Pesticide Residues Determination in Vegetables from South Western Region of Saudi Arabia


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ABSTRACT
BACKGROUND: Pesticides are used widely before and after harvesting to protect the crop against many plant diseases and harms. Exposure of human to pesticides residues either by direct or indirect contact result in many health risk. In the present study, multi-residue extraction method QuEchERS (Quick, Easy, Cheap, Effective, Rugged and Safe) were used and followed by Gas Chromatography/ Mass Spectrometry (GC/MS) for analysis. The samples were collected from different local market in Jazan region including (Tomato, Eggplant, Chili, Okra, Molokhya). RESULTS: Our results revealed detection of 15 different pesticides residues in all vegetables samples collected with average rate 26.2 % (21% exceeded the MRLs). Tomato samples were found to be contaminated with 14 pesticides with different concentrations out of 15 pesticides detected in overall vegetables samples. Ten moderately toxic pesticides, three slightly toxic while two non-toxic pesticides were detected in all samples tested. None of the pesticides detected in our study is classified as highly toxic. Only Metalaxyl-M and Propiconazole were found to be below the quantification limit (< 0.1 ppm). CONCLUSION: Combined usage of QuEchERS method and GC/MS provide high accuracy, sensitivity as well as time and cost consuming. We recommend applying this method for routine monitoring for pesticides in vegetables as well as fruits in Jazan area, KSA.

KEYWORDS: Vegetables, QuEchERS, GC/MS, Pesticides.

INTRODUCTION

Pesticides are widely used to protect plant and crop in agriculture from pests and plant diseases. There are different categories of pesticides such as insecticides, fungicides, and herbicides. The European Union reported in 2010 that 208,000 tons of pesticides were used which are biologically active with specific inherent toxicity [1].
Furthermore, the usage of pesticides been accompanied by human health risk and the environmental contamination because of their toxicity [1]. As well, the chronic effect of pesticides exposure resulted in carcinogenic, genotoxic effects and endocrine disturbances [2]. Bioaccumulation of pesticides in the food chain affects mammal’s health by negatively. Global public health attention regarding the misuse of pesticides and there are many reports recorded about pesticide residue in the grains [3], vegetables [4], milk [5] and fish [6].

Human requirements for fibers, vitamins and minerals are available in the fruits and vegetables which considered the main diet for them [7]. World health organization [WHO, 2003] recorded that 30% of consumption food products consists of fruits and vegetables which are more contaminated by pesticides than other products of animal origin [8, 9]. As well, fruits and vegetables were consumed in a raw or semi-processed way, so it contains a higher amount of pesticides residues than other food types.

Screening and detection of pesticides residue in the food are becoming fundamental and challenging. The annual report of the EFSA, European Food Safety Authority, declared that detection of pesticides residues is needed due to the herb matrix contain more than five types of pesticides in each sample and exceeds the Maximum Residue Limit (MRLs) [10]. Various governments and international agencies conducted the maximum residue limits (MRLs) for food, normally at μg kg⁻¹ [11].

Recently, use of pesticides has been increased. Therefore, there are many legal limits for pesticides residues in the plants which set by the European Commission (EC) and United Stated Department of Agriculture to control pesticides usage. Who has reported that three million pesticide poisonings occur annually and result in 220,000 deaths worldwide [12]. In public health safety and trade, pesticide residue determination in all food products, especially in raw vegetables and fruits, is a very demanding task.

One of the most global obstacles that there are no fixed limits on pesticides use. Also, a basic conflict between exporting and importing countries [13]. Furthermore, the low concentration of pesticides and the excessive amount of interfering substances and adversely affect the analysis results [14].

Therefore, efficient analytical methods required to determine the pesticides residue limit. The Quick, Easy, Cheap, Effective, Rugged and Safe (QuEchERS) method is considered one of the most efficient multi-residue analytical technique including wide polarity range. The QuEChERS method replaces many complicated analytical steps which conducted in the traditional methods. This method is relying on an extraction with acetonitrile and partitioning with salt [15].

First published data about QuEchERS for pesticide extraction was in 2003 [16]. It is used for all food stuff [17-20] and others like soil [21]. There are various applications for QuEchERS in the field rather than pesticides including, pharmaceutical uses (drug analysis; hormones and steroids), mycotoxin analysis and environmental studies of toxic compounds such as acrylamide [22-24].

The QuEChERS method is the most successful routine lab test and famous for more than ten years. It requires few steps and minimum solvent in comparing with conventional sample preparation techniques. It characterized by 95% recovery range and repeatability of <5%, and cost-effective strategy (<30 min and $1 per sample). It was recommended to be used in international standard methods such as The AOAC official method 2007.01 and the CEN's, European Committee for Standardization, standard method EN 15662 [25].

To the extent of our knowledge, data are lacking regarding the pesticide contamination in the vegetables we are consuming in Jazan region. There is no official information about the safety of these vegetables as the majority of them are cultivated locally. Also, because pesticides are used at any stage of the plant life cycle, pesticides can produce residue in any part of the vegetable, either in the inner part or outer surface.

MATERIAL AND METHODS

Study area:
Jazan City, South Province of Saudi Arabia.

Sampling:
As a total, 183 samples were purchased from Jazan district, KSA. Five different vegetable samples including (Tomato, Chili, Eggplant, Okra, Molokhia) were collected randomly from open local markets such as Sabya, Samtah, Al-Arada, and Jazan city.

Sample processing:
The selected edible part (100 – 120 g) from each fresh vegetable was homogenized in a blender. Ten grams of the homogenized vegetable were weighed in 50 ml falcon tubes. Acetonitrile solution (15 ml) was added to each sample and vortexed vigorously for 2 minutes.
Internal standard and QuEChERS powder kit (UCT, USA) was added to each tube slowly and vortexed for more 2 minutes. Then, all samples were centrifuged at 3000 rpm for 5 minutes. The supernatant (organic layer) was concentrated by using nitrogen evaporator. One milliliter of the concentrated supernatant was then filtered through 0.45 µm syringe filter and transferred to GC vials and be ready for analysis.

On the other hand, for green vegetables containing a huge amount of chlorophyll, the supernatant was transferred to chlorophyll clearance tubes (UCT, USA) then vortexed for 30 seconds and centrifuged at 3000 rpm for 5 minutes. The clear solution was transferred to GC vials for analysis.

**RESULTS AND DISCUSSION**

Our results revealed a detection of 15 different pesticides residues in all vegetables samples collected. The average percentage was 26.2 % (21% exceeded the MRLs). These results agree with a previous study by Frederick, 2011 who reported the contamination of Cabbage by 21 pesticides. He found that about two-thirds of the total sample number was contaminated with pesticides exceeding the MRLs [27]. Qualitative detection of pesticides in our samples was done by GC-MS analysis and confirmation by retention time, specific ion fragments as well as the help of pesticides library available in the software of GC-MS (Figure 1).

Molokhia samples were the most contaminated one among all vegetables tested where 20 out 40 of samples (50%) are contaminated with the potentially carcinogenic pesticide Cypermethrin as well as Carbaryl and Metalaxyl-M. On the other hand, 19 out of 43 Tomato samples (44.2%) were found to be contaminated with 14 pesticides detected in overall vegetables samples. One sample only out of 30 Eggplant was contaminated with Pencomazle. While only 6 out of 40 Okra samples (15%) were found to be contaminated by Carbaryl. Thirty Chili samples tested were all negative except 2 samples contaminated with Pyridaben.

**Fig. 1:** Qualitative detection of pesticides in samples depending on peak retention time & pesticide library
available in GC-MS software. Similarity Index (SI) of more than 80% is accepted.

It is evident from our results that tomato was the only vegetable samples in our study found to be contaminated with almost all pesticides detected which may be due to use of several types of pesticides during tomato cultivation. The case was not the same as with the other vegetables tested where we found contamination by only one type of pesticide as in case of Eggplant, Chili and Okra. Molokheya samples were found to be contaminated by three pesticides but Cypermethrin found to contaminate 77% of these samples. Cypermethrin is one of the pyrethroid pesticides and is known to be a potent carcinogen compound. Cypermethrin and Carbaryl were found as the most frequent detected pesticides compared to others in our study (Figure 2).

A similar study from Egypt reported the contamination of 55% of samples tested with pesticides residues. Some of them are considered hazardous to human such as Malathion [28]. Selim, 2011 reported the detection of pesticides residues in 24.69% of total tested samples collected from Riyadh main supermarket, 18.34% of these pesticides exceeded the MRLs [29].

![Fig. 2: Frequency of detected pesticides in all samples tested.](image)

Quantitative estimation of pesticides residues detected in vegetables samples were done using a calibration curve prepared from standard mixture of pesticides 100, 100, 500 and 2000 μg/kg (ppb) as well as the specific target ions for each pesticide (Figure 3). The R and R² values were not less than 0.998 and 0.997, respectively. Cancer development is another adverse effect of pesticides on human’s health. Triadimenol and cypermethrin concentration were the highest among all the quantified pesticides residues with a value of 2.9 and 2.3 mg/dl (ppm), respectively. Diazinone and chlorfenapyr concentration were less but still above the MRLs where as other pesticides were found to be at low to moderate levels compared to their corresponding MRLs (Figure 4).

![Fig. 3: Quantitative determination of pesticides detected in all samples depending on calibration curve prepared and target ions specific for each pesticide (Examples: Diazinone and Kresoxim-Methyl).](image)
Cancer development is another adverse effect of pesticides on human’s health. The analysis of cancerous kidney biopsies showed elevated levels of pesticides higher than those found in healthy kidney biopsies [30]. Many epidemiological pieces of evidence are linking the development of various cancers, especially children cancers, to the exposure of parents or children to pesticides. Different types of cancers in children are caused by pesticides exposure such as leukemia, brain tumors, lymphoma, neurological, colorectal cancer, cancer of the germ cell, Hodgkin’s disease, eye cancer, tumors of the kidney, liver, thyroid cancer and skin cancer. On the other hand, pesticides cause many cancers to adults such as multiple myeloma, non-Hodgkin’s lymphoma, leukemia breast, lung, ovarian, pancreatic, prostate, bladder, kidney, stomach, colon, rectal, lip, connective tissue, brain and testis cancers [31].

Among the 15 pesticides detected in our samples, 10 are classified as moderately toxic (Diazinone, Malathion, Chlorfenapyr, Carbaryl, Cypermethrin, Bifenthrin, Triadimenol, Propiconazole, Pyridaben and Metalaxyl-M) and 3 pesticides are classified as slightly toxic (Kresoxim-Methyl, Myclobutanil and Difenoconazole) while Penconazole and Procymidone are classified as non-toxic pesticides (Figure 5). However, none of the pesticides detected in our study is classified as highly toxic [32].

Nine pesticides were found to be above MRL, 7 of them are moderately toxic while only 2 are non-toxic (Figure 4). On the other hand 4 pesticides are below MRL, only Pyridaben is a moderately toxic and the other 3 are slightly toxic. Two moderately toxic pesticides; Metalaxyl-M and Propiconazole were found to be below the quantification limit (< 0.1 ppm).

**Fig. 4:** Average concentration of quantified pesticides as mg/kg (ppm).

**Fig. 5:** Classification of pesticides detected according to acute toxicity.
Conclusion:

Our results revealed a detection of 15 different pesticides residues in all vegetables samples collected. The average percentage is 26.2 % (21% exceeded the MRLs). Molokhia samples were the most contaminated one among all vegetables tested where 20 out 40 of samples (50%) are contaminated with the potentially carcinogenic pesticide Cypermethrin as well as Carbaryl and Metalaxyl-M. On the other hand, 19 out of 43 Tomato samples (44.2%) were found to be contaminated with 14 pesticides out of 15 pesticides detected in overall vegetables samples. One sample only out of 30 Eggplant was contaminated with Penconazole (3.3%).

Only 6 out of 40 Okra samples (15%) were found to be contaminated by Carbaryl, while the thirty Chili samples tested were all negative except 2 samples contaminated with Pyridaben (6.7%). However, none of the pesticides detected in our study is classified as highly toxic. Ten pesticides are moderately toxic. Three pesticides are slightly toxic while only two are non-toxic.

Recommendation:

- Creation a governmental program to design regulations for permanent monitoring and control of using pesticide in agriculture.
- Encourage and support the organic agriculture as the way to avoid problems of pesticide.
- Finally, we recommend routine pesticides screening regimens to be performed on regular periods to monitor control and minimize uncontrolled pesticides usage in agriculture.

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REFERENCES


