Evaluation of Some Pesticide Residues in Fruits import by High Performance Liquid Chromatography

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ABSTRACT

Pesticide residues have been found in various fruits and vegetables. This study collected 24 samples and reported a method based on High Performance Liquid Chromatography (HPLC). For determination of pesticide residues used in Some fruits which were collected from different markets of Baghdad city to make( 24 ) samples from (peel , core, mixture) for each type of fruits markets as: Oranges (Egypt, Africa), Pomegranate (Egypt), Mango (Kenya), Pears (China), Plum fruits (Africa), Kiwi (Turkey). That detective of (5 ) different pesticides ( Diazinon, malathion, chlorpyrifos, parathion and cypermethrin ) . The results were detected of multi-residues of pesticides on the fruit in (peel, core, mixture) may be in the limit of Maximum residue limits (MRL) or higher of it . The pesticides detected that exceeding the limits are: cypermethrin in Kiwi (peel, core and mixture) at (0.204,0.038,0.537) in pomegranate detected in (peel and mixture) at (0.509,0.189) mg/kg, Diazinondetected in Egyptian orange in (peel,core and mixture) at (0.031,0.207,0.099) mg/kg. Malathion and Parathion was not detected at any type of fruits and that results was compared with codex of FAO/WHO(2013). That data is important to monitor residues in food and to fill gaps in current knowledge would be helpful in assessing human exposure risks from ingestion of contaminated Fruits Imported to our country.

INTRODUCTION

In Iraq with decreases the percentage of agricultural in comparison with others countries so that it seems to find different kind of fruits and vegetable in our markets and may be in lower prices than our crops from different countries. Fruits and vegetables are essential to a nutritious and healthy diet;Fruits, nuts, and vegetables play a significant role in human nutrition, especially as sources of vitamins (C, A, B6, thiamine, niacin ,E), minerals, and dietary fiber Some components of fruits and vegetables are strong antioxidants and function to modify the metabolic activation and detoxification/disposition of carcinogens, or even influence processes that alter the course of the tumor cell (Kader,2004), however, the health benefits are compromised by consistent contamination with some chemicals as pesticide residues (Tahir etal,2009).

The Food and Agriculture Organization (FAO) has defined pesticide as: any substance or mixture of substances used for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the...
production, processing, storage, transport, or marketing of food, agricultural commodities (Marrazza, 2014). Exposure of the general population to pesticide most commonly occurs through consumption of treated food sources. Persistent chemical pesticides can be magnified through the food chain that have been detected in products ranging from meat and fish, to vegetable oils, various fruits and vegetables (Marrazza, 2014). That Some of these pesticides contain chemical organophosphorous compounds. Organophosphorous (OP); compounds are derived from phosphoric and thiophosphoric acids. Individual OP pesticides vary widely in acute toxicity, but collectively they are among the most acutely toxic of all pesticides to mammals. Most organophosphorous to control operators who use (OP) every day in their work (Chloride, 2013).

The aim of this study is to detect the presence of pesticide residues in fruits, mainly how they are introduced, measured, degraded and their risk assessment. Show the Fruits are important components of the human diet since they provide essential nutrients.

The aim of this study is

1- To describe the presence of pesticide residues in fruits, mainly how they are introduced, measured, degraded and their risk assessment.
2- Show the Fruits are important components of the human diet since they provide essential nutrients.

3- Assessment of penetration for these pesticides on fruits and measuring pesticide residue in body fruits (peel, core and mixture).
4- The affecting exposure of fruits to pesticides pre and post – Harvesting the crop and acceptable daily intake (ADI) for human health.
5- Extraction and Detection of some kind of organophosphate pesticides which mostly used on the selected fruits.
6- Measuring pesticide residues to ensure that in fruits do not exceed maximum residue levels (MRLs) of FAW/WHO codex (MRL, 2013).

MATERIALS AND METHODS

Sample Collection
The Import fruits were collecting from different markets in Baghdad, Iraq, different time and chooses from different countries. After collecting the fruits were washed with deionized water three times to clean them from dust. These fruits are - Oranges (Egypt, Africa), Pomegranate (Egypt), Mango (Kenya), Pears (China), Plum fruits (Africa), Kiwi (Turkey).

Chemicals and Solvents
Standard solutions of pesticides are (Parathion, malathion, Diazinon, Cypermethrin, Chlorpyrifos) and the solvent are (Acetonitrile, Deionize water, Anhydrous sodium sulphate, Ethyle acetate). All Chemicals and solvents in this study are grad-HPLC and obtained from Sigma – Aldrich Company (Germany) by OMA international scientific office in Baghdad.

Preparation of Stock Standard Solution
Preparation of standard stock and working solution are carried out by the following method. To prepare 50 ppm stock standard of any substances, 12.5 ml was transferred into a volumetric flask of 50 ml and diluted to mark by using Acetonitrile solvent …and so on … until prepare 1 ppm of standard of any substances from 5ppm we need 10 ml was transferred in to volumetric flask of 50 ml and diluted with acetonitrile and to prepare 0.5 ppm from 25ppm we need 1 ml was transferred in to volumetric flask of 50 ml and diluted to mark 50ml by using acetonitrile that called Calibration Curve of standard. (Islam et al, 2009).

Preparation of Fruits
Sample (100g) of peel of fruit deep around (2-3 mm) and (100gm) of inside the fruit, peel of banana and peel skin of grape, other fruits and inside were mixed were cut into small pieces and homogenized by means of a kitchen blender (Islam et al, 2009).

Extraction Method
The blended fruits sample was mixed with anhydrous sodium sulphate (50 g) and extracted with ethyl acetate (200 ml) in conical flask using an Ultra-Turrax for 4-5 min. The content was allowed to settle down for about half an hour and the ethyl acetate extract was
then filtered through a Buchner-funnel fitted with a filter paper covered by (20g) of anhydrous sodium sulfate. After filtration, the extract was evaporated to dryness and re-dissolved in (5 ml) of acetonitrile (MeCN) and finally the volume was reduced to about (0.5 ml) using stream of liquid nitrogen. The extract was then transferred to a graduated test tube and the final volume was adjusted at exactly (1 ml) by adding a few drops of acetonitrile. Solutions were then centrifuged and filtered.

The clean organic layers were taken and analyzed by a high performance liquid chromatography having UV/Visible detector (Islam et al., 2009). The Mobile phase: linear gradient of solvent A (De-ionized water) : solvent B (acetonitrile) was (70:30, v/v).

**HPLC systems:** A Shimadzu LC-2010A HT, High performance liquid chromatography having UV/visible detector was used for identification and quantification of pesticides.

**Identification and quantification**

The compound was identified by comparing its retention time with respect to technical grade reference standard. The quantitative determination was carried out with the help of a chromatographic curve drawn from chromatographic experiments with standard solution. For quantification an external chromatographic curve with four different concentrations of each pesticide, with matrix matching were made. The standard solutions for the chromatographic curves were prepared in control matrix because samples may possess co-extractants in the matrix which may affect the peak area of the unknown samples. As explained in table (1) and illustrated in figure (A):

The figure (A) shown the chromatographic curve of pesticides standard at concentration 0.5 mg/kg. And in the same condition:

**Peak (1) at 1.18 shown the Rt. Of Malathion.**

**Peak (2) at 2.44 shown the Rt. Of Diazinon.**

**Peak (3) at 3.51 shown the Rt. Of Chlorpyrifos.**

**Peak (4) at 4.35 shown the Rt. Of Cypermethrin.**

**Peak (5) at 5.18 shown the Rt. Of Parathion**

| Seq. | Subjects      | Retention time : minute | Area : µvolt |
|-----|---------------|-------------------------|--------------|
| 1   | Malathion     | 1.18                    | 35393        |
| 2   | Diazinon      | 2.44                    | 88758        |
| 3   | Chlorpyrifos  | 3.51                    | 80299        |
| 4   | Cypermethrin  | 4.35                    | 65314        |
| 5   | Parathion     | 5.18                    | 41999        |

Concentration of pesticide = \( \frac{\text{area of sample}}{\text{area of standard}} \times \text{conc. of standard} \times \text{dilution factor} \), sample mg/kg.
Determination of each part of fruits with pesticides:
After having the chromatography report by an HPLC instrument that records the Rt. Of each pesticide with detected and the area of samples depend on the retention time. Then making the calculation equation that previously mentions to get the concentration of residue on each part of fruits than compared the results of whole fruits with FAO/WHO Codex(2013). That shown in table (2, 3, 4) for each parts of fruits (peel, core and mixture).

Table 2. Effect of different pesticide residues in concentration (mg/kg) on different samples of the fruits that measured (Peel):

| Sample          | Pesticides  | LSD value |
|-----------------|-------------|-----------|
|                 | Malathion   | Diazion   | Chlorpyrifos | Cypermethrin | Parathion |
| Egypt orange    | 0.00        | 0.207 ↑   | 0.232        | 0.00         | 0.00      | 0.035 *  |
| African orange  | 0.00        | 0.00      | 0.609        | 0.00         | 0.00      | 0.156 *  |
| Kiwi            | 0.00        | 0.398     | 0.00         | 0.204 ↑      | 0.00      | 0.048 *  |
| Mango           | 0.00        | 0.00      | 0.00         | 0.00         | 0.00      | 0.074 *  |
| Plum fruit rough| 0.00        | 0.00      | 0.345        | 0.00         | 0.00      | 0.043 *  |
| Plum fruit smooth| 0.00      | 0.00      | 0.241        | 0.00         | 0.00      | 0.033 *  |
| Pears           | 0.00        | 0.00      | 0.00         | 0.493        | 0.00      | 0.103 *  |
| Pomegranate     | 0.00        | 0.00      | 0.00         | 0.509 ↑      | 0.00      | 0.075 *  |
| LSD value       | 0.00 NS     | 0.094 *   | 0.147 *      | 0.063 *      | 0.00 NS   | ----     |

* (P<0.05), NS: Non-significant. ↑: Above the MRL

Table 3. Effect of sample and pesticides in concentration mg/kg of pesticides residue on fruits that measured (Core):

| Sample            | Pesticides            | LSD value |
|-------------------|-----------------------|-----------|
|                   | Malathion             | Diazinon  | chlorpyrifos | Cypermethrin | Parathion |
| Egypt orange      | 0.00                  | 0.031 ↑   | 0.01         | 0.00         | 0.00      | 0.011 *  |
| African orange    | 0.00                  | 0.00      | 0.070        | 0.00         | 0.00      | 0.037 *  |
| Kiwi              | 0.00                  | 0.00      | 0.00         | 0.038 ↑      | 0.00      | 0.014 *  |
| Mango             | 0.00                  | 0.24      | 0.00         | 0.00         | 0.00      | 0.054 *  |
| Plum fruit rough  | 0.00                  | 0.00      | 0.031        | 0.00         | 0.00      | 0.014 *  |
| Plum fruit smooth | 0.00                  | 0.00      | 0.031        | 0.00         | 0.00      | 0.011 *  |
| Pears             | 0.00                  | 0.00      | 0.00         | 0.00         | 0.00      | 0.00 NS  |
| Pomegranate       | 0.00                  | 0.00      | 0.00         | 0.038        | 0.00      | 0.012 *  |
| LSD value         | 0.00 NS               | 0.063 *   | 0.022 *      | 0.015 *      | 0.00 NS   | ----     |

* (P<0.05), NS: Non-significant. ↑: Above the MRL
Table 4. Effect of sample and pesticides in concentration mg/kg of pesticides residue on fruits that measured (Mixture)

| Sample            | Pesticides             | LSD value |
|-------------------|------------------------|-----------|
|                   | Malathion | Diazinon | chlorpyrifos | Cypermethrin | Parathion |
| Egypt orange      | 0.00       | 0.099↑   | 0.031        | 0.00         | 0.00      | 0.036 *   |
| African orange    | 0.00       | 0.00     | 0.141        | 0.00         | 0.00      | 0.048 *   |
| Kiwi              | 0.00       | 0.00     | 0.00         | 0.537↑       | 0.00      | 0.129 *   |
| Mango             | 0.00       | 0.270    | 0.00         | 0.00         | 0.00      | 0.051 *   |
| Plum fruit rough  | 0.00       | 0.00     | 0.1334       | 0.00         | 0.00      | 0.042 *   |
| Plum fruit smooth | 0.00       | 0.00     | 0.198        | 0.00         | 0.00      | 0.066 *   |
| Pears             | 0.00       | 0.00     | 0.00         | 0.118        | 0.00      | 0.044 *   |
| Pomegranate       | 0.00       | 0.00     | 0.00         | 0.186↑       | 0.00      | 0.072 *   |
| LSD value         | 0.00 NS    | 0.0594 * | 0.047 *      | 0.107 *      | 0.00 NS   | ----       |

* (P<0.05), NS: Non-significant, ↑: Above the MRL

DISCUSSION

Our results showed that Malathion and Parathion were non-significant at any parts of fruits (peel, core and mixture).Diazinon was recorded on Egyptian orange in all parts (peel, core and mixture) at a concentration (0.207, 0.031, 0.099) mg/kg in tables 2-4 that the results were exceeding the limits of MRL (0.01, 0.031, 0.099) mg/kg respectively. That was different from (Gad Alla et al. 2013, 2015) who reported that no pesticides were detected in (peel, core, and mixture) at a concentration of (0.01 mg/kg). In Mango that we found Diazinon in (peel, core, and mixture) at concentration (0.398, 0.24, 0.270)mg/kg all these results were below the limits of MRL (1.3 mg/kg)While (Sivaperumal et al. 2015) exceeded the limits of Diazinon in mango at a concentration of (1.8mg/kg). Chlorpyrifos was detected in Egyptian orange in (peel, core, and mixture) at (0.232, 0.01, 0.031)mg/kg, all these results which were below the limits of MRL (1.0 mg/kg), That was admitted by (Gad Alla et al. 2013, 2015) also had detected Chlorpyrifos in Egyptian orange below the limits at a concentration of (0.06, 0.02) mg/kg respectively and disagreement with (Latif et al. 2011) that his results were above the limits of MRL at concentration (1.8 mg/kg). That's coinciding with (Faraget al. 2011) also found chlorpyrifos in orange at a concentration of (0.040 mg/kg.). In African orange that detected Chlorpyrifos in all parts (peel, core, and mixture) at (0.609, 0.070, 0.141) mg/kg these results were below the limits of MRL (1.0 mg/kg) that was Compatible with (Latif, et al. 2011) who detected chlorpyrifos also below the limits at (0.040 /kg). And Chlorpyrifos was shown in Plums (rough and smooth) at all parts (peel, core and mixture). In Rough plum at (0.345, 0.031, 0.1334) mg/kg and in Smooth plum at concentration (0.241, 0.031, 0.198) mg/kg. all these results were below the limits of MRL (0.5 mg/kg). Our results were similar with (Syed et al., 2014) who detected Chlorpyrifos in plum at (0.013 mg/kg) also below the limits.

Cypermethrin which also detected in all parts of Kiwi at concentration (0.204, 0.038, 0.537) mg/kg. Only in core was below the limits of MRL (0.07 mg/kg) but its over the limits in peel and mixture of Kiwi. Cypermethrin also found in (peel and Mixture) of Pears within limits of MRL (0.7 mg/kg) at concentration (0.493, 0.118) mg/kg. not detected in core, Our results agreed with (Gad Alla et al. 2013 and Bempah et al. 2011). They found Cypermethrin in pears within the limits at a concentration of (0.07, 0.008) mg/kg, respectively. And Cypermethrin also detected on Pomegranate in all parts (peel, core and mixture) at concentration (0.509, 0.038, 0.189) mg/kg. That exceeding the limits of MRL (0.05 mg/kg) in both (peel and mixture). Our results are different from (Savant et al., 2010) who reported that no pesticides were detected in Pomegranate.

CONCLUSION

The results of the present study indicated that samples of imported fruits that had been analyzed in the laboratory contained multi-residue of pesticides, some of them were exceeding the maximum limits allowed for residues. The reason for that could be attributed to the presence of other pesticide groups which were used in the origin countries and not included in this experiment. There is an urgent need to establish quality control laboratory equipped with some more advanced instruments such as Gas chromatography Sectorometry (GC-MS) and HPLC-MS to check all agriculture commodities imported to Iraq and to establish a local guide for the post-harvest period and the permissible level for each pesticide group.
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