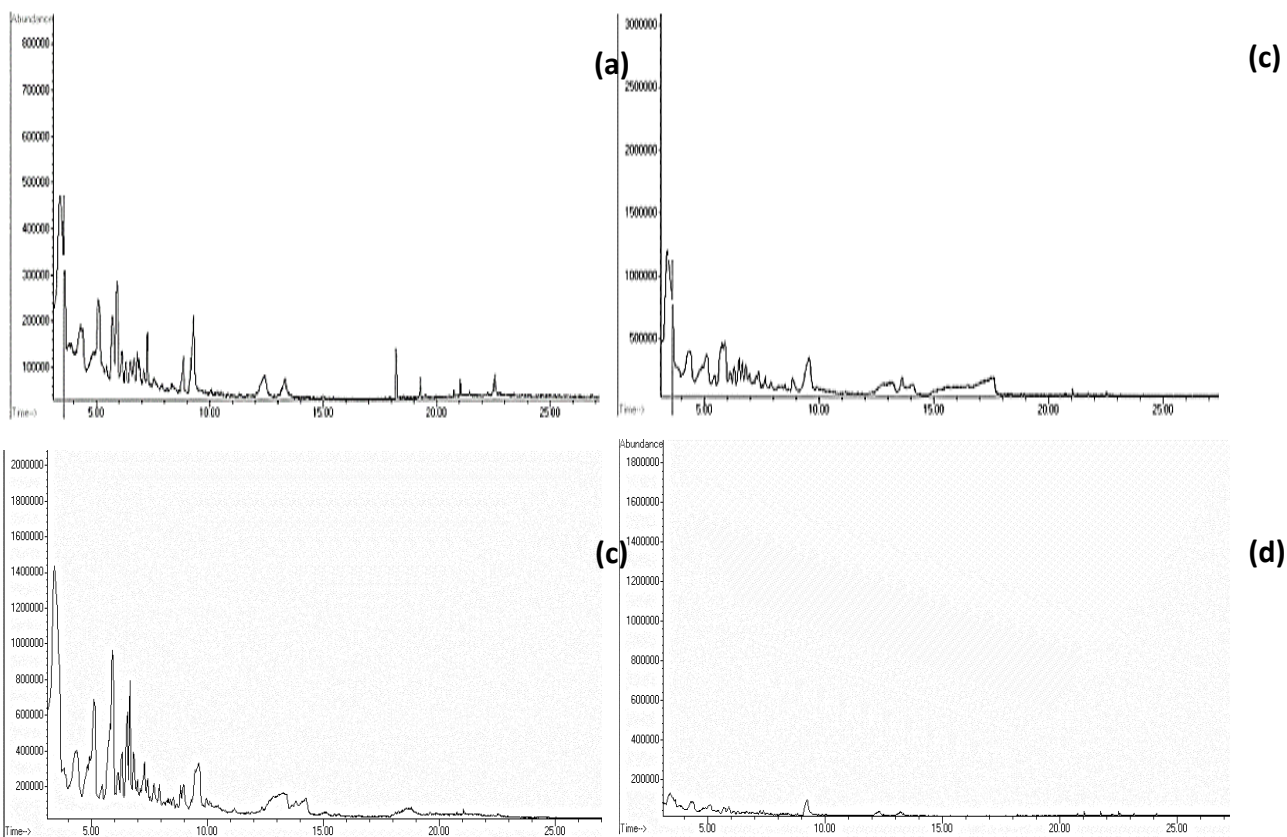


Figure 5(a). GC/MS Spectra of Ginger Sample (b) GC/MS Spectra of Chikoo Sample (c) GC/MS Spectra of Orange Sample (d) GC/MS Spectra of Carrot Sample.

None of the peaks shows any pesticide compound in the sample of orange (Fig 5c). The carrot sample was found to have many compounds, including, Sorbitol, Galactitol, D-Mannitol, 1,5-Anhydroglucitol, 2,5-O-Methylene-D-mannitol, Lactose, Levetiracetam, Linuron, Coniine, Clofibrate, Phorate, etc. None of the peaks show pesticide compounds in the carrot sample (Fig 5d).

The Banana sample was found to have many compounds, including, Melezitose, d-Mannose, Sucrose, Lactose, Maltose, L-Glucose, Galactocellulose, Zidovudine, Levetiracetam, Linuron, Ethambutol, Duloxetine, etc. None of the peaks shows any pesticide compound in the banana sample (Fig 6a). The Potato sample had many compounds, including dl-Alanine, melezitose, d-mannose, lactose, sucrose, nickel tetracarbonyl, alanine 2-amino-1-(o-

hydroxyphenyl) propane, galacto-cellulose, acetic acid, formic acid, L-glucose, etc. None of the peaks shows any pesticide residues in the potato samples (Fig 6b). The tomato sample were found to have many compounds, including dl-Alanine, ethyl hydrogen oxalate, nickel tetracarbonyl, alanine, 2-amino-1-(o-hydroxyphenyl) propane, hydroxyurea, D-alanine, amphetamine-3-methyl,) acetic acid, formic acid, sucrose, L-glucose, etc. None of the peaks shows any pesticide compound in the sample of tomato (Fig 6c). The onion sample was found to have many compounds, including dl-alanine, alanine, 1,2-propanediamine, carbon dioxide, glycyl-dl-alanine, and nickel tetracarbonyl) ethanol, 2-(methylamino), hydrogen cyanide, etc. None of the peaks shows any pesticide compound in the onion sample (Fig 6d).

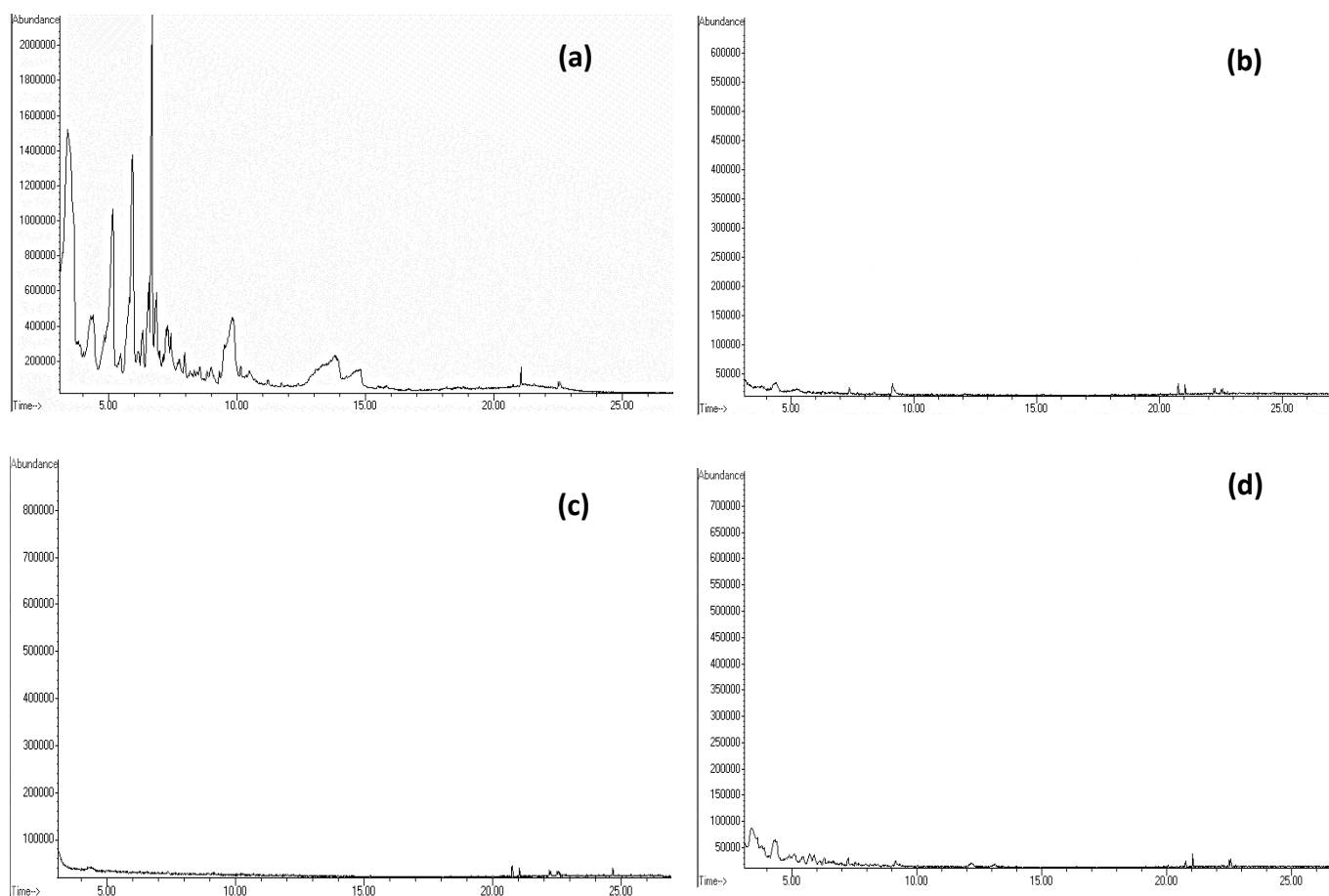


Figure 6(a). GC/MS Spectra Banana of Sample (b) GC/MS Spectra of Potato Sample (c) GC/MS Spectra of Tomato Sample (d) GC/MS Spectra of Onion Sample.

DISCUSSION

In recent decades, the production of fruits and vegetables has increased to meet the ever-increasing population demand. However, there is always a high rate of pesticide detection in fruits and vegetables because insects, pests and diseases rapidly influence the growth, development and post-harvest storage of

fruits and vegetables [16]. In this study, the pesticide residues were detected in GC-MS and the residues were found in only a few fruits and vegetables collected from the local market of Multan. The residues were detected in cabbage, pear, and lemon. On the other hand, the fruits, including apple, guava, orange, strawberry, sapodilla, and banana, were

residue-free. The vegetables were residue-free, including cauliflower, ginger, onion, pumpkin, carrot, chilies, potato, tomato, and turnip. The most often identified pesticides were Carbamate, Linuron, and Phorate. Even though these pesticides are moderately dangerous, they have been linked to human neurotoxicity. However, if the residue of pesticides is modest, the continued use of contaminated fruits and vegetables may cause long-term health hazards to human beings. In addition to the residue, pesticides are not readily soluble. They may accumulate in the tissues of human organs and become carcinogenic with the passage of time. Like farmers, they mostly grow corn or maize before cabbage in the same field and apply a high amount of Carbofuran, which contains Carbaryl [17]. Carbaryl is a carbamate-family compound that is mainly applied as an insecticide. Carbamates do not last as long as chlorinated insecticides do. Carbaryl is poisonous to insects, although quickly digested and removed from vertebrates. It is preferred for food crops because it is neither fat-concentrated nor expressed in milk. Linuron is a phenyl urea herbicide used to suppress the growth of grass and weeds to promote crop growth. It was observed in pear fruit. As an androgen receptor (AR) antagonist, Linuron has been discovered to cause reproductive damage in animals and is thus classified as an endocrine disruptor [18]. The lemon sample shows the residue of the phorate based pesticides. Phorate is organophosphate used as an insecticide and it is used to control chewing and sucking pests. Long-term exposure to this pesticide can be lethal [17]. Other than the phorate, non-pesticide compounds, including Sucrose, d-mannose, L-lyxose, melezitose, lactose, L-glucose, d-glycerol-d-ido-heptose were also present in the sample of lemon. Various pesticide residues in different samples may be due to the frequent spray of pesticides, herbicides, and insecticides used to control insects, pests, and weeds in the farmer's field. The issue of pesticide residue is particularly found in greenhouses and controlled systems where the insect may proliferate in the closed system compared to conventional cultivation [19] and these vegetables are being sold in local market which carry them a load of pesticide. This research is in line with other studies in which multi-residue pesticides are reported in different fruits and vegetables [16; 20; 21; 24]. The study's finding reveals that farmers don't follow safety measures while spraying pesticides in the field, which could be a reason for pesticide residue in the fruits and vegetables. Moreover, the other possible reason for residue is due to the spray by local vendors to control the insect pest in the local fruit and vegetable markets. Pesticides were identified in a significant proportion of fruit and vegetable samples consequently. The existence of high pesticide levels in

some of the samples shows that pesticides were used arbitrarily, which potentially causes health concerns not just for farmers but also for the public. Pesticide usage is common and excessive in Pakistani agriculture, particularly greenhouse vegetable cultivation [22], so it is now frequently detected in fruits and vegetables from the local market. Due to a lack of feasible alternative pest management measures, farmers with many pest complexes depend only on pesticides to solve pest issues. Research shows 58% of Multan's smallholder vegetable growers use pesticides to protect their vegetables from insects, pest, weed and disease. The pesticide treatment frequency varied in vegetable crops from twice a month to once a week [23]. Priority should be given to developing pesticide reduction measures in agriculture, such as farmer training for responsible and safe pesticide usage and promoting alternatives to chemical pest management and biological control. To encourage safe pesticide use, regulatory authorities must create intervention approaches to strengthen the current pesticide legislation's enforcement mechanisms at the farm and retail levels. Pesticide label guidelines, especially those concerning pre-harvest periods, must be observed. It is also critical to educate the public, who may be directly or indirectly exposed to pesticides, to the hazards of these chemicals and how to avoid them. Consumers should be informed of feasible strategies for decreasing pesticide contamination in fresh farm food, especially raw fruits, and vegetables. Finally, due to Pakistan's rising pesticide usage trend, frequent pesticide residue testing in agricultural output is required to assure consumer safety.

CONCLUSION

In present study we detected the pesticides in the fruits and vegetables collected from the local market of Multan, Pakistan. Pakistan's most significant concerns include food shortages and hunger due to overpopulation. Fruits and vegetables are essential components of vitamins and mineral nutrients in the daily diet. Insect pest infestation is the most significant barrier to fruit and vegetable production. As a result, pesticide usage is now an unavoidable aspect of agricultural pest management. Many nations throughout the globe, including Pakistan, have been discovered to use chemical pesticides extensively to develop crops, with a significant amount of these pesticides being uptake by plant leaves upon application. Our findings revealed that pesticide residue in cabbage, lemon, and pear were detected. Among more than 300 pesticides in Pakistan, only Carbaryl, Linuron, and Phorate were detected in fruits and vegetables. Therefore, the knowledge collected regarding residue levels of pesticides in fruits and

vegetables among framers would increase public awareness and policymakers in taking the necessary actions to minimize residue levels of pesticides among fruits and vegetables in Pakistan's Multan region.

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AUTHOR'S Contribution

AH supervised the research, AM conducted the research and analyzed the samples, TUH and NF assisted in planning the research, UR and AF contributed to the writing of this manuscript.

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