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Determination of pesticide residues above and below MRL in selected vegetable of south Punjab, Pakistan

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Abstract

The increased production of food goods to satisfy the requirements of the people has led to the widespread use of pesticides. It is important to note that the use of these pesticides poses a risk of contaminating both the land and the food supply. The present study was carried out to determine selected pesticides namely Imidacloprid, Chlorpyrifos, Cypermetrin and Lufeuron residual in vegetables (Tomato, Okra, Cabbage, Cauliflower, Green Pepper, Carrot, Radish and Bottle Gourd) collected from fields and shops in villages and commercial market of south Punjab. The four districts of south Punjab were selected for sample collection. The names of selected districts were Khanewal, Multan, Muzaffargarh and Vehari. These samples were prepared and subjected to high pressure liquid Chromatography (HPLC) for detection of pesticide residues. The results showed that around 48 percent of vegetable samples have high MRL, 32 percent of vegetable samples have low MRL and 20 percent of vegetable samples show no results. Pesticides are used to control illnesses and pests, despite the fact that they are utilized. However, pesticide residues are still present in vegetables, which have the potential to cause consumers to experience adverse health

effects. Because of this, it is strongly advised that continual monitoring of pesticide residues be carried out.

Keywords

Pesticide Residues, Vegetables, South Punjab of Pakistan, MRL, Human health risks, HPLC

Introduction

Food safety and food security are becoming more important issues on a worldwide scale as a result of the growing population and the accompanying need for food production to meet the needs of the expanding population(Varzakas & Smaoui, 2024). Despite the fact that the use of pesticides plays a large part in the enhancement of crop yields in the horticulture sector, the presence of chemical residues from these pesticides that exceed the maximum residue levels or limits (MRLs) has a substantial impact on the health of both humans and animals(Bempah *et al.*, 2016; Mebdoua *et al.*, 2017; Mac Loughlin *et al.*, 2018; Vázquez *et al.*, 2019). Moreover, the use of pesticides to horticulture crops not only pollutes the environment but also leaves terminal degradation products in food, which may have an impact on human beings that is carcinogenic, teratogenic, and immunosuppressive(Osman *et al.*, 2010; Yawar *et al.*, 2012; Khan *et al.*, 2020). The presence of increased levels of pesticide residues was discovered in a variety of commodities (Yuan *et al.*, 2014), which are responsible for a number of illnesses and health hazards.(Bhandari *et al.*, 2018). An investigation conducted in India provided essential information on the residual status of widely used pesticides (chlorpyrifos and carbofuran) in some vegetables that are often consumed in Hyderabad. The findings of this investigation indicated the presence of potentially hazardous pesticides, which are related to the excessive application of these pesticides and need to be regulated [16]. The use of pesticides in excess of what is permitted has led to the buildup of a significant quantity of organophosphate and organochloride residues in fruits and vegetables that are suitable for human consumption (Latif *et al.*, 2011; Kumari & John, 2019). Pesticides that are most commonly used include parathion methyl and dimethoate, which are both organophosphates. These pesticides are considered to be genotoxic because their consumption causes DNA damage in human lymphocytes (Ündeğer & Başaran, 2005). Additionally, residues of chlorpyrifos are known to have an indirect impact on neurodevelopment (Eaton *et al.*, 2008), and

they are also considered to be carcinogenic for living beings (Kaur *et al.*, 2019). These residues are rapidly becoming a component of our food supply chain system (Soomro *et al.*, 2008; Bempah *et al.*, 2011), and they have a negative impact on the human blood circulatory system after fresh product has been consumed. In fruits and vegetables, it is presumed that neonicotinoids and carbendazim are used widely to combat sucking insects and a variety of fungal diseases, respectively. It has been reported that large residues of these pesticides have been found in a number of countries (Sheikh *et al.*, 2012; Mohamed *et al.*, 2018). Agriculture is the second most important contributor to Pakistan's gross domestic product (GDP), which is a nation that is reliant on agriculture(Usman, 2016). There has been a significant rise in the use of pesticides in Pakistan over the course of the last twenty years(Khan *et al.*, 2015). It is widely acknowledged that pesticides play a crucial part in ensuring that agricultural output remains high, and as a result, they are regarded as the most essential component of contemporary farming(Lykogianni *et al.*, 2021). The widespread use of pesticides, on the other hand, has rapidly become a major source of worry in agricultural production that is very input intensive(Rajak *et al.*, 2023). However, because to the unanticipated long-term and chronic harmful consequences that pesticides have on human health and the environment, it is difficult to maintain a dependence on pesticides.

Organophosphates, organochlorines, pyrethroids, and carbamates are some of the pesticides that are often used because they are effective against fruits and vegetables(Osaili *et al.*, 2023). Despite the fact that many nations have outlawed the use of certain organochlorine pesticides, these herbicides are still being used in several developing countries such as Pakistan(Aryal & Aryal, 2023). Because monitoring is insufficient and farmer knowledge is lacking, there is a significant cause for worry over the absence of law enforcement and the presence of adulterated pesticides. The quantities of pesticide residues found in various fruits and vegetables have been proven to vary, according to a number of studies.

In addition, exposure to pesticide residues not only poses a threat to the health of farmers, but also of those who are directly involved in the handling of pesticides(Abaineh *et al.*, 2023). This is because they come into contact with the pesticide residues as a result of improper maintenance of spraying equipment, unsafe handling, storage, and disposal practices, as well as a lack of personal

protective equipment or an inability to use it appropriately. Additionally, pesticides are prevalent in all ecosystems that are dependent on agriculture; nevertheless, the main danger to human health comes from the exposure to residues that are found in primary and derived agricultural products9. There is a spectrum of effects that pesticides have, ranging from acute to chronic, on the human population, biodiversity, and the crops that are the principal beneficiaries of these chemicals. A seventy percent rise in the risk of cancer and birth problems has been attributed to the usage of pesticides, according to many reports.

People who have embraced organic diets have been seen to have a notable lack of these effects. Furthermore, biodiversity is negatively impacted as a result of the introduction of pesticides into the food chain, which also leads to a reduction in the production of beneficial plants that are used as animal fodder(Khan *et al.*, 2023). Because they are eaten in their raw form, fruits and vegetables are a source of worry. Additionally, the quality of the soil deteriorates as a result of the continual use of pesticides, which leads to the depletion of minerals in the soil as well as the yield of the soil. Numerous studies have focused their attention on the residuals of pesticides in fruits and vegetables. Imidacloprid, Cypermetrin, Chlorpyrifos, and Lufenuron were the four pesticides that were investigated in this research. The plants that were examined were Tomato, Okra, Cabbage, Cauliflower, Green Pepper, Carrot, Radish, and Bottle Gourd. The purpose of this study was to evaluate the presence of these pesticides in these vegetables. An HPLC analysis was performed on the samples that were collected in order to identify certain pesticides.

Material and Methods

The purpose of this experiment was to gather random samples from various places in South Punjab, including farms and marketplaces in the districts of Multan, Khanewal, Muzaffargarh, and Vehari that were chosen. By following the provided technique, we were able to perform an analysis on all of the samples and identify any pesticide residues that were either over or below the maximum residue limits (MRLs).

3.1.Procurement of Samples

Before collecting samples, the markets and fields of selected districts were chosen based on the constant availability of vegetables. The names of the selected districts were Multan, Khanewal, Muzaffargarh, and Vehari and the selected vegetables were Carrots, Tomatoes, Okra and Green Pepper for the summer seasons and cauliflower, cabbage, Radish, and Bottle gourd for the winter seasons. For field samples, the harvesting period of individual vegetables was noted to maintain smooth sampling. After that vegetable samples were randomly procured from the fields and markets of selected districts. One specific day was chosen for each district to maintain homogeneity in samples and polyethylene zip bags were used to retain the fresh vegetable samples. The procured samples were immediately transferred and stored at 4°C in the processing laboratory at the Faculty of Food Science and Nutrition, Bahauddin Zakariya University Multan, Pakistan.

3.2.Extraction, Clean up, and Concentration

The 500g sample was blended for about 2 minutes to achieve homogeneity. A 3-gram sample were extracted and placed in a polypropylene tube. Then, 12 milliliters of distilled water was added to the tube. This would be accomplished by using a vortex for 2 minutes. Next, a mixture of 15 milliliters of acetonitrile with 1 percent acetic acid, 6 grams of anhydrous magnesium sulfate, and 1.5 grams of anhydrous sodium acetate should be introduced into the tube. The tube will next be subjected to centrifugation at a force of 4000 rpm for about 5 minutes. Following centrifugation, 8 ml of the liquid remaining above the sediment will be transferred to a separate tube. A total of 1.5 grams of anhydrous magnesium sulfate and 0.4 grams of primary secondary amine will be included. The sample was subjected to shaking using a vortex for 2 minutes, followed by centrifugation at a force of 4000 rpm for about 5 minutes. This process was repeated. One ml supernatant was then be transferred in a dark container and were diluted with 4 ml of a combination comprising methanol and water in an 85:15 ratio V/V. 20 microliter of this solution were put into HPLC. After that, all the samples were evaluated for the presence of pesticide residues by utilizing a High-Performance Liquid Chromatograph (HPLC) with modest modification.

3.3. Determination of pesticides by HPLC

Analyses of pesticides were performed using HPLC that was geared up with an Ultra Violet visible detector model SPD-10A, delivery pumps, a column oven model CTO-10A, a system control unit model SCL 10A, and an injector model 20 l. Through the Communication Bass Module, the signals of the HPLC were to be anticipated. The analytical column was made of Discovery Supelco C18 (with dimensions of 250 by 4.5 millimeters and a particle size of 5 micrometers). The analytical column had a wavelength that was always maintained at 246 nm. The mobile phase of the HPLC column is a combination of acetonitrile, double-distilled water, and phosphoric acid with a pH of 4.0. This mixture is maintained at a constant temperature of 25 degrees Celsius. The flow rate of the mobile phase was kept constant at 1.5 mL/min. The

temperature of the mobile phase in the column was fixed at 30 degrees Celsius (Michel & Buszewski, 2002; Baig *et al.*, 2009).

Figure Number 1=HPLC at Pesticide Quality Control Laboratory, Multan

Results

Figure 2 represents the Punjab province area of Pakistan in which four districts were selected for study. The names of districts were Multan, Khanewal, Muzafargarh and Vehari. Comparison of retention time of standard chromatogram, with the samples chromatogram retention time was done to assess the presence of pesticides in agriculturally incurred samples. Selected pesticides namely Cypermethrin, Chlorpyrifos, Imidacloprid and Lufenuron (Table: 1) were detected in all samples used in this study i.e. Tomato, Okra, Cabbage, Cauliflower, Green Pepper, Carrot, Radish, Bottle Gourd (Table: 2) in various concentrations. These detected concentrations in all samples of vegetables were then compared with Maximum Residue Limits (MRLs). Samples were analyzed in triplicate and the concentration of pesticides with maximum residue limits by European Union (EU) standards are illustrated in Table 3, 4, 5 and 6.

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Figure No 2= Punjab Province district wise map

Table 1:

Pesticide selected for detection in fruit and vegetables samples

Sr.No	Pesticide Name	Chemical Name	Molecular Formula
01	Imidacloprid	1-(6-chloro-3- pyridylmethyl)-N-O2 nitroimidazolidin-2-ylideneamine	C9 H10ClN5
02	Chlorpyrifos	0,0-diethyl 0-(3,5,6-trichloro -2-pyridinyl)-phosphorothioate	$C_9H_{11}Cl_3NO_3PS$
03	Lufenuron	1-[2,5-Dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy) phenyl]-3-(2,6-difluorobenzoyl)	C17H8Cl2F8N2O3
04	Cypermetrin	cyano(3-phenoxyphenyl)methyl 2,2-dimethyl-3-(2-methyl-1-propenyl) cyclopropanecarboxylate	$C_{22}H_{19}Cl_2NO_3$

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Table 2:

Background information related to selected vegetables from South Punjab Districts like Multan, Khanewal, Muzafergarh and Vehari.

Commodity	Botanical Name	Family	Area For Market	G.Avg Edu	N.P.S
Okra	Abelmoschus esculentus	Malvaceae	PUA	Primary	3±1
Green Pepper	Capsicum annuum	Amaranthaceae	PUA	Secondary	2±1
Tomato	Solanum lycopersicum	Solanaceae	PUA	Primary	3±1
Cauliflower	Brassica oleracea	Brassicaceae	PUA	Secondary	2±1
Cabbage	Brassica oleracea	Brassicaceae	PUA	Primary	3±1
Carrot	Daucus carota subsp	Apiaceae	PUA	Secondary	4±1
Radish	Raphanus sativus	Brassicaceae	PUA	Primary	3±1
Bottle Gourd	Lagenaria siceraria	Cucurbitaceae	PUA	Secondary	2±1

PUA=Peri urban Area
G.Avg Edu=Growers Average Education
N.P.S=Number of Pesticide Spray

Table 3:

Comparison of detected pesticide concentration in vegetables with MRL values (Codex Standards) at Khanewal District.

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Sr.No	Vegetables	Pesticides	Detected Concentrations (Mg/Kg)	MRL(mg/kg)
01	Okra	Imidacloprid	1.40	0.5
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.8	0.5
		Lufenuron	ND**	0.01
02	Green Pepper	Imidacloprid	1.25	0.9
		Chlorpyrifos	0.08	0.01
		Cypermethrin	0.9	0.5
		Lufenuron	ND**	0.8
03	Tomato	Imidacloprid	ND**	0.3
		Chlorpyrifos	ND**	0.01
		Cypermethrin	ND**	0.5
		Lufenuron	ND**	0.4
04	Carrots	Imidacloprid	ND**	0.01
		Chlorpyrifos	ND**	0.01
		Cypermethrin	ND**	0.01
		Lufenuron	ND**	0.01
05	Cabbage	Imidacloprid	0.005	0.01
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.9	1
		Lufenuron	ND**	0.01
06	Cauliflower	Imidacloprid	0.09	0.01
		Chlorpyrifos	0.04	0.01
		Cypermethrin	ND**	0.5
		Lufenuron	0.03	0.01
07	Radish	Imidacloprid	0.005	0.01
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.03	0.05
		Lufenuron	ND**	0.01
08	Bottle Gourd	Imidacloprid	0.3	0.4
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.4	0.2
		Lufenuron	0.1	0.15

MRL=Maximum Residue Level
ND**= Not Detected

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Figure Number 3
This figure show the detected concentrations of four pesticides (Imidacloprid, Chlorpyrifos, Lufenuron and Cypermethrin) in eight vegetables (Okra, Cauliflower, Cabbage, Green Pepper, Carrots, Radish, Tomato and Bottle Gourd) compare with their MRL value(Codex Standard) at Khanewal District.

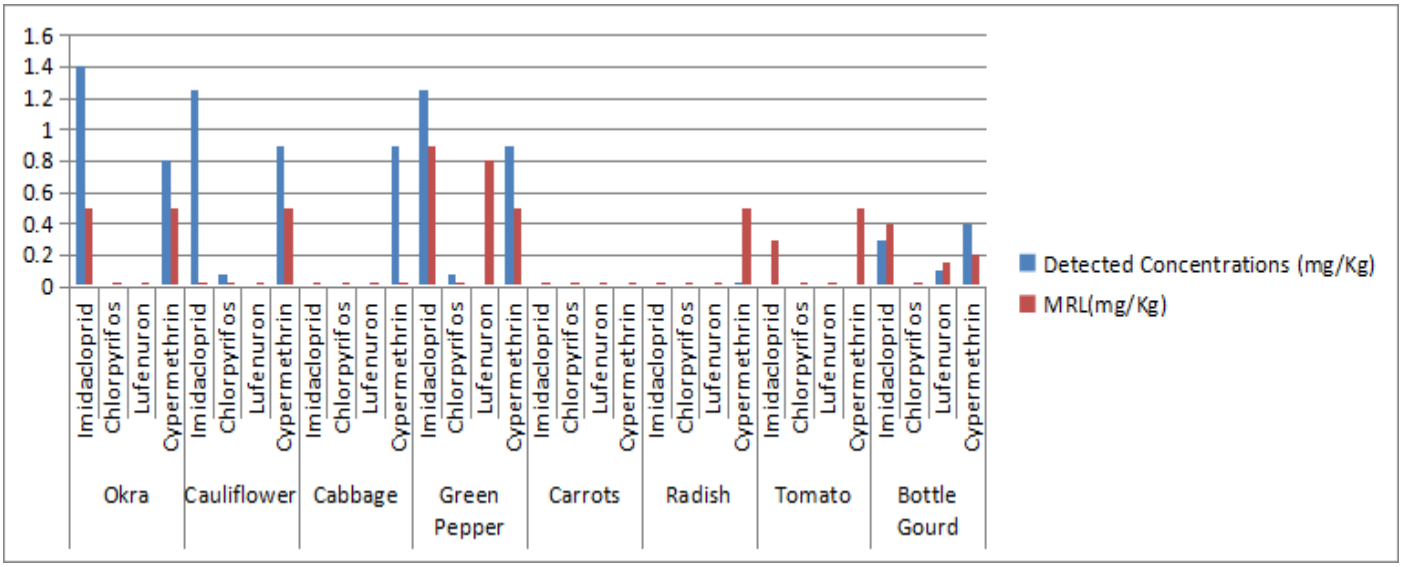

Table 4:
Comparison of detected pesticide concentration in vegetables with MRL values (Codex Standard) at Multan District.

Sr.No	Vegetables	Pesticides	Detected Concentrations (Mg/Kg)	MRL (mg/kg)
01	Okra	Imidacloprid	1.85	0.5
		Chlorpyrifos	ND**	0.01
		Cypermetrin	0.6	0.5
		Lufenuron	ND**	0.01
02	Green Pepper	Imidacloprid	2.08	0.9
		Chlorpyrifos	0.05	0.01
		Cypermethrin	0.7	0.5

		Lufenuron	ND**	0.8
03	Tomato	Imidacloprid	0.9	0.3
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.6	0.5
		Lufenuron	ND**	0.4
04	Carrots	Imidacloprid	0.02	0.01
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.03	0.01
		Lufenuron	ND**	0.01
05	Cabbage	Imidacloprid	0.003	0.01
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.7	1
		Lufenuron	ND**	0.01
06	Cauliflower	Imidacloprid	0.07	0.01
		Chlorpyrifos	0.005	0.01
		Cypermethrin	ND**	0.5
		Lufenuron	0.02	0.01
07	Radish	Imidacloprid	ND**	0.01
		Chlorpyrifos	ND**	0.01
		Cypermethrin	ND**	0.05
		Lufenuron	ND**	0.01
08	Bottle Gourd	Imidacloprid	0.5	0.4
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.1	0.2
		Lufenuron	0.18	0.15

MRL=Maximum Residue Level

ND**=Non Detected

Figure Number 3
This figure show the detected concentrations of four pesticides (Imidacloprid, Chlorpyrifos, Lufenuron and Cypermethrin) in eight vegetables (Okra, Cauliflower, Cabbage, Green Pepper, Carrots, Radish, Tomato and Bottle Gourd) compare with their MRL value (Codex Standard) at Multan District.

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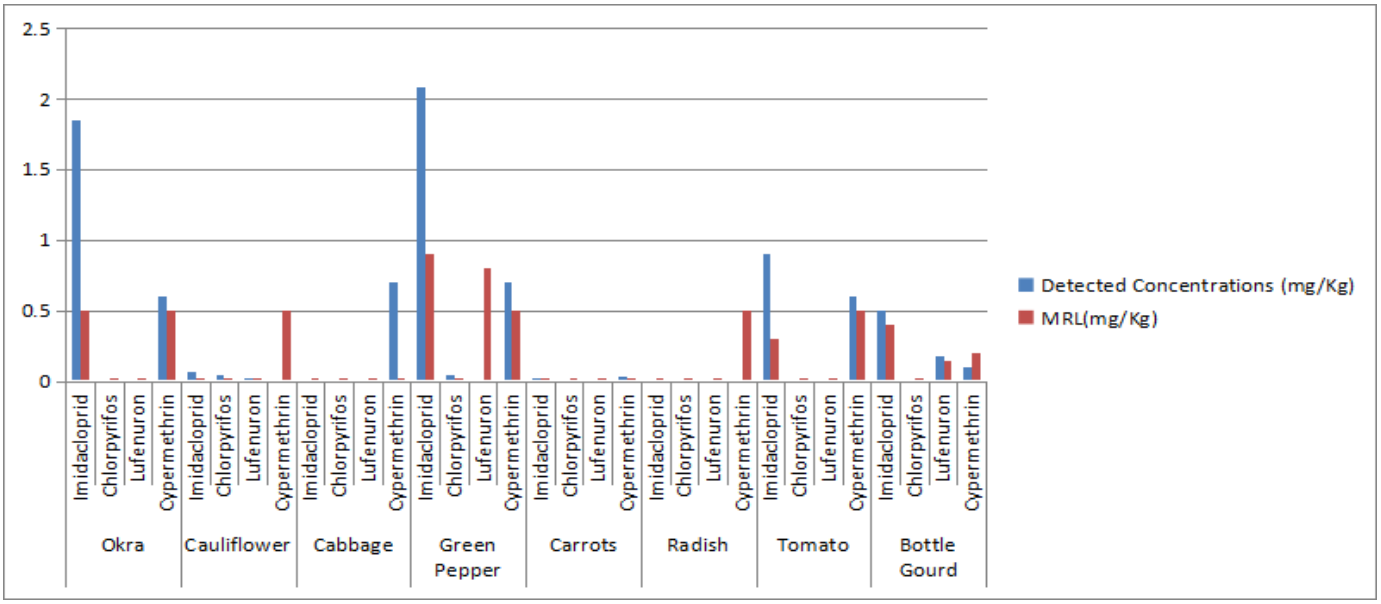

Table Number 5:

Comparison of detected pesticide concentration in vegetables with MRL Values (Codex Standard) at Vehari District.

Sr.No	Vegetables	Pesticides	Detected Concentrations (Mg/Kg)	MRL (mg/kg)
01	Okra	Imidacloprid	2.10	0.5
		Chlorpyrifos	ND**	0.01
		Cypermetrin	0.3	0.5
		Lufenuron	ND**	0.01
02	Green Pepper	Imidacloprid	1.10	0.9
		Chlorpyrifos	0.06	0.01
		Cypermethrin	0.4	0.5
		Lufenuron	ND**	0.8
03	Tomato	Imidacloprid	0.2	0.3
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.2	0.5
		Lufenuron	ND**	0.4
04	Carrots	Imidacloprid	ND**	0.01
		Chlorpyrifos	ND**	0.01
		Cypermethrin	ND**	0.01
		Lufenuron	ND**	0.01
05	Cabbage	Imidacloprid	ND**	0.01

		Chlorpyrifos	ND**	0.01
		Cypermethrin	ND**	1
		Lufenuron	ND**	0.01
06	Cauliflower	Imidacloprid	0.005	0.01
		Chlorpyrifos	0.003	0.01
		Cypermethrin	ND**	0.5
		Lufenuron	0.005	0.01
07	Radish	Imidacloprid	0.005	0.01
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.06	0.05
		Lufenuron	ND**	0.01
08	Bottle Gourd	Imidacloprid	0.2	0.4
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.3	0.2
		Lufenuron	0.2	0.15

MRL=Maximum Residue Level
ND**=Non Detected

Figure Number 5
This figure show the detected concentrations of four pesticides (Imidacloprid, Chlorpyrifos, Lufenuron and Cypermethrin) in eight vegetables (Okra, Cauliflower, Cabbage, Green Pepper, Carrots, Radish, Tomato and Bottle Gourd) compare with their MRL value (Codex Standards) at Vehari District.

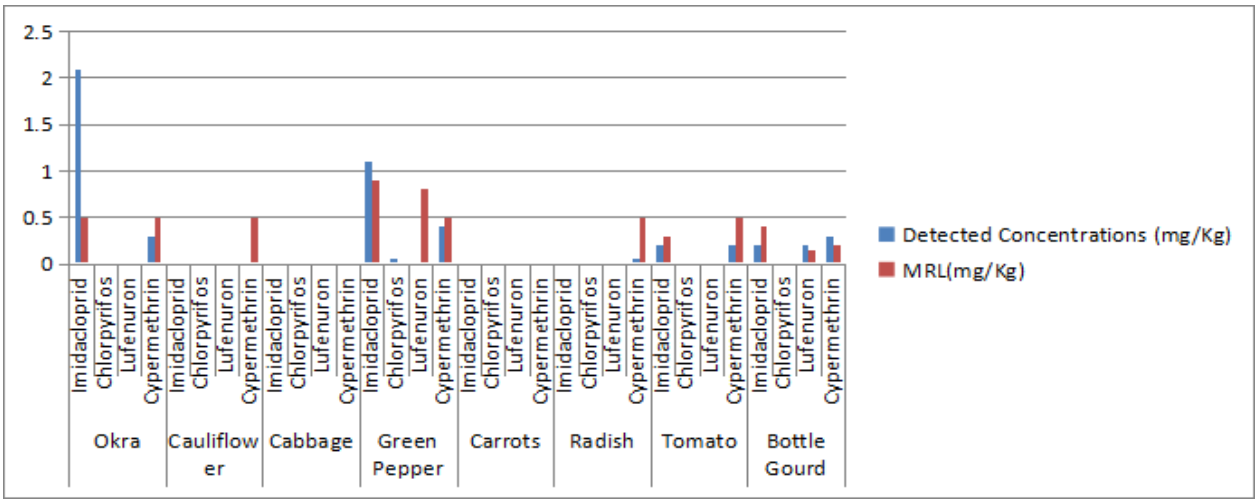

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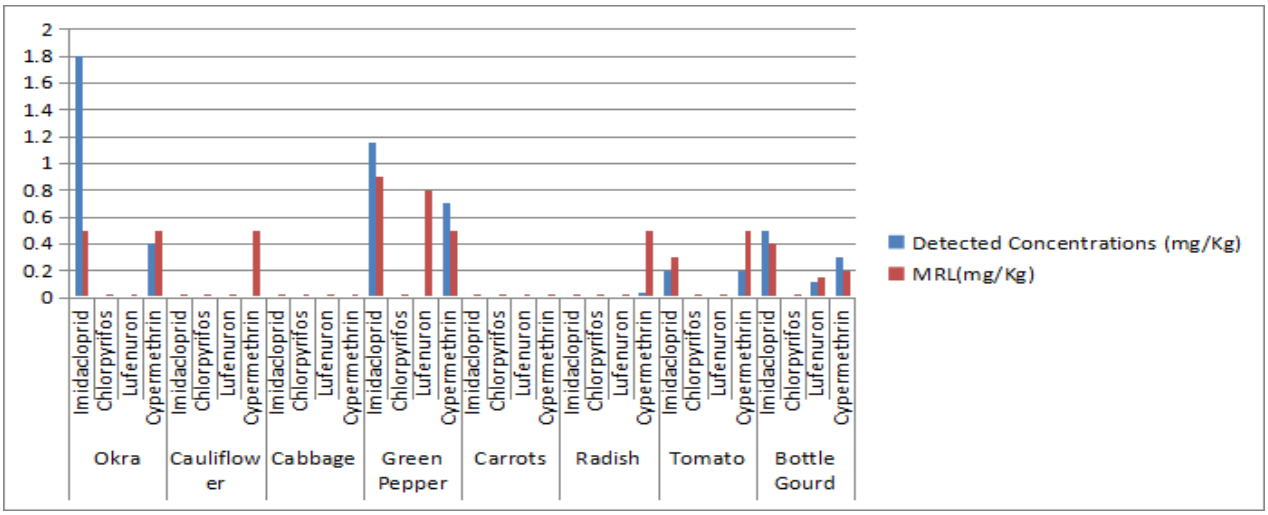
Table 6:
Comparison of detected pesticide concentration in vegetables with MRL Values (Codex Standard) at Muzaffargarh District.

Sr.No	Vegetables	Pesticides	Detected Concentrations (Mg/Kg)	MRL(mg/kg)
01	Okra	Imidacloprid	1.80	0.5
		Chlorpyrifos	ND**	0.01
		Cypermetrin	0.4	0.5
		Lufenuron	ND**	0.01
02	Green Pepper	Imidacloprid	1.15	0.9
		Chlorpyrifos	0.005	0.01
		Cypermethrin	0.7	0.5
		Lufenuron	ND**	0.8
03	Tomato	Imidacloprid	0.2	0.3
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.2	0.5
		Lufenuron	ND**	0.4
04	Carrots	Imidacloprid	0.007	0.01
		Chlorpyrifos	ND**	0.01
		Cypermethrin	ND**	0.01
		Lufenuron	ND**	0.01
05	Cabbage	Imidacloprid	ND**	0.01
		Chlorpyrifos	ND**	0.01
		Cypermethrin	ND**	1
		Lufenuron	ND**	0.01
06	Cauliflower	Imidacloprid	0.005	0.01
		Chlorpyrifos	0.005	0.01
		Cypermethrin	ND**	0.5
		Lufenuron	0.005	0.01
07	Radish	Imidacloprid	0.005	0.01
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.04	0.05
		Lufenuron	ND**	0.01
08	Bottle Gourd	Imidacloprid	0.5	0.4
		Chlorpyrifos	ND**	0.01
		Cypermethrin	0.3	0.2
		Lufenuron	0.12	0.15

MRL= Maximum Residue Level
ND=** Non Detected

Figure No 6

This figure show the detected concentrations of four pesticides (Imidacloprid, Chlorpyrifos, Lufenuron and Cypermethrin) in eight vegetables (Okra, Cauliflower, Cabbage, Green Pepper, Carrots, Radish, Tomato and Bottle Gourd) compare with their MRL value (Codex Values) at Muzaffarabad District.



Discussion

There are four districts of south Punjab selected for pesticides detection. At Khanewal district, Okra, Green Pepper and cauliflower show above the MRL While Cabbage, Radish, Bottle Gourd Show below the MRL and tomatoes, carrots show no results. At Multan district, okra, green pepper, tomatoes, carrots, cauliflower, Bottle Gourd show above the MRL While cabbage Show below the MRL and Radish show no results. At Vehari district, Okra, Green Pepper and Bottle Gourd show above the MRL While cauliflower, tomatoes, radish show below the MRL and cabbage, carrots show no results. At Muzaffargarh district, Okra, Green Pepper and Bottle Gourd show above the MRL While carrots, tomatoes, radish, cauliflower show below the MRL and cabbage show no results.

In order to prevent insect infestations in food crops, several types of pesticides are used. However, if the maximum legal limit for the consumption of pesticides is increased, then it may lead to the development of a variety of diseases(Kubiak-Hardiman *et al.*, 2023). The results of several pesticides that were researched suggested that toxins are absorbed and transported into the various organs of the body(Kaur *et al.*, 2024). The metabolizing enzymes are responsible for both the bio-activation and detoxification of the toxins into the various tissues of the body(Ali *et al.*, 2024).

The use of pesticides without proper judgment has been a cause for worry about food safety on a worldwide scale, particularly in poor nations(Patil *et al.*, 2023). This is despite the fact that the use of pesticides is practically an essential component of commercial vegetable supply chains. Our findings demonstrated that pooled samples of vegetables gathered from fields and markets in south Punjab were contaminated with a variety of pesticides that were more or less in excess of the MRLs standards (Siddique *et al.*, 2021). It is well known that pesticide residues that are in excess of the MRLs level have a negative impact on human health (Lozowicka *et al.*, 2014; Mittal *et al.*, 2014; Shafi *et al.*, 2014; Mohamed *et al.*, 2018; Kumari & John, 2019). Regardless of the fresh food that was examined, the highest levels of pesticide residues were found in the case of imidacloprid, which is a pesticide that is often used for the purpose of reducing sucking insects. Imidacloprid is a member of the neonicotinoid group, which may also include chlorpyrifos, lufenuron, and cypermethrin. These chemicals were found in the vegetables that were examined. Additionally, previous local study studies shown that several fruits and vegetables sold at the Punjab market were discovered to be polluted with a variety of pesticides (Randhawa *et al.*, 2016; Qamar *et al.*, 2017). These pesticides were not removed from the surfaces of the fruits and vegetables by simply washing them with tap water(Akhtar *et al.*, 2018).

Conclusion

Different vegetables (okra, green pepper, tomato, cabbage, cauliflower, carrots, radish and bottle gourd) collected from south Punjab districts namely Multan, Khanewal, Vehari and Muzaffargarh markets and fields were found contaminated with pesticides. The pesticides detected in these vegetables were Cypermethrin, Chlorpyrifos, Imidacloprid and Leufenuron. Some vegetables samples show above the MRL values and some vegetables shows low MRL according to the codex standard. Some vegetables shows no results. Our results showed that around 48 percent of vegetable samples have high MRL, 32 percent of vegetable samples have low MRL and 20 percent of vegetable samples show no results.

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