INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

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EVALUATION OF SOME PESTICIDE RESIDUES IN IMPORTED FRUITS IN IRAQ BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT
Pesticide residues have been found in various fruits and vegetables. The study 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 through divided the fruits to some parts (peel , core and mixture) for each type of fruits markets as: Oranges (Egypt, Africa), Pomegranate (Egypt), Mango (Kenya), Pears (China), Plum fruits (Africa), Kiwi (Turkey). For detection of 5 different pesticides (diazinon, malathion, chlorpyrifos, parathion and cypermethrin) . The results detected multi-residues of pesticides on the fruits in (peel, core, mixture) may be in the limit of MRL (Maximum residue limits) or over of it of FAO/WHO codex. The pesticides detected were Cypermethrin between 0.01-1.038 mg/kg , Chloropyrofis between 0.01- 0.609 mg/kg , Malathion as 0.346mg/kg , Diazinon between 0.013-0.63 mg/kg , while Parathion was not detected at any type of fruits. In conclusions, it is very imperative to monitor the residues in imported to reduce the human exposure risks.

KEYWORDS: Pesticides, HPLC, diazinon, malathion, chlorpyrifos, parathion, cypermethrin.

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 vegetables in our markets and may be in lower prices than our crops; from different countries FAO/WHO codex (MRL, 2013). 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 act to modify the metabolic activation and detoxification / disposition of carcinogens, or even influence the 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 *et al*., 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 has 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 compounds are insecticides, although there are also a number pest control operators who use (OP) every day in their work (Chloride, 2013).

The work activities involving organophosphorous pesticides (OP) which require special attention when assessing exposure include:

1-Manufacture and packaging
2-Transport, storage and distribution
3-Handling used containers, for example, in scrap recovery
4-Agricultural and horticultural activities like mixing, loading and applications where direct handling of the chemical occurs.
5-Veterinary activities like cattle and sheep diving

The use of pesticides is widespread in fruit production for pre and post-harvest protection and many chemical substances may be applied in order to control undesirable mold or insects. A survey was carried out to evaluate levels of pesticide residues in fruit (Ortelli *et al.*, 2005), that numerous post-harvest treatments, including dipping and treatment with a water-emulsion wax containing fungicide is extensively used for preventing moisture loss during storage, shipment and marketing. As many pesticides are designed to inhibit various enzymes within insects and other pests, utilizing these enzymes for detection purposes seemed a logical route. In this manner, enzymes such as acetylcholinesterase, but cholinesterase and others were investigated for their ability to detect pesticides in the environment (Marrazza, 2014). For the identification and quantification of pesticides is generally

based on a high-performance liquid chromatography (HPLC) (Marrazza, 2014).

For this reason this study focusing on the detection of the residues of some pesticides which have major used in an agriculture in some kind of fruits import to Iraq.

MATERIALS & METHODS

Samples Collection

The Imported fruits from different countries were collected from different markets in Baghdad-Iraq, at different time. After collecting the fruits were washed with deionized water three times to clean them from dust. These fruits are:-

1. Plum fruits from South Africa. *Prunus Africana* (Canningham and Mbenkum,1993)
2. Pomegranate from Eygpt. *Punica granatum* (Taufer *et al.*, 2012)
3. Kiwi fruits from Turkey *Actinidia deliciosa (Ferguson,1999)*
4. Orange fruits from Egypt and South Africa. *Citrus reticulate* (USDA, 2011*)*
5. Mango from Kenya (South Africa). *Mangifera indica (Morton,1987)*
6. Pears from China. *Pyrus pyrifolia* (Baily *et al.*, 1976).

Chemicals &Solvents

Standard solutions of pesticides are Parathion, Malathion, Diazinon, Cypermethrin, and Chlorpyrifos, while the solvent are Acetonitrile, Deionize water, anhydrous sodium sulphate and Ethyle acetate. All chemicals and solvents are grad-HPLC and obtained from Sigma–Aldrich Company (Germany) by OMA International Scientific Office in Baghdad city.

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 standared of any substances from 5ppm then 10ml were transferred in to volumetric flask of 50 ml and diluted with acetonitrile and to prepare 0.5 ppm from 25ppm ; 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 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 (50g) 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 (5ml) of acetonitrile (MeCN) and finally the volume was reduced to about (0.5ml) using stream of liquid nitrogen (Islam *et al.*, 2009). The extract was then transferred to a graduated test tube and the final volume was adjusted at exactly (1ml) 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 Deionzied water : solvent B was acetonitrile (70: 30,v/v).

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

Statistical Analysis

Statistical analysis was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA with least significant differences (LSD) post hoc test was performed to assess significant difference among means. $P < 0.05$ was considered statistically significant.

RESULTS

The compounds were identified by comparing its retention time with respect to technical grade reference standard. The quantitative determination was carried out with the help of achromatographic curve drawn from chromatographic experiments with standard solution. For quantification an external calibration 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 explain in table (1) and illustrated in figure (A):

TABLE 1: shown the set of standard solutions of pesticides each standard was measured at concentration 0. 5 mg/kg. to calculated with fruits

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

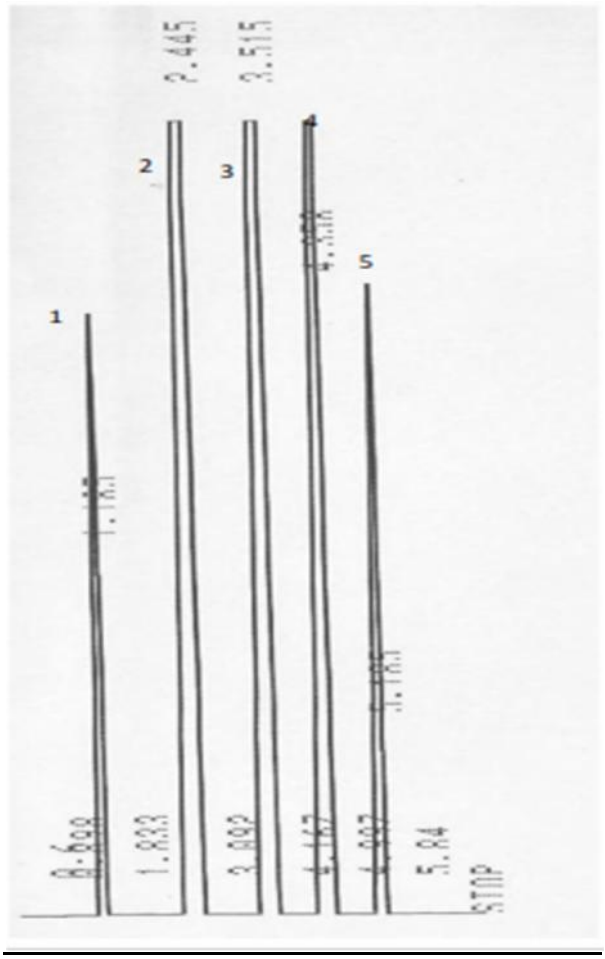

FIGURE A: shown the chromatographic curve of pesticides standard at concentration 0.5mg/kg.. And in the same condition .

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 Chloropyrifos.

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

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

Determination of Pesticides

After selecting the optimum working parameters as previously described, a series of standard solutions are prepared using deionized water and acetonitrile the peak area measurements are obtained as a function of different concentration of standard pesticides. The calibration curve shows also, the analytical features of this method demonstrate a typical regression as follows:

Concentration of = (area of sample)/ (area of standard) × conc. of standard × 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 retention time, Rt. Of each pesticide with detected and the area of samples depend on the (RT). Then making the calculation equation that previously mentioned 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, 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	0.00	0.00	0.204 ↑	0.00	0.048 *
Mango	0.00	0.398	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, 031, 0.099) mg/kg respectively. That was different from (Gad Alla *et al.*, 2015) that they detected Diazinon within limits at concentration (0.01 mg/kg). In Mango that we found Diazinon in (peel, core, mixture) at concentration (0.398, 0.24, 0.270) mg/kg., all these results were below the limits of MRL (1.3mg/kg) While (Sivaperumal *et al.*, 2015) exceeded the limits of Diazinon in mango at a concentration of (1.8mg/kg). Chloropyrifos was detected in Egyptian orange in (peel, core, 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 Chloropyrifos 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 (Farag *et al*. (2011) also found chloropyrifos in orange at a concentration of (0.040 mg/kg.). In African orange that detected Chloropyrifos in all parts (peel, core, 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 chloropyifos also below the limits at (0.040 /kg), and Chloropyrifos 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 Chloropyrifos 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 (0.07mg/kg) but it's 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. and 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. 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 Sectrometry (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.

ACKNOWLEDGEMENT: Researcher gratefully acknowledges Assist. Prof. Dr. Ali Sh. Sultan for his advice and critical reading of the manuscript.

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