

Detection and Estimation of Pesticides in Seasonal Fruits and Vegetables

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Abstract

The massive use of pesticides to kill insects and herbs poses the most serious danger to human health. To ensure the safe level of pesticides in food items, three vegetables i.e. Cabbage, Cauliflower and Spinach were selected from winter season and three fruit samples i.e. Orange, Guava, and Pomegranate were also selected from winter. Three vegetables belonging to summer season were Lady finger, Capsicum and Brinjal. And the three fruit samples of Summer season were Strawberry, Grapes and Mango. Each of the twelve samples was purchased from three main Fruit markets of Lahore city (BadamiBagh, KoatLakhpat and Shahdara), so a total of 36 samples were selected for pesticide residue analysis. Extraction of samples was carried out using Acetonitrile, Sodium Chloride and phosphate buffer. Extracted samples were tested through HPLC fitted with UV-Visible detector using analytical standards (imported by M/s Four Brothers Cemics (Pvt.) Limited Lahore, from M/s. Chem Service). As the above mentioned

vegetables and fruits are attacked by the insects like Aphids, Caterpillars and Cutworms at the time of flowering and ripening, so the selected insecticides for residual analysis were Acetamiprid, Imidacloprid, Emamectin-Benzoate, Chlorpyrifos and Azoxystrobin. Strawberry, Spinach and Lady finger samples collected from Badami Bagh Market showed the presence of Acetamiprid, Imidacloprid and Emamectin-Benzoate residues as 0.84mg/kg, 0.26mg/kg and 0.94mg/Kg respectively. Grapes, Cabbage and Cauliflower samples collected from KoatLakhpat (Kahna) Market showed the presence of Chlorpyrifos, Acetamiprid and Imidacloprid residues as 0.32mg/kg, 0.67mg/kg and 0.54 mg/Kg respectively. Capsicum sample collected from Shahdara Market showed the presence of Emamectin-Benzoate residues as 0.58mg/kg. Pesticides pose a serious threat to all living organisms if they are present in food items more than the maximum residue limits as given by GAIN (Global Agriculture Information Network).

such research studies are worth carrying in the countries like Pakistan.

Keywords

Acetonitrile, Extraction, HPLC, Pesticide

1. INTRODUCTION

With the explosive increase in world's population, the demand of food is increasing day by day and about 45% of the total crops of the world are thought to be destroyed by plant diseases and pests (Kolani *et al.*, 2016). Therefore, to meet the food needs of people, farmers are using pesticides massively for increasing crops by killing insects and eliminating diseases (Mahugija *et al.* 2017). No doubt that pesticide is that group of chemicals which is highly important in respect of increasing crop yield but this fact also can't be ignored that pesticides can put adverse and serious chronic effects over environment and human health (Jallow *et al.*, 2017). Most of the applied pesticides (34.2%) are categorized as highly toxic and about 35% are categorized as moderately toxic pesticides (Damalas *et al.*, 2011). Organochlorines, Pyrethroids, Organophosphates and Carbamates are the common pesticides which are sprayed on fruits and vegetables. A huge research work has been done for determining the amount of pesticides in vegetables and fruits (Mebdoua *et al.*, 2017).

Lack of knowledge about the toxicity, handling and application of pesticides is the big cause of those problems which are linked with toxic effects of pesticides. Among the farmers only 58% have handful knowledge about the application of pesticides. About 16% farmers are made to learn how to handle and use pesticides but only 13% of them gain and apply information regarding this (Damalas *et al.*, 2017). Exposure to

the pesticides puts many chronic and acute toxic effects over human health. Not only the farmers are victim of adverse effects of pesticides through direct exposure but the real threat to human health is in the form of intake of pesticide residues which persist in environment and agricultural products (Jallow *et al.*, 2017). The careless and extensive use of pesticides results in the accumulation of poisonous material in food stuff (Damalas *et al.*, 2017). Infant methemoglobinemia, Immunologic abnormalities, lung damage, developmental and reproductive disorders and chronic neurotoxicity are the most highlighted effects of pesticides. Many types of cancer especially hematopoietic cancers originate due to the massive intake of pesticides (Sankhla *et al.*, 2018).

There are many pesticides which are banned in lot of countries but these are still being used in developing countries like Pakistan (Montory *et al.*, 2017). Pakistan is ranked as 2nd in south Asian countries that are using pesticides extensively (Yadav *et al.*, 2015; Waheed *et al.*, 2017). Many researchers have compiled research work about the pesticides residues in fruits and vegetables (Islam *et al.*, 2015) and (Mebdoua *et al.*, 2017). The pesticides are of large varieties and the amount of their residues also largely varies, therefore their continuous monitoring is necessary in an area. The objective of this study is the determination of pesticidal residues of Acetamiprid, Imidacloprid, Chlorpyrifos, Emamectin-Benzoate and Azoxystrobin in the seasonal fruits and vegetables because these pesticides are used in Pakistan at a dose of 100-200 mL per acre before the time of flowering and ripening.

The massive use of pesticides to kill insects and herbs poses the most serious danger to human health. So, to ensure the safe level of pes-

ticides in food items, the monitoring of these compounds is important. In this research work the estimation of certain pesticides in various seasonal fruits and vegetables has been estimated and reported. For carrying out the research project 12 samples were selected, among of which 6 were from winter season and 6 belonged to summer season. Three vegetables i.e. Cabbage, Cauliflower and Spinach were selected from winter season and three Fruit samples i.e. Orange, Guava, and Pomegranate were also selected from winter. Three vegetables belonging to summer season were Ladyfinger, Capsicum and Brinjal. And the three Fruit Samples of Summer season were Strawberry, Grapes and Mango. Each of the twelve samples was purchased from three main Fruit markets of Lahore City (BadamiBagh, KoatLakhat and Shahdara). Pesticides pose a serious threat to all living organisms if they are present in food items more than the maximum residue limits (MRL) as described by GAIN (Global Agriculture Information Network). The maximum residue limits of Acetamiprid, Imidacloprid and Emamectin-Benzoate in Strawberry, Spinach and Ladies finger are 2.0mg/Kg, 5.0mg/Kg and 0.05mg/Kg respectively. The maximum residue limits of Chlorpyrifos, Acetamiprid and Imidacloprid in Grapes, Cabbage and Cauliflower are 0.5mg/Kg, 0.5mg/Kg and 1.0mg/Kg respectively. And the maximum residue limit of Emamectin-Benzoate for Capsicum is 0.2mg/Kg. So, this research study was carried-on and such research studies are highly recommended after regular intervals.

2. MATERIALS AND METHODS

Analyses were performed using reagents and chemicals as Highly purified analytical stan-

dards of Pesticides purchased from Chem Service, Acetonitrile (HPLC Grade), provided by Merck, Water (HPLC Grade), Provided by Merck, Methanol (HPLC Grade), provided by Merck, Distilled Water, Tri-ethyl Amine, Phosphoric acid, Buffer Solution (Phosphate), NaCl and Na₂SO₄.

2.1. Area and Collection of Samples

Three vegetables i.e. Cabbage, Cauli-flower and Spinach were selected from winter season and three fruit samples i.e. Orange, Guava, and Pomegranate were also selected from winter.

Three vegetables belonging to summer season were Ladyfinger, Capsicum and Brinjal. And the three Fruit Samples of Summer season were Strawberry, Grapes and Mango. Each of the twelve samples was purchased from three main Fruit markets of Lahore City (Badami Bagh, Koat Lakhat and Shahdara). So a total of 36 samples were selected for pesticide residue analysis.

2.2. Pretreatment and Extraction of Samples

The collected fruit and vegetable samples were washed under a stream of water. The soil and dust over the samples was removed by brushing gently. Each sample was chopped with a knife and 50 grams of each sample was blended in a warring laboratory blender. Then each sample was air dried and kept in oven to dry it at 105°C for the time period 48 hours. These dried fruit and vegetable samples were then grinded into a fine powder (Akhtar *et al.*, 2018).

2.3. Extraction

The extraction of samples was carried out according to the extraction procedure described by (Hussain *et al.*, 2010).

For each sample, the same Extraction Procedure was followed with a little modification. 40 grams of each sample were mixed with 40 mL distilled water. The mixtures were left to stand for 20 minutes. After it, 100 mL Acetonitrile was added to each sample. The samples were crushed and homogenized in a blender. After making homogenized, the samples were filtered under suction vacuum. The residues on filter paper were mixed with 40 mL more Acetonitrile and were filtered again under suction. The filtrates of both filtrations were mixed and volume was increased to 200 mL by adding Acetonitrile. 40mL from this solution was taken and added with 20g Sodium Chloride + 40 ml phosphate buffer having strength 0.5mol/L. After mixing well, the solutions were left to stand for the separation of aqueous layer. The organic layer was dried over Anhydrous Sodium Sulphate and filtered.

2.4. Analytical Technique

For Analysis, High Performance Liquid Chromatograph (Waters 1525 HPLC-binary pump system) Equipped with UV-Visible detector 2489 was used. HPLC column was with the following dimensions: stainless steel, 150 x 4.6 mm (i.d.), packed with symmetry C18 (5 μ m).

2.5. Chromatographic operation

1- Prior to the analysis of sample, HPLC column was washed by passing filtered HPLC grade Water through it for 10 minutes. After it, HPLC grade Methanol was run through the column for 20 minutes. Then a solution containing 50% HPLC grade Acetonitrile + 50% HPLC grade Water was used to was the column for 20 minutes.

2- For each analysis, the respective mobiles

phase was purged through HPLC for 2 minutes at a flow rate of 5mL per minute. Then it was passed through the column at least for 20 minutes until the system was equilibrated (Flat base line). Under the chromatographic operating conditions, when the base line of the instrument became stable, a glass syringe of 100 μ l was fitted with a syringe filter of 0.45 μ m pore size. Using this glass syringe, five consecutive injections of the calibrating solutions of pesticide standards were injected one by one. After injecting the Calibration solutions, three injections of the sample were run through HPLC column and the response of all injected species shown by detector was noted.

2.6. Preparation of Stock Solutions and working standard

1000 ppb Stock solution of each pesticide was prepared in its respective make up phase using analytical standards purchased from Chem Service, USA. Below is the list of pesticides and their respective make up phases as written below:

Imidacloprid: 95% HPLC grade Acetonitrile + 5% HPLC grade Methanol.

Ememectin-Benzoate: 9.8% HPLC grade Water + 0.2% Triethylamine + 90 HPLC grade Methanol.

Chlorpyrifos: 70% Acetonitrile (HPLC grade) + 30% Water (HPLC grade).

Azoxystrobin: (60% HPLC grade Acetonitrile + 40% HPLC grade Water).

For drawing Calibration Curves, five working standard solutions of each Analytical standard were prepared from their respective stock solutions. Strength of working standards for each pesticide was 200 ppb, 400 ppb, 600 ppb, 800

ppb and 1000 ppb which were prepared using dilution formula.

2.7. Sample preparation for analysis

Each extracted sample (following washing, cleaning, chopping and blending) was taken in a 100 ml volumetric flask. The makeup phase of the each respective pesticide was used to make volume up to the mark. After making up the volume, flask of each sample was kept in ultrasonic bath for 2 minutes.

2.8. Pesticides Detection and Quantification

An internationally published method developed and described by “Collaborative International Pesticides Analytical Council” was used for the detection and quantification of Acetamiprid and Imidacloprid (CIPAC 2005,

CIPAC 2010). **Emamectin-Benzoate, Azxystrobin and Chlorpyrifos** were detected using In-House validated methods developed by Four Brothers Chemicals Lab Lahore, accredited with Pakistan National Accreditation (PNAC, 2015).

3. RESULTS AND DISCUSSION

The quantitative analysis of pesticides in seasonal fruits and vegetables revealed that **Strawberry** purchased from Badami Bagh Fruit Market, was found containing an average level of Acetamiprid as 0.84 mg/Kg. After the detection of Acetamiprid in Strawberry, its three samples were prepared and tested against calibration standards. The calibration standard curves of all tested pesticides are given in Figure 1.

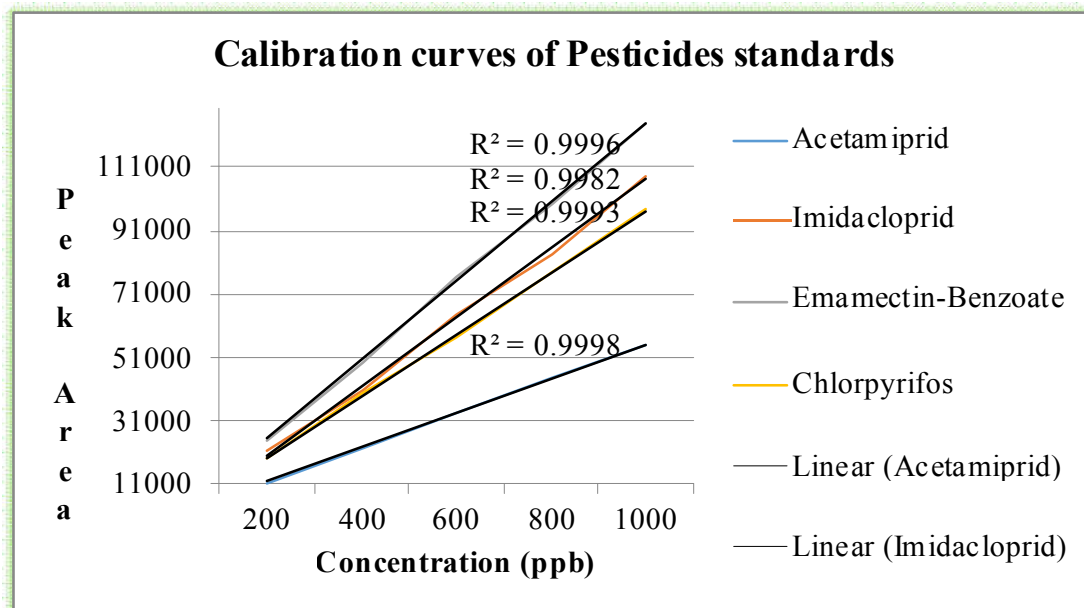


Fig.1: Calibration Curves of Pesticide Standards

The strengths and the respective peak areas of injected calibration standards is shown in table 1. Acetamiprid contents in first, second and third sample of strawberry was 0.79 mg/kg, 0.88 mg/kg

and 0.85 mg/kg respectively. Table 2 also shows the concentration of Acetamiprid in all the 3 prepared samples of Strawberry. Acetamiprid was also present in **cabbage** sample which was purch-

sed from Koat Lahpat market. An average level of Acetamiprid detected in cabbage was 0.67 mg/Kg. Acetamiprid amount in 1st, 2nd and 3rd sample of cabbage was 0.63 mg/kg, 0.66 mg/kg and 0.72 mg/kg respectively. Table 3 shows the concentration of Acetamiprid in all the 3 prepared samples of Cabbage.

Table 1. Pesticides Calibration Standards and their peak areas

Concentration (ppb)	Peak Areas by HPLC			
	Acetamiprid	Imidacloprid	Emamectin-Benzaote	Chlorpyrifos
200	11256	21202	25002	19409
400	22469	40472	49538	39380
600	33628	64145	76360	56876
800	44320	83560	99411	77860
1000	54968	108569	125174	97698

Table 2. Concentration of Acetamiprid in Strawberry purchased from Badami Bagh Market

No. of Samples	Peak Area of Samples	Concentration of Acetamiprid (mg/Kg)
1	43659	0.79
2	48751	0.88
3	46987	0.85
Mean:		0.84
S.D.:		0.0458

Table 3. Concentration of Acetamiprid in Cabbage purchased from Koat Lakhpat Market

No. of Samples	Peak Area of Samples	Concentration of Acetamiprid (mg/Kg)
1	34992	0.63
2	36621	0.66
3	39896	0.72
Mean:		0.67
S.D.:		0.0458

Grapes purchased from Kahna-Kacha Fruit Market of Lahore, showed the Presence of Chlorpyrifos. Grapes were found containing an average level of Chlorpyrifos as 0.32 mg/Kg. After the detection Chlorpyrifos in Grapes, its three samples were prepared and tested for a

Pesticides. First, Second and Third sample showed the Chlorpyrifos contents as 0.36 mg/kg, 0.34 mg/kg and 0.26 mg/kg respectively. Table 4 shows chromatographic peak areas and concentration of Chlorpyrifos in all the three prepared samples of Grapes.

Table 4. Concentration of Chlorpyrifos in Grapes purchased from Kahna-Kacha (KoatLakhpat) Market

No. of Samples (mg/Kg)	Peak Area of Samples	Concentration of Chlorpyrifos
1	34825	0.36
2	32889	0.34
3	25099	0.26
		Mean: 0.32
		S.D.: 0.0529

Ladyfinger purchased from Badami Bagh Fruit Market of Lahore was found contaminated with Emamectin-Benzoate. An average level of Emamectin-Benzoate in ladyfinger was 0.94 mg/Kg. After the detection Emamectin-Benzoate, three samples were prepared of lady finger also. Emamectin-Benzoate contents in first, second and third sample of ladyfinger were 0.97 mg/kg, 0.92 mg/kg and 0.93 mg/kg respectively. Table 5 shows the concentration and peak areas

of Emamectin-Benzoate in all prepared samples of Ladyfinger. Emamectin-Benzoate was present in **Capsicum** also. This sample of Capsicum was purchased from local market of Shahdara and it showed an average level of Emamectin-Benzoate as 0.58 mg/Kg. Amount of Emamectin-Benzoate detected in first, second and third sample of capsicum was 0.58 mg/kg, 0.64 mg/kg and 0.51 mg/kg respectively. The resulted concentration and peak areas have been shown in Table 6.

Table 5. Concentration of Emamectin-Benzoate in Ladyfinger purchased from Badami Bagh Market

No. of Samples	Peak Area of Samples	Concentration of Emamectin (mg/Kg)
1	121410	0.97
2	115244	0.92
3	117099	0.93
		Mean: 0.94
		S.D.: 0.0265

Table 6: Concentration of Emamectin-Benzoate in Capsicum purchased from Badami BaghMarket

No. of Samples	Peak Area of Samples	Concentration of Emamectin (mg/Kg)
1	72621	0.58
2	80122	0.64
3	63849	0.51
Mean:		0.58
S.D.:		0.0651

Spinach (purchased from Badami Bagh Fruit Market) and **Cauliflower** (purchased from Koat Lakhpat Market) showed the Presence of Imidacloprid. An average level of Imidacloprid in Spinach and Cauliflower was 0.26 mg/Kg and 0.54 mg/Kg respectively. The

Resulted values of Imidacloprid in all prepared samples Spinach and Cauliflower along with their peak areas have been given in table 7 and table 8 respectively. A detailed comparison of pesticides which were detected in selected fruit samples has been shown in figure 2.

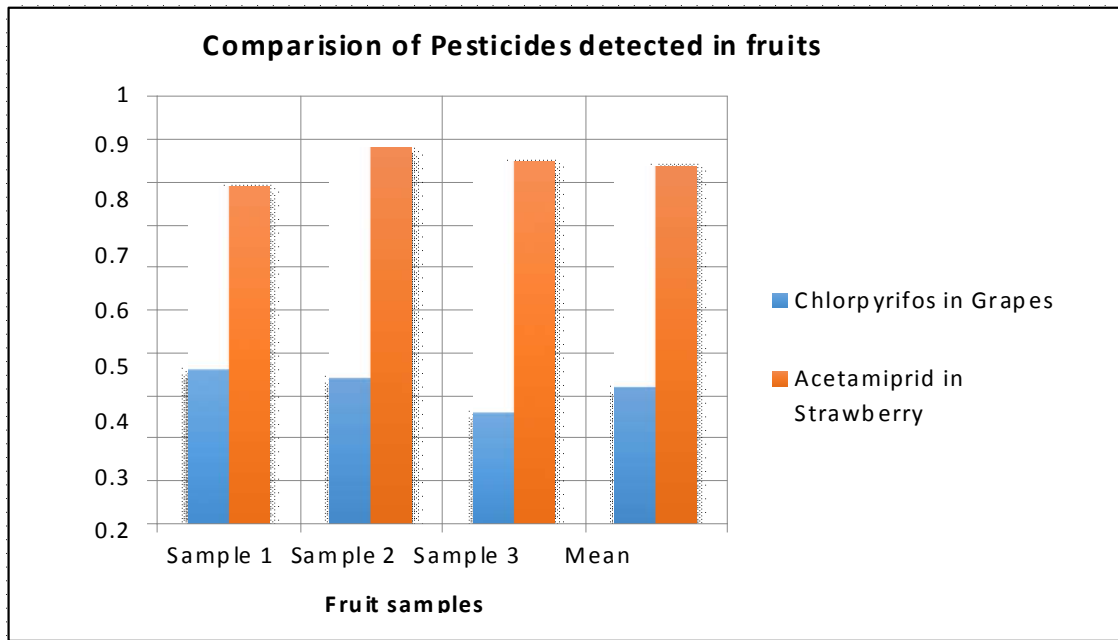


Fig.2: Pesticides in Grapes (Koat Lakhpat Market) and Strawberry (Badami BaghMarket)

Table 7. Concentration of Imidacloprid (mg/kg) in Spinach purchased from Badami Bagh Market

No. of Samples (mg/Kg)	Peak Area of Samples	Concentration of Imidacloprid
1	27622	0.27
2	28756	0.28
3	23248	0.23
Mean:		0.26
S.D.:		0.0265

Table 8: Concentration of Imidacloprid in Cauliflower purchased from Kahna-Kacha (Koat Lakhpat) Market

No. of Samples	Peak Area of Samples	Concentration of Imidacloprid (mg/Kg)
1	64957	0.61
2	57320	0.54
3	50687	0.48
Mean:		0.54
S.D.:		0.0651

The the detailed comparison of pesticides which were detected in selected vegetables samples has been shown in figure 3. Many health risks are associated with the pesticides if these are used in quantities which are more than the permissible levels. A number of research studies state that in Pakistan the fruits and vegetables are found to be containing lethal residues pesticides like Profenofos, Chlorpyrifos, Carbofuran and Ensosulfa-Methyl (Hayat *et al.*, 2018).

For carrying out this research project, twelve samples of fruits and vegetables were selected. Among of these six were from winter season and six belonged to summer season.

Three vegetables i.e. Cabbage, Cauliflower and Spinach were selected from winter season and three Fruit samples i.e. Orange, Guava, and Pomegranate were also selected from winter. Three vegetables belonging to summer season were Ladyfinger, Capsicum and Bingil. And the three Fruit Samples of Summer season were Strawberry, Grapes and Mango. Each of the twelve samples was purchased from three main Fruit markets of Lahore City (Badami Bagh, Koat Lakhpat and Shahdara). So a total of 36 samples were selected for pesticide residue analysis. At the end of research work, the results were compiled and compared with results of resembling studies carried on in the recent past.

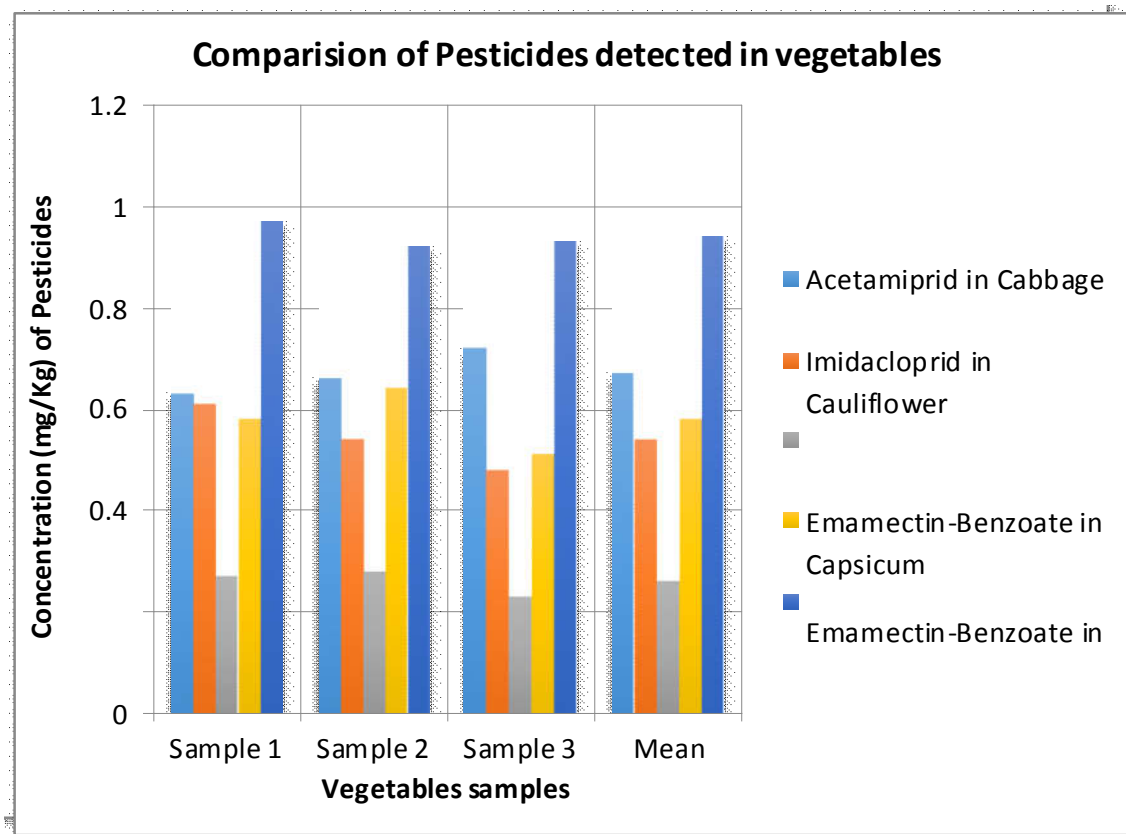


Fig.3: Pesticides in Cabbage, Cauliflower, Spinach, Capsicum and Ladyfinger purchased from Koat Lakhpat, Koat Lakhpat, Badami Bagh, Shahdara and Badami Bagh Markets respectively.

Strawberry, Spinach and Ladyfinger samples collected from Badami Bagh Market showed the presence of Acetamiprid, Imidacloprid and Emamectin-Benzoate residues as 0.84mg/kg (MRL= 0.5 mg/kg), 0.26mg/kg (MRL = 2 mg/kg) and 0.94mg/Kg (MRL = 0.02 mg/kg) respectively. Strawberry and Ladyfinger were found to be containing the pesticides higher than maximum residual limit (MRL).

Grapes, Cabbage and Cauliflower samples collected from Koat Lakhpat (Kahna) Market showed the presence of Chlorpyrifos, Acetamiprid and Imidacloprid residues as 0.32-

mg/kg (MRL= 1 mg/kg) , 0.67mg/kg (MRL = 0.4 mg/kg) and 0.54 mg/Kg (MRL = 0.5 mg/kg) respectively. Grapes and Cabbage were found to be containing the pesticides higher than maximum residual limit (MRL). Capsicum sample collected from Shahdara Market showed the presence of Emamectin-Benzoate residue as 0.58mg/kg (MRL = 0.02 mg/kg).

In 2018 (Akhtar et al., 2018) detected that Imidacloprid was present in Guava samples, collected from Lahore. Concentration of Imidacloprid detected by them was 1.65 mg/kg (MRL=0.5 mg/Kg) which exceeded MRLs.

Moreover they determined that various pesticides i.e. Bifenthrin, Difenconazole and Paraquat were also present in Guava and Egg plant. Guava was found to be contaminated with bifenthrin (5.13 mg/kg) (MRL = 0.03 mg/Kg), Egg plant (3.53 mg/kg) (MRL = 0.2 mg/Kg). In 2014 (Shafi et al., 2014) conducted a study on pesticide residues in different Fruit samples collected from various fruit markets of Lahore city. They found Bifenthrin concentration in Guava fruit (0.06 mg/kg).

In 2013, a research study was conducted by Punjab Forensic Agency and it was detected that a significant residual contents of pesticides present in various vegetables. They resulted that following pesticides were in highest concentrations: Imidacloprid in Ladyfinger (0.131 mg/kg), Chlorpyrifos and Bifenthrin in Potato (0.124 mg/kg) and (0.109 mg/kg) respectively, in Egg Plant Cypermethrin was (0.090 mg/kg), in Tomato Carbofuranin was (0.120 mg/kg), In Cucumber Lambda cyhalothrinin was (0.114 mg/kg). They also concluded that carbofuran and cypermethrin tend to be having more frequency to contaminate different vegetables samples (Munawar et al., 2013).

The present research study is of great importance to make people know that how hygienic fruits and vegetables they are eating. Acetamiprid is the pesticide of common use in Pakistan. The male reproductive system is negatively affected by the toxicity of Acetamiprid in high dose. Apoptosis, Hormonal disruptions and oxidative stress are linked with the toxicity of Acetamiprid (Arýcan et al., 2020). Imidacloprid disturbs the homeostasis of animals (Kobir et al., 2020). The spatial memory, auditory nerves and vocal system of insectivorous bats was found to be abnormal with the exposure to Imidacloprid (Wu et al., 2020).

It was studied that prolonged exposure to Emamectin-Benzoate increased the testicular cells and damaged the DNA of male mice (Zhang et al., 2020). Emamectin-Benzoate has a high potential in creating genotoxic effects on lung cells and damaging the DNA (Niu et al., 2020). El- Wakf et al. carried Toxicological studies about Chlorpyrifos on rats in 2018. They found that prolonged exposure of rats to Chlorpyrifos resulted in the elevated heart beat and blood pressure in rats. Histopathological changes were also observed in myocardial fibers in the form of their degeneration and disorganization (El-Wakf et al., 2018).

It can be deduced from the above discussion that Pesticides are being engulfed by Human beings along with contaminated fruits and vegetables. When the pesticides become a part of food chain, they show their adverse effects in living bodies in the form of many acute and chronic diseases. To lessen the toxic effects of Pesticides in living organisms and in environment, it is suggested that farmers must go regular official training sessions about the application and health risks of pesticides. Spray of pesticides on the crops should be selective to kill the destructive pests only. To know the output of all such precautionary measurements, continuous monitoring of pesticide residues in fruits, vegetables and crops is necessary as was done in the present research work.

4. CONCLUSION

Chromatographic studies of pesticides were carried on a mean of 12 samples of seasonal fruits and vegetables, collected from three local markets of Lahore. Five samples of Vegetables: Cabbage, Cauliflower, Spinach, Capsicum and Ladyfinger; and two sample of fr-

uits: Strawberry and Grapes showed the presence of Pesticides. To lessen the toxic effects, the Pesticides should be sprayed uniformly in the field of crops and the Pesticides formulations should be chemically synthesized in such a medium where these could degrade to the less toxic constituents after a certain period.

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