

Research Article

Monitoring of Pesticide Residues in Some Vegetables From Locally Markets in Gharbia Governorate, Egypt.

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Article info: -

- **Received:** 4 November 2024
- **Revised:** 20 November 2024
- **Accepted:** 28 November 2024
- **Published:** 1 December 2024

Abstract:

Pesticide residues on vegetables are one of the biggest concerns for consumers who need assurance about food safety. The aim of this study was to assess the level of pesticide contamination in several commonly consumed local horticultural products, including tomatoes, onions, potatoes, spinach, and peppers, in Gharbia Governorate, Egypt. Approximately 175 horticultural plant samples were analyzed using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, followed by GC-MS/MS and LC-MS/MS. Out of the total 175 samples analyzed, 39 samples (22.28%) were free of pesticide residues, 136 samples (77.72%) were contaminated. Among the 136 contaminated samples, 45 samples (33.08%) contaminated sample exceeded the maximum residue limits (MRLs). The most commonly detected pesticides were chlorantraniliprole, chlorpyrifos, cyprodinil, metalaxyl, azoxystrobin, chlorfenapyr, imazalil, chlorpropham, acetamiprid, flutolanil, indoxacarb, imidacloprid, and lambda-cyhalothrin. Farmers should also look for other alternative methods of pest control. Consumers should be careful about processing and preparation steps such as washing and peeling to reduce the risk of pesticide residues in fresh produce.

Keywords:

Vegetable Crops, Pesticide Residues, Monitoring, QuEChERS, GC.MS/MS, LC.MS/MS.

1. Introduction

Worldwide agricultural productivity increases thanks to the use of pesticides, yet some chemical residues may have unfavorable effects on the environment and public health. Pesticide residues left over after improper application to crops have become a serious global issue. Vegetables are abundant sources of fiber, vitamins, minerals, and other nutrients, and they also have healthy antioxidant properties. They are frequently employed to fulfill the needs of a balanced diet and excellent health. However, given that the majority of them are consumed raw, it is predicted that they have a greater degree of pesticide residue than other food categories Bhandari et al., (2019).

Therefore, monitoring pesticide residues is the main way to protect consumers from the possible harmful effects of pesticides. Accordingly, the primary method to protect consumers against the potential negative effects of pesticides is to monitor pesticide residues. Therefore, it can be affirmed that the pesticide content in food does not exceed the maximum residue limit (MRL) established by some international organizations, such as WHO and FAO. The possibility of vegetables being contaminated before being transported to the market often occurs due to failure to harvest in time, incorrect use of the application method, failure to comply with etiquette requirements, and violations of law Shwetha et al.,(2020). Determination of pesticide residues in vegetables helps assess the potential risk of these products to human health and provides information about pesticides used in agricultural activities. Within the framework of the foregoing, some of the main vegetables are collected in the markets of Gharbia Governorate to monitor pesticide residues on agricultural crops in the local markets to ensure their safety and that they are free of pesticide residues to preserve the health and confirm the quality of Egyptian agricultural products and the extent to which they are free or contain residues in order to satisfy the Egyptian government Ibrahim et al., (2018).

The main objective of this study was to detect pesticide residues in selected vegetables collected from different markets in Gharbia Governorate and estimate the ratio of detected pesticides to total pesticides analyzed in depth.

2. Materials and Methods

2.1. Samples collection.

A total of one hundred seventy five vegetable samples, i.e., 50 tomatoes, 50 potatoes, 30 onions, 30 peppers, and 15 spinach, have been gathered from Egyptian nearby markets in Gharbia Governorate positioned inside the regions after eight centers inside the Gharbia governorate: Tanta, Kafr El-zayat, El Mahalla Elkobra, Basion, Zefta, Smanod, Kotor, and El Santa, during the seasons at 2021 and 2022. Samples were analyzed straight away upon arrival in the laboratory or stored at temperatures between 0 and 5°C. Approximately 2 kg of every product is thoroughly combined and prepared in line with the approach generally encouraged for coping with by the Codex Alimentarium Commission (CAC).

2.2. Chemicals and reagents:

Acetonitrile and methanol of HPLC grade were bought from Merck (Darmstadt, Germany). Ultrapure water was prepared using a Millipore system. Anhydrous magnesium sulfate was also purchased from Merck (Darmstadt, Germany). Anhydrous magnesium sulfate was activated by heating at 400 °C for 4 hours in an oven, cooling, and storing in a desiccator before use. Primary secondary amine (PSA, 40 µm Bondesil) sorbents were obtained from Agilent Technologies (Santa Clara, CA). Analytical grade sodium chloride and sodium sulphate were purchased from El Nasr Pharmaceutical Chemical Company (Cairo, Egypt). Pesticide reference standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany) with purities >95%.

2.3. Extraction Sample and clean up.

Samples were transported to the laboratory and stored at -20°C till evaluation. Between 2 and 5 kg of each pattern was cut into small cubes and homogenized for five minutes at high velocity in a laboratory homogenizer and extracted in step with the described method and 10 grams of every sample was homogenized adjusted in a 50 ml Teflon- tube. Extraction and purification were performed using the optimized (QuEChERS) approach (Anastassiades et al. 2007). 10 ml of 1.0% acetonitrile acidified with acetic acid was mixed with the sample and shaken vigorously for 1 min. The entire extract was decanted onto glass wool. A glass funnel containing 4 g of anhydrous magnesium sulfate and 1 g of sodium chloride was attached, and the mixture was shaken vigorously for 1 minute. 1 g of dehydrated sodium citrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added. The filtrate was then shaken vigorously with a vortex mixer at maximum speed for 1 minute. Next, 4 g anhydrous MgSO_4 , 1 g NaCl , 1 g sodium citrate dihydrate, and 0.5 g disodium citrate sesquihydrate were added, and the mixture was vortexed vigorously for 2 mins and centrifuged at 5000 rpm for 4 mins. A 3ml aliquot of the supernatant was transferred to a new clean 5-ml centrifuge tube and purified by dispersive solid-phase extraction with 75 mg of PSA and 500 mg of magnesium sulfate. The mixture was then centrifuged at 5000 rpm for 3 mins. Analysis of pesticide residues. Plant extracts contained in autosampler vials were analyzed for pesticide residues by LC-MS/MS and GC-MS/MS.

2.4. Instrumentation

2.4.1. LC-MS/MS

LC-MS/MS analysis was performed using an Exion HPLC device coupled to a QTRAP mass spectrometer (QTRAP 6500+, AB SCIEX). The chromatographic column used was a Synergy C18, 2.5 μm Fusion-RP 100Å, 3.0 x 50 mm column (Phenomenex). The column temperature was maintained at 40°C . The mobile phase consisted of 5 mM ammonium formate at pH 4 in water/methanol (90:10, v/v) (phase A) and 5 mM ammonium formate at pH 4 in methanol (phase B). The gradient elution of the mobile phase was programmed as follows: 0 min, 100% A; 1-15 mins from 100% to 0% A; 15-18 minutes 0% A; 18-20 mins a 100% A. Total running time was 20 minutes. The flow rate was set at 0.3 mL/min. The injection volume was 2 μL . The LC-MS/MS was operated using ESI in positive ion mode with MRM as scanning mode. Sources and gas parameters were optimized as follows: ion spray voltage 5500 v for ESI (+); ion source temperature 400°C ; curtain 20 psi air; collision gas medium; nebulizer gas and auxiliary gas 35 psi. The Analyst software (Version 1.7.1, Applied Biosystems) was used for the instrument setting, data acquisition and processing. Retention time, precursor and product ions quantification and confirmation, collision energy potentials (CE), collision exit potential (CEP), decluttering potential (DP) and entrance potential (EP) were determined for each tested compound.

2.4.2. GC-MS/MS

An Agilent 7980A gas chromatograph with quadrupole 7000B tandem mass spectrometer and an electron impact (EI) interface was used to perform analysis with an HP-5MS 5% phenyl methyl siloxane capillary column (30 μm long x 0.25 mm ID x 0.25 μm thick film). The temperature programming of the GC oven was initially kept at 70°C for 2 min and then

increased to 150°C at $25^{\circ}\text{C}/\text{min}$ (held for 0 min), and raised to 200°C at a rate of $3^{\circ}\text{C}/\text{min}$ (held for 0 min), then rose from 200 to 280°C at $8^{\circ}\text{C}/\text{min}$ (holding for 10 min). Samples were injected in non-split mode and the run time was 16 min.

2.5. Method validation and quality control

Quality assurance standards were followed to validate the performance of the standard approved by the Finnish Accreditation Service (FINAS) method through ISO/IEC Guide 17025. The reproducibility expressed as a relative standard deviation was less than 20%. The quantification limit started at 0.01 mg kg^{-1} and was dependent on the pesticide type and detection unit. Measurement uncertainty expressed as relative standard deviation (at 95% confidence level) was less than the default value set by the EU ($\pm 50\%$). Blank samples were fortified with pesticide mixture and analyzed as a set of samples. The average recovery percentage of these pesticides at different concentrations varied from 70% to 120%.

3. Results and Discussion

3.1. Pesticide residues in different vegetables

3.1.1. Tomatoes

Table (1) and Figure (1) shown the levels of pesticide residues detected in tomato samples and the percentage of samples that exceeded the maximum residue levels. Of the 50 samples, 5 tomato samples (10%) did not contain detectable pesticide residue, the remaining 45 samples (90%) were contaminated, of which 18 samples (40%) exceeded the established MRLs set by the Codex Alimentarius. out of 45 tomato samples were not detected. Meet regulations MRL is established by Codex Alimentarius. The most frequent residues in tomato samples were imidacloprid, chlorantraniliprole, lufenuron, azoxystrobin, myclobutanil, cypermethrin and chlorpyrifos, repeated 17, 16, 15, 13, 12, 11 and 10 times, respectively. However, the highest pesticide residue concentration exceeding the MRL was chlorantraniliprole, detected in 22% of tomatoes from markets in Gharbia province, followed by chlorpyrifos with 17.77%.

These results are consistent with similar trends reported by Ibrahim et al. (2022) who determined the contamination levels of pesticide residues in some locally consumption of horticultural products such as tomatoes in the following Governorates of Egypt : Dakahlia, Ismailia, Fayoum, Alexandria, Cairo, Gharbia, Kafr. El-Sheikh, Sharkia, Port Said, Beheira, Minya, Menoufia, Zagazig and Giza.

Approximately 175 horticultural plant samples analyzed using the (QuEChERS) method, followed by GC-MS/MS and LC-MS/MS analysis. Of the total 175 samples analyzed, 35 samples (20%) were free of pesticide residues, 140 samples (80%) were contaminated, and 59 samples (42%) were contaminated above the minimum residue limit. multi (MRL). The most frequently detected pesticides were chlorpyrifos, cypermethrin, and carbendazim.

3.1.2. Potatoes

Table (2) and Figure (2) shown the levels of pesticide residues detected in potato samples and the percentage of samples exceeding the maximum residue levels MRLs. Out of 50 potato samples, no pesticide residue was detected in 10 potato samples (20%), while the remaining 40 samples (80%) were contaminated, 10 samples (25%) out of 40 potato samples were contaminated. west violates violatede established MRLs set by Codex

Alimentarius.

The most common residues in potato samples were chlorpropham, chlorpyrifos, imazalil, azoxystrobin, cyprodinil, flutolanil, flutriafol and chlorfenacryras, repeated 20, 18, 17, 15, 15, 13 and 12 times, respectively. However, the highest pesticide residue concentration exceeding the MRL was cyprodinil detected in 17.5 % of potatoes from markets in Gharbia province, followed by metalaxyl with 7.5%.

These results have the same trend as those of Jallow et al. (2017), who found that analyzed a total of 150 different fresh fruit and vegetable samples, including potatoes, were analyzed for the presence of 34 pesticides using Fast, Easy, Inexpensive, Effective, Robust, and Safe (QuEChERS) extraction, followed by gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Pesticide residues exceeding maximum residue limits (MRLs) were detected in 21% of samples, and 79 % of samples contained no residues of the studied pesticides or had residues below the MRLs. Multiple residues appeared in 40% of the samples containing from 2 to 4 pesticides and 4 samples were contaminated with more than 4 pesticide residues. Of the pesticides examined, 16 were detected, with imidacloprid, deltamethrin, cypermethrin, malathion, acetamiprid, monocrotophos, chlorpyrifos-methyl and diazinon exceeding their MRL values. Aldrin, an organochlorine pesticide, was detected in an apple sample with residues below the MRL.

3.1.3. Onion

Table (3) and Figure (3) shown the levels of pesticide residues detected in onion samples and the proportion of samples exceeding the maximum residue levels MRLs. Of the 30 samples, 10 onion samples did not contain detectable pesticide residue (33.33%), the remaining 20 samples (66.66%) were contaminated, of which 2 samples (10%) exceeded the MRL set by the Codex Alimentarius.

The most common residues in onion samples were acetamiprid, azoxystrobin, tebuconazole, cyprodinil, metalaxyl, difenoconazole, deltamethrin, pyrimethanil and copper oxychloride, which were detected 15, 12, 11, 10, 10, 5, 4, 4 and 2 times, respectively. The highest pesticide residue concentration exceeding the MRL was deltamethrin, detected in 10% of onions from markets in Gharbia province.

These results were the same trend to Ahn et al., (2012) who found that A total of 250 samples fruit and vegetables contains onions collected from traditional markets and supermarkets in 6 cities in Korea. 132 pesticides excluding herbicides were analyzed by a multi-residue method using GC-MS/MS and HPLC. Detected 17 types of pesticides from 42 samples, including 32 conventional agricultural products, 1 organic type, 4 types without pesticides and 5 types with low pesticide content.

3.1.4. Pepper

Table (4) and Figure (4) shown the level of pesticide residues detected in pepper samples and the proportion of samples exceeding the MRLs. Out of the 30 samples, 8 pepper samples did not contain detectable pesticide residue (26.66%), while the remaining 22 samples (36.36%) were contaminated, including 3 samples (13.63%) exceeded the MRL established by Codex Alimentarius. The most common residues found in pepper samples were acetamiprid, lambda-cyhalothrin,

boscalid, chlorpyrifos, chlorfenapyr, difenoconazole, metalaxyl, thiamethoxam, and clothianidin, which were repeated 13, 11, 10, 10, 7, 7, 6, 3, and 2 times, respectively. However, the highest pesticide residue concentration exceeding the MRL was chlorfenapyr, detected in 13.63% of pepper from markets in Gharbia province.

These results have the same trend as those of Jallow et al. (2017), who analyzed 150 different fresh fruit and vegetable samples for the presence of 34 pesticides using (QuEChERS) extraction, followed by gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Pesticide residues exceeding the maximum residue limit (MRL) were detected in 21% of samples, and 79% of samples contained no residues of the studied pesticides or samples with residues below the MRL. Multiple residues appeared in 40% of samples containing from 2 to 4 types of pesticides and 4 samples were contaminated with more than 4 types of pesticide residues. Of the pesticides studied, 16 were detected, of which imidacloprid, deltamethrin, cypermethrin, malathion, acetamiprid, monocrotophos, and chlorpyrifos-methyl.

3.1.5. Spinach

Table (5) and Figure (5) shown the levels of pesticide residues detected in the spinach samples and the percentage of samples exceeding the maximum residue levels. Among the 15 spinach samples, 6 samples did not contain detectable pesticide residue (40%), while the remaining 9 samples (60%) were contaminated, of which 2 samples (22.22%) Exceeded the MRL established by Codex Alimentarius.

The most frequent residues in spinach samples were indoxacarb, acetamiprid, fludioxonil, dimethomorph, Spintoram, difenoconazole, propamocarb-Hcl, fenhexamide, metalaxyl, and cyprodinil, repeated by 7.5, 5, 4, 3, 3, 2, 2 and 1 time, respectively. However In turn, the highest pesticide residue concentration exceeding the MRL was Propamocarb-Hcl, detected in 22.22% of Spaniards in food markets.

These results are consistent with those of Mebdoua et al. (2017), who analyzed 160 samples of 13 types of domestically produced and imported fresh fruits and vegetables including Spinach for the presence of pesticide residues using multiresidue extraction followed by gas chromatography and mass spectrometry. In 42.5% of tested samples, no residues were detected, while 12.5% samples contained pesticide residues exceeding the maximum residue limit.

The results of this study are consistent with the study conducted by Doghem et al (2001). Who identified many pesticide residues in 1579. Egyptian fruit and vegetable samples were collected from eight local markets of six governments in 1996 to find 53 pesticide residues, including includes organic phosphate and organic nitrogen compounds as well as some synthetic pyrethroids. They found that 23.9% of samples contained detectable residues for each crop, with contamination ranging from 0 to 96% across the samples analyzed.

4. Conclusions

Research provides important information about the contamination of some fresh vegetables with pesticides collected from the local Egyptian markets of Gharbia Governorate (8 centers) in Egypt.

The results indicated that certain vegetable samples

were concentrated with pesticide residues at concentrations above the MRL. Hence, there is a need for continuous survey and monitoring programs for pesticide residues in food commodities to protect the end-user from indiscriminate exposure to pesticides. It is recommended to conduct educational programs for farmers on the control and safer use of pesticides. Regulatory policies on pesticides should also be done to protect farmers, and consumers, health. Moreover, an extension program

should be put in place for the farmers to increase their awareness of the safe use and application of pesticides and the importance of adhering to the pre-harvest interval period. Farmers should also look for other alternative methods of pest control. Consumers should be careful about processing and preparation steps such as washing and peeling to reduce the risk of pesticide residues in fresh produce.

Table 1. Monitoring pesticide residues in tomatoes.

Commodity	Total No. of samples	No. of contaminated samples	Contamination %	The detected pesticides	MRL mg/kg	Frequency	Min mg/kg	Max mg/kg	Mean mg/kg	No. of violated	Violated %	Total violated sample	Total violated sample %
Tomatoes	50	45	90%	Abamectin	0.05	7	0.006	0.02	0.013	0	0	18	40%
				Azoxystrobin	3	13	0.002	0.07	0.036	0	0		
				Bifenazate	0.5	5	0.01	0.09	0.05	0	0		
				Chlorantraniliprole	0.6	16	0.09	0.59	0.24	10	22		
				Chlorfenapyre	0.4	8	0.005	0.08	0.045	0	0		
				Chlorpyrifos	0.01	10	0.01	0.1	0.055	8	17.77		
				Cypermethrin	0.2	11	0.001	0.1	0.055	0	0		
				Difenoconazole	2	12	0.04	0.06	0.05	0	0		
				Imidacloprid	0.5	17	0.009	0.05	0.059	0	0		
				Indoxacarb	0.5	10	0.02	0.06	0.04	0	0		
				Lufenuron	0.4	15	0.02	0.07	0.045	0	0		
				Malathion	0.5	8	0.008	0.03	0.019	0	0		
				Methomyl	1	8	0.01	0.5	0.255	0	0		
Myclobutanil	0.3	12	0.04	0.07	0.055	0	0						
Tebuconazole	0.7	10	0.003	0.01	0.006	0	0						

Table 2. Monitoring pesticide residues in potatoes.

Commodity	Total No. of samples	No. of Contaminated samples	Contamination %	The detected pesticides	MRL mg/kg	Frequency	Min mg/kg	Max mg/kg	Mean mg/kg	No. of violated	Violated %	Total violated sample	total violated sample %
Potatoes	50	40	80%	Azoxystrobin	7	15	0.1	1.02	0.56	0	0	10	25%
				chlorfenapyr	0.01	12	0.002	0.01	0.006	0	0		
				chlorpropham	10	20	0.662	3.177	1.919	0	0		
				chlorpyrifos	2	18	0.001	0.93	0.46	0	0		
				clethodim	0.5	10	0.01	0.2	0.105	0	0		
				cyprodinil	0.01	15	0.002	0.5	0.251	7	17.5		
				Deltamethrin	0.01	10	0.005	0.02	0.015	0	0		
				Difenoconazole	4	12	0.02	0.1	0.06	0	0		
				Dinethoate	0.05	5	0.007	0.06	0.035	0	0		
				Fludioxonil	5	13	0.02	0.1	0.06	0	0		
				Flutolanil	0.1	15	0.009	0.018	0.0135	0	0		
				Flutriafol	0.01	13	0.002	0.01	0.006	0	0		
				Imazalil	9	17	1.57	4.68	3.125	0	0		
				Malathion	0.02	10	0.003	0.01	0.0065	0	0		
				Metalaxyl	0.05	9	0.006	0.2	0.103	3	7.5		
				Propamocab-Hcl	0.03	5	0.05	0.1	0.075	0	0		
Pyrimethanil	0.05	11	0.003	0.05	0.026	0	0						
Trifloxystrobin	0.02	12	0.005	0.01	0.008	0	0						

Table 3. Monitoring pesticide residues in onions.

Commodity	Total No. of samples	No. of contaminated samples	Contamination %	The detected pesticides	MRL mg/kg	Frequency	Min mg/kg	Max mg/kg	Mean mg/kg	No. of violated	Violated %	Total violated sample	Total violated sample %
Onions	30	20	66.66%	Acetamiprid	0.02	15	0.004	0.01	0.007	0	0	2	10%
				Azoxystrobin	10	12	0.011	0.2	0.15	0	0		
				Copperoxychloride	5	2	0.007	0.03	0.018	0	10		
				Cyprodinil	0.3	10	0.02	0.08	0.05	0	0		
				Deltamethrin	0.05	4	0.01	0.04	0.05	0	0		
				Difenoconazole	0.1	5	0.002	0.05	0.026	0	0		
				Metalaxyl	2	10	0.01	1	0.55	0	0		
				Pyrimethanil	0.2	4	0.03	0.01	0.02	0	0		
				Tebuconazole	0.15	11	0.005	0.01	0.0075	0	0		

Table 4. Monitoring pesticide residues in peppers.

Commodity	Total No. of samples	No. of contaminated samples	Contamination %	The detected pesticides	MRL mg/kg	Frequency	Min mg/kg	Max mg/kg	Mean mg/kg	No. of violated	Violated %	Total violated sample	Total violated sample %
Peppers	30	22	73%	Acetamiprid	2	13	0.008	0.11	0.059	0	0	3	13.63%
				Boscalid	10	10	0.05	0.32	0.185	0	0		
				Chlorfenapyr	3	7	0.5	6	3.25	3	13.63%		
				Chlorpyrifos	20	10	0.001	0.1	0.05	0	0		
				Clothianidin	5	2	0.002	0.01	0.006	0	0		
				Difenoconazole	5	7	0.03	0.62	0.325	0	0		
				Lambda-cyhalothrin	3	11	0.15	0.82	0.485	0	0		
				Metalaxyl	10	6	0.02	0.55	0.285	0	0		
				Thiamethoxam	7	3	0.02	0.41	0.215	0	0		

Table 5. Monitoring pesticide residues in spinach

Commodity	Total No. of samples	No. of contaminate samples	Contamination %	The detected pesticides	MRL mg/kg	Frequency	Min mg/kg	Max mg/kg	Mean mg/kg	No. of violated	Violated %	Total violated sample	Total violated sample %
Spinach	15	9	60%	Acetamiprid	0.9	5	0.03	0.2	0.115	0	0	2	22.22%
				Cyprodinil	0.02	1	0.001	0.001	0.001	0	0		
				Difenoconazole	0.6	3	0.01	0.01	0.5	3	0		
				Dimethomorph	0.01	4	0.004	0.01	0.007	0	0		
				Fenhexamid	0.01	2	0.005	0.01	0.0075	0	0		
				Fludioxonil	10	5	0.01	0.05	0.03	0	0		
				Indoxacarb	0.02	7	0.005	0.001	0.003	0	0		
				Metalaxyl	0.5	2	0.002	0.02	0.011	0	0		
				Propamocarb-Hcl	0.01	3	0.006	0.1	0.053	2	22.22		
Spinetoram	0.02	4	0.007	0.01	0.0085	0	0						

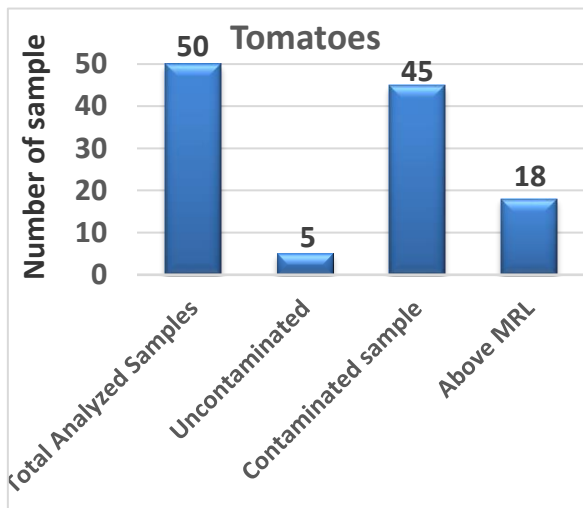


Figure 1. The number of samples that contain pesticide residues detected in Tomatoes.

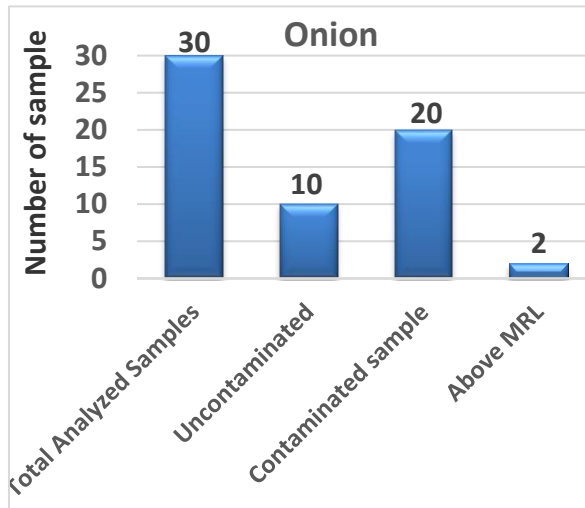


Figure 3. The number of samples that contain pesticide residues detected in onions.

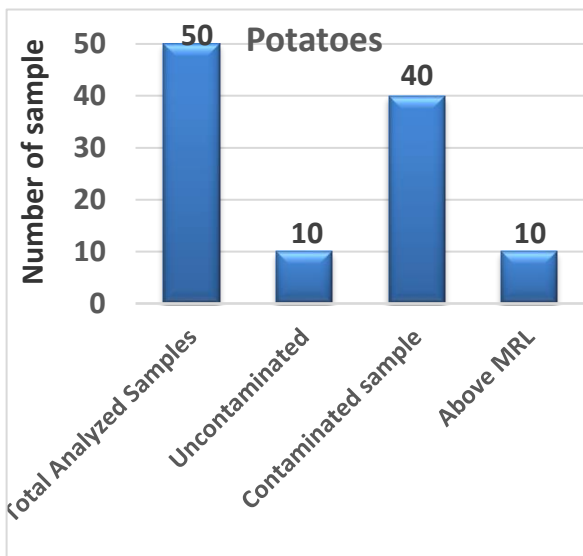


Figure 2. The number of samples that contain pesticide residues detected in Potatoes.

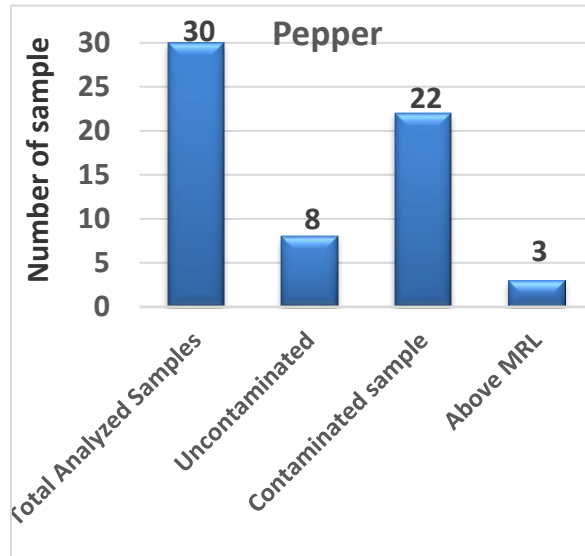


Figure 4. The number of samples that contain pesticide residues detected in Pepper.

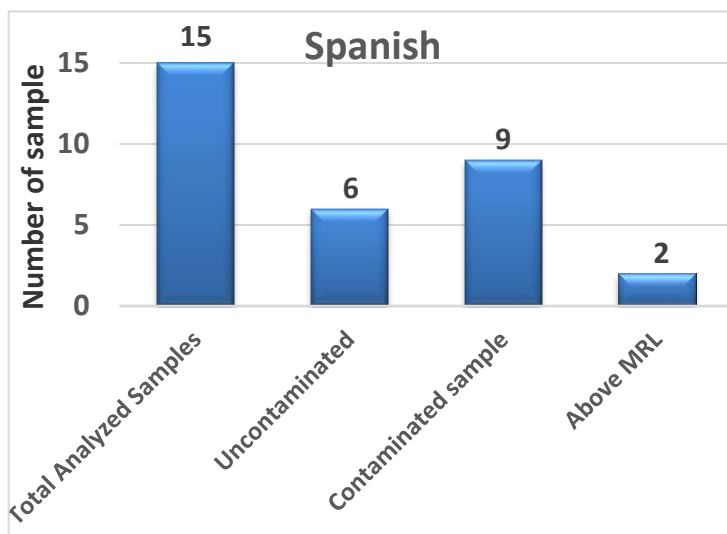


Figure 5. The number of samples that contain pesticide residues detected in Spinach.

5. Reference

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